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The acetyl-CoA synthetase gene *ACS2* of the yeast *Saccharomyces cerevisiae* is coregulated with structural genes of fatty acid biosynthesis by the transcriptional activators Ino2p and Ino4p

Margit Hiesinger, Christian Wagner, Hans-Joachim Schüller*

Institut für Mikrobiologie, Biochemie und Genetik, Lehrstuhl Biochemie, Universität Erlangen/Nürnberg, Staudtstr. 5, D-91058 Erlangen, Germany

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Abstract The yeast *Saccharomyces cerevisiae* contains two acetyl-CoA synthetase genes, *ACS1* and *ACS2*. While *ACS1* transcription is glucose repressible, *ACS2* shows coregulation with structural genes of fatty acid biosynthesis. The *ACS2* upstream region contains an ICRE (inositol/choline-responsive element) as an activating sequence and requires the regulatory genes *INO2* and *INO4* for maximal expression. We demonstrate in vitro binding of the heterodimeric activator protein Ino2p/Ino4p to the *ACS2* promoter. In addition, the pleiotropic transcription factor Abf1p also binds to the *ACS2* control region. The identification of *ACS2* activating elements also found upstream of *ACC1*, *FAS1* and *FAS2* suggests a role of this acetyl-CoA synthetase isoenzyme for the generation of the acetyl-CoA pool required for fatty acid biosynthesis.

Key words: Acetyl-CoA synthetase; *Saccharomyces cerevisiae*; Fatty acid biosynthesis; transcriptional regulation; *INO2*

1. Introduction

The activated C_2-metabolite acetyl-coenzyme A (acetyl-CoA) is required for several anabolic pathways of cellular metabolism. Since metabolic demands for acetyl-CoA may vary with the current nutrient supply of a cell, a regulatory balance among acetyl-CoA consuming and acetyl-CoA producing pathways appears reasonable. In the yeast *Saccharomyces cerevisiae*, biosynthesis of acetyl-CoA is mainly achieved by the acetyl-CoA synthetase (ACS) reaction, while oxidative decarboxylation by the mitochondrial pyruvate dehydrogenase complex appears to be of minor importance (reviewed in [1]). Even under glycolytic growth conditions, *S. cerevisiae* converts pyruvate into acetate, catalyzed by the subsequent action of pyruvate decarboxylase (PDC), acetaldehyde dehydrogenase and ACS [2]. In the presence of a fermentable carbon source, acetyl-CoA may be mainly used as a precursor of fatty acid and sterol biosynthesis. On the other hand, an additional pool of acetyl-CoA is required for the glyoxylate cycle (citrate synthase and malate synthase reactions) when cells grow with a non-fermentable substrate such as ethanol or acetate.

Previously, two ACS isoenzymes showing distinct immunological, biochemical and regulatory properties have been characterized in *S. cerevisiae* [3,4]. These findings agree with the recent isolation of two structural genes, *ACS1* [5,6] and *ACS2* [7], which exhibit a sequence identity of 57% at the protein level. While *ACS1* is transcriptionally repressed by high concentrations of glucose (or other easily fermentable sugars), a severe derepression occurs with limiting amounts of sugar or in the presence of a non-fermentable carbon source such as acetate or ethanol [6,8]. Thus, *ACS1* must be considered as the structural gene which contributes to the biosynthesis of acetyl-CoA under gluconeogenic growth conditions while a function of *ACS2* for fatty acid biosynthesis appears plausible.

Transcription of the yeast fatty acid synthase genes *FAS1* and *FAS2* is activated by ICRE motifs (inositol/choline-responsive element; [9]). Synthetic promoters containing the ICRE as the sole upstream activation site (UAS) mediate a differential gene expression, regulated by the availability of phospholipid precursors inositol and choline. An ICRE motif was also found in the control region of the acetyl-CoA carboxylase gene *ACC1/FAS3* [10]. In general, ICRE sequences are involved in the coordinate transcriptional activation of phospholipid biosynthesis, affecting genes such as the inositol-1-phosphate synthase gene *INO1* [11] and the phosphatidylserine synthase gene *PSS1* ([12]; summarized in [13]). ICRE-dependent gene activation requires functional regulatory genes *INO2* and *INO4*, both encoding proteins with a basic helix-loop-helix (bHLH) motif characteristic of DNA-binding proteins. Indeed, interaction of the heterodimeric Ino2p/Ino4p complex with the ICRE could be shown [14]. In addition to Ino2p/Ino4p, *FAS* genes are also activated by pleiotropic transcription factors such as Rap1p, Abf1p and Reb1p [15,16]. Thus, pathway-specific factors as well as general activator proteins are required for the efficient transcription of genes involved in fatty acid biosynthesis. In this work, we identify Ino2p/Ino4p as an activating factor of *ACS2* gene expression. Thus, acetyl-CoA necessary for fatty acid biosynthesis may be provided by the *ACS2* encoded acetyl-CoA synthetase isoenzyme.

2. Materials and methods

2.1. Yeast strains, media and growth conditions

Strains of *S. cerevisiae* used for this work are listed in Table 1. Yeast transformation was performed by an established procedure [17]. Gene disruption cassettes to obtain *Δino2::LEU2*, *Δino4::LEU2*, *Δino4::URA3* and *Δacs1::LEU2* null mutations, respectively, have been described [6,14,18]. Strains were grown in synthetic complete media at 30°C under selective conditions. Carbon sources were added as indicated. For inositol/choline repression, cells were grown with 200 μM inositol+2 mM choline. To achieve derepressing conditions, cells were grown with 5 μM inositol+5 μM chol-

*Corresponding author. Fax: +49 (9131) 858254.
E-mail: jschuell@biologie.uni-erlangen.de

Abbreviations: ACS, acetyl-coenzyme A synthetase; ICRE, inositol/choline-responsive element; ORF, open reading frame; UAS, upstream activation site

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ine. Fatty acid supplemented media contained 0.01% hydrolyzed butter+1% Tween 40.

2.2. Northern blot hybridization

Isolation of total cellular RNA and fractionation of denatured RNA (20 μg) has been described [6]. The 1.1 kb KpnI/SacI fragment containing part of the *ACS2* reading frame was ^{32}P-labelled by nick-translation. Hybridization was done under stringent conditions. As a reference, a labelled fragment with the actin gene *ACT1* was used.

2.3. Plasmid constructions

Initially, the complete *ACS2* gene was amplified by the polymerase chain reaction and subsequently cloned as a 3.1 kb PstI/SacI fragment (kindly provided by S. Kratzer). The *ACS2-lacZ* fusion gene was obtained by transfer of a 2.0 kb PstI/KpnI fragment into YEp356R [19]. The resulting episomal plasmid pMH4 contains 953 bp of the *ACS2* upstream region together with the N-terminal 339 codons, ligated in frame to *lacZ*. The *ACS2-lacZ* fusion cassette of pMH4 was transferred into the integrative plasmid YIp352 [20] to give pJS455. Minimal promoter constructs containing the ICRE(*ACS2*) motif were obtained by insertion of fragment ACS2-PrA (5′ tcgaagatctAATTATTCATATGCGTTTC 3′; authentic *ACS2* nucleotides in capital letters, ICRE underlined) into pJS205 (ΔUAS-*CYC1-lacZ URA3* 2μm; [9]). The resulting plasmid pMH24N contains a single ICRE(*ACS2*) motif in its natural orientation upstream of the *CYC1-lacZ* reporter gene.

2.4. Gel retardation experiments

Probes used for gel retardation analyses were ACS2-PrA (synthetic DNA fragment, −347/−329) and ACS2-PrB (EcoRI/SmaI fragment, −326/−80), respectively. Probes were end-labelled by a fill-in reaction using α-[^{32}P]-dATP. The Ino2p/Ino4p complex incubated with ACS2-PrA was synthesized in *E. coli* as previously described [14]. To study protein/ACS2-PrB interactions, total cellular protein from wild-type strain JS91.15-23 was used. Conditions of protein/DNA interaction as well as native gel electrophoresis have been described [6]. To compete for the binding of pleiotropic factors to the ACS2-PrB probe, a 100-fold molar excess of unlabelled synthetic DNA fragments containing binding sites of Abf1p (BAF12), Rap1p (RAP12) or Reb1p (OF5; [15]) was used. Similarly, binding to ACS2-PrA was competed for with FBF56 (ICRE[*INO1*, −240]; [9]) or the non-functional ICRE variant FBF-CGN (tcgagTTTTCGCATGCagatct).

2.5. Enzyme assays

Specific β-galactosidase activity was assayed by monitoring ONPG hydrolysis as previously described [9]. Acetyl-CoA synthetase activity was determined according to [21].

3. Results and discussion

3.1. ACS2 transcription is not affected by the glucose concentration of the medium

In order to investigate the possible influence of the carbon source supply on *ACS2* transcription, we isolated RNA from wild-type cells grown under conditions of glucose repression (2% glucose) or derepression (0.2%). We have previously shown that *ACS1* mRNA was almost not detectable in the presence of 2% glucose, while maximal expression occurred with 0.2% glucose [6]. In contrast to *ACS1* transcription, a Northern blot hybridization showed no influence of the glucose concentration on the amount of the *ACS2* transcript with respect to the constitutively expressed actin mRNA (Fig. 1). This finding agrees with recent results described in [8]. We also performed β-galactosidase assays in extracts of repressed or derepressed transformants, containing an *ACS2-lacZ* reporter construct (see below). Again, no substantial influence of the carbon source (2% glucose, 0.2% glucose or 3% ethanol) on *ACS2* expression was found (data not shown). Thus, we consider *ACS2* as being constitutively expressed with respect to the carbon source.

Table 1
Strains of *S. cerevisiae* used in this work

Strain	Genotype
JS91.15-23	*MATα ura3 leu2 his3 trp1 can1 MAL3 SUC3*
SS92.3-1	*MATα ura3 leu2 his3 trp1 can1 MAL3 SUC3 Δino2::LEU2*
MBY1	*MATα ura3 leu2 his3 trp1 can1 MAL3 SUC3 Δopi1::HIS3*
JS96.8-1	*MATα leu2 his3 trp1 can1 MAL3 SUC3 ura3::ACS2-lacZ::URA3*
JS96.10-1	*MATα leu2 his3 trp1 can1 MAL3 SUC3 ura3::ACS2-lacZ::URA3 Δino2::LEU2*
JS96.11-1	*MATα leu2 his3 trp1 can1 MAL3 SUC3 ura3::ACS2-lacZ::URA3 Δino4::LEU2*
SKH1	*MATa ura3 leu2 trp1 can1 MAL3 SUC3 Δacs1::LEU2*
MHY5	*MATa ura3 leu2 trp1 can1 MAL3 SUC3 Δacs1::LEU2 Δino4::URA3*

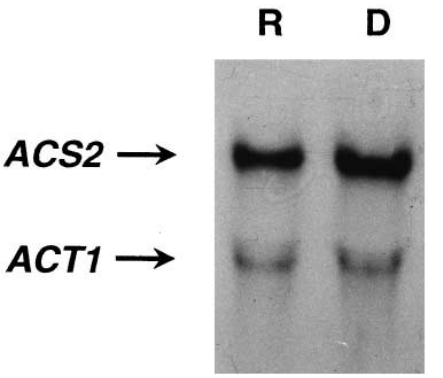

Fig. 1. Northern blot hybridization of an *ACS2* probe against RNA from the wild-type strain JS91.15-23, grown under glucose-repressing (R, 2% glucose) or -derepressing (D, 0.2% glucose) conditions. 20 μg of total cellular RNA was loaded on a denaturing agarose gel, blotted and subsequently hybridized against a ^{32}P-labelled 1.1 kb KpnI/SacI fragment representing the C-terminus of the *ACS2* reading frame. As a reference, the constitutively expressed actin gene *ACT1* was used.

3.2. The ACS2 promoter contains an inositol/choline-responsive element (ICRE)

We analyzed the 406 bp *ACS2* control region (as defined by the non-coding interval between *YLR154C* and *ACS2*, cf. Fig. 2) by a comparative search for binding sites of known transcription factors. Interestingly, we identified a sequence (TATTCATATGC, −344/−334 with respect to the translational start codon) with significant similarity to the ICRE motif (inositol/choline-responsive element), previously described as a UAS of structural genes required for phospholipid biosynthesis ([9,22]; reviewed in [13]). In addition, three putative binding sites of the pleiotropic transcription factor Abf1p were also identified (see below). All *cis*-acting elements as revealed by the in silicio analysis are depicted in Fig. 2.

In order to investigate the UAS character of the putative ICRE, the synthetic DNA fragment ACS2-PrA (−347/−329; comprising ICRE[*ACS2*]) was inserted into the upstream region of the heterologous ΔUAS-*CYC1-lacZ* reporter gene on plasmid pJS205 (2 μm *URA3*; [9]). The resulting plasmid pMH24N contains a single ICRE insert upstream of the TATA box of the reporter gene. After transformation of pMH24N into the wild-type strain JS91.15-23, transformants were grown in the presence of repressing or derepressing concentrations of inositol+choline. As is shown in Fig. 3, expres-

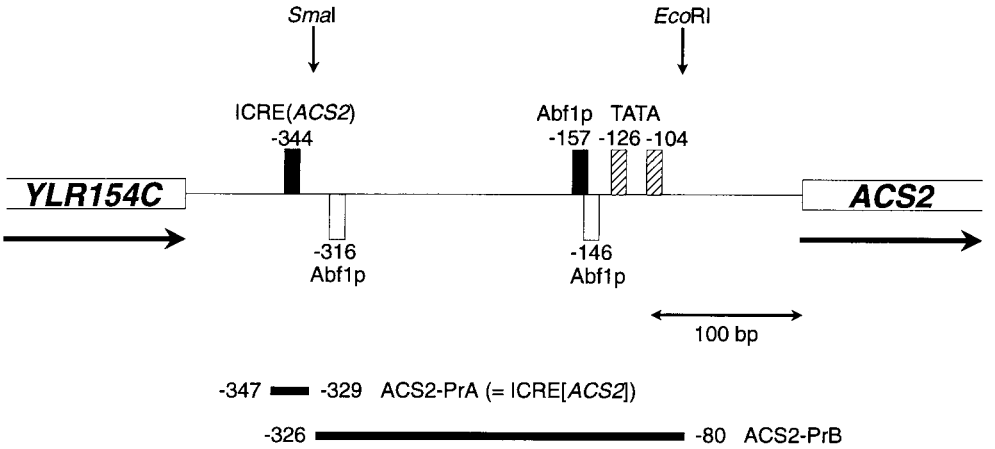

Fig. 2. Control region of the *ACS2* gene. Direction of transcription of *ACS2* (=*YLR153C*) and the hypothetical ORF *YLR154C* is indicated by arrows. TATA elements are shown as hatched boxes. Positions of putative transcription factor binding sites are indicated by filled (consensus elements) or open boxes (slight deviations from the consensus sequence). Only the position of the most 5′ nucleotide of the binding site is specified. The ICRE acts as a binding site for the heterodimeric activator Ino2p/Ino4p. Bars indicate the position of promoter fragments ACS2-PrA and ACS2-PrB used for protein binding studies.

sion of the reporter gene increased about 12-fold under conditions of inositol/choline limitation. Activation by ICRE(*ACS2*) was independent of its orientation (not shown), as previously found for ICRE variants upstream of *FAS1*, *FAS2* and *INO1* [9]. A synergistic activation (about 40-fold) was found in the presence of two ICRE(*ACS2*) inserts (data not shown). Gene activation by ICRE(*ACS2*) was completely abolished in a Δ*ino2* mutant, lacking the ICRE-binding factor Ino2p [14]. On the contrary, a constitutive expression of the reporter gene was observed in the Δ*opi1* mutant strain, defective for a negative regulator of *INO1* transcription [23]. In conclusion, a synthetic minimal promoter driven by the ICRE(*ACS2*) motif is differentially activated, responding to the concentration of phospholipid precursors. Regulated expression of the ICRE(*ACS2*)-*CYC1-lacZ* reporter gene requires *trans*-acting factors, previously shown to affect structural genes of phospholipid biosynthesis.

In order to confirm the direct interaction of the Ino2p/Ino4p activator complex with the ICRE(*ACS2*) motif, a gel retardation experiment with the labelled fragment ACS2-PrA was performed. Ino2p and Ino4p were synthesized by heterologous expression in *E. coli*. As depicted in Fig. 4, a strong retardation signal was obtained in the presence of the Ino2p/Ino4p complex (lane 2). Specificity of the Ino2p/Ino4p-ICRE interaction was confirmed by competition with an excess of unlabelled DNA fragments ACS2-PrA (lane 3) or FBF56 (comprising an ICRE of the *INO1* promoter; [9]). No competition was observed with a mutant ICRE variant (lane 5). Thus, the transcription factor Ino2p/Ino4p of phospholipid biosynthetic genes binds specifically to the *ACS2* promoter.

3.3. *Influence of Ino2p and Ino4p on the complete ACS2 promoter*

Previous work had revealed that *ACS2* is essential for the proliferation of *S. cerevisiae* in the presence of high concentrations of glucose [7]. Since *ino2* and *ino4* mutants show growth under these conditions, additional factors must be required for *ACS2* activation. In order to determine the importance of Ino2p and Ino4p for *ACS2* expression quantitatively, we investigated the influence of *ino2* and *ino4* mutations on a chromosomally integrated *ACS2-lacZ* reporter gene. The integrative *URA3* plasmid pJS455 contains 953 bp of the *ACS2* upstream region together with 1017 bp of its coding region, fused in frame to *lacZ*. Plasmid pJS455 was transformed into the wild-type strain JS91.15-23, thus giving JS96.8-1. The isogenic strains JS96.10-1 and JS96.11-1 were derived from JS96.8-1 by subsequent introduction of *ino2* and *ino4* deletion mutations, respectively. Growth of the regula-

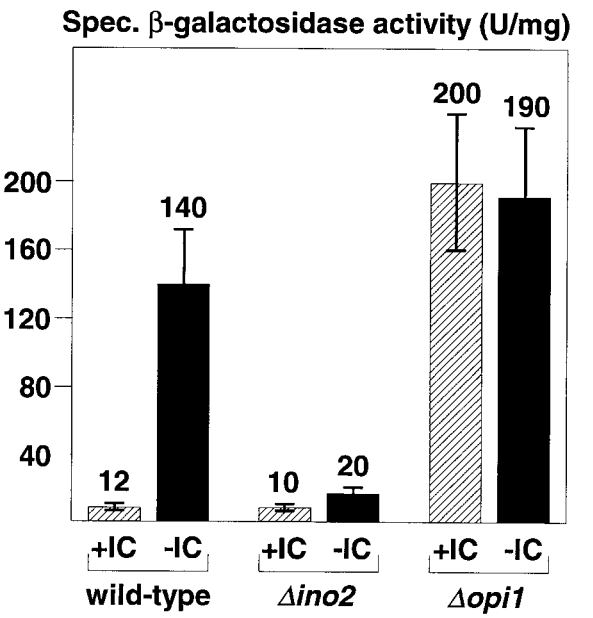

Fig. 3. Inositol/choline-dependent expression of an ICRE(*ACS2*)-*CYC1-lacZ* reporter gene in the wild-type and in *ino2* or *opi1* regulatory mutants. Strains JS91.15-23 (wild-type), SS92.3-1 (Δ*ino2*) and MBY1 (Δ*opi1*) were transformed with the episomal plasmid pMH24N (2 μm *URA3*), carrying a single ICRE(*ACS2*) insert upstream of the *CYC1-lacZ* reporter gene. Transformants were grown under inositol/choline-repressing (+IC, 200 μM inositol+2 mM choline; hatched columns) or -derepressing conditions (−IC, 5 μM inositol+5 μM choline; black columns). Specific β-galactosidase activities are given in nmoles ONPG hydrolyzed per min per mg of protein (U/mg). Transformants of the ΔUAS-*CYC1-lacZ* reporter plasmid pJS205, grown under derepressing conditions, showed a specific β-galactosidase activity of 15 U/mg. Error bars represent the standard deviation of the mean value.

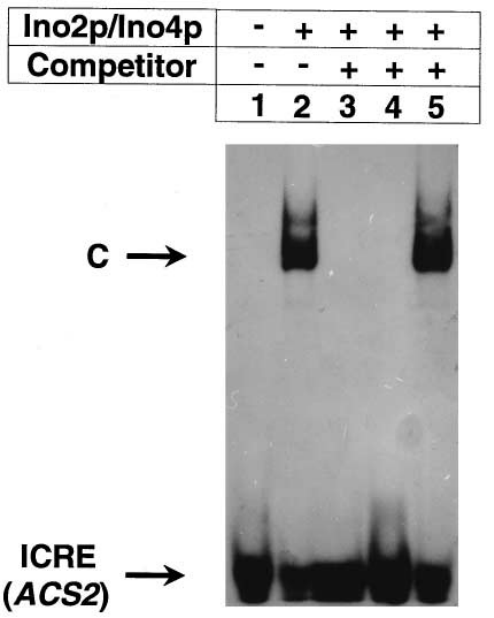

Fig. 4. In vitro binding of the Ino2p/Ino4p complex to the ICRE(*ACS2*) probe. The ^{32}P-labelled synthetic DNA fragment ACS2-PrA (−347/−329 of the *ACS2* upstream region, 5000 cpm) was incubated with 0.2 μg of the affinity-purified Ino2p/Ino4p complex and subsequently run on a 4% native polyacrylamide gel. For competition studies, a 200-fold molar excess of unlabelled synthetic DNA fragments ACS2-PrA (lane 3), FBF56 (=ICRE[*INO1*, −240], lane 4) and FBF-CGN (mutant ICRE variant, lane 5) was used, respectively. C, Ino2p/Ino4p-ICRE(*ACS2*) complex.

tory wild-type under conditions of inositol/choline repression decreased the *ACS2-lacZ* gene expression to about 70% of the derepressed level (Fig. 5). *ACS2-lacZ* activation in the *ino2* and *ino4* null mutants grown under either conditions was reduced to about 35% of the derepressed wild-type level. Similarly, expression of the fatty acid synthase genes *FAS1* and *FAS2* in *ino2* and *ino4* mutants was reduced to about 40% of the wild-type level [14,18]. Thus, functional *INO2* and *INO4* genes are required for maximal expression of *ACS2*. In agreement with this finding, the specific acetyl-CoA synthetase activity of 19 mU/mg measured in the glucose-grown Δ*acs1* mutant strain SKH1 decreased to 10 mU/mg in the Δ*acs1* Δ*ino4* double mutant MHY5. Nevertheless, a substantial expression of *ACS2* (about 35–50% of the wild-type level, depending on the assay) was detectable even in *ino2* and *ino4* mutant strains. The residual ACS activity in the Δ*acs1* Δ*ino4* double mutant still allowed growth on a synthetic medium supplemented with inositol and choline but containing 1% acetate as the sole carbon source. Nevertheless, the doubling time of the mutant strain MHY5 in this medium was significantly reduced when compared with the isogenic wild-type (12.3 h vs. 8.1 h). Supplementation of the acetate medium with fatty acids slightly accelerated growth of MHY5 (doubling time 10.2 h).

3.4. Binding of Abf1p to the ACS2 promoter

Our search for *cis*-acting elements within the 406 bp *ACS2* control region gave evidence for three putative binding sites of the essential transcription factor Abf1p. Abf1p has been characterized as a pleiotropic factor which is required for the transcriptional activation of several house-keeping genes (e.g. genes of ribosomal proteins such as *RPL3* and *RPS33* [24]; genes encoding enzymes of glycolysis such as *PGK1* and *PYK1* [25]; or genes of enzymes involved in fatty acid biosynthesis such as *FAS1* and *ACC1/FAS3* [15,16]). Among these sites, the element at positions −157/−145 (ATCGCACCGCACG, filled box in Fig. 2) perfectly matches the Abf1p consensus binding site (RTCRYNNNNNACG; [24,26]), while the elements −304/−316 (ATCGGCAATAACG) and −134/−146 (ATCAGGCTAAACG, open boxes) show a single mismatch. Interestingly, the putative Abf1p binding sites −157/−145 and −134/−146 overlap by two nucleotides. A similar situation was previously described for the intergenic region separating the divergently transcribed genes *YPT1* and *TUB2* [26].

In order to verify the interaction of Abf1p with the *ACS2* upstream region, we performed a gel retardation experiment with probe ACS2-PrB (−326/−80), comprising the putative Abf1p binding sites. As is shown in Fig. 6, two Abf1p-dependent retardation signals were detected (lane 2). Competition with an excess of the unlabelled Abf1p binding site of the *FAS1* promoter (BAF12; lane 3) released the labelled probe from the retarded complex. On the contrary, competition with binding sites of the pleiotropic factors Rap1p (RAP12; lane 4) or Reb1p (OF5; lane 5) did not alter the pattern of protein/DNA interaction. Presumably, efficient binding of Abf1p occurs at the consensus element −134/−146, leading to a single site occupied by Abf1p. In addition, a less efficient binding at one of the sites deviating from the Abf1p consensus may also occur, resulting in a double occupation of the probe. In con-

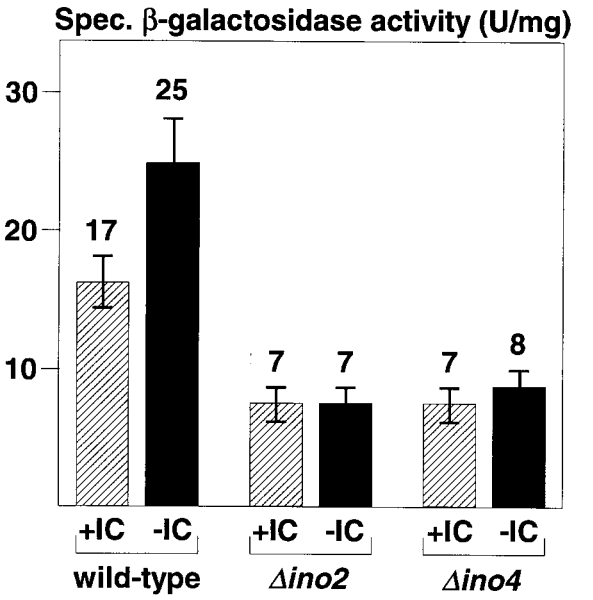

Fig. 5. Influence of regulatory mutations *ino2* and *ino4* on the activation of an *ACS2-lacZ* reporter gene. Wild-type JS96.8-1 as well as the mutant strains JS96.10-1 (*ino2*) and JS96.11-1 (*ino4*) contain the plasmid pJS455 with the reporter gene integrated at the *ura3* locus. Cells were grown under inositol/choline-repressing (+IC, 200 μM inositol+2 mM choline; hatched columns) or -derepressing conditions (−IC, 5 μM inositol+5 μM choline; black columns). Specific β-galactosidase activities are given in nmoles ONPG hydrolyzed per min per mg of protein (U/mg). Error bars represent the standard deviation of the mean value.

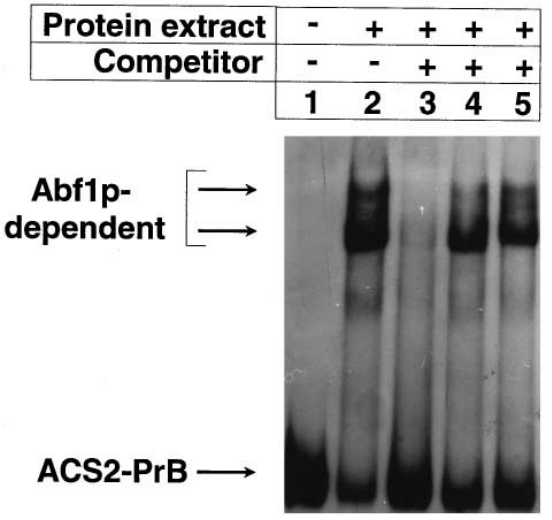

Fig. 6. In vitro binding of Abf1p to an *ACS2* promoter probe. The ^{32}P-labelled DNA fragment ACS2-PrB (−326/−80 of the *ACS2* upstream region, 5000 cpm) was incubated with 20 μg of cellular protein, prepared from wild-type cells grown in synthetic complete medium with 2% glucose. For competition studies, a 100-fold molar excess of unlabelled synthetic DNA fragments BAF12 (Abf1p binding site of the *FAS1* promoter, lane 3), RAP12 (Rap1p consensus binding site, lane 4) and OF5 (Reb1p binding site of the *FAS1* promoter, lane 5) was used, respectively. Abf1p-dependent protein/DNA complexes are indicated by arrows.

trast to Abf1p, pleiotropic transcription factors Rap1p and Reb1p may not be required for *ACS2* activation.

In conclusion, these data show that the *ACS2* control region contains a regulated UAS (the ICRE) as well as constitutively activating promoter elements (presumably the Abf1p binding site(s)). Thus, *ACS2* is transcriptionally activated by factors which are also required for the expression of *FAS1*, *FAS2* and *ACC1/FAS3* [9,15,16]. The acetyl-CoA synthetase encoded by *ACS2* may be considered as the ACS isoenzyme which is involved in the production of acetyl-CoA as a precursor of fatty acid biosynthesis.

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References

[1] Pronk, J.T., Steensma, H.Y. and van Dijken, J.P. (1996) Yeast 12, 1607–1633.
[2] Flikweert, M.T., van der Zanden, L., Janssen, W.M.T.M., Steensma, H.Y., van Dijken, J.P. and Pronk, J.T. (1996) Yeast 12, 247–257.
[3] Satyanarayana, T., Mandel, A.D. and Klein, H.P. (1974) Biochim. Biophys. Acta 341, 396–401.
[4] Klein, H.P. and Jahnke, L. (1979) J. Bacteriol. 137, 179–184.
[5] De Virgilio, C., Büerckert, N., Barth, G., Neuhaus, J.M., Boller, T. and Wiemken, A. (1992) Yeast 8, 1043–1051.
[6] Kratzer, S. and Schüller, H.-J. (1995) Gene 161, 75–79.
[7] van den Berg, M.A. and Steensma, H.Y. (1995) Eur. J. Biochem. 231, 704–713.
[8] van den Berg, M.A., de Jong-Gubbels, P., Kortland, C.J., van Dijken, J.P., Pronk, J.T. and Steensma, H.Y. (1996) J. Biol. Chem. 271, 28953–28959.
[9] Schüller, H.-J., Hahn, A., Tröster, F., Schütz, A. and Schweizer, E. (1992) EMBO J. 11, 107–114.
[10] Hasslacher, M., Ivessa, A.S., Paltauf, F. and Kohlwein, S.D. (1993) J. Biol. Chem. 268, 10946–10952.
[11] Lopes, J.M., Hirsch, J.P., Chorgo, P.A., Schulze, K.L. and Henry, S.A. (1991) Nucleic Acids Res. 19, 1687–1693.
[12] Kodaki, T., Nikawa, J.-i., Hosaka, K. and Yamashita, S. (1991) J. Bacteriol. 173, 7992–7995.
[13] Greenberg, M.L. and Lopes, J.M. (1996) Microbiol. Rev. 60, 1–20.
[14] Schwank, S., Ebbert, R., Rautenstrauß, K., Schweizer, E. and Schüller, H.-J. (1995) Nucleic Acids Res. 23, 230–237.
[15] Schüller, H.-J., Schütz, A., Knab, S., Hoffmann, B. and Schweizer, E. (1994) Eur. J. Biochem. 225, 213–222.
[16] Chirala, S.S., Zhong, Q., Huang, W. and Al-Feel, W. (1994) Nucleic Acids Res. 22, 412–418.
[17] Soni, R., Carmichael, J.P. and Murray, J.A.H. (1993) Curr. Genet. 24, 455–459.
[18] Schüller, H.-J., Schorr, R., Hoffmann, B. and Schweizer, E. (1992) Nucleic Acids Res. 20, 5955–5961.
[19] Myers, A.M., Tzagoloff, A., Kinney, D.M. and Lusty, C.J. (1987) Gene 45, 299–310.
[20] Hill, J.E., Myers, A.M., Koerner, T.J. and Tzagoloff, A. (1986) Yeast 2, 163–167.
[21] Frenkel, E.P. and Kitchens, R.L. (1977) J. Biol. Chem. 252, 504–507.
[22] Schüller, H.-J., Richter, K., Hoffmann, B., Ebbert, R. and Schweizer, E. (1995) FEBS Lett. 370, 149–152.
[23] White, M.J., Hirsch, J.P. and Henry, S.A. (1991) J. Biol. Chem. 266, 863–872.
[24] Dorsman, J.C., Doorenbosch, M.M., Maurer, C.T.C., de Winde, J.H., Mager, W.H., Planta, R.J. and Grivell, L.A. (1989) Nucleic Acids Res. 17, 4917–4923.
[25] Chambers, A., Stanway, C., Tsang, J.S.H., Henry, Y., Kingsman, A.J. and Kingsman, S.M. (1990) Nucleic Acids Res. 18, 5393–5399.
[26] Halfter, H., Müller, U., Winnacker, E.-L. and Gallwitz, D. (1989) EMBO J. 8, 3029–3037.