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Genetic and Biochemical Characterization of the Galactose Gene Cluster in *Kluyveromyces lactis*

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We isolated and identified mutant strains of *Kluyveromyces lactis* that are defective for the Leloir pathway enzymes galactokinase, transferase, and epimerase, and we termed these loci *GAL1*, *GAL7*, and *GAL10*, respectively. Genetic data indicate that these three genes are tightly linked, having an apparent order of *GAL7-GAL10-GAL1*. This same gene order has been observed in *Saccharomyces cerevisiae*. Strains harboring *gal7* mutations have elevated levels of β-galactosidase, coded by an unlinked gene, galactokinase, and epimerase activities under uninduced conditions. We investigated the genetic basis of this constitutive gene expression and found no recombinants between the constitutive and Gal⁻ phenotypes among 76 tetrads, suggesting that either *GAL7* or a tightly linked gene codes for a regulatory function. This is the second gene that has been shown to specifically coregulate expression of the genes coding for β-galactosidase and the Leloir pathway enzymes.

The yeast *Kluyveromyces lactis* is able to use lactose or galactose as its sole carbon source. Lactose utilization is accomplished by inducing both lactose transport and an intracellular β-galactosidase (EC 3.2.1.23) more than 100-fold above a moderate basal level (10, 11). Galactose is catabolized by inducing enzymes of the Leloir pathway: ATP-α-D-galactose-1-phosphotransferase ("galactokinase," EC 2.7.1.6), uridine diphosphoglucose-D-galactose-1-phosphate uridylyltransferase, ("transferase," EC 2.7.7.10), and uridine diphosphogalactose-4-epimerase ("epimerase," EC 5.1.3.2). The structural gene for β-galactosidase has been identified and designated *LAC4* (34), but the genes for the Leloir enzyme activities are not known. The structural genes for lactose and galactose utilization appear to be under a common regulatory circuit. For example, strains harboring a *lac10* defect are constitutive for lactose transport, β-galactosidase, and the Leloir enzymes (12), whereas strains harboring a *lac9* defect are uninducible for all of these enzymes (unpublished data).

The discovery of a common regulatory circuit for both lactose and galactose utilization has prompted a search for mutants defective in the remaining structural genes. Many mutants unable to grow on lactose, designated Lac⁻, have previously been isolated (33). They define seven complementation groups designated *LAC3* through *LAC9*. Mutants having mutations in several of these loci, designated Gal⁻, were also unable to grow on galactose, raising the possibility that some may be defective in one of the Leloir enzymes. The present report provides evidence that *LAC5* and *LAC8* as well as a new locus, *LAC11*, code for transferase, epimerase, and galactokinase, respectively. These genes were found to be tightly linked. Because of similarities to the galactose gene cluster in *Saccharomyces cerevisiae*, the *K. lactis* genes *LAC5*, *LAC8*, and *LAC11* have been renamed *GAL7*, *GAL10*, and *GAL1*, respectively.

MATERIALS AND METHODS

Strains. Strains were derived from wild-type strain Y1140 (**a** *lac1 LAC2*) as previously discussed or as UV-induced revertants isolated in this study (33; Table 1). Revertants were selected from cultures that had been grown to stationary phase in YMPD (see below) after irradiation to ca. 70% survival with two germicidal lamps (Sylvania G15T8). Revertants of independent origin only were studied.

Media. The complex medium YMPD contained 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1.0% dextrose. Minimal medium contained double-strength yeast nitrogen base without amino acids (Difco Laboratories and 1% carbon source (dextrose, galactose, or lactose). Minimal medium was supplemented with 10 μg of adenine, uracil, methionine, tryptophan, histidine, or lysine per ml as necessary. Media were made solid by adding 1.5% agar. The medium used for mating and sporulation was ME, consisting of 5.0% malt extract and 3% agar.

Preparation of cell-free extracts. Overnight cultures were diluted into 50 ml of YMPD and grown from an absorbance of 0.5 to 2 at 600 nm. They were centrifuged and suspended in 2 ml of chilled buffer (4°C) containing 50 mM sodium phosphate at pH 7.5, 5% glycerol, 1 mM EDTA, and 1 mM phenylmethane-sulfonyl fluoride (added just before use). Glass beads (Glasperlen, 1 ml of 0.5-mm diameter; VWR Scientific Inc.) were added, and cells were broken by vortexing vigorously for eight 30-s pulses, with chilling to 4°C between pulses. Debris was pelleted by centrifugation at 10,000 × *g* for 20 min, and the supernatant fluid was decanted, stored at 4°C, and used within 1 week for enzyme assays.

Enzyme assays. β-Galactosidase activity and alkaline phosphatase activity were measured by using chromogenic substrates as previously described (12, 33). A radiometric assay was used to measure galactokinase activity (1, 5). Galactose-1-phosphate uridylyltransferase activity, uridine diphosphogalactose-4-epimerase activity, and phosphoglucomutase activity were determined by monitoring reactions coupled to an appropriate dehydrogenase and by measuring the initial rate of NADPH or NADP production at 340 mm (5, 12, 32). Assays for transferase and epimerase activity have been modified to include a 5-min preincubation of cell-free extract at room temperature with reaction mixture, which lacks galactose-1-phosphate, for the transferase assay, or UDP-galactose, for the epimerase assay. Assays were then initiated by adding these substrates to the reaction mixture. Transferase and epimerase activity assays were

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TABLE 1. Yeast strains

Strain	Genotype[a]
Y1140	a wild type
2A610	α *ade1-1*
AS1D	a *gal7-10 ade1-1*
AS44	a *gal7-11ts ade1-1*
AS128	α *gal7-25 ade1-1*
MS12	a *gal10-1 met1-1*
MS15	α *gal10-5 lys1-1*
5R115	a *gal7-10 gal1-115 ade1-1*
5R191	a *gal7-10 gal1-191 ade1-1*
5R209	a *gal7-10 gal1-209 ade1-1*
5R176	a *gal7-10 gal1-176 ade1-1*
5R199	a *gal7-10 gal1-199 ade1-1*
5R216	a *gal7-10 gal1-216 ade1-1*
8R29	a *gal10-1 gal1-329 met1-1*
8R39	a *gal10-1 gal1-339 met1-1*
8R165	a *gal10-1 gal1-465 met1-1*
22A295	α *gal1-209 ade1-1 trp1-1*
5C170	α *his2-1 trp1-1 ura1-1*
9D170	α *his2-1 trp1-1 ura1-1*
3B212	a *ade3-1 met2-2*
3C212	a *ade3 met2-2 trp1*
10A212	α *ade3-1 met2-2*
11B170	α *trp1-1 ura1-1*

[a] *gal7* and *gal10* were previously designated *lac5* and *lac8*, respectively (33).

conducted at 22°C, whereas all other assays were conducted at 30°C. The protein concentrations of cell-free extracts were determined by the procedure of Lowry et al. (21) with ovalbumin as a standard. Specific activities are expressed as nanomoles per minute per milligram of protein.

Thermal inactivation of transferase activity. Cell-free extracts were heated at 44°C in closed tubes in a water bath while controls were kept at 4°C. After various times of heating, samples were removed, cooled to 4°C, and assayed for activity within 30 min.

Analysis of Leloir intermediates. YMPD cultures were inoculated and grown for ca. 16 h. After achieving an absorbance of 1 at 600 nm, they were harvested by centrifugation and suspended in 25 ml of YMPD (three-fourths strength) containing 7 mM barbiturate acetate buffer (7 mM sodium acetate; 7 mM sodium barbiturate; 29 mM NaCl, pH 4.7), 40 mM D-galactose, and 31 μCi of D-[1-^{14}C]galactose (45 mCi/mmol; Research Products International) incubated for 1 h at 30°C. Cells were harvested by filtration (polycarbonate, 0.4-μm pore size by 25 mm; Nuclepore Corp.), washed three times with 10 ml of 0.02% sodium azide in buffer (7 g of $Na_2HPO_4 \cdot 7H_2O$, 4 g of NaCl, and 3 g of KH_2PO_4 per liter), and suspended in 5 ml of 0.02% sodium azide. Extracts were made by adding 12.5 ml of ethanol and heating at 70°C for 30 min. The supernatant fluid was decanted after centrifugation, concentrated to about 0.2 ml by flash evaporation, diluted into 2.5 ml of water, and loaded on a 1-ml anion exchange column (AG1-X2, Bio-Rad Laboratories) to separate sugars according to charge. The neutral sugars (galactose) were in the void volume. Monophosphorylated sugars (galactose-1-phosphate) were eluted with 0.2 M triethylammonium formate in 5 N formic acid, and diphosphorylated sugars (UDP-galactose) were eluted with 2 M triethylammonium formate adjusted to pH 1 with formic acid. These three fractions were concentrated by flash evaporation. The neutral fraction was examined by descending chromatography on Whatman 3MM paper with butanol-ethanol-H_2O (40:11:19) (15) for 48 h at 22°C. Galactose, glucose, and lactose were standards chromatographed simultaneously and detected with diphenylamine stain (31). The monophosphate fraction was electrophoresed on EDTA-treated Whatman 3MM paper at 18 V/cm in 0.1 M acetyltrimethylammonium borate (pH 9.8) for 4 h at 22°C (30). Galactose-1-phosphate, glucose-1-phosphate, and glucose-6-phosphate were used as standards. They were detected with acid molybdate stain (4). The diphosphate fraction was chromatographed (descending) on EDTA-treated Whatman 3MM paper in 0.5 M morpholinium tetraborate–methyl ethyl ketone–ethanol (30:20:70, pH 8.6) containing 1 mM EDTA for 48 h at 22°C (6). UDP-galactose and UDP-glucose were used as standards, and they were detected by UV light. Lanes containing the labeled samples were cut into 1-cm pieces and counted by liquid scintillation spectroscopy. Data were plotted as nanomoles of compound per milligram of cell protein. Protein was assayed by the method of Stewart (35) in samples taken before the 70%-ethanol extraction. Ovalbumin was used as the standard.

Genetic analysis. Haploid strains were mated on ME by mixing equal amounts of 24-h-old cells grown on YMPD at 22°C (original mating plate). Each haploid strain had one or more unique auxotrophic markers. After 2 to 3 days of incubation at 22°C, diploids were selected for marker complementation by replica plating on unsupplemented minimal dextrose medium and allowed to grow for 2 days at 22°C. By simultaneously replica plating on minimal lactose or galactose media, we obtained complementation data for the Lac and Gal phenotypes. (Note: extended growth on selective medium frequently results in the appearance of diploids which lose some or all their auxotropic or lactose markers. Thus complementation analysis is always conducted with newly constructed diploids.) Diploids were induced to sporulate by subsequent replica plating onto ME and by incubation for 3 to 5 days at 22°C (asci also formed on minimal medium and the original mating plate owing to the transitory diploid phase). Mutations were mapped by standard tetrad analysis (26).

RESULTS

Enzyme activities in mutant strains. *S. cerevisiae* strains defective in either transferase or epimerase activity are sensitive to galactose (Gal^s), since they are unable to grow in the presence of galactose even though another utilizable carbon source is available (13). Presumably, sensitivity is due to a buildup of galactose-1-phosphate, the product of the galactokinase reaction, and subsequent inhibition of some essential metabolic step. Previously isolated mutant strains of *K. lactis* have the Gal^s phenotype (33), and we have, therefore, examined their Leloir enzyme activities (Table 2). Two allelic *gal7* strains exhibited no transferase activity, and the temperature-sensitive strain had reduced transferase activity; they all had nearly normal induced activities for the remaining enzymes. The two allelic *gal10* stains lacked only epimerase activity. Alkaline phosphatase activity was used as a control because it is not affected by the addition of inducer. These results suggest that *GAL7* codes for transferase, and *GAL10* codes for epimerase.

The data in Table 2 indicate that the basal levels of β-galactosidase, galactokinase, and epimerase have increased in *gal7*-defective strains, indicating that the strains have a constitutive phenotype. The constitutive phenotype could be mediated through a buildup of galactose-1-phosphate or some other metabolite, which acts as an inducer (9). However, this does not appear to be the case since double mutants defective in both kinase and transferase (strains 5R115,

TABLE 2. Enzyme activities[a] in strains defective in *gal7* and *gal10*

Strain	*gal* genotype	No. of determinations	β-Galactosidase	Galactokinase	Transferase	Epimerase	Phosphoglucomutase	Alkaline phosphatase
Y1140	Wild type	7	2,340	46	50	6.0	97	51
			407	9.8	6.4	1.2	91	45
AS1D	*gal7-10*	5	1,934	36	0.0	3.4	135	61
			2,375	39	0.0	6.1	110	32
AS44	*gal7-11*ts	2	1,713	54	4.8	6.1	140	28
			1,267	33	1.6	7.7	123	42
AS128	*gal7-25*	1	2,869	44	0.0	5.0	183	60
			4,223	78	0.0	8.3	137	36
MS12	*gal10-1*	6	741	18	21	0.0[b]	133	28
			343	7.2	4.2	0.0	128	46
MS15	*gal10-5*	2	1,738	26	30	0.0[b]	112	60
			927	9.6	29	0.0	84	32

[a] Specific enzyme activities (nanomoles per minute per milligram) were determined on cell-free extracts prepared from log-phase cells grown on YMPD (20 mM glucose) medium at 36°C. The value above the bar is for cells induced by the addition of 40 mM galactose for two doublings (4 to 6 h of growth), whereas the value below the line is for uninduced cells. Typically the values had a 20 to 30% standard deviation.

[b] These values are below the detection limit of 0.2 nmol/min per mg.

5R209, 5R176, 5R191, 5R199, and 5R216) block the production of galactose-1-phosphate but remain constitutive (Table 3). It is not clear yet whether the constitutive phenotype is due to a mutation in *gal7* or in a closely linked regulatory gene.

The data in Table 2 also show that strains defective in *gal10* (MS12 and MS15) are not induced as fully as wild-type strains and that enzyme activities in strains defective in *gal7* (AS1D, AS44, and AS128), which are initially constitutive, fall somewhat after the addition of exogenous inducer. These strains fail to divide after lactose or galactose is added to the medium. The reason for low enzyme activities under induced conditions is not clear, but several phenomenon, including reduced protein synthesis and protein turnover, could be involved.

Strains defective in the other *lac* loci, *lac3*, *lac6*, and *lac7*, possess normal uninduced and induced levels of the enzyme activities listed in Table 2 (data not shown), and the nature of their defects remains unknown. Thus, no mutants defining the galactokinase and phosphoglucomutase loci were found among our original Lac$^-$ strains (33). As shown previously (12), phosphoglucomutase activity is not induced in wild-type cells, nor is it constitutive in *gal7* strains, implying that it is not regulated by galactose.

Thermostability of transferase activity in revertants of gal7. Mutants defective in structural genes and their revertants frequently produce proteins with physical properties that are distinguishable from those of wild-type strains. To substantiate further our claim that *GAL7* codes for transferase, we isolated UV-induced Gal$^+$ revertants of *gal7-10* and examined them for a change in the thermostability of their transferase activity. Two revertants, 5R31 and 5R51, contained transferase activity with greatly reduced thermostability (Fig. 1). To show that these two revertants were specific for transferase activity, the thermostability of their β-galactosidase activity was measured and was shown to be identical to that of wild-type strains (data not shown). When the revertants were backcrossed to Lac$^+$ strains, no recombinants were found in 30 tetrads. Failure to find recombinants suggests that the revertants are due to a second site mutation within the transferase gene, in addition to the original *gal7* mutation. These data along with those from the previous section strongly imply that *GAL7* codes for transferase. We cannot, however, rule out the possibility that this locus codes for an enzyme that modifies transferase activity.

UV-induced Gal$^+$ revertants of *gal10-1* were also isolated and examined for a change in the thermostability of their epimerase activity. Because of their low levels of epimerase activity, only revertants with a large alteration in thermostability would be significant, and thus far, only revertants with a slight reduction in thermostability have been found.

Analysis of Leloir intermediates. Strains defective in transferase or epimerase activity were expected to have larger pools of galactose-1-phosphate and UDP-galactose, respectively, relative to those of wild-type strains. To test these expectations, log-phase cells were labeled for 1 h with [1-^{14}C]galactose, and sugars were extracted with 70% ethanol and analyzed as described above. The major compound detected in the neutral fraction (Fig. 2A) from wild-type cells (Y1140) comigrated with galactose; larger amounts of it were found in both mutant strains (AS1D *gal7-10* and MS12 *gal10-1*).

In the monophosphate fraction (Fig. 2B), no detectable galactose-1-phosphate was found in wild-type cells. When this fraction was electrophoresed for less time, several rapidly migrating peaks were observed (data not shown). Presumably these unidentified labeled compounds were monophosphate sugars made after the Leloir pathway. These peaks were not detected in mutant strains. A very large peak (Fig. 2B, right-hand scale) that comigrated with galactose-1-phosphate was found in the *gal7* strain. There also appeared to be a small buildup of galactose-1-phosphate in the *gal10* strain.

In the diphosphate fraction (Fig. 2C), no material with a mobility of UDP-galactose or UDP-glucose was found in wild-type cells, but small amounts were present in mutant

TABLE 3. Enzyme activities in *Gal*^{r} revertants

Strain	*gal* genotype	β-Galacto-sidase	Galacto-kinase	Trans-ferase	Epimerase	Alkaline phos-phatase
Y1140	Wild type	2,119[a]	53	122	1.7	33
		217	3.3	3.4	0.7	26
5R115	*gal7-10 gal1-115*	2,947	1.0	0.0	3.6	19
		1,450	0.2	0.0	3.3	30
5R191	*gal7-10 gal1-191*	968	0.9	0.0	0.7	37
		1,131	0.0	0.0	2.8	31
5R209	*gal7-10 gal1-209*	3,051	0.6	0.0	4.0	24
		1,583	0.0	0.0	3.4	27
5R176	*gal7-10 gal1-176*	3,438	0.3	0.0	3.3	24
		3,727	0.1	0.0	6.9	18
5R199	*gal7-10 gal1-199*	3,369	1.1	0.0	3.3	44
		5,516	0.9	0.0	5.1	28
5R216	*gal7-10 gal1-216*	3,335	0.0	0.0	5.2	23
		3,189	0.0	0.0	5.0	21
8R29	*gal10-1 gal1-329*	2,106	3.8	56.5	0.0[b]	23
		169	0.2	3.5	0.0	42
8R39	*gal10-1 gal1-339*	2,178	0.5	57.5	0.0[b]	27
		161	0.05	4.1	0.0	45
8R165	*gal10-1 gal1-465*	1,369	0.4	44.6	0.0[b]	66
		120	2.6	2.7	0.0	33
22A295	*gal1-209*	3,006	0.2	53.6	3.9	45
		69	0.2	4.5	1.1	52

[a] Specific enzyme activities (nanomoles per minute per milligram) were determined on cell-free extracts prepared from log-phase cells grown on YMPD (20 mM glucose) medium at 36°C. The value above the bar is for cells induced by the addition of 40 mM galactose for 4 h, whereas the value below the bar is for uninduced cells.

[b] These values are below the detection limit of 0.2 nmol/min per mg.

strains. The major compound labeled in the *gal10* strain comigrated with UDP-galactose. Smaller amounts of UDP-galactose and UDP-glucose appeared in the *gal7* strain, presumably owing to partial transferase activity.

In summary, wild-type cells have undetectable pools of galactose-1-phosphate and UDP-galactose. The major Leloir intermediate accumulated in a *gal7*-defective strain was galactose-1-phosphate, whereas in a *gal10*-defective strain, UDP-galactose accumulated. These data support our hypothesis that *gal7* strains are defective in transferase activity and that *gal10* strains are defective in epimerase activity since the intermediate accumulated in each strain is the substrate for the missing enzyme activity.

Isolation of mutants defective in galactokinase activity. Mutants defective in galactokinase should be selectable according to the following considerations. Sensitivity to galactose (Gal^s) in transferase or epimerase mutants presumably acts through the accumulation of galactose-1-phosphate, the product of galactokinase. Consequently, a mutant defective in either transferase or epimerase activity could become resistant to galactose (Gal^r) by losing its galactokinase activity. In *S. cerevisiae*, the predominant class of Gal^r revertants selected from epimerase-minus strains are those defective in galactokinase activity (13).

When UV-induced Gal^r revertants were isolated from *gal7* or *gal10* mutant strains of *K. lactis*, the major class had reduced galactokinase activity in addition to their original lack of transferase or epimerase activity (Table 3). Since other inducible enzymes were unaffected in Gal^r revertants, the loss of galactokinase activity is most likely owing to a mutation in the galactokinase gene, designated *GAL1*, rather than to a mutation in a gene that regulates enzyme induction.

To determine whether *GAL1* defines a new locus, complementation analysis was performed. The original Gal^r reverants were first backcrossed to Gal$^+$ strains to separate the original mutation from that in *gal1*. A recombinant spore bearing only the galactokinase defect, 22A295 (Table 3), was used as a source for mating to mutants in other loci, particularly *gal7* and *gal10*, and diploids were then tested for their ability to grow on galactose. The results indicate that *gal1* complemented *gal7* and *gal10* and represented a new gene.

Linkage among *GAL7*, *GAL10*, and *GAL1*. Tetrad analysis based on the Gal phenotype revealed tight genetic linkage among *GAL1*, *GAL7*, and *GAL10* (Table 4). These data have not been corrected for gene conversion because not all gene conversions are equally detectable. No wild-type recombinants were found between *gal7* and *gal10* in 196 tetrads,

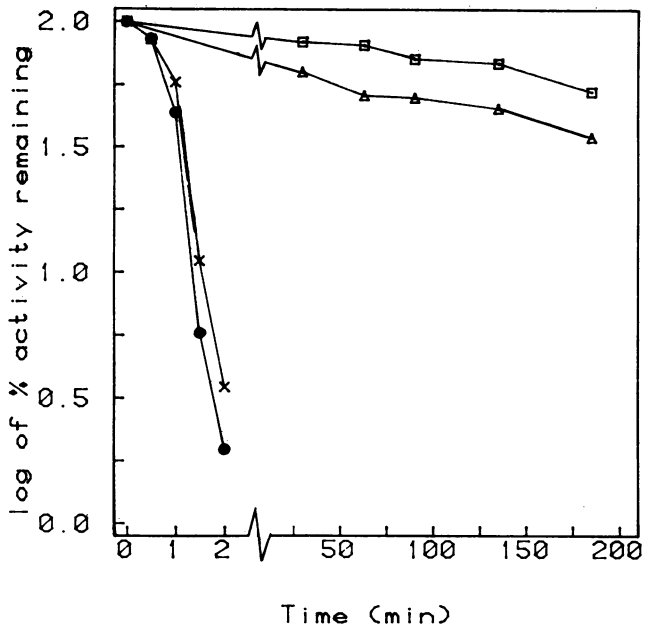

FIG. 1. Thermostability of transferase activity. The loss of transferase activity in cell-free extracts upon heating at 44°C was determined as described in the text. Symbols: wild-type strains Y1140 (△) and 2A610 (□); revertant strains 5R31 (x) and 5R51 (●).

indicating tight linkage of less than 0.3 cM (map distance was calculated by the method of Perkins [30]). *Gal7* maps 1.8 cM and *gal10* maps 1.5 cM from *gal1*. These data favor a gene order of *gal7-gal10-gal1*. The orientation of these genes relative to the centromere is unknown.

DISCUSSION

Data supporting the hypothesis that *GAL1*, *GAL7*, and *GAL10* code for the Leloir enzymes galactokinase, transferase, and epimerase, respectively, are summarized below. (i) When examined for Leloir enzymatic activities, *gal7*-defective strains lacked only transferase activity, *gal10*-defective strains lacked only epimerase activity, and *gal1*-defective strains lacked galactokinase activity. (ii) Mutants bearing *gal7* or *gal10* defects are sensitive to galactose, Gal^s, a characteristic of strains defective in transferase and epimerase activities in *S. cerevisiae* (13), *Escherichia coli* (19, 39), and *Salmonella* (14, 27). (iii) Two Gal^r revertants of *gal7* have transferase activity with reduced thermostability. (iv) Analysis of Leloir intermediates revealed a buildup of galactose-1-phosphate in *gal7* strains and of UDP-galactose in *gal10* strains, as expected.

Tetrad analyses indicate that *GAL1*, *GAL7*, and *GAL10* are tightly linked and form a gene cluster that is unlinked to other known *lac* loci. The gene order appears to be *gal10-gal7-gal1*. However, this order is not firmly established by our data because gene conversions have not been excluded from tetrad data, and there is no statistical significance in the number of recombinants in crosses involving *gal1* and *gal7* or *gal1* and *gal10*. Differences in the genetic background of these strains could easily account for the differences in the number of recombinants. Numerous tetrads from a three-point cross or extensive fine-structure mapping are needed to rigorously establish gene order by conventional genetic methods. Alternatively, a physical map of the region is now being determined by using a cloned DNA fragment (Webster and Dickson, unpublished data).

The clustering of genes raises the possibility that they form an operon. However, no polycistronic mRNAs have been demonstrated in yeasts (23). Instead, gene clusters are commonly explained by a single gene coding for a polyfunctional protein (e.g., *his4ABC* [3], *ade3* [17, 28], *trp5* [22], and *dur1,2* [8]). Alternatively, gene clusters can be composed of linked genes that each produce a discrete polypeptide (e.g., *gal7-gal10-gal1* (36, 37) and *dal1-dal4-dal2* [7]). Based upon these results and the following evidence, we hypothesize that the *K. lactis* galactose cluster consists of three genes rather than a single gene encoding a polyfunctional protein. No polar mutations defective for two or more Leloir activities have been found in at least six mutants from each gene, as would be expected for a polyfunctional gene. A polyfunctional peptide defective in one domain might alter physical properties in another domain. However, the Leloir enzyme present in *gal1*, *gal7*, or *gal10* strain has galactokinase, transferase, and epimerase activities that are as thermostable as those in wild-type strains. Moreover, in the case of two *gal7* revertants, whose transferase thermostability is grossly reduced, the galactokinase thermostability behaves like that in wild-type strains.

Strains harboring a *gal7* mutation, besides being defective in transferase activity are constitutive for the other lactose-inducible enzyme activities. This regulatory effect is not mediated via galactose-1-phosphate because *gal1-gal7* double mutants remain constitutive (Table 3). No segregation has been observed between the constitutive and the Gal^- Gal^s phenotypes in 76 tetrads examined, indicating close genetic linkage between these phenotypes. It is not clear whether *GAL7* codes for a polyfunctional protein possessing both a transferase and a regulatory function or whether the two functions reside in separate genes. To resolve this issue, the cloned galactose gene cluster is being subcloned to see if the constitutive and Gal^- Gal^s phenotypes can be physically separated (Webster and Dickson, unpublished data).

The yeast *S. cerevisiae* is not closely related to *K. lactis*. For example, they cannot mate, and they utilize unique carbon sources (38). Yet, both yeasts have a gene cluster coding for the Leloir enzymes. In *S. cerevisiae* the gene order is centromere-*gal7-gal10-gal1* (2, 36), and each gene produces a unique transcript (37). Thus, the clustering and ordering of the galactose genes appears to have been conserved either from a common ancestor or by coevolution in these two yeasts. It will be interesting to determine if the coding and regulatory portions of these genes have been conserved also. A detailed comparison can begin since the *S. cerevisiae* cluster (36) and the *K. lactis* cluster (Webster and Dickson, unpublished data) have been cloned.

Besides the structural similarity of the galactose gene clusters, there are regulatory features which are similar between these two yeasts. In *S. cerevisiae*, the galactose gene cluster is under the control of a positive regulatory element coded by *GAL4* and a negative regulatory element coded by *GAL80* (13). In *K. lactis*, the galactose gene cluster is under the control of a positive regulatory element, *LAC10* (12), and a negative regulatory element, *LAC9* (unpublished data). In *S. cerevisiae GAL4* and *GAL80* also coregulate *MEL1*, which codes for an α-galactosidase and allows growth on melibiose (18). In *K. lactis*, *LAC10* and *LAC9* regulate lactose transport (10) and *LAC4*, which codes for β-galactosidase (12; unpublished data). Both yeasts can be induced with galactose or the appropriate disaccharide, melibiose for *S. cerevisiae* or lactose for *K. lactis*. It will be

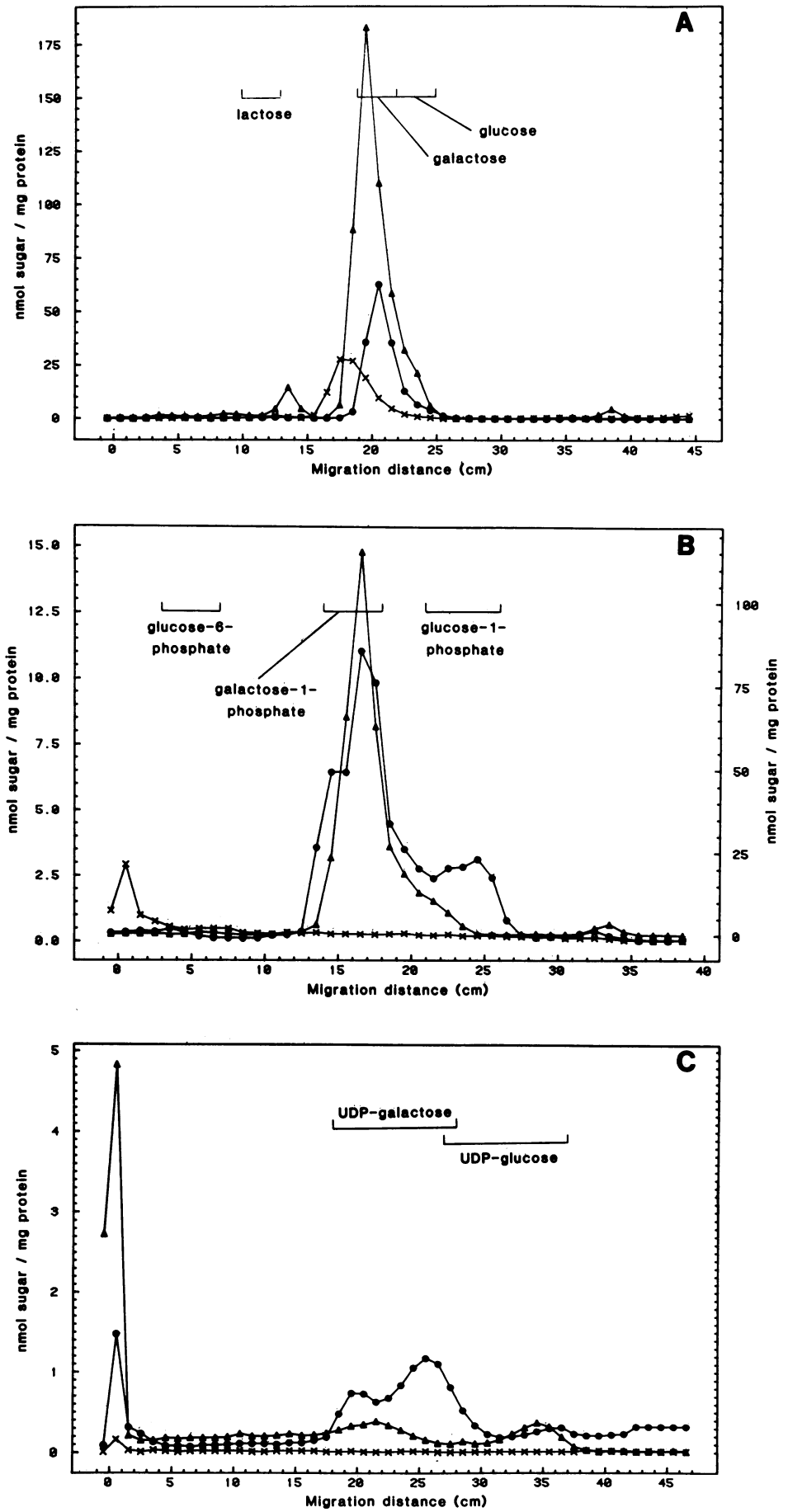

FIG. 2. Analysis of Leloir intermediates in mutant strains. (A) Chromatogram of the neutral sugars. (B) Electrophoresis of the monophosphorylated sugars. The right scale is for AS1D. (C) Chromatogram of the diphosphorylated sugars. Symbols: wild-type Y1140 (x), *gal7* mutant strains AS1D (▲), *gal10* mutant strain MS12 (●).

TABLE 4. Linkage of *GAL7*, *GAL10*, and *GAL1*

Cross	Tetrad frequency[a]			Map distance[b] (cm)
	PD	T	NPD	
gal7-10 × *gal10-1*	162	0	0	
gal7-10 × *gal10-5*	20	0	0	
gal7-25 × *gal10-5*	14	0	0	
	196	0	0	<0.3
gal1-115 gal7-10 × ± +	19	1	0	
gal1-191 gal7-10 × ± +	36	3	0	
gal1-209 gal7-10 × ± +	39	1	0	
gal1-209 × *gal7-10*	14	0	0	
gal1-176 gal7-10 × ± +	12	0	0	
gal1-199 gal7-10 × ± +	23	0	0	
gal1-216 gal7-10 × ± +	22	0	0	
gal1-230 gal7-10 × ± +	30	0	0	
	195	5	0	1.8
gal1-209 × *gal10-1*	41	1	0	
gal1-209 × *gal10-5*	83	0	0	
gal1-465 gal10-1 × ± +	15	2	0	
gal1-329 gal10-1 × ± +	39	3	0	
gal1-339 gal10-1 × ± +	23	0	0	
	201	6	0	1.5

[a] Tetrad analysis based on Gal phenotype. Tetratypes included both reciprocal recombination and gene conversion events.
[b] Map distance calculated according to Perkins (30).

interesting to determine whether any of the regulatory elements between these two yeasts are functionally equivalent. For instance, can the cloned *GAL4* gene of *S. cerevisiae* (16, 20) complement a *lac9* strain of *K. lactis*?

Several differences do exist between these two regulatory systems. No counterpart to the *GAL3* regulatory locus of *S. cerevisiae* has been found in *K. lactis*. Likewise, regulatory loci equivalent to *LAC12*, a positive regulatory element (unpublished data), and *GAL7* have not been observed in *S. cerevisiae*. The lactose-galactose utilization system of *K. lactis* is not permanently repressed by glucose (11), whereas the melibiose-galactose utilization system of *S. cerevisiae* is repressed. Glucose repression is mediated by the *GAL82*, *GAL83*, and *REG1* loci (24, 25). *K. lactis* strains provide a biological system for determining whether glucose repression is conferred by regulatory genes, structural genes, or both.

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