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Genetic and Physical Localization of the Acetyl-Coenzyme A Synthetase Gene *ACS1* on Chromosome I of *Saccharomyces cerevisiae*

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The *ACS1* gene, encoding acetyl-coenzyme A synthetase, was mapped genetically at the left arm of chromosome I between *pURA3* and *PYK1* at 19 and 28 cM respectively. Comparison with the physical map defined a recombinational 'hot-spot' in this region in addition to the one between *CDC24* and *PYK1*.

KEY WORDS — *Saccharomyces cerevisiae*; chromosome I; genetic mapping; acetyl-coenzyme A synthetase; *ACS1*.

Many genes involved in primary metabolism of *Saccharomyces cerevisiae* have been cloned and much has been learned about their regulation. Until recently the gene encoding acetyl-coenzyme A synthetase (EC 6.2.1.1) was an exception. The enzyme catalyses the conversion of acetate into acetyl-coenzyme A and is essential for the yeast when growing on C-2 components, i.e. acetate and ethanol.

It has been found that acetyl-coenzyme A synthetase is also used during growth on glucose at elevated sugar concentrations (Postma *et al.*, 1989). Together with pyruvate decarboxylase and acetaldehyde dehydrogenase it forms a metabolic bypass of the mitochondrially located pyruvate dehydrogenase complex (Holzer and Goedde, 1957).

It is not clear, however, if the same enzyme is involved in the bypass and growth on C-2 components. Two forms of acetyl-coenzyme A synthetase have been described, which appear under different physiological conditions and are located in different subcellar compartments (Satyanarayana *et al.*, 1980; Klein and Jahnke, 1979).

Recently a *S. cerevisiae* gene was cloned which showed high homology with acetyl-coenzyme A synthetase genes from the fungi *Aspergillus nidulans* and *Neurospora crassa* and from the archaebacterium *Methanotrix soehngenii*. Disruption of this gene resulted in mutants which lacked the strong increase in acetyl-coenzyme A synthetase activity found in

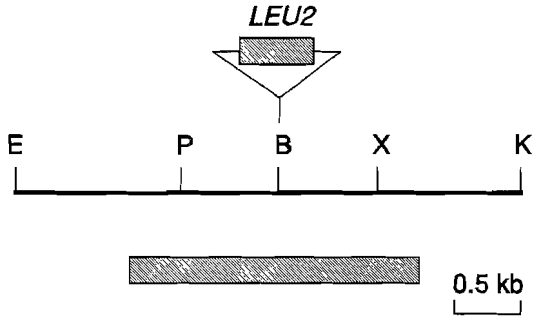

Figure 1. Disruption of the *ACS1* gene. The *LEU2* gene was cloned into the *Bam*HI site in the centre of the *ACS1* open reading frame (De Virgilio *et al.*, 1992). B = *Bam*HI, E = *Eco*RI, K = *Kpn*I, P = *Pst*I, X = *Xba*I. The box shows the position of the open reading frame.

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Table 1. Linkage and recombination frequency between *pURA3*, *ACS1* and *PYK1*.

Gene pair	PD	NPD	T	Genetic distance (cM)	Physical distance (kb)	Recombination frequency (cM/kb)
pURA3–ACS1						
DK387-1BxMM67	31	0	11			
DK387-9CxMM67	31	1	9			
Total	62	1	20	19	9·5	2·00
pURA3–PYK1						
DK387-1BxMM67	17	2	22			
DK387-9CxMM67	20	3	15			
Total	37	5	37	42	40·5	1·04
ACS1–PYK1						
DK387-1BxMM67	22	1	18			
DK387-9CxMM67	23	1	14			
Total	45	2	32	28	31	0·90

Standard methods were used for crosses, sporulation and dissection of asci (Sherman *et al.*, 1974). The *pURA3* marker and the *acs1::LEU2* and *pyk1-102* alleles were scored by the Ura$^+$, Leu$^-$ and temperature-sensitive phenotypes respectively. In all but four (not included in the table) asci these markers segregated 2 : 2. Distances were calculated using Perkin's formula, $50 \times (T+6 \times NPD)/(PD+NPD+T)$, where PD is parental ditype, NPD non-parental ditype and T tetratype.

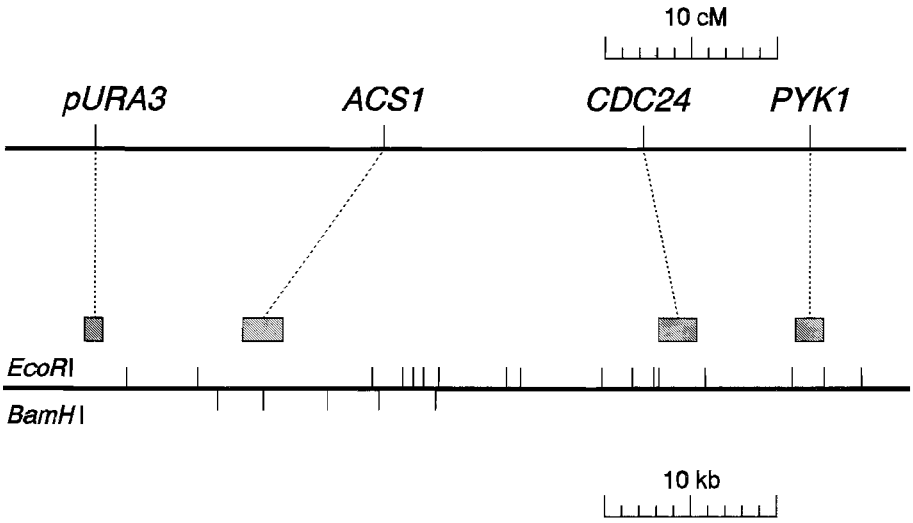

Figure 2. Genetic and physical maps of part of the left arm of chromosome I. Top bar represents the genetic map, bottom bar the physical map on which the *Eco*RI sites, above the line, and *Bam*HI sites, under the line, are shown. Boxes give the positions of the open reading frames. For comparison, the *CDC24* gene was included. It was placed on the genetic map at 11 cM from *PYK1* (Kaback *et al.*, 1989) and 7·5 kb from *PYK1* on the physical map (Coleman *et al.*, 1986).

wild-type yeast cells upon entering stationary phase (De Virgilio *et al.*, 1992).

Pulsed-field gel electrophoresis located the gene, named *ACS1*, on chromosome I (De Virgilio *et al.*, 1992). A 1·4 kb *Xba*I-*Pst*I probe corresponding to the middle of the gene hybridized to a 9·0 kb *Eco*RI fragment and to 3·0 kb and 2·2 kb *Bam*HI fragments on Southern blots of chromosomal DNA. By comparison with the physical map of chromosome I for these restriction enzymes (Kaback *et al.*, 1989), we could place the *ACS1* gene on the left arm of the chromosome between the *pURA3* marker and *CDC24*.

We checked this localization by genetic crosses. Since the Acs$^-$ phenotype was hard to score, the gene was disrupted by inserting a 2·2 kb *Bgl*II fragment containing the *LEU2* gene in the *Bam*HI site in the centre of the transcribed region of the *ACS1* gene as shown in Figure 1 (De Virgilio *et al.*, 1992). The resulting *acs1::LEU2* allele was scored by the Leu$^+$ phenotype. Strain MM67 (*Matα ura3 leu2 his4 bar1 acs1::LEU2*) (De Virgilio *et al.*, 1992) was crossed to two segregants of strain DK387 (*MAT***a**/*MATα ade1*/+ *pyk1-102*/+ *pURA3*/0 *trp1*/+ *ade2*/+ *leu2*/+ *ura3-52*/*ura3-52 his3*/*his3*) (Kaback *et al.*, 1989). Both segregants, DK387-1B and DK387-9C, contained the *pURA3* marker and the *pyk1-102* allele, which is a temperature-sensitive allele. In addition, both had mating-type **a** and were Leu$^-$.

The results of the crosses are shown in Table 1. They confirm that the *ACS1* gene is located between *pURA3* and *PYK1* on the left arm of chromosome I. The mapping distances are 19 and 28 cM respectively. It may further be seen that the distance between *pURA3* and *PYK1* corresponds to previous data (Kaback *et al.*, 1989; Mortimer *et al.*, 1989).

Comparison of the genetic and physical distances between the three markers (Figure 2) shows that the recombination frequency in the interval between *pURA3* and *ACS1* is approximately 2 cM/kb, which is much higher than 0·3 cM/kb, the average for the whole yeast genome, or the 0·6 cM/kb found for the smaller chromosomes (Kaback *et al.*, 1989; Mortimer and Schild, 1985). The recombination frequency in the *ACS1–PYK1* interval is also slightly elevated. Since most of the elevated recombination frequency between *ACS1* and *PYK1* might be caused by the recombinational 'hotspot' previously located between *CDC24* and *PYK1*, the recombination frequency between *ACS1* and *CDC24* might be normal, as has been reported for the total *pURA3–CDC24* interval (Kaback *et al.*, 1989). However, our data define another 'hotspot' between *pURA3* and *ACS1*, suggesting the *ACS1–CDC24* interval has a lower than average recombination frequency. These data seem to support the notion that the relatively high average recombination frequency for chromosome I in comparison to larger chromosomes is due to several interspersed recombinational 'hot spots' (Kaback *et al.*, 1989).

REFERENCES

Coleman, K. G., Steensma, H. Y., Kaback, D. B. and Pringle, J. R. (1986). Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: Isolation and characterization of the *CDC24* gene and adjacent regions of the chromosome. *Mol. Cell. Biol.* **6,** 4516–4525.

De Virgilio, C., Burckert, N., Barth, G., Neuhaus, J.-M., Boller, T. and Wiemken, A. (1992). Cloning and disruption of a gene for growth on acetate but not on ethanol: the acetyl-coenzyme A synthetase gene of *Saccharomyces cerevisiae*. *Yeast*, **8,** 1043–1051.

Holzer, H. and Goedde, W. H. (1957). Zwei Wegen von Pyruvate zu Acetyl coenzyme A in Hefe. *Biochem. Z.* **329,** 175–191.

Kaback, D. B., Steensma, H. Y. and de Jonge, P. (1989). Enhanced meiotic recombination on the smallest chromosome of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **86,** 3694–3698.

Klein, H. P. and Jahnke, L. (1979). Effect of aeration on formation and localization of the acetyl-coenzyme A synthetases of *Saccharomyces cerevisiae*. *J. Bacteriol.* **137,** 179–184.

Mortimer, R. K. and Schild, D. (1985). Genetic map of *Saccharomyces cerevisiae*, edition 9. *Microbiol. Rev.* **49,** 181–212.

Mortimer, R. K., Schild, D., Contopoulos, C. R. and Kans, J. (1989). Genetic map of *Saccharomyces cerevisiae*, edition 10. *Yeast* **5,** 321–403.

Postma, E., Verduyn, C., Scheffers, W. A. and van Dijken, J. P. (1989). Enzymic analysis of the Crabtree effect in glucose-limited cultures of *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **55,** 368–477.

Satyanarayana, T., Chervenka, C. H. and Klein, H. P. (1980). Subunit specificity of the two acetyl-CoA synthetases of yeast as revealed by an immunological approach. *Biochim. Biophys. Acta* **614,** 601–606.

Sherman, F., Fink, G. R. and Lawrence, C. W. (1974). *Methods in Yeast Genetics* (revised edn 1983). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.