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Elements Involved in Catabolite Repression and Substrate Induction of the Lactose Operon in *Lactobacillus casei*

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In *Lactobacillus casei* ATCC 393, the chromosomally encoded lactose operon, *lacTEGF*, encodes an antiterminator protein (LacT), lactose-specific phosphoenolpyruvate-dependent phosphotransferase system (PTS) elements (LacE and LacF), and a phospho-β-galactosidase. *lacT*, *lacE*, and *lacF* mutant strains were constructed by double crossover. The *lacT* strain displayed constitutive termination at a ribonucleic antiterminator (RAT) site, whereas *lacE* and *lacF* mutants showed an inducer-independent antiterminator activity, as shown analysis of enzyme activity obtained from transcriptional fusions of *lac* promoter (*lacp*) and *lacpΔRAT* with the *Escherichia coli gusA* gene in the different *lac* mutants. These results strongly suggest that in vivo under noninducing conditions, the lactose-specific PTS elements negatively modulate LacT activity. Northern blot analysis detected a 100-nucleotide transcript starting at the transcription start site and ending a consensus RAT sequence and terminator region. In a *ccpA* mutant, transcription initiation was derepressed but no elongation through the terminator was observed in the presence of glucose and the inducing sugar, lactose. Full expression of *lacTEGF* was found only in a *man ccpA* double mutant, indicating that PTS elements are involved in the CcpA-independent catabolite repression mechanism probably via LacT.

Lactobacillus casei is a lactic acid bacterium (LAB) found in many food products, such as fermented vegetables, milk, and meat, as well as in the human body and other natural environments. Recently, *L. casei* has been used in new fermented milk products with original flavors for which certain health benefits are claimed.

During milk fermentation, lactose is fermented by LAB through different pathways that differ in intermediary metabolites and their bioenergetics. However, it is the transport and phosphorylation mechanism that will determine the metabolism of the translocated disaccharide. Three lactose transport mechanisms have been identified in LAB: lactose-galactose antiporters, lactose-H^+ symport systems, and the lactose-specific phosphoenolpyruvate-dependent phosphotransferase system (PTS) (19). The lactose-specific PTS (Lac-PTS) is bioenergetically the most efficient one since the sugar is translocated and phosphorylated in a single step. This system has been described only for *Streptococcus mutans*, *Lactococcus lactis*, *L. casei*, and the non-LAB *Staphylococcus aureus* (1–3, 11, 12, 18, 19, 23, 31, 37).

L. casei ATCC 393 has two lactose assimilation mechanisms, the chromosomal Lac-PTS and a permease/β-galactosidase system encoded by plasmid pLZ15 (13, 21). In *L. casei* ATCC 393[pLZ15$^-$], the genetic structure and nucleotide sequence of lactose assimilation genes differs from that in *S. mutans*, *L. lactis*, and *Staphylococcus aureus* (22). In *L. casei*, the lactose genes are transcribed as an operon, where the genes of the tagatose-6-phosphate pathway are not included, as they are in other *lac* operons described (19). The cluster *lacTEGF* encodes for a regulatory protein (*lacT*), lactose-specific PTS proteins (*lacE* and *lacF*), and a phospho-β-galactosidase (P-β-Gal) (*lacG*). The promoter region contains a *cre* element overlapping the −35 region, which is followed by a highly conserved sequence, the ribonucleic antiterminator (RAT) sequence, and a terminator structure. It has previously been reported (22, 34) that the expression of the *lac* operon in *L. casei* ATCC 393[pLZ15$^-$] is subject to dual regulation: carbon catabolite repression (CR) and induction by lactose through transcriptional antitermination. Most CR was shown to be mediated by the general regulatory protein CcpA that regulates *lac* operon expression, possibly by binding to the *cre* element at the lactose promoter (*lacp*). However, an additional CcpA-independent CR effect was observed, which was related to a functional glucose-specific PTS (EII^{Man}). The second regulatory mechanism involves induction by lactose and is mediated by LacT, a protein that belongs to the BglG family of transcriptional antiterminators (3, 22). This later mechanism is remarkably different from the induction system found in the *lac* operon in *L. lactis*, where gene expression is controlled by LacR with tagatose-6-phosphate the likely inducer (18, 19). There is only one other antiterminator protein described in LAB, BglR from *L. lactis* (10).

Antitermination activity has been extensively studied in homologous proteins, such as BglG from *Escherichia coli*, which regulates β-glucoside utilization genes (24, 32, 33, 43, 45). However, antiterminators seem to be more frequently found in gram-positive bacteria. In *Bacillus subtilis*, four different antiterminators have been described: SacT, SacY, LicT, and GlcT, which control the expression of genes related to sucrose, β-glucan, or glucose assimilation (7, 17, 40, 42, 46, 47, 50). The antitermination activity of all of these proteins is dependent on their binding to a RAT sequence, resulting in the unwinding of a neighboring terminator structure in their respective mRNAs (9). BglG from *E. coli* has been found to be phosphorylated by the β-glucoside PTS transporter, BglF (EII^{Bgl}), which is encoded in the *bgl* operon. Phosphorylated BglG is monomeric and has no antitermination activity. However, in the presence of β-glucosides, BglG is dephosphorylated, which in turn promotes dimer formation and subsequently full antitermination activity (4–6, 43, 44).

The antiterminator protein SacY controls expression of the *sacB* gene in *B. subtilis*; it functions similarly to BglG, and the

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TABLE 1. *L. casei* strains and plasmids used in this study

Strain or plasmid	Description	Reference or source
L. casei strains		
BL23	ATCC 393[pLZ15$^-$]	B. Chassy (University Illinois, Urbana)
BL23D	*man*	49
BL71	*ccpA*	34
BL72	*man ccpA*	22
BL153	BL23 *lacE*	This work
BL154	BL23 *lacT*	This work
BL155	BL23 *lacF*	This work
Plasmids		
pUC18	Am^r	Boehringer Mannheim
pRV300	Erm^r from pAMβ1	30
pBluescriptII SK$^+$	Am^r	Stratagene
pT7Blue-T-vector	Am^r	Novagen
pNZ272	*gusA* Cm^r	36
pNZlac	pNZ272 containing the *cre* element of *lacp* fused to *gusA*	34
pNZRAT	pNZ272 containing the *cre* element and RAT-terminator of *lacp* fused to *gusA*	This work
pMJ39	*lacE* with 0.965-kb deletion in pRV300	This work
pMJ41	*lacT* with a frameshift at *Pst*I site in pRV300	This work
pMJ45	*lacF* with a frameshift at *Sph*I site in pRV300	This work
pMJ33	pBluescript containing a fragment of *lacp* (Fig. 1)	This work
pMJ64	pBluescript containing a fragment of *lacE* and *lacG* (Fig. 1)	This work

PTS component involved in this case is SacX (EII^{Scr}) (15, 26). Tortosa et al. (48) found two conserved domains (P1 and P2) common to a number of transcriptional regulators, and through elegant in vitro experiments they demonstrated phosphorylation of SacY by HPr-His-P. Also, SacT and LicT were shown to be activated by phosphorylation by the general components of the PTS (HPr and EI) in *B. subtilis* (8, 16, 27–29). Recently, Stülke et al. (47) have described the conserved domains common to PTS-controlled transcriptional regulators as the PTS regulation domains (PRDs). They proposed that the PRD closer to the N terminus (PRD-I) is related to the negative control played by the specific sugar permeases, whereas the PRD closer to the C terminus (PRD-II) shows a positive regulation by HPr.

To establish the role of the *lac* genes in the regulation of the *lac* operon in *L. casei* ATCC 393[pLZ15$^-$], different mutants (*lacE*, *lacT*, and *lacF*) were obtained by double crossover and then used to monitor the expression of *E. coli* β-glucuronidase gene (*gusA*) as reporter under the control of *lacp*. Transcriptional analysis was also performed in the three *lac* mutants, in a *man* (encoding EII^{Man}) mutant and in the *ccpA man* double mutant. These experiments confirmed that the RAT-terminator/LacT interaction is involved in the CcpA-independent CR mechanism and demonstrated that the antiterminator activity of LacT is also negatively regulated by the lactose-specific enzymes, EII^{Lac}.

MATERIALS AND METHODS

Plasmids, bacterial strains, and growth conditions. The *L. casei* strains and plasmids used in this work are listed in Table 1. *L. casei* cells were grown in MRS medium (Oxoid) and MRS fermentation broth (Adsa-Micro; Scharlau S.A., Barcelona, Spain) plus 0.5% carbohydrate at 37°C under static conditions. *E. coli* DH5α was grown with shaking at 37°C in Luria-Bertani (LB) medium. Bacteria were plated on media solidified with 1.5% agar. When required, the concentrations of antibiotics used were 100 μg of ampicillin, 300 μg of erythromycin, or 10 μg of chloramphenicol per ml to select *E. coli* transformants and 5 μg of erythromycin or 5 μg of chloramphenicol per ml for *L. casei*.

Recombinant DNA procedures. Genomic DNA from *L. casei* strains was purified by using a Purogene DNA isolation kit (Gentra Systems, Inc., Minneapolis, Minn.) as described by the manufacturer. Restriction and modifying enzymes were used according to the recommendations of manufacturers. General cloning procedures were performed as described by Sambrook et al. (41).

To obtain plasmid pNZRAT, the promoter *lacp* was amplified with primers lac11 (5′-TAGCACTGATCATTAAA-3′) and lac33 (5′-TTGCACTGGGAGG GGAT-3′), using *L. casei* DNA as the template, and the PCR product was cloned into the *Sma*I site of pUC18. The orientation of the insert was checked by PCR. A clone with the appropriate orientation was digested with *Eco*RI and *Pst*I, and the resulting fragment was cloned into *Pst*I/*Eco*RI-digested pNZ272 vector (36). The plasmid obtained, pNZRAT, carries a transcriptional fusion of the *L. casei lacTEGF* promoter, including the RAT sequence and terminator structure, with the *gusA* gene of *E. coli*. pNZlac (34) carries a transcriptional fusion of the *lac* promoter, lacking the RAT-terminator region, with the *gusA* gene. *L. casei* strains were transformed by electroporation with a Gene-Pulser apparatus (Bio-Rad Laboratories, Richmond, Calif.) as described elsewhere (38).

RNA isolation and Northern blot analysis. *L. casei* strains were grown in MRS fermentation medium with different sugars to an optical density at 550 nm of 0.8 to 1. Cells from a 10-ml culture were collected by centrifugation, washed with 50 mM EDTA, and resuspended in 1 ml of Trizol (Gibco BRL). One gram of 0.1-mm-diameter glass beads was added, and the cells were broken by shaking in a Fastprep apparatus (Biospec, Bartlesville, Okla.) two times for 45 s. RNA was isolated as described by Gibco BRL, separated by formaldehyde-agarose gel electrophoresis, and transferred to Hybond-N membranes (Amersham).

RNA probes were obtained as follows. Probe Ppt was obtained from plasmid pMJ64. A fragment from nucleotides (nt) −162 to +125 with respect to the transcriptional start site of the *lac* operon was cloned between the *Eco*RV and *Bam*HI sites of pBluescriptII SK$^-$. Probe PIIgal was derived from pMJ33 carrying an internal fragment of the *lac* operon cloned in the *Eco*RV site of pT7Blue-T-vector (Novagen). Antisense RNAs were synthesized in vitro from linearized plasmids with T3 and T7 RNA polymerase, respectively, using the reagents from the Boehringer digoxigenin-RNA labeling kit as recommended by the manufacturer. Hybridization, washing, and staining were done as described by the supplier.

Enzymatic assays. P-β-Gal and β-glucuronidase activities were assayed as previously described (14, 36) in permeabilized *L. casei* cells (49).

Construction of *L. casei lac* mutants. Mutations in *lacT*, *lacE*, and *lacF* genes were obtained by Campbell-like recombination using integrative plasmid pRV300 (30).

Three plasmids, pMJ41, pMJ39, and pMJ45, were constructed by cloning a fragment of the genes *lacT*, *lacE*, and *lacF*, respectively, in the integration vector. pMJ41 contained a PCR product obtained by using primers lac11 (5′-TAGCA CTGATCATTAAA-3′) and lac2 (5′-CAACGATATAAGCGCAGATC-3′). The fragment was made blunt ended and digested with *Xba*I and then cloned in pRV300 digested with *Spe*I and *Eco*RV, and an internal *Pst*I site was made blunt ended with the Klenow fragment of *E. coli* DNA polymerase, thus generating a frameshift mutation in the cloned fragment. A 1-kb *Hin*dIII/*Bgl*II fragment from pLZ613 (1) was introduced into pRV300, and the *Sph*I site was treated as was the *Pst*I site described above to give pMJ45. pMJ39 carried a 1.7-kb insert that had a 965-bp deletion in *lacE*. For its construction, two PCR fragments spanning the regions upstream and downstream of the desired deletion, which also had newly created *Sac*I sites (underlined regions below), were digested with *Sac*I and ligated. The oligonucleotides used as primers in the PCRs were lac11 (5′-TAG CACTGATCATTAAA-3′) and lac25 (5′-CGATATGAGCTCAGATC-3′) for one fragment and lac26 (5′-CAACGAGCTCAACAAAC-3′) and lac6 (5′-CTT

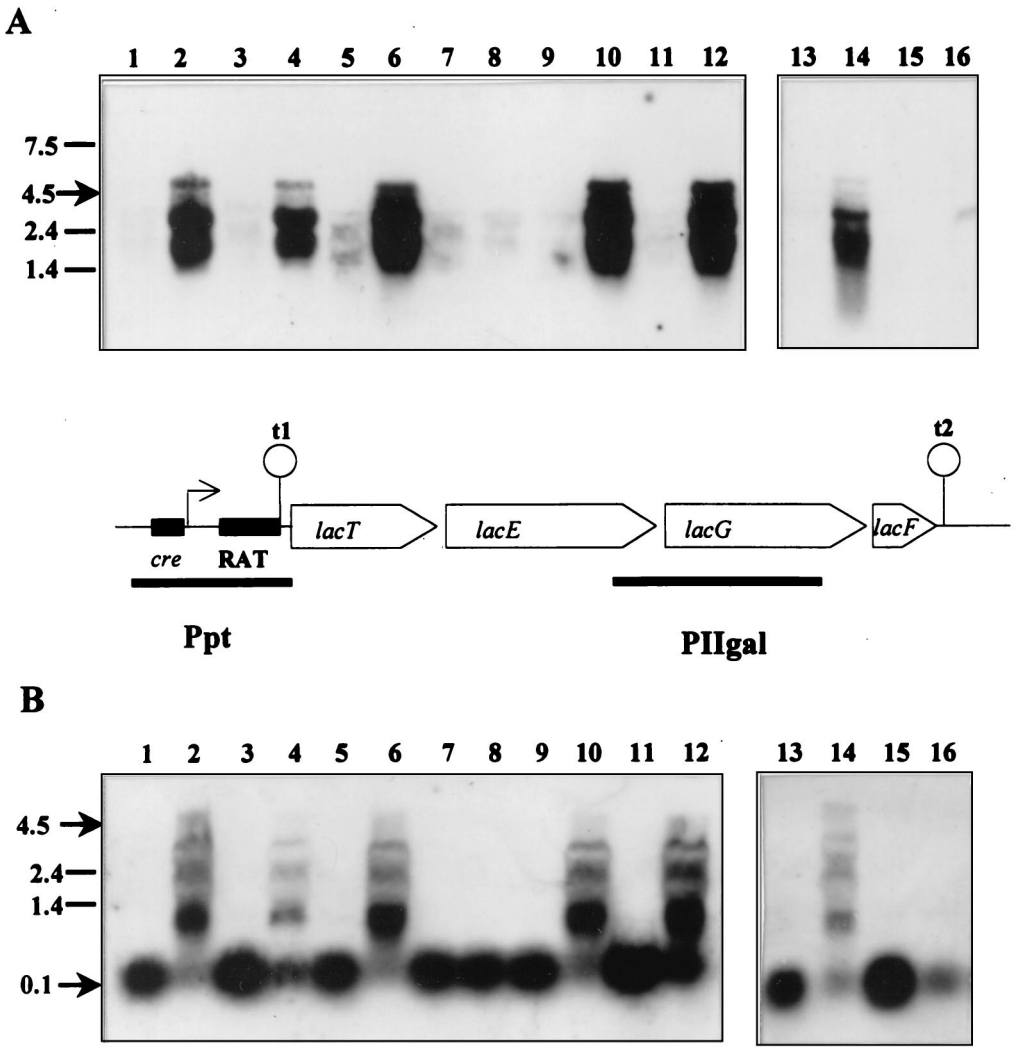

FIG. 1. Northern blots prepared with RNA from different *L. casei* mutants impaired in the catabolite repression signal transduction. The probes used were PIIgal (A) and Ppt (B); the strains used were BL23D (*man*) (lanes 1 to 4), BL71 (*ccpA*) (lanes 5 to 8), BL72 (*man ccpA*) (lanes 9 to 12), and BL23 (wild type) (lanes 13 to 16). Cells were grown on glucose (lanes 1, 5, 9, and 13), lactose (lanes 2, 6, 10, and 14), ribose (lanes 3, 7, 11, and 15) or glucose plus lactose (lanes 4, 8, 12, and 16). The diagram shows the structure of the *lac* operon and the relative positions of the RNA probes, PIIgal and Ppt, used in this experiment.

GCTGTCTAAATAGCC-3′) for the other fragment. The ligation product was cloned as a *Kpn*I-digested blunt-end fragment into *Kpn*I/*Eco*RV-digested pRV300. These three plasmids were used to transform *L. casei* to erythromycin resistance (Erm^r), as integration of the DNA fragments into *L. casei* chromosome occurred through a single crossover by Campbell-like recombination. For each transformation, one Erm^r colony was grown for 200 generations without antibiotic. Strains that had undergone a second recombination event due to the excision of the vector could be detected as Erm^s. The proper first and second recombination events were confirmed by Southern blot hybridization of the chromosomal DNA, and the phenotype of the appropriate mutants was analyzed.

RESULTS

Transcriptional analysis of *lac* operon in EII^{Man} and CcpA-deficient mutants. In a previous study (22), P-β-Gal was measured in BL23, BL23D (*man*), BL71 (*ccpA*), and BL72 (*man ccpA*) grown on glucose, lactose, and glucose plus lactose. Only the double mutant, BL72, showed full derepression of P-β-Gal activity when grown on the two latter sugars, whereas BL23D (*man*) had 24% of the activity found during growth on lactose. To investigate this behavior at the molecular level, Northern blot experiments were performed. With probe PIIgal (Fig. 1A), a signal corresponding to a transcript of 4.5 kb was obtained in all strains when the cells were grown on lactose (Fig. 1A, lanes 2, 6, 10, and 14). This size is in agreement with the expected length (4.8 kb) of a transcript which runs from the transcriptional start site of *lac* operon to the *rho*-independent terminator t_2 (3, 22). Additional bands corresponding to the 23S and 16S rRNAs were also noticed, which is sometimes the case in Northern blot experiments (25, 35, 39). In the wild-type strain, the 4.5-kb transcript was detected only on lactose-grown cells. When a mixture of glucose and lactose was used to grow all strains, transcription of the complete *lac* operon occurred only in the *man* mutant and *ccpA man* double mutant, although the intensity of the signal was lower in BL23D (Fig. 1A, lanes 4 and 12). However, the 4.5-kb mRNA was never found in glucose- or ribose-grown cells. These results correlate perfectly with the P-β-Gal activities described elsewhere (22).

RNAs from the same sources were used in another Northern blot with the Ppt probe from the region between nt −162 and +125 with respect to the transcriptional start site of *lac* operon (Fig. 1). A transcript of about 100 nt was detected in all sam-

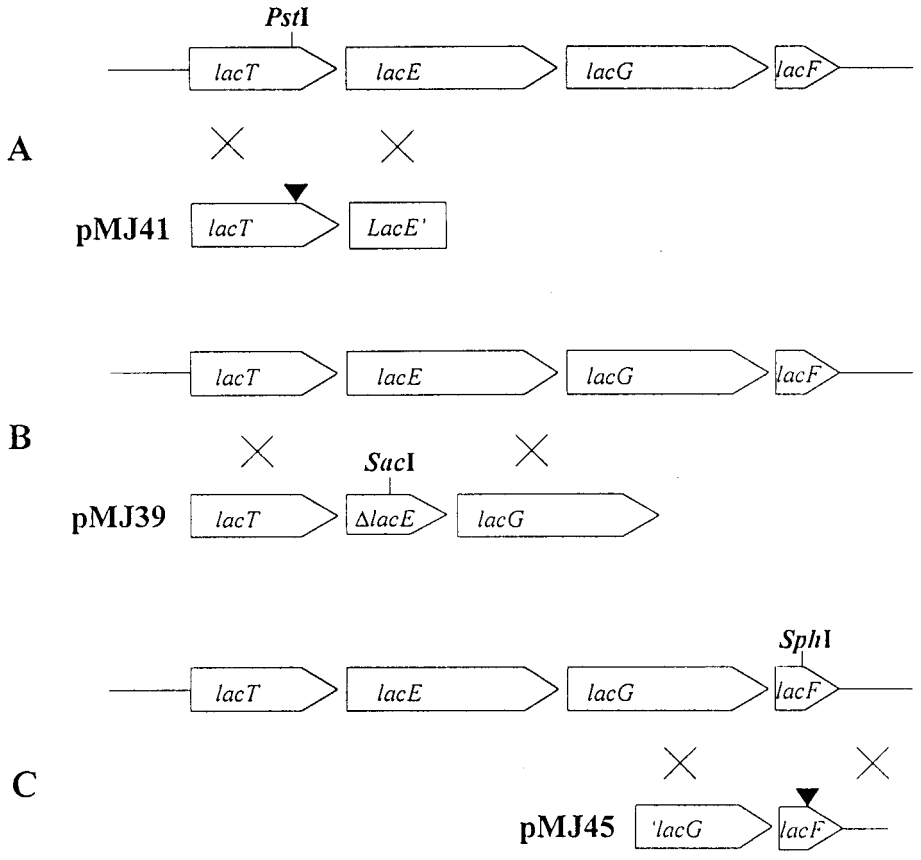

FIG. 2. Construction of *lacT* (A), *lacE* (B), and *lacF* (C) mutants of *L. casei*. Plasmids pMJ41, pMJ39, and pMJ45 carrying a frameshift in the *Pst*I site, a 0.965-kb deletion in *lacE*, and a frameshift in the *Sph*I site, respectively, were used to transform the wild-type strain. The different mutants were selected after double-crossover events.

ples where large mRNA species were not found. This indicates that in the absence of inducer (e.g., ribose-grown cells) transcription stopped at the terminator structure downstream of the promoter (Fig. 1B, lanes 3, 7, 11, and 15). In the wild type, the amount of this 100-nt transcript clearly decreased under repressing conditions (Fig. 1B; compare lanes 13, 15, and 16). However, in BL71 (*ccpA*), the intensities of the 100-nt RNA obtained under repressing and nonrepressing conditions were nearly identical (Fig. 1B, lanes 5, 7, and 8). This result corroborates previous data (22, 34) and further suggests that CcpA protein mediates catabolite repression at the level of transcription initiation, by binding to the *cre* site of *lacp*. However, the absence of a functional CcpA protein is not enough to overcome glucose repression, as full derepression of *lac* operon was found only in glucose-plus-lactose-grown cells of the BL72 (*man ccpA*) mutant. Possibly there is an additional CcpA-independent catabolite repression mechanism that involves the transport of glucose by the PTS and also probably the LacT protein.

Construction of *L. casei lac* mutants and expression studies using the *gusA* gene as reporter. The lactose operon, *lacTEGF*, of *L. casei* BL23 encodes the regulatory protein LacT, lactose-specific EIIA and EIICB PTS elements (LacF and LacE), and P-β-Gal (LacG) (22). To establish the role of *lac* gene products in induction of the *lacTEGF* operon, mutants BL154 (*lacT*), BL153 (*lacE*), and BL155 (*lacF*) were obtained by a double-crossover event (Fig. 2). Although all mutants turned out to be impaired in lactose fermentation, P-β-Gal activity, encoded by *lacG*, could be used to report the expression of the gene cluster. Consequently, lactose induction of this activity was determined in the *lac* mutants and in the wild-type strain grown on ribose and ribose plus lactose. In BL23, expression of the *lac* operon was induced by lactose (9 and 17.9 nmol/min/mg [dry weight], respectively), whereas BL155 (*lacF*) showed higher P-β-Gal activity under both conditions (27.7 and 29.5 nmol/min/mg [dry weight], respectively). These results indicate that LacF is involved in the induction of the *lac* operon. No activity was detected in BL154 (*lacT*), which would be impaired in the antiterminator protein, indicating a lack of induction in the presence of lactose. Surprisingly, no P-β-Gal activity was detected in BL153.

The effect of the mutations in *lacE*, *lacT*, and *lacF* was also studied with the β-glucuronidase reporter system in plasmids pNZlac and pNZRAT (Table 2). In plasmid pNZlac, which lacks the RAT-terminator area but contains the *cre* element in *lacp*, β-glucuronidase activity was detected in similar amounts in all strains grown on ribose but was negligible when strains were grown on glucose. Moreover, no induction by lactose was found in the wild type. With pNZRAT, carrying the whole promoter, a remarkable decrease of activity occurred in the *lacT* mutant with respect to the other strains, consistent with the antiterminator nature of LacT. The fact that β-glucuronidase activity in BL23 grown on ribose was 20-fold higher than that detected in the *lacT* mutant suggests that antitermination

TABLE 2. Expression of different transcriptional fusions in *L. casei* strains

Strain	Relevant genotype	Sugar	β-Glucuronidase activity[a] (nmol/min/mg [dry wt])	
			pNZlac	pNZRAT
BL23		Lactose	101.3 ± 7.2	2,318.5 ± 443.5
		Ribose	139.7 ± 15.6	1,025.0 ± 17.5
		Glucose	6.3 ± 1.8	15.2 ± 2.3
BL153	*lacE*	Ribose	144.4 ± 7.9	2,964.0 ± 262.0
		Glucose	7.3 ± 1.9	10.5 ± 0.6
BL154	*lacT*	Ribose	94.7 ± 4.9	47.6 ± 13.4
		Glucose	9.5 ± 0.8	5.5 ± 2.2
BL155	*lacF*	Ribose	120.3 ± 7.5	2,860.0 ± 545.0
		Glucose	15.1 ± 5.4	8.0 ± 2.4

[a] Measured with *p*-nitrophenyl-β-D-glucuronic acid in permeabilized cells grown with 0.5% sugar to an optical density at 550 nm of 0.8. The values (means and standard deviations) are from three independent experiments.

mediated by LacT also occurs to some extent in the absence of lactose and without glucose in the wild-type strain. The highest β-glucuronidase activity was found in BL153 (*lacE*) and BL155 (*lacF*) grown under noninducing conditions, indicating a negative effect of EII^{Lac} on the antiterminator activity of LacT. No release of glucose repression was observed in the different strains transformed with pNZlac or pNZRAT, but there was a greater repression when the RAT-terminator element was present in the fusion used, except in the *lacT* strain, suggesting that there is a glucose repression effect mediated by the LacT protein. In the case of strain BL153 (*lacE*), divergent results were found between the homologous (P-β-Gal) and heterologous (*gusA*) reporter systems. However, the disruption of *lacE* might have caused a translational defect in *lacG*, as the operon seems to be transcribed at a high rate under nonrepression conditions (Fig. 3A, and B, lanes 6 and 7).

Influence of Lac-PTS elements on transcription of the lactose operon. We analyzed in vivo the role of lactose-specific proteins by means of Northern blotting using the same RNA probes (PIIgal and Ppt) as before. With the PIIgal probe, strong signals were obtained on ribose- and ribose-plus-lactose-grown cells of BL155 (*lacF*) (Fig. 3A, lanes 14 and 15), in agreement with the P-β-Gal activity detected (see above). Very intense signals were also observed in BL153 (Fig. 3A, lanes 6 and 7). The remarkable difference between the latter result and that with the wild-type strain (Fig. 3A, lanes 2 and 3) indicates that in *lacE* and *lacF* mutants, lactose induction is not required for the antitermination activity. This suggests that in vivo, LacE or LacF interacts with the antiterminator, LacT. Again, there is an excellent correlation between the hybridization patterns obtained with both probes. In the presence of glucose, no large mRNA was detected, while the Ppt probe showed that 100-nt mRNA was observed in all samples, albeit with slight changes in intensity (Fig. 3). A smaller size of the full transcript was noticed in ribose-grown cells of BL153, confirming the deletion generated by recombination (Fig. 3B, lanes 6 and 7). In BL154 (*lacT*), no 4.5-kb mRNA or any other transcript larger than 100 nt could be detected under any conditions (Fig. 3 and B, lanes 10 to 13), indicating that RNA polymerase always stops at the terminator site when LacT is absent.

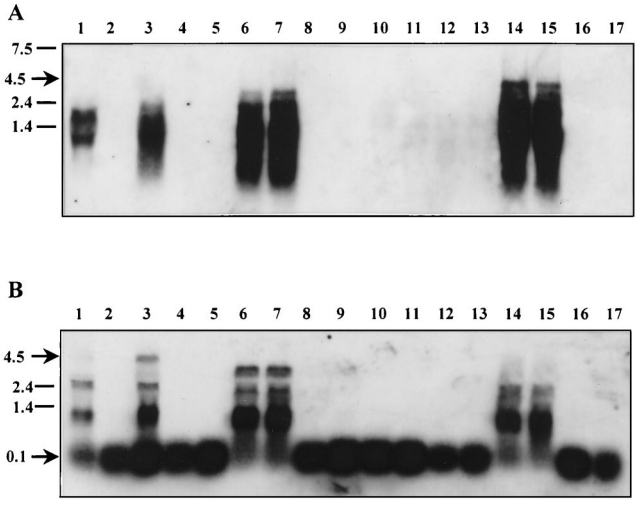

FIG. 3. Northern blot analysis of RNA from the wild-type strain and different *lac* mutants. The probes used were PIIgal (A) and Ppt (B); the strains used were BL23 (wild type) (lanes 1 to 5), BL153 (*lacE*) (lanes 6 to 9), BL154 (*lacT*) (lanes 10 to 13), and BL155 (*lacF*) (lanes 14 to 17). Cells were grown on lactose (lane 1), on ribose (lanes 2, 6, 10, and 14), ribose plus lactose (lanes 3, 7, 11, and 15), glucose (lanes 4, 8, 12, and 16), or glucose plus lactose (lanes 5, 9, 13, and 17).

DISCUSSION

The lactose operon, *lacTEGF*, in *L. casei* ATCC 393 is located on the chromosome and encodes the transcriptional antiterminator LacT, lactose-specific PTS proteins, and P-β-Gal (22). Previous reports suggested that this operon could have a regulatory system different from that described for the lactose operons in *L. lactis*, *S. aureus*, and *S. mutans* (19). In this work, we describe the construction and analysis of *lacE*, *lacF*, and *lacT* mutants showing the involvement of EII elements of the Lac-PTS in modulation of the *lac* operon of *L. casei*.

Lactose induction: modulation of LacT activity of EII^{Lac}. Regulation by antitermination has been described for several operons in low-GC, gram-positive bacteria, such as *sacPA*, *sacB*, *bgl*, *licTS*, and *glc* of *B. subtilis* (7, 17, 40, 42, 46, 47, 50). In gram-negative bacteria, this system has been found in the *bgl* operon of *E. coli* and the *arb* operon of *Erwinia chrysanthemi* (20, 24, 32, 33, 43, 45). Antiterminator proteins of these systems have been assigned to the BglG family on the basis of sequence homology, cross-complementation, and the finding that their DNA targets share a consensus RAT sequence (40). LacT from *L. casei* shows homology to proteins of this family and has been shown to complement the *sacB* system of *B. subtilis* (3). Indeed, the His residues which are potentially phosphorylated in PRD-I (H101 and H159) and PRD-II (H210 and H273) of SacY are conserved in LacT (47). In wild-type *L. casei* ATCC 393, a transcript corresponding to the whole operon *lacTEGF* was detectable only in the presence of lactose. Taking into account the evidence shown here and knowledge gained from homologous systems, the general mechanism by which LacT controls transcription of the *lac* operon would be as follows. LacT binds to the RAT sequence and prevents formation of the terminator in the presence of lactose. In the absence of the inducer, the antiterminator would be inactive, and transcription would start and proceed only until the RNA polymerase reaches the *rho*-independent terminator. Hence, only a small transcript spanning a region of about 100 nt (from the transcription start site to the terminator) could be detected. As expected, in a *lacT* mutant constitutive termination (the presence of 100-nt transcript in all conditions) was observed, consistent with the model for LacT.

The antiterminator proteins BglG in *E. coli* and SacY in *B. subtilis* are regulated through phosphorylation by sugar-specific

PTS components or Hpr (40, 47, 48). We examined the role of LacE (EIICB) and LacF (EIIA) in the regulation of LacT antiterminator activity, by inactivation of the *lacE* and *lacF* genes. Transcriptional studies showed an inducer-independent antiterminator activity in the *lacE* and *lacF* mutants, indicating that their corresponding proteins in the wild type may be involved in the inactivation of the antiterminator LacT. The model described for *B. subtilis* antiterminators (40, 47) could apply here, as in the absence of functional II^{Lac} elements, the antiterminator LacT was active, possibly because it cannot be phosphorylated at one of the conserved PTS regulation domains (PRD-I) (47). In the wild type, the phosphoryl group would be transferred to the incoming inducing sugar, with the same effect. This is the first report in which sugar-specific PTS elements from a lactic acid bacterium have been conclusively shown to be involved in the induction mechanism via an antiterminator protein.

Involvement of LacT in catabolite repression. Preliminary reports showed that lactose utilization was repressed by glucose, since *L. casei* ATCC 393 displayed a likely diauxie (plateau lasting 15 to 20 h) when grown on both sugars (49). It was then shown that transcription of the *lac* operon was subject to CcpA-mediated CR (22, 34). However, an additional CcpA-independent CR mechanism was suggested when P-β-Gal activity was monitored in mutants lacking CcpA and EII^{Man} individually and in a *ccpA man* double mutant (22, 34). This additional CR mechanism was dependent on a functional glucose-PTS transporter. In the *bgl* and *lev* operons of *B. subtilis*, HPr-dependent phosphorylation of the regulator proteins controls a CcpA-independent CR effect (27, 28, 40). However, the experiments with *lacp-gusA* fusions suggest that the RAT sequence and LacT are involved in the proposed CcpA-independent catabolite repression mechanism. In *L. casei*, Northern blot analysis showed that transcription initiation of *lacTEGF* operon is fully derepressed in the *ccpA* mutant, but that there was no elongation beyond the terminator in the presence of glucose. The double mutant BL72 (*man ccpA*) exhibits full expression of the lactose operon when grown on glucose plus lactose; therefore, LacT is active. This strain is impaired in the glucose-PTS transporter, which probably links the EII^{Man} or other PTS elements with the glucose repression mediated by LacT, which was observed in the *ccpA* mutant.

The model of PTS-mediated control of PRD-containing regulators described by Stülke et al. (47), which also includes the mentioned antiterminators, could explain the regulation of LacT activity. In this model, PRD-I would be phosphorylated by inducer-specific EII components of the PTS in the absence of inducer and PRD-II would be phosphorylated by HPr in absence of glucose (or repressing carbohydrates). Consequently, the antiterminator LacT would exist in three forms: (i) active, when dephosphorylated in PRD-I and phosphorylated in PRD-II; (ii) inactive (noninduced), when phosphorylated in both domains; and (iii) inactive (CR), when PRD-II is dephosphorylated by HPr when grown on glucose. The presence (nonphosphorylated PRD-I) or absence (phosphorylated PRD-I) of the inducer, lactose, would not affect this later form. However, we cannot exclude the possibility that phosphorylation of PRD-II can be carried out by the sugar-specific PTS transporter, EII^{Man}. Clearly the analysis of defined *L. casei* mutants lacking the HPr gene (*ptsH*) or defective *ptsH* and either *lacF* or *ccpA* could lead to a further understanding of this regulation system, as could studies on the phosphorylation of LacT.

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