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The *YJL185C, YLR376C* and *YJR129C* genes of *Saccharomyces cerevisiae* are probably involved in regulation of the glyoxylate cycle

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The ER24 *aci* (acidification) mutant of *Saccharomyces cerevisiae* excreting protons in the absence of glucose was transformed with a multicopy yeast DNA plasmid library. Three different DNA fragments restored the wild-type phenotype termed Aci$^-$ because it does not acidify the complete glucose medium under the tested conditions. Molecular dissection of the transforming DNA fragments identified two multicopy suppressor genes *YJL185C, YJR129C* and one allelic *YLR376C*. Disruption of either of the three genes in wild-type yeast strain resulted in acidification of the medium (Aci$^+$ phenotype) similarly to the original ER24 mutant. These data indicate the contribution of the ER24 gene product Ylr376Cp and of the two suppressor gene products Yjl185Cp and Yjr129Cp to a complex regulation of the glyoxylate cycle in yeast.

Keywords: S*accharomyces cerevisiae*, acidification mutants, multicopy suppressor, glyoxylate cycle, yeast

INTRODUCTION

The yeast *Saccharomyces cerevisiae* growing on complete Kok medium containing glucose and bromocresol purple forms gray colonies on a violet background (Kok *et al.*, 1975; Goffeau, 2000). At least 17 non-allelic single-gene *aci* (acidification) mutants whose colonies are surrounded by a yellow acidic zone have been isolated. This determined the Aci$^+$ mutant phenotype (Gonchar *et al.*, 1990; Boniewska-Bernacka *et al.*, 1998; Grochowalska *et al.*, 2003). The mutant cells show abrupt proton liberation when suspended in distilled water. Gas chromatography analysis indicated that the Krebs/glyoxylate cycle intermediates are the proton carriers excreted in the medium and that these intermediates preexisted in the cells at the start of the acidification test (Machnicka *et al.*, 2004). We report here a detailed study of the ER24 mutant that belongs to the complementation group III of the *aci* mutants (Grochowalska *et al.*, 2003).

MATERIAL AND METHODS

Yeast strains and plasmids. Strains and plasmids used in this work are shown in Table 1 and Table 2, respectively. For cloning we used *Escherichia coli* strain DH5*α supE44 ΔlacU169 (φ80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi1 relA1* (Hanahan, 1983).

Media. YPD (bactopeptone 1%, yeast extract 1%, dextrose 2%), mineral Go medium (yeast nitrogen base 0.67%, glucose 2%), Kok screening medium (Kok *et al.*, 1975) (glucose 1%, bactopeptone 1%, yeast extract 2%, bromocresol purple 10 mg/ml), LB (yeast extract 1%, bactotryptone 1%, glucose 0.1%, NaCl 0.5%) after Sambrook *et al.* (1989), Semi synthetic medium of Sigler *et al.* (1981). The plating media contained 2% bactoagar. Amino acids were added, if necessary.

Growth conditions. The strains were held on slants at 4ºC and cultivated at 28ºC in liquid medium with shaking. The acidifying mutants were screened

Abbreviations: Aci$^-$, wild-type phenotype; Aci$^+$, mutated phenotype; *aci,* mutated gene; *ACI,* wild-type gene.

Table 1. Strains used in this work

Strain of ***Saccharomyces cerevisiae***	Markers or description	Origin
D273-10B/A_1	*MATα met6*	Grenson M., Université Libre de Bruxelles, Belgium
F87-24B	*MATa his3*	Grenson M., Université Libre de Bruxelles, Belgium
ER24	MATα *met6 Δura3 aci1*	this study
tER24-1	*MATα met6 ACI1*	ER24 transformant
tER24-2	*MATα met6 ACI1*	ER24 transformant
tER24-3	*MATα met6 ACI1*	ER24 transformant
YJL185C	*MATa ura3-52 his3Δ1 leu2-3_112 trp1-289 YJL185c::KANMX4*	Disruptant of gene *YJL185C* (Euroscarf)
YLR376C	*MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 YLR376c::kanMX4*	Disruptant of gene *YLR376C* (Euroscarf)
YJR129C	*MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 YJL129c::kanMX4*	Disruptant of gene *YJR129C* (Euroscarf)

on Kok medium as forming colonies surrounded by yellow zones (Kok *et al.*, 1975).

Proton extrusion. Proton extrusion by yeast cells was estimated according to Haworth *et al.* (1991) with a modification by Sigler *et al*. (1991) on a computer-linked pH-meter (Radomski *et al.*, 1995).

Genetic manipulations. Strain construction, crossing and tetrad analysis were carried out by standard genetic techniques (Sherman *et al.*, 1987). Tetrads were dissected with a Singer micromanipulator. For chromosomal mapping, the mutants were crossed with a collection of 16 cir^0 tester strains, each containing plasmid DNA integrated near the centromere of suitable chromosomes (Wakem & Sherman, 1990).

Cloning strategies. *aci* strains with the Δ*ura3* disruption (introduced by recombination)

Table 2. Plasmids used in this work

Plasmid	Characterization	Origin
pFL44L	Multicopy, shuttle vector, Amp^R *URA3*	Bonneaud *et al.* (1991)
pFL44S	Multicopy, shuttle vector, Amp^R *URA3*	Bonneaud *et al.* (1991)
pFL38	Centromeric vector, Amp^R *URA3*	Bonneaud *et al.* (1991)
pER24-1/No1	pFL44L with a 3638 bp fragment of chromosome X comprising genes *YJL185C, YJL184W*	this study
pER24-3/No1	pFL44L with 5964 bp fragment of chromosome XII with genes *tK(UUU)L, FBP1, YLR376C, YLR375W(STP3), YLR374C, VID22*	this study
pER24-3/No2	Isolated from yeast gene bank plasmid pFL44L with 5415 bp fragment of chromosome X with genes *YJR128W, SNR3, YJR129C, STR2*	this study
p24-1A	Constructed from plasmid pER24-1/No1 by deletion of *Sal*I-*Sal*I fragment (959 bp), includes gene *YJL184W*	this study
p24-1B	Construct plasmid pER24-1/No1 by deletion of *Pvu*II-*Pvu*II fragment (1720 bp), includes gene *YJL185C*	this study
p24-3A	Construct plasmid pER24-3/No1 by deletion of *Bam*HI-*Bam*HI fragment (1926 bp), includes gene *YLR374C*	this study
p24-3B	*Kpn*I-*Xba*I fragment (2521 bp) from plasmid pER 24-3/No1 ligated to pFL44S, includes gene *FBP1*	this study
p24-3C	Construct *Bam*HI-*Bam*HI fragment (1926 bp) from plasmid pER24-3/No1 ligated with pFL44S, includes gene *YLR376C*	this study
p24-3D	Construct *Xba*I-*Hin*dIII fragment (2505 bp) from plasmid pER24-3/No1 ligated with pFL44S, include genes *STP3* and *YLR374C*	this study
p24-3E	Construct plasmid pER24-3/No2 by deletion of *Pvu*II-*Pvu*II fragment (1740 bp), includes gene *YJR128W*	this study
p24-3F	Construct plasmid pER24-3/No2 by deletion of *Sph*I-*Sph*I fragment (2121 bp), includes gene *STR2*	this study
p24-3G	Construct *Bam*HI-*Xho*I fragment (1857 bp) from plasmid pER24-3/No2 ligated with pFL44S, includes gene *YJR129C*	this study

were transformed with a yeast DNA library on the plasmid shuttle vector pFL44L (Bonneaud *et al*., 1991) using the lithium acetate procedure (Gietz & Woods, 1998). The transformants selected for uracil prototrophy were screened for the Aci$^-$ phenotype on Kok medium.

Plasmid DNA was isolated from the yeast transformants and cloned in *Escherichia coli*. Restriction fragments from the cloned plasmids were isolated from low melting point agarose gel (Sambrook *et al*., 1989). Sequencing of about 100 nucleotides from the ends of yeast DNA fragments was carried out using the dideoxy chain termination procedure (Sanger *et al*., 1977).

Physical mapping of the plasmid DNA isolated from the transformants was performed according to the recommendations of Sherman *et al*. (1987).

The stability of plasmids in the transformants was tested by mitotic segregation. Yeast cells were serially cultivated for about 24 generations in complete YPD medium. After each passage a sample of about one hundred cells was plated on Kok medium and the fraction of Aci$^+$ colonies was determined (Kok *et al*., 1975).

RESULTS

Genetics of the *aci1* mutant ER24

The mutant ER24 excreted acids during growth on glucose complete medium. It was unable to grow on glycerol, acetate, ethanol, or citrate and this feature co-segregated 2:2 with the Aci$^+$ character in 20 tetrads from a cross with the isogenic wild-type stain F87-24B (not shown).

The mutated genes responsible for acidification are recessive, as the diploid obtained from a cross to the isogenic strain F87-24B *MATa his3* did not excreting acids. The inability to grow on glycerol is not caused by cytochrome defects which were present (Claisse *et al*., 1992).

In order to localize the *aci1* gene causing the ER24 phenotype, the Sherman collection of *cir*^{0} tester strains for each chromosomes of both *MATα* and *MATa* were crossed to the ER24 mutant in which *ura3* was introduced by recombination (Wakem & Sherman, 1990).

The diploids obtained were grown in complete YPD medium for at least 24 generations and an increased frequency of Aci$^-$ colonies was observed in the diploids of the mutant ER24 and chromosome X or XII tester strains, indicating that *ER24* was located on one of these two chromosome (not shown).

Proton extrusion test

When cells of the wild-type strains D273-10B/A1 or F87-24B were suspended in distilled water, the pH remained at a constant level of 5 to 6. Addition of glucose led to a decrease of pH (Fig. 1a and b). A further decrease of pH was stimulated by addition of potassium ions.

The same experiment was carried out with the ER24 mutant (Fig. 1c). In this case addition of yeast cells to distilled water led to an abrupt decrease of pH, and the following addition of glucose or potassium ions was without effect.

Acidification by the ER24 mutant without glucose addition does not occur at the cost of endogenous substrates as starvation for 16 h does not deprive the mutant of the acidification ability (not shown).

The glucose-independent acidification co-segregated with the Aci$^+$ character as shown in Fig. 1.d–h. According to the recesiveness of the *aci* mutations the heterozygous diploid excretes protons upon glucose addition although some abrupt decrease of pH immediately after addition of yeast cells to distilled water is also observed. In the tetrad, the two Aci$^+$ spore clones extruded protons upon addition of water and glucose had no effect. The two Aci$^-$ spores excrete acids upon glucose addition although as in the case of the diploid, the addition of water alone cause some slight abrupt pH decrease.

Isolation and cloning of genes complementing the mutant phenotype of ER24

In order to isolate genes restoring wild-type (Aci$^-$) phenotype in the ER24 mutant we used a yeast bank gene library on multicopy plasmid pFL44L obtained thanks to courtesy of Dr. Francois Lacroute (Gif-sur-Yvette, France).

The *Δura3* marker was incorporated by recombination into the mutant used a recipient in the transformation. The recombinant *aci1 Δura3* cells were transformed with the DNA bank and the transformants ER24 selected for Ura prototrophy were screened for the Aci$^-$ phenotype. Three transformants of ER24 were isolated and designated tER24-1, -2 and -3.

Test of mitotic segregation

In order to check the stability of the plasmids carrying the genes restoring the Aci$^+$ phenotype, mitotic segregation of the transformed markers was determined.

After about 24 generations on complete medium (without selective pressure) approx. 80% of

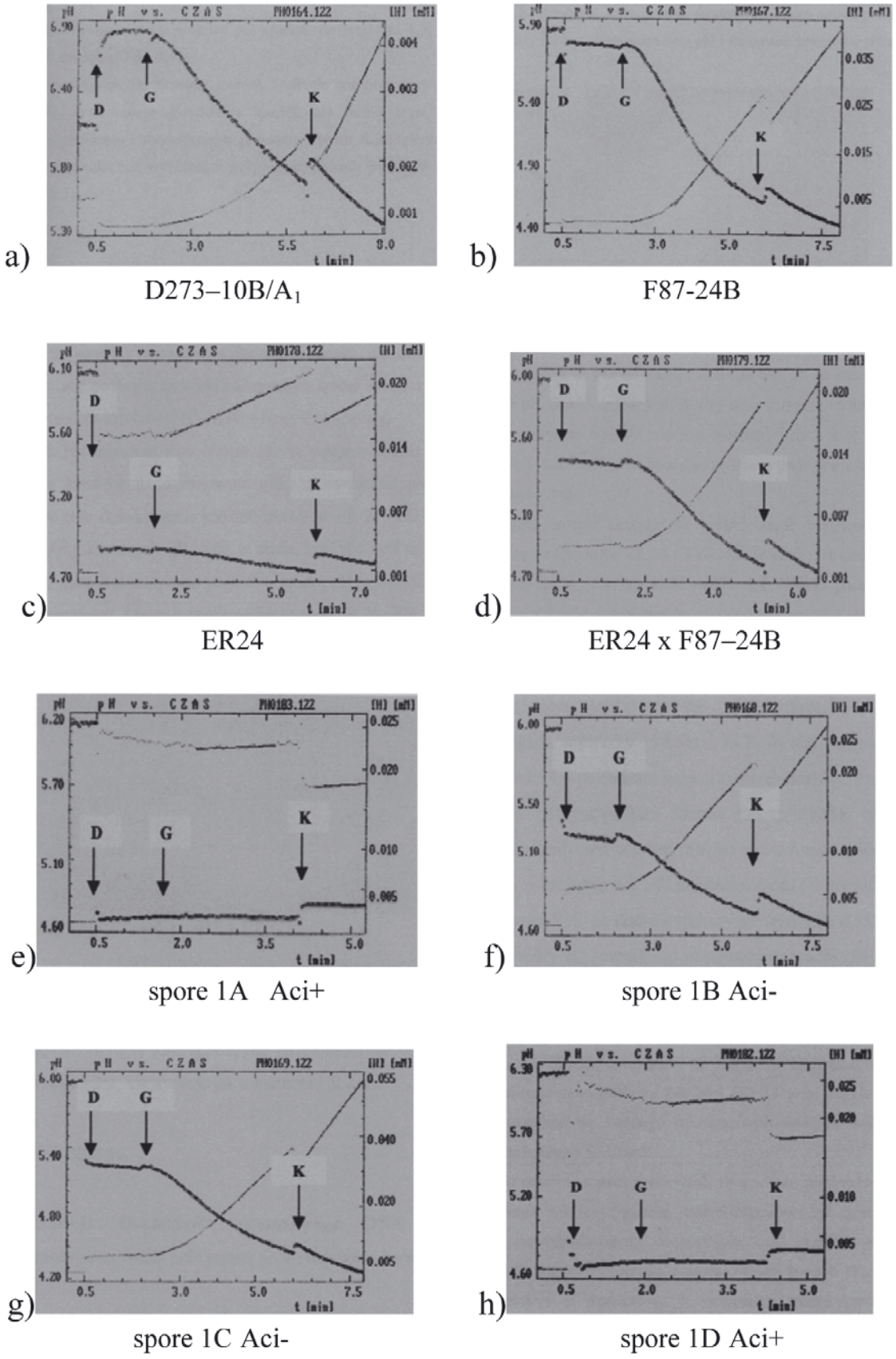

Figure 1. Acidification traces after successive addition of yeast cells (D) from the original strain D273-10B/A_1 (a), the isogenic strain F87-24B (b), the ER24 mutant (c), the diploid ER24 x F87-24B (d), the spore clones of the tetrad (e, spore1A Aci^+; f, spore1B Aci^-; g, spore1C Aci^-; h, spore 1D Aci^+), 50 mM glucose (G) or 20 mM KCl (K) to distilled water.

Table 3. Phenotypes of transformants of non-allelic mutants from different complementation groups with multicopy genes *YJL185C, YLR376C* and *YJR129C*

Aci phenotype of transformant obtained from mutants:																	
Complementation group	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
Aci⁻ Mutants	EG7	EG13	ER24	EG37	EG40	EG47	EG74	EG77	EG84	EG87	EG85	EG43	EG51	EG8	EG20	EG30	EG16
Genes *aci*																	
YJR129C	Aci⁻	Aci⁻	Aci⁻	Aci⁻	Aci⁻	Aci⁻	0	Aci⁻	Aci⁺	Aci⁻	Aci⁺	Aci⁺	Aci⁺	Aci⁺	Aci⁻	Aci⁺	Aci⁻
YJL185C	Aci⁻	Aci⁺	Aci⁻	Aci⁻	Aci⁺	0	Aci⁻	Aci⁻	Aci⁺	0	Aci⁺	Aci⁺	Aci⁺	Aci⁺	Aci⁺	Aci⁺	Aci⁻
YJR376C	Aci⁻	Aci⁻	Aci⁻	Aci⁻	Aci⁻	Aci⁻	Aci⁻	Aci⁻	Aci⁺	Aci⁻	Aci⁺	Aci⁺	Aci⁺	Aci⁺	Aci⁺	Aci⁺	Aci⁺

the cells retained the complete plasmids. This indicates that the transformants were rather stable.

Sequencing and identification of yeast genome fragments

Plasmid DNA was isolated from the transformants. The plasmids were cloned in *E. coli* strain DH5α and for each yeast transformant three bacterial clones were isolated. The plasmids were restriction mapped with *Eco*RI and *Hin*dIII. For further investigation we chose three plasmids that differed in restriction maps (pER24-1/No1, pER24-3/No1, pER24-3/No2). After retransformation of the plasmids chosen into the ER24 strain, one hundred of transformants obtained (selected for Ura prototrophy) appeared Aci⁻.

The fragments of the yeast genome carried on the plasmids were cut out, separated by electrophoresis and their 3′ and 5′ ends were sequenced. The obtained nucleotide sequences were identified using the BLAST program (Altschul *et al*., 1990).

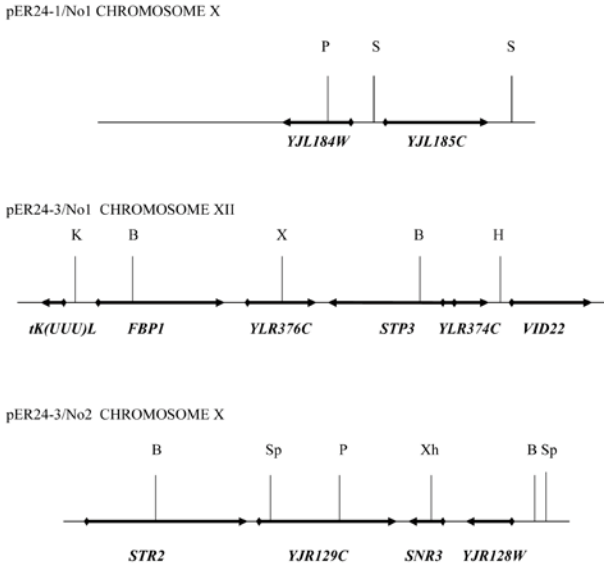

Figure 2. Molecular map of the genes restoring the wild phenotype in the ER24.
Restriction enzymes and cut sites: P (*Pvu*II), S (*Sal*I), K (*Kpn*I), B (*Bam*HI), X (*Xba*I), H (*Hin*dIII), Sp (*Sph*I), Xh (*Xho*I).

Plasmid tER24-1/No1 contained a 3638 bp fragment from chromosome X (81639 to 85276) with ORFs: *YJL185C* (hypothetical ORF), and *YJL184W* (hypothetical ORF) (Fig. 2).

Plasmid tER24-3/No1 contained a 5964 bp fragment from chromosome XII (876262 to 870299) with ORFs: tK(UUU)L (tRNA), *YLR377C* (*FBP1* — fructose-bisphosphatase), *YLR376C* (hypothetical ORF), *YLR375W* (*STP3* — involved in pre-tRNA splicing and in uptake of branched-chain amino acids), *YLR374C* (hypothetical ORF), and *YLR373C* (*VID22* — glycosylated integral membrane protein localized to the plasma membrane) (Fig. 2).

Plasmid tER24-3/No2 contained a 5415 bp fragment from chromosome X (667453 to 662038) with ORFs: *YJR128W* (hypothetical ORF), *SNR3* (small nucleolar RNA), *YJR129C* (hypothetical ORF) and *YJR130C* (*STR2* — cystathionine gamma-synthase) (Fig. 2).

Identification of the gene(s) restoring wild-type phenotype in the ER24 mutant

As cloned, the yeast genomic fragment of plasmid tER24-1/No1 restoring the wild-type Aci⁻ phenotype contained two complete genes. In order to determine which of these genes is responsible for the suppression, we successively cut out the genes *YJL184W* and *YJL185C* with the restriction enzyme *Sal*I (Fig. 2), inserted them into empty plasmid pFL44L and transformed the ER24 mutant (Table 2). The Aci⁻ (wild-type) phenotype was found only in transformants that obtained the *YJL185C* gene.

As cloned, the yeast genomic fragment of plasmid tER24-3/No1 restoring the wild-type Aci⁻ phenotype contained six genes: *tK(UUU)L, FBP1, YLR376C, STP3, YLR374C* and *VID22* (Table 2, Fig. 2) and inserted them separately into empty plasmid pFL44L. In

order to determine which of them was responsible for the suppression, we successively cut out the genes with restriction enzymes (Fig. 2). Out of the four constructs obtained only one with the *YLR376C* gene restored the wild-type Aci$^-$ phenotype in the ER24 mutant.

Plasmid pER24-3/No2 restoring the wild-type Aci$^-$ phenotype in ER24 contained yeast DNA carrying four genes: *STR2, YJR129C, SNR3* and *YJR 128W* (Fig. 2). The genes were separately subcloned in pFL44L. Restoration of the wild-type phenotype was obtained only with the plasmid carrying the *YJR129C* gene (Fig. 2).

Disruption of the *YJL185C, YLR376C* and *YJR129C* genes

Each of the genes restoring the wild-type phenotype in ER24, *YJL185C, YLR376C* and *YJR129C*, was disrupted in the wild-type strain. The disruptants (from Euroscarf) were unable to grow on glycerol and produced the acid yellow halo on Kok medium. In the liquid acidification test, each mutant extruded protons without glucose stimulation. The disruptants were recessive as they gave wild-type Aci$^-$ diploids when crossed with the wild-type strain D273-10B/A_1. In the diploids the phenotype of abrupt proton extrusion in the absence of glucose gave monogenic 2:2 segregation. In other words, they showed the typical mutated Aci$^+$ phenotype.

The suppression appears non-specific as each of the wild type genes *YJL185C, YLR376C* and *YJR129*C on a multicopy plasmid restored the Aci$^-$ wild-type phenotype in several other non-allelic *aci* mutants (Table 3). This will be explained in more detail in a next publication.

In order to determine which of the three genes is allelic to ER24 (*aci1*) mutation, we carried out complementation experiments with three disruptants. The disruptants, named YJL185C, YLR376C and YJR129C, were crossed with the original ER24 mutant and the obtained diploids were tested for the acidification phenotype. One of the analyzed diploids, ER24 x YLR376, was Aci$^+$ indicating allelism of YLR376 with the ER24 mutant *aci1* gene. To confirm this finding, the *YLR376* gene was introduced into the centromeric plasmid pFL38 and the original ER24 mutant was transformed with the obtained construct. The resulting transformant was Aci$^-$, as expected. The other two genes (*YJL185C* and *YJR129C*) when similarly tested, appeared to be multicopy, but not single-copy, suppressors of the mutant Aci$^+$ phenotype.

DISCUSSION

The *aci1* mutant ER24 was transformed with a genomic DNA library on the multicopy shuttle plasmid pFL44L. We found that transformants carrying the *YJL185C, YLR376C* or *YJR129C* genes from chromosomes X, XII and X, respectively, restored the wild-type Aci$^-$ phenotype in the ER24 mutant.

The suppression is unspecific as each of this three genes restores the wild-type phenotype of many other non-allelic *aci* mutants. When disrupted in a wild-type strain, each of the genes gives the Aci$^+$ phenotype and prevents growth on glycerol. According to the test of complementation one of these genes appeared allelic with the ER24 mutation. The other two genes appeared to be multicopy suppressors of the ER24 mutant Aci$^+$ phenotype.

Their function is far from being fully understood. Out of several possibilities considered also in our previous publication (Machnicka *et al.*, 2002) we propose the following two most likely interpretations. Either the multicopy suppressors modify the components of the cellular membrane in such a way that leakage of acid intermediates is prevented or they function in a complex regulatory mechanism that turns on the glyoxylic acid cycle when the keto acid substrates for amino acid synthesis are not required. The second possibility may be more likely, at least for the *YLR376C* and *YJR185C* genes. Indeed, while Yjl129Cp is still of unknown function, recent evidence suggests possible regulatory functions for Ylr185Cp either in chromatin action or in protein trafficking and for Yjr376Cp at the level of DNA maintenance. The protein Ylr185Cp is a putative methyltransferase. Its expression is drastically increased in Histone 4 mutants and this protein may be involved in microtubule biogenesis as a member of the folding of a prechaperone complex (Huh *et al.*, 2003). Ylr376Cp/Psy3p has been reported to be part of a large novel protein complex involved in error-free DNA repair (Shor *et al.*, 2005). How such proteins may regulate the expression and or activity of the glyoxilic and tricarboxilate cycles remains to be determined. The described genes seem to represent a new family of genes encoding a new family of proteins.

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REFERENCES

Altschul SH, Gish W, Miller W, Myers W, Lipman J (1990) Basic local aligment search tool. *J Mol Biol* **215**: 403–410.

Boniewska-Bernacka E, Lachowicz TM, Kotylak Z (1998) Genetical and biochemical characterization of Krebs cycle mutants in yeast. *Acta Microbiol Polon* **47**: 131–140.

Bonneaud N, Ozier-Kalogeropoulos O, Li G, Labouesse M, Minvielle-Sebastia L, Lacroute F (1991) A family of low and hight copy replicative, integrative and single-stranded *S. cerevisiae*/*E. coli* shutte vectors. *Yeast* **7**: 609–613.

Claisse M, Lachowicz TM, Sybirny A, Gonchar M, Boniewska E, Witkowska R (1992) Isolation and characterization of Krebs cycle mutants of yeast *Saccharomyces cerevisiae*. *Yeast* **8**: 105.

Gietz RD, Woods RA (1998) Transformation of yeast by the lithium acetate/single-stranded carrier DNA/PEG method. *Methods Microbiol* **20**: 53–66.

Goffeau A (2000) Four years of post-genomic life with 6000 yeast genes. *FEBS Lett* **480**: 37–41.

Gonchar M, Lachowicz TM, Sybirny A, Witkowska R, Zakowska Z (1990) Genetics, physiology and biochemistry of some *Saccharomyces cerevisiae* mutants excreting acidic products. In *Genetics of Respiratory Enzymes in Yeast*. pp 53–64. Wroclaw University Press, Wrocław.

Grochowalska R, Machnicka B, Wysocki R, Lachowicz TM (2003) The genetic characteristics *Saccharomyces cerevisiae* aci(+) mutants. *Cell Mol Biol Lett* **8**: 261–268.

Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* **166**: 557–580.

Haworth R, Lemire B, Crandall D, Cragoe E, Fliegel L (1991) Characterization of proton fluxes across the cytoplasmic membrane of the yeast *Saccharomyces cerevisiae*. *Biochim Biophys Acta* **1089**: 78–89.

Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK (2003) Global analysis of protein localization in budding yeast. *Nature* **425**: 686–691.

Kok J, Muller JL, Slater EC (1975) EPR studies on the respiratory chain of wild type *Saccharomyces cerevisiae* and mutants with deficiency in succinate dehydrogenase. *Biochim Biophys Acta* **912**: 441–450.

Machnicka B, Grochowalska R, Lipiński T, Boniewska E, Słomińska L, Lachowicz TM (2002) An identification of the products excreted by *Saccharomyces cerevisiae* aci$^+$ mutants (acidifying growth media). *Cell Mol Biol Lett* **7**: 304.

Machnicka B, Grochowalska R, Boniewska-Bernacka E, Słominska L, Lachowicz TM (2004) Acid excreting mutants of yeast *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* **325**: 1030–1036.

Radomski R, Radomska M, Szajnowska K, Wisialski Z (1995) Microcomputer-controlled electrochemical universal meter. *Computers Chem Res* **7**: 40–46.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning. A Laboratory Manual.* 2nd edn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sanger F, Nicklen S, Coulson A (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**: 5463–5467.

Sherman F, Fink GR, Hicks JB (1987) *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor NewYork.

Shor E, Weinstein J, Rothstein R (2005) A genetic screen for top3 suppressors in *Saccharomyces cerevisiae* identifies *SHU1*, *SHU2*, *PSY3* and *CSM2*: Four genes involved in error-free DNA repair. *Genetics* **169**: 1275–1289.

Sigler K, Hofer M (1991) Mechanism of acid extrusion in yeast. *Biochim Biophys Acta* **1071**: 375–391.

Sigler K, Knotkova A, Kotyk A (1981) Factors governing substrate induced generation and extrusion of protons in the yeast *Saccharomyces cerevisiae*. *Biochim Biophys Acta* **643**: 572–582.

Wakem L, Sherman F (1990) Chromosomal assignment of mutations by specific chromosome loss in the yeast *Saccharomyces cerevisiae*. *Genetics* **12**: 333–340.