Yeast Mapping Reports

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 subcellular 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 BamHI site in the centre of the ACS1 open reading frame (De Virgilio et al., 1992). B = BamHI, E = EcoRI, K = KpnI, P = PcoI, X = XbaI. The box shows the position of the open reading frame.](image-url)
Table 1. Linkage and recombination frequency between \textit{pURA3}, \textit{ACSI} and \textit{PYKI}.

<table>
<thead>
<tr>
<th>Gene pair</th>
<th>PD</th>
<th>NPD</th>
<th>T</th>
<th>Genetic distance (cM)</th>
<th>Physical distance (kb)</th>
<th>Recombination frequency (cM/kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{pURA3-ACSI}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DK387-1BxMM67</td>
<td>31</td>
<td>0</td>
<td>1</td>
<td>11</td>
<td></td>
<td></td>
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<tr>
<td>DK387-9CxMM67</td>
<td>31</td>
<td>1</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>1</td>
<td>20</td>
<td>19</td>
<td>9.5</td>
<td>2.00</td>
</tr>
<tr>
<td>\textit{pURA3-PYKI}</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>DK387-1BxMM67</td>
<td>17</td>
<td>2</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DK387-9CxMM67</td>
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<td>3</td>
<td>15</td>
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</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>5</td>
<td>37</td>
<td>42</td>
<td>40.5</td>
<td>1.04</td>
</tr>
<tr>
<td>\textit{ACSI-PYKI}</td>
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<tr>
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<td>1</td>
<td>18</td>
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<td>Total</td>
<td>45</td>
<td>2</td>
<td>32</td>
<td>28</td>
<td>31</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Standard methods were used for crosses, sporulation and dissection of asci (Sherman et al., 1974). The \textit{pURA3} marker and the \textit{acs1::LECZ} and \textit{pyk1-102} alleles were scored by the Ura\textsuperscript{+}, Leu\textsuperscript{+} and temperature-sensitive phenotypes respectively. In all but four (not included in the table) asci these markers segregated 2:2:2:2. 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 \textit{EcoRI} sites, above the line, and \textit{BamHI} sites, under the line, are shown. Boxes give the positions of the open reading frames. For comparison, the \textit{CDC24} gene was included. It was placed on the genetic map at 11 cM from \textit{PYK1} (Kaback et al., 1989) and 5.8 kb from \textit{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 ACSI, on chromosome I (De Virgilio et al., 1992). A 1.4 kb XbaI-PstI probe corresponding to the middle of the gene hybridized to a 9.0 kb EcoRI fragment and to 3.0 kb and 2.2 kb BamHI 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 ACSI gene on the left arm of chromosome 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 BglII fragment containing the LEU2 gene in the BamHI site in the centre of the transcribed region of the ACSI gene as shown in Figure 1 (De Virgilio et al., 1992). The resulting acsl::LEU2 allele was scored by the Leu" phenotype. Strain MM67 (Mata ura3 leu2 his4 bar1 ascl::LEU2) (De Virgilio et al., 1992) was crossed to two segregants of strain DK387 (MATa/ MATa ade1 + pykl-102 + pURA3::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 pykl-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 ACSI 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 ACSI 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 ACSI—PYK1 interval is also slightly elevated. Since most of the elevated recombination frequency between ACSI and PYK1 might be caused by the recombinational 'hotspot' previously located between CDC24 and PYK1, the recombination frequency between ACSI 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 ACSI, suggesting the ACSI—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


