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LYC1 is the structural gene for lysine N-6-acetyl transferase in yeast

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Abstract. In the yeast *Yarrowia lipolytica*, the *LYC1* locus controls the first step of the lysine degradation pathway which is catalyzed by lysine N-6-acetyl transferase (LAT). This gene was cloned by complementation of the *lyc1-100* mutation. Its position in the cloned insert was determined by conversion mapping and by complementation. The *LYC1* gene encodes a 391 amino-acid polypeptide which has no homolog in protein databases. The required upstream region extends over 960 bp. When placed under the control of the *GAL10* promoter in *Saccharomyces cerevisiae*, *LYC1* drives the expression of lysine acetyl transferase activity, thus providing strong evidence that it is the structural gene encoding this enzyme.

Key words: Yeast – *Yarrowia lipolytica* – Lysine acetyl transferase – Lysine catabolism

Introduction

Lysine is degraded by a number of yeasts species but by different pathways (Hammer et al. 1991). The industrial yeast *Yarrowia lipolytica* is representative of those using the catabolic pathway with N-acetylated derivatives of lysine. This yeast is able to use this amino acid as a nitrogen, as well as a carbon, source. Its lysine catabolism was genetically described by Gaillardin et al. (1976). Eight complementation groups were defined by mutant studies. Mutations affecting the first step of the pathway defined the *LYC1* gene. These mutations were assumed to be impaired in the activity of lysine acetyl transferase (LAT).

The switching from lysine synthesis to lysine degradation is a complex regulatory event. It involves a shutdown of the biosynthesis of lysine, especially of the last enzyme, saccharopine dehydrogenase (SDH). In *lyc*$^-$ mutants, however, SDH levels remain high under repressing conditions for the wild-type (Gaillardin et al. 1979). As the bulk of intracellular lysine is stored in a vacuole away from the cytoplasmic degradative enzymes, switching also implies a modification of the flux of lysine between the cytoplasm and this organelle (Beckerich et al. 1986). Finally, induction of degradative enzymes should also be observed.

We made an effort to genetically and biochemically characterize LAT and the *LYC1* gene. An assay method was set up for LAT and properties of this enzyme were determined (Lambert et al. submitted): LAT catalyzes the acetylCoA-dependent acetylation of lysine into N-6-acetyl-lysine and displays a "ping-pong" mechanism typical of an enzyme with two substrates, acetylCoA and lysine. The most notable inhibitors were saccharopine, a lysine precursor, and 5-aminovalerate, a lysine degradation product, both involved in lysine metabolism. LAT expression was shown to be controlled by nitrogen catabolic repression and the highest expression levels of LAT were observed when 5-aminovalerate and lysine were used as a nitrogen source. In vitro assay indicated that *lyc1* mutants were defective in LAT activity. However revertants such as *LYC1-018* displayed an alteration in the regulation of the intracellular pools of lysine during the course of the induction of lysine degradation (Beckerich et al. 1984), but were not modified for the LAT kinetic parameters as compared to a reference *LYC1*$^+$ strain (unpublished data). It was not clear, therefore, whether the *LYC1* gene was the structural gene for LAT or a regulatory gene controlling transition from lysine anabolism to catabolism.

In this paper, cloning, sequencing, and heterologous expression of the *LYC1* gene are reported and provide evidence that this gene indeed encodes lysine acetyl transferase.

Materials and methods

Media and strains. Strains used in this work are described in Table 1. The recipient strain ML111-04 was selected for its high transformability among *lyc1-100*, *LEU2-35* segregants from a cross between ML100-04, the original *lyc1-100* strain, and ML106-4. Cul-

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Table 1. List of strains and plasmids

Strain/plasmid	Genotype/description	Source
E. coli		
HB 101	*hsdR*$^-$, *hsdM*$^-$, *recA13*, *SupE44*, *LacZ4*, *leuB6*, *proA2*, *thi-1*, Sm^R	B. Bachman
SCS 1	F$^-$, *recA1*, *endA1*, *gyrA96*, *thi*, *hsdR17* (r_k^-, m_k^+), *supE44*, *relA1*	Stratagene
Y. lipolytica		
W29	Wild-type	Our collection
ML111-04	*Mat B*, *ura2–21*, *leu2–35*, *lyc1-100*, *xpr2–34*	Our collection
ML106-4	*Mat B*, *ade1–1*, *leu2–35*, *xpr2–34*, *LYC1*	Our collection
S. cerevisiae		
AH 220	*Mat a*, *leu2–3*, *leu2–112*, *his3–11*, *his3–15*, *trp1*, *pho3*, *pho5*	
Plasmids		
pINA62	A 5.2-kb *Sal*I fragment carrying *LEU2* of *Y. lipolytica* in *Sal*I pBR322	Gaillardin et al. 1987
pINA237	pBR322 with *LEU2* of *Y. lipolytica* and *ARS18* inserted in the *Eco*RI site	A.M. Ribet, our collection
pINA333	pBR322 with *LEU2* of *Y. lipolytica*, *ARS18* and a *MluI* linker at *Bam*HI	S. Blanchin (our laboratory)
D8	pINA62 with an insert carryind *LYC1* recovered from *Y. lipolytica*	This work
pML4	*Eco*RI - *Nhe*I fragment of D8 cloned in *Eco*RI - *Sph*I PBR322	This work
YEp52′	Derivative of YEp52 with the polylinker of M13 tg131 dowstream from *GAL10* promoter	P. Durrens (personal comm.)
pML35	A *Bal*I-*Bcl*I fragment carrying the *LYC1* gene in *Sph*I-*Bcl*I YEp52′	This work

ture media used in maintenance as well as in growth assays have been described in Gaillardin et al. (1973). Routinely the cells were grown on minimal media consisting of Difco YNB without amino acids and without ammonium sulphate supplemented with 1% glucose as a carbon source and with 0.1% L-proline or 0.1% L-lysine as a nitrogen source. For *GAL10* induction experiments, *S. cerevisiae* transformants were grown on YNB without amino acids and without ammonium sulphate containing 2% galactose, 300 mg/l threonine, 20 mg/l tyrosine, 30 mg/l lysine, 20 mg/l phenylalanine, 25 mg/l tryptophan and 20 mg/l adenine, to improve growth.

Transformation of Y. lipolytica. The method of transformation of linearized plasmids with LiCl was derived from that described by Davidow et al. (1985) for *Y. lipolytica*. The modifications introduced by Xuan et al. (1988) were adopted. Special care was devoted to the freshness of the stem culture and to the stage of cell harvest (about 1.2×10^8 cells/ml). Replicative plasmids were transformed by electroporation using the method of Meilhoc et al. (1990) and a GHT 1287 electropulsator (Jouan, France). Electrodes were 2 mm spaced and the parameters of the pulse were 675 mV and 15 ms. Cells were plated on the solid medium described by Meilhoc et al. (1990).

Molecular techniques. All restrictions, ligations, and hybridization experiments were performed according to standard protocols (Sambrook et al. 1989). Labelled compounds were supplied by Amersham-France.

Sequencing. Restriction fragments were subcloned in the polylinker of Bluescript plasmids (Stratagene) and sequence analysis was performed by the dideoxy chain-termination method (Sanger et al. 1977) on double-stranded plasmids according to the protocol of Zhang et al. (1988) using T7-modified DNA polymerase (Sequenase, US Biochemicals). The sequencing reactions were primed either with commercial primers supplied by Stratagene or with internal primers synthesized on a Cyclone Biosearch synthesiser and purified by polyacrylamide-gel electrophoresis. Sequence analysis was performed using the GCG package (Devereux et al. 1984). The *LYC1* sequence is registered in the EMBL database with the accession number X63548.

Lysine acetyl transferase assay conditions. One-litre cultures in MM were shaken overnight at 28 °C in 5-l Erlenmeyer flasks. The cells were then harvested in exponential phase by centrifugation and washed three times with distilled water at 4 °C. The cells were disrupted in a Braun shaker cell-homogenizer with glass beads in 0.1 M Tris-HCl buffer pH 7.5 containing 20% glycerol (v/v), 2.5 mM EDTA, 1 mM dithiothreitol and 1 mM phenylmethylsulphonylfluoride (PMSF). The cell homogenizate was centrifugated for 45 min at 30 000 rpm in a Beckman type 50 rotor. The supernatant was dialysed overnight against 0.02 M Tris-HCl pH 7.5 buffer (containing EDTA, glycerol and PMSF as described above). Protein concentration was estimated by the method of Bradford (1976). The crude dialysed extract can be stored for months at −20 °C. The standard incubation mixture was: 100 mM Tris-HCl pH 8.0, 2.5 mM EDTA, 1 mM dithiothreitol, 2% bovine serum albumin, 0.2 to 0.4 µCI of randomly-labelled ^{14}C-lysine (CB-16, CEA, France) and crude extract (about 0.15 to 0.2 mg of proteins) in a total volume of 200 µl. The incubations were carried out at 30 °C in Eppendorf tubes. They were prewarmed for 90 s. The reaction was started by the addition of 1.5 mM acetylCoA (final concentration). The incubation was stopped by the addition of 50 µl of 25% TCA (trichloracetic acid). The tubes were centrifuged for 2 min in an Eppendorf centrifuge. The supernatant was analysed by thin layer chromatography. Ten-microliter aliquots were placed on silica gel plates (Merck DC-Alufolien Kieselgel 60/Kieselguhr F254 precoated) previously activated for 15 min at 80 °C. Ten-microliters of 5 mM cold acetyl-lysine was added to every spot as a migration marker. The chromatography was run for 4 h using 70% isopropanol: 30% H_2O as a solvent. In this solvent system, the Rf for lysine was 0 and the Rf for N-6-acetyl-lysine was about 0.6. After drying the plates at 80 °C, they were sprayed with 0.1% nynhydrin and after 5 min at 80 °C the spot of N-6-acetyl-lysine was cut out and counted in a Packard Minaxi liquid scintillation counter.

Results and discussion

Cloning by complementation in Yarrowia lipolytica

Mutants deficient in LAT activity have so far been isolated only in *Y. lipolytica* and map in the *LYC1* gene (Gaillardin et al. 1976). Cloning of the *LYC1* gene was attempted using a gene library consisting of a *Sau*3A partial-digest of total DNA from *Y. lipolytica* inserted into the unique *Bam*HI site of the integrative vector pINA62 (Xuan et al. 1988). This plasmid carries the