The Lactate-Proton Symport of *Saccharomyces cerevisiae* Is Encoded by *JEN1*

MARGARIDA CASAL,[1] SANDRA PAIVA,[1] RAQUEL P. ANDRADE,[1] CARLOS GANCEDO,[2] AND CECÍLIA LEÃO[1]*

Centro de Ciências do Ambiente, Departamento de Biologia, Universidade do Minho, 4719 Braga Codex, Portugal,[1] and Instituto de Investigaciones Biomédicas Alberto Sols, CSIC-UAM, 28029 Madrid, Spain[2]

A mutant of *Saccharomyces cerevisiae* deficient in the lactate-proton symport was isolated. Transformation of the mutant with a yeast genomic library allowed the isolation of the gene *JEN1* that restored lactate transport. Disruption of *JEN1* abolished uptake of lactate. The results indicate that, under the experimental conditions tested, no other monocarboxylate permease is able to efficiently transport lactate in *S. cerevisiae*.

Saccharomyces cerevisiae is able to utilize short-chain monocarboxylic acids as sole carbon and energy sources under aerobic conditions. Transport across the plasma membrane is the first step in the metabolism of these substrates. Monocarboxylate proton symporters with different specificities have been described: acetic acid or ethanol-grown cells present a permease which is shared by acetate, propionate, and formate (5), while cells grown with lactic acid have an additional permease which transports lactate, pyruvate, acetate, and propionate (4, 6). The capacity to transport monocarboxylates was not present when the cells were grown in glucose (6). In agreement with the physiological results indicating the existence of different monocarboxylate permeases in *S. cerevisiae*, four open reading frames (ORFs) with important similarities to mammalian monocarboxylate permeases were found in the genome of *S. cerevisiae* (13, 16). However, to our knowledge, no direct evidence relating a concrete gene product with a definite monocarboxylate transport activity is available. We have obtained yeast mutants unable to transport lactic acid and have cloned a gene that restores this transport capacity. The results presented in this work indicate that the product of the gene *JEN1* is a permease for lactate and suggest that, under the experimental conditions analyzed, no other system for lactate transport was operating in *S. cerevisiae*.

S. cerevisiae W303-1A (*MAT***a** *ade2 leu2 his3 trp1 ura3*) (20) was used as starting material. Its isogenic strain of the opposite mating type was used in crosses. Yeasts were grown in 1% (wt/vol) yeast extract–1% (wt/vol) peptone (YP) or in 0.7% (wt/vol) Difco yeast nitrogen base (YNB) supplemented with adequate quantities of nutrients required for growth. Carbon sources were 2% (wt/vol) glucose, 0.5% (vol/vol) DL-lactic acid (pH 5.0), 0.5% (wt/vol) sodium pyruvate (pH 5.0), 0.5% (vol/vol) acetic acid (pH 5.0), 1% (wt/vol) glycerol, and 1% (wt/vol) ethanol. To prepare solid media, 2% (wt/vol) agar was added. Growth was at 28°C, and liquid cultures were grown with shaking. Cultures were always harvested during the exponential phase of growth. *Escherichia coli* XL-Blue and DH5a were used for plasmid amplification and preparation (19). Uptake experiments were carried out with yeast suspensions prepared from cells growing exponentially in YP-lactate washed twice with chilled water and resuspended in water. Conical centrifuge tubes containing 30 ml of 0.1 M KH_2PO_4 buffer at pH 5.0 and 10 ml of the yeast suspension (25 to 45 mg [dry weight]/ml) were incubated for 4 min at 25°C. Next, the reaction was started by the addition of 10 ml of an aqueous solution of labelled DL-[1-^{14}C]lactic acid (sodium salt; Amersham) (3,000 dpm/nmol) at pH 5.0. The reaction was stopped by dilution with 5 ml of ice-cold water. Sampling times were 0, 5, 10, and 20 s, so that uptake rates remained linear. The reaction mixtures were filtered immediately through GF/C membranes (Whatman, Inc., Clifton, N.J.), and the filters were washed with 10 ml of ice-cold water and transferred to scintillation fluid (Opti-Phase HiSafe II; LKB FSA Laboratory Supplies, Loughborough, United Kingdom). Radioactivity was measured in a Packard Tri-Carb 2200 CA liquid scintillation spectrophotometer, with disintegrations per minute correction. For nonspecific ^{14}C adsorption, labelled lactate was added at zero time after the cold water. To determine the transport kinetics best fitting the experimental initial uptake rates and to estimate the kinetic parameters, a computer-assisted nonlinear regression analysis (GraphPAD Software, San Diego, Calif.) was used. All the experiments were repeated at least three times. Proton uptake was measured in a thermostated chamber at 25°C with a standard pH meter connected to a recorder. To the chamber, 4.5 ml of 10 mM KH_2PO_4 and 0.5 ml of cell suspension were added, and the pH was adjusted to the desired value to obtain a baseline. The lactate solution, adjusted to the same pH value, was added and the subsequent alkalization was monitored. Rates of proton uptake were calculated from the slope of the initial part of the pH trace. Calibration was performed with HCl.

A commercial *S. cerevisiae* genomic library in YEp13 (ATCC 37323) was used. Transformation of yeast cells and molecular biology operations were performed according to the methods of Ausubel et al. (1). Disruption of the cloned gene was done by replacing the 1,758-bp *Hpa*I-*Eco*47III sequence by a 1,472-bp *Pvu*II-*Pvu*II fragment from vector YDpH (2) carrying the *HIS3* gene. A 2.7-kb *Xba*I-*Sca*I fragment was used to replace the genomic copy of the gene by the one-step disruption

* Corresponding author. Mailing address: Centro de Ciências do Ambiente, Departamento de Biologia, Universidade do Minho, Campus de Gualtar, 4719 Braga Codex, Portugal. Phone: 351-53-604310. Fax: 351-53-678980. E-mail: cleao@bio.uminho.pt.

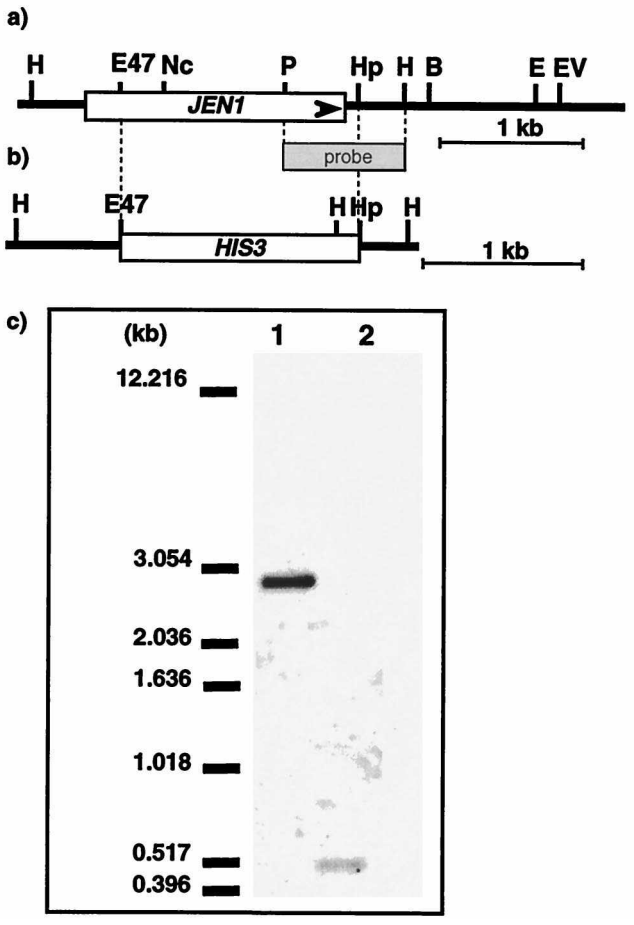


FIG. 1. Scheme of the yeast genomic DNA region containing *JEN1* and Southern blot analysis of its disruption. (a) Schematic restriction map of the yeast genomic DNA fragment contained in the plasmid pT12. This plasmid, which contains the complete sequence of *JEN1* (YKL 217w), was isolated from the original YEp13 genomic library. (b) Disruption of *JEN1* was carried out as described in the text. The 2.7-kb *Sac*I-*Xba*I fragment was used to replace the genomic wild-type copy. (c) Southern blot analysis of the *JEN1* disruption. Genomic DNA was digested with *Hin*dIII, subjected to electrophoresis in a 0.8% agarose gel, transferred to Hybond-N membrane, and hybridized with the 855-bp probe indicated in panels a and b. Lanes: 1, DNA from wild type; 2, DNA from BLC 203 strain carrying the *jen1::HIS3* disruption. Sizes of the fragments are indicated at left.

method (18) (Fig. 1). Northern blot analysis of *JEN1* expression was performed according to standard procedures (19).

Isolation and characterization of a mutant unable to grow on DL-lactate. A suspension of about 10^7 yeast cells/ml was irradiated with a UV germicidal lamp, plated on YP-glycerol, and replica plated onto YNB-lactate. Four colonies unable to grow in this medium were found, but only one appeared affected in its capacity to transport lactate. A diploid isolated from a cross of this mutant with a wild-type strain grew on lactate, indicating that the mutation was recessive. The segregation of the negative growth on lactate was 21:22 in 10 complete tetrads analyzed, indicating that the phenotype was caused by a nuclear, monogenic mutation. The original mutant was named BLC 55, and a spore from a cross presenting the lactate-negative phenotype was termed BLC 142. In both cases, faint, residual growth on lactate was always observed. A strain mutated in the *PCK1* gene, encoding phosphoenolpyruvate carboxykinase, or a double mutant with alterations in the *CYB2* and *DLD* genes (12), encoding the D- and L-lactate ferricytochrome *c* oxidoreductases, respectively, gave a complete absence of growth in the same medium. Strains BLC 55 and BLC 142 grew in glycerol, ethanol, or acetate as did the wild type, but they did not grow in pyruvate.

Cells from the BLC 142 mutant grown in glucose and incubated in YP-lactate for 4 h showed at 2.0 mM lactate a transport activity that was only one-fifth that of the wild type. Moreover, the transient extracellular alkalization indicative of proton uptake was absent after addition of DL-lactate or pyruvate (not shown). The observed lactate uptake could be due to diffusion of the undissociated acid. This behavior was similar to that found in glucose-repressed cells of the wild type, where no measurable activity of the lactate permease is found (6).

Isolation of the gene responsible for the lactate-negative phenotype. The gene whose mutation caused inability to grow in lactate was isolated by functional complementation of strain BLC 142. The yeast was transformed to leucine prototrophy, and transformants were screened for growth on lactate. Among 20,000 transformants, 20 were able to grow efficiently on DL-lactate. When these clones were grown under nonselective conditions, the ability to grow in lactate and the Leu^+ phenotypes were lost together in one clone. Plasmid DNA was prepared from this transformant and used to transform again the original BLC 142 strain. The plasmid (pT12) restored the ability to grow in lactate, the permease activity, and the capacity to alkalinize the medium after addition of the acid (not shown). By restriction analysis, a yeast genomic DNA insert of 4.4 kb was identified in pT12 (Fig. 1a). It seems, therefore, that the DNA on the plasmid carries the gene encoding the lactate permease. A 0.9-kb *Eco*RI-*Bam*HI yeast genomic DNA fragment contained in the pT12 plasmid was sequenced. It was found to have a sequence of nucleotides in the vicinity of ORF YKL217w (21). This ORF corresponds to a gene termed *JEN1* that in the YPD database is said to be implicated in the transport of lactate. The restriction analysis of pT12 indicated that it contained *JEN1*. This gene has been described as a protein member of the major facilitator superfamily belonging to the sialate:H^+ family (13, 15). It is also apparently similar to the *E. coli* genes encoding the osmoregulatory proline-betaine transporter proP and the a-ketoglutarate transporter (10). It is likely that different search algorithms recognize diverse features and produce different assignments within a superfamily, in this case the major facilitator superfamily (15).

Effects of the disruption of the *JEN1* gene. To ascertain the physiological role of the *JEN1* gene, we disrupted it and placed the disrupted copy into the genome (Fig. 1b). Southern blot analysis demonstrated that the integration was correct (Fig. 1c). The disruptant, BLC 203, did not grow on lactate and did not show measurable permease activity (Fig. 2a). In contrast, transport of acetate was operational in the mutant BLC 203 under all derepressed conditions tested (data not shown), confirming previous results which indicated that the transport system for acetate was always present in derepression conditions but could not transport lactate.

The mutant BLC 203 transformed with plasmid pT12 was able to grow in YP-lactate and recovered the activity of the lactate carrier (Fig. 2b). From the data in Fig. 2b, the following kinetics parameters were estimated: for the wild-type strain V_{max} was estimated to be 0.40 nmol s^{-1} and K_m was estimated to be 0.69 mM; for the BLC 203 transformed strain, V_{max} was estimated to be 0.36 nmol s^{-1} mg (dry weight) and K_m was estimated to be 0.68 mM. The fact that

the transformed strain did not transport lactate faster than the wild-type strain could be due either to a lack of appropriate expression or to problems with insertion of the protein in the membrane. To distinguish between these possibilities, a North-

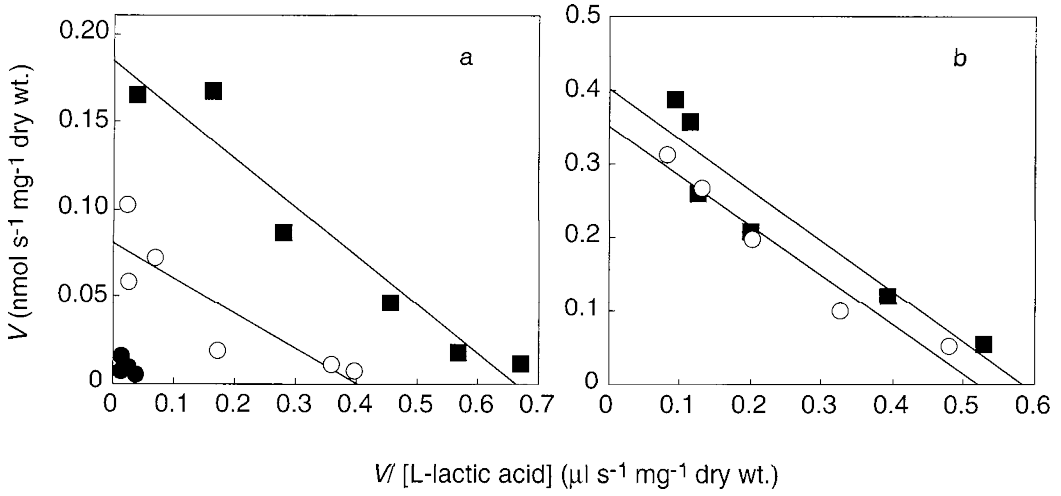


FIG. 2. Eadie-Hoffstee plots of the initial uptake rates of lactate by the wild type and a *jen1::HIS3* disrupted strain. Uptake studies were done at pH 5.0 as described in the text. (a) Cells growing exponentially in YNB-glucose were harvested by centrifugation, washed twice with deionized water, and incubated in YP-lactate for 4 h. (b) Cells growing exponentially in YP-lactate. Symbols: n, wild type; F, mutant BLC 203; E, mutant BLC 203 transformed with plasmid pT12.

ern analysis of the expression of *JEN1* was performed (Fig. 3). After induction in YP-lactate medium, the wild-type strain exhibited a strong signal against a *JEN1* probe while no signal was detected in noninduced cells. Also, no signal was visible with strain BLC 203, which carries an interrupted *JEN1* gene. BLC 203 cells transformed with pT12 plasmid recovered *JEN1* expression. However, in these cells, the level of *JEN1* mRNA after induction was of the same order of magnitude as that found in the wild-type strain. This finding points to the existence of some regulatory element in the promoter that is missing in the plasmid; in fact, it has only about 500 bp in front of the putative ATG. The results of the Northern analysis indicate therefore that the lack of increased transport activity in the transformants is due to an inefficient expression.

The transport of lactate is an important step in the metabolism of several organisms. In mammals, it is a key step in the reutilization by liver and kidney of lactate produced in muscle glycolysis (the Cori cycle). A family of monocarboxylate transporters that also transport pyruvate and other ketoacids exists with different tissue specificities (17). In erythrocytes, three different systems with different mechanisms appear to operate in lactate transport, although proton symport accounts for about 90% of it (7). In *E. coli*, the *lct* locus, involved in lactate metabolism, encompasses three overlapping genes, one of which (*lctP*) appears to encode a lactate transporter (8). However, to our knowledge no further information on this system is available. Among yeasts, the mechanisms of lactate transport are different; while *Candida utilis* (11), *S. cerevisiae* (4, 6), and *Torulaspora delbrueckii* (3) transport lactate by proton symports, *Kluyveromyces marxianus* does it via a uniport (9). It will be interesting to compare the structures of the respective proteins involved in the process.

Our initial aim was to isolate the genes encoding the H^+ symport that takes up lactate in *S. cerevisiae*. The results presented here clearly show that the gene *JEN1* encodes a lactate permease and that in its absence diffusion alone does not allow efficient growth of the yeast in lactate.

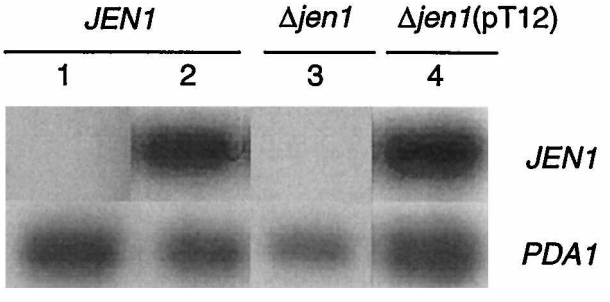


FIG. 3. Northern blot analysis of *JEN1* transcripts from strains differing in locus state. *JEN1*, wild type; D*jen1*, disruptant mutant BLC 203; D*jen1*(pT12), BLC 203 strain transformed with pT12 plasmid. Total cellular mRNA was prepared from cells growing exponentially in YNB-glucose (lane 1) and subsequently incubated in YP-lactate for 4 h (lanes 2, 3, and 4) (14). Total RNA (20 mg) was separated on 1.5% agarose morpholinepropanesulfonic acid (MOPS)-formaldehyde gels and blotted to nylon membranes by vacuum transference. Hybridization was carried out using the 844-bp *Nco*I-*Pst*I fragment as a probe for *JEN1* (Fig. 1a), and *PDA1* (22) served as internal standard.

We acknowledge the help of Maria J. Lafuente in the DNA sequencing and analysis and the critical reading of the manuscript by Juana M. Gancedo.

This study was supported by Portuguese grant PRAXIS/2/2.1/BIO/1068/95. Work in the laboratory of C.G. was supported by grant PB97-1213-CO2-01 from the Spanish CICYT. M.C. had a FEBS short-term fellowship during the early stages of this work. S.P. received a fellowship from PRAXIS XXI-BM4229. R.P.A. received a fellowship from PRAXIS XXI-BD15737/98.

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