**Adr1 and Cat8 synergistically activate the glucose-regulated alcohol dehydrogenase gene \( ADH2 \) of the yeast \*Saccharomyces cerevisiae \*

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Glucose-repressible alcohol dehydrogenase II, encoded by the \( ADH2 \) gene of the yeast \*Saccharomyces cerevisiae \*, is transcriptionally controlled by the activator Adr1, binding UAS1 of the control region. However, even in an \( adr1 \) null mutant, a substantial level of gene derepression can be detected, arguing for the existence of a further mechanism of activation. Here it is shown that the previously identified UAS2 contains a distantly related variant of the carbon source-responsive element (CSRE) initially found upstream of gluconeogenic genes. In a mutant defective for the CSRE-binding factor Cat8, derepression of an \( ADH2\text{-}\text{lacZ} \) fusion was reduced to about 12% of the wild-type level. Gene expression in a \( cat8 \text{ adr1} \) double mutant decreased almost to the basal level of the glucose-repressed promoter. CSREADH2 present in a single copy turned out to be a weak UAS element, while a significant synergism of gene activation was found in the presence of at least two copies. Its importance for regulated gene activation was confirmed by site-directed mutagenesis of the CSRE in the natural \( ADH2 \) control region. Direct binding of Cat8 to CSREADH2 could be shown by electrophoretic retardation of the corresponding protein/DNA complex in the presence of a specific antibody. In contrast to what was shown previously for CSRE sequence variants, no significant influence of the isofunctional activator Sip4 on CSREADH2 was detected. In conclusion, these results show a derepression of \( ADH2 \) by synergistically acting regulators Adr1 (interacting with UAS1) and Cat8, binding to UAS2 (\( \equiv \text{CSREADH2} \)).

**Keywords:** gluconeogenesis, transcriptional regulation, UAS element

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**INTRODUCTION**

Utilization of ethanol as sole carbon source by the yeast \*Saccharomyces cerevisiae \* correlates with a more than 100-fold derepression of alcohol dehydrogenase II encoded by the \( ADH2 \) gene (Ciriacy, 1975). In contrast, \( ADH2 \) is completely repressed in the presence of a fermentable substrate. Two \textit{cis}-acting elements (UAS1 and UAS2), both of which are necessary for maximal derepression, have been identified in the \( ADH2 \) control region (Shuster \textit{et al}., 1986; Yu \textit{et al}., 1989; Donoviel \textit{et al}., 1995). UAS1 turned out to be a binding site of Adr1 (Thukral \textit{et al}., 1991) which has been genetically identified as a positive regulator of \( ADH2 \) (Ciriacy, 1975, 1979). Adr1 contains two zinc finger motifs of the Cys\textsubscript{2}–His\textsubscript{2} type (Blumberg \textit{et al}., 1987) and interacts as a monomer with each halfsite of the palindromic UAS1 element. Mutagenesis of individual positions within UAS1 allowed derivation of the sequence TTGGRGA or its reverse complement as the preferred Adr1-binding site (Cheng \textit{et al}., 1994). In addition to its DNA-binding domain, Adr1 also contains four transcription activation domains (TADI–IV) which contact several coactivators and basal transcription factors such as Ada2, Gcn5 and TFIIB as well as subunits of TFIID (Cook \textit{et al}., 1994; Chiang \textit{et al}., 1996; Komarnitsky \textit{et al}., 1998). Although several regulators of UAS1/\( ADH2 \)-dependent gene expression have been described [the Cat1/Snf1/\( Ccr1 \) protein kinase (Ciriacy, 1977; Denis, 1987), cAMP-dependent protein kinases Tpk1, 2, 3 (Cherry \textit{et al}., 1989) and the protein phosphatase complex...
Glc7+Reg1/Hex2 (Dombek et al., 1993, 1999), the molecular mechanism(s) leading to carbon source control is still controversial (Vallari et al., 1992). At least, regulation of Adr1 activity may occur at the post-translational level, affecting its DNA-binding domain (Sloan et al., 1999).

In contrast to UAS1 and its corresponding trans-acting factor, Adr1, UAS2 is much less understood. A DNA-binding factor which is specifically required for UAS2-dependent gene activation and directly interacts with UAS2 has not yet been identified. We have previously analysed the genetic control of structural genes involved in the glyoxylate cycle and gluconeogenesis [ICL1, FBP1 (Schöler & Schüller, 1993, 1994), MLS1 (Casparry et al., 1997) and MDH2 (Roth & Schüller, 2001)]. This led to the identification of a common cis-acting element, designated CSRE (carbon source-responsive element; consensus CCRTYSRNCCG; reviewed by Entian & Schüller, 1997; Gancedo, 1998), which could confer glucose-sensitive gene regulation to a synthetic minimal promoter. CSRE-dependent gene activation requires a functional CAT8 gene (Hedges et al., 1995; Rahner et al., 1996), encoding a transcription factor with a C-terminal transcription activation domain and derepressing conditions (der.; 0% glucose – 2% ethanol). Specific \( \beta \)-galactosidase activities are given in nmol ONPG hydrolysed min\(^{-1}\) (mg protein\(^{-1}\)). SD, Standard deviation of the mean value; AF, activation factor.

**METHODS**

**Yeast strains, media and growth conditions.** Strains of *S. cerevisiae* used in this work were derived from the regulatory wild-type JS91.15-23 (MAT\( ^{a} \) ura3 leu2 his3 trp1 can1). Regulatory mutants *EMY1 (∆cat8::HIS3)*; *Rahner et al., 1996)*; *MHY14 (∆sip4::kanMX4)*, *SRB24 (∆cat8::HIS3 ∆sip4::kanMX4)*; *JS96.20-1 (∆cat1::LEU2; Hiesinger et al., 2001)*; *JS95.14-1 (∆adr1::LEU2)* and *JS95.16-1 (∆cat8::LEU2 ∆cat8::HIS3)*; *Bronio & Schüller, 1997)* were obtained by introduction of the null alleles indicated. Synthetic complete medium used for selective growth of transformants was described previously (Schüller & Entian, 1987). For the preparation of protein extracts, yeast transformants were grown in synthetic medium under repressing (2% glucose) or derepressing conditions (0.2% glucose + 2% ethanol) until a cell density of about 10\(^{7}\) cells ml\(^{-1}\) was obtained.

**Plasmid constructions.** To obtain pK7 variants, the synthetic DNA fragment ADH2-UAS2 (5′-tgaTCTCCCTCTGCGGGAACACGGGGCATgtacc-3′, together with the constitutively synthesized Cat8 and Sip4 variants containing heterologous activation domains (Vincent & Carlson, 1998; Rahner et al., 1999; Hiesinger et al., 2001).

Previously, we identified a CSRE motif together with an Adr1-binding site in the upstream region of the acetyl-CoA synthetase gene ACS1 (Kratzer & Schüller, 1997). While characterizing a cat8 null mutant, we obtained evidence for a Cat8-dependent activation of ADH2 (Rahner et al., 1996). However, the hypothesis of coregulation of ACS1 and ADH2 by Cat8 and Adr1 was rejected by Donoviel & Young (1996) who reported a marginal reduction of UAS2-dependent gene expression in a cat8 mutant. In this work we identify a functional and completely Cat8-dependent variant of the CSRE within UAS2. We also demonstrate in vitro binding of Cat8 to UAS2 and therefore suggest a combined derepression of ADH2 by Cat8 and Adr1.

**Fig. 1.** Carbon-source-dependent gene activation by UAS2 (= CSRE\( \text{ADH2} \)). (a) UAS2 of the ADH2 control region (–319/–292, with respect to the translational start) contains overlapping CSRE variants on complementary DNA strands (indicated by arrows). (b) The synthetic DNA fragment ADH2-UAS2 was inserted into the promoter test plasmid pJS401 (UASAS-ICL1-lacZ URA3 2\( \mu \)), which contains the basal ICL1 promoter but is devoid of all upstream regulatory elements. Orientation and copy number of ADH2-UAS2 with respect to the TATA box (black square) is indicated by arrows. Plasmids were transformed into the wild-type strain JS91.15-23. Transformants were grown under repressing (rep.; 2% glucose) and derepressing conditions (der.; 0.2% glucose + 2% ethanol). Specific \( \beta \)-galactosidase activities are given in nmol ONPG hydrolysed min\(^{-1}\) (mg protein\(^{-1}\)). SD, Standard deviation of the mean value; AF, activation factor.
complementary oligonucleotide; authentic ADH2 upstream sequence in capital letters) was inserted into promoter testing plasmid pJS401 (∆ICL1-lacZ URA3 2 Δ; Caspary et al., 1997), cut at its single XhoI site. Copy number and orientation of inserts were determined by DNA sequencing. The complete ADH2 control region (−926/+3) was amplified as a HindIII/BamHI fragment by PCR and subsequently inserted into lacZ fusion vector YEp356R (Myers et al., 1986) to give pKW13 (ADH2-lacZ URA3 2 Δ). Point mutations within UAS2 (alteration of four nt essential for a functional CSRE) were generated with the QuikChange site-directed mutagenesis kit (Stratagene), using mutagenic primers ADH2-Mut1 (5'-CTTCTCTGCGGACCCGGGCTACT-3'; artificial SalI site underlined) and ADH2-Mut2 (reverse complement). The desired mutation in plasmid pKW14 (ADH2-CSRE lacZ URA3 2 Δ) was confirmed by DNA sequencing. Plasmids pAR33, pAR34 and pMH33, encoding deregulated variants of CAT8 and SIP4, have been described previously (carbon source-independent MET25 promoter, constitutive Ino2 or VP16 activation domains together with a FLAG epitope; Rahner et al., 1999; Hiesinger et al., 2001).

**RESULTS**

**UA52 of ADH2 contains overlapping CSRE-related sequences**

Activation of the glucose-regulated alcohol dehydrogenase gene ADH2 under derepressing conditions is mediated by Adr1, binding to the UAS1 promoter motif (Yu et al., 1989; Thukral et al., 1991). However, deletion of either UAS1 (ADH2) or ADR1 still allowed a substantial derepression of ADH2, arguing for an additional UAS element and trans-activator. Although UAS2 was identified immediately upstream of UAS1 (Yu et al., 1989), the corresponding regulatory factor remained unknown. Since our previous characterization of a cat8 deletion mutant provided evidence for reduced expression of an ADH2-lacZ fusion (Rahner et al., 1996), we looked for a Cat8-binding site (= CSRE) within UA52. Indeed, UA52 contains two sequence motifs on complementary DNA strands (Fig. 1a) which almost completely overlap and are distantly related to the previously defined CSRE consensus (CCRTYNR-CCG; Schöler & Schüller, 1994; Caspary et al., 1997; Roth & Schüller, 2001). We thus inserted the synthetic DNA fragment ADH2-UA52 into the upstream region of an ICL1-lacZ reporter gene, devoid of its natural regulatory sequences (pJS401; Caspary et al., 1997). As is shown in Fig. 1(b), only a twofold stimulation of basal gene expression was detected in the presence of a single copy insertion in either orientation. In contrast, strong activation was found with at least two copies, arguing for a marked synergism (>300-fold activation with three copies; cf. plasmid pKW7NNR). Importantly, UA52-dependent gene activation absolutely required a functional CAT8 gene, supporting our view of UA52 as a functional CSRE variant (Table 1). The Cat8-related activator Sip4, which partially contributes to activation by CSRE_{FBP} and CSRE_{ICL} (Vincent & Carlson, 1998; Hiesinger et al., 2001), was dispensable for CSRE_{ADH2}-mediated gene derepression. A decrease in gene expression to about half of the wild-type level was also found in the adr1 mutant. Absence of the Cat1 (Snf1) protein kinase which acts as a positive upstream regulator of Cat8 also led to a total loss of activity.

To confirm the specific contribution of Cat8 to UA52-dependent gene activation, we assayed the influence of a regulatory CAT8 variant (MET25-CAT8-IN02_TAD fusion gene, plasmid pAR33; promoter and activation domain not affected by carbon source) on reporter plasmid pKW7NNR. When introduced into the cat8 sip4 double mutant SRH24, the modified Cat8 activator could strongly stimulate the reporter gene even under conditions of glucose repression (>50-fold, compared to wild-type; Table 2). Under derepressing conditions, the wild-type level of gene expression was completely restored. We also investigated the influence of a deregulated SIP4 variant (MET25-SIP4-VP16 fusion gene, plasmid pMH33) which could efficiently activate a CSRE_{ICL}-driven reporter gene (Hiesinger et al., 2001). However, with this construct, UA52-dependent gene activation remained at 8% of the derepressed wild-type level (Table 2). In conclusion, these results suggest that Cat8 is indeed an important activator of UA52 (= CSRE_{ADH2})-dependent gene expression, while Sip4 at best mediates some minor influence on this CSRE variant.

**Site-directed mutagenesis of CSRE_{ADH2}**

In addition to UA52-containing synthetic promoter constructs, we also investigated ADH2-lacZ fusion genes containing either the complete natural upstream region (−926/+3) or a mutant CSRE_{ADH2} variant (pKW13 and pKW14, respectively; cf. Fig. 2). With the ADH2 wild-type promoter, deletion of either ADR1 or CAT8 led to a significant decrease of β-galactosidase activity (to 19.5 and 12.5% of the reference, respectively; Fig. 2). In the adr1 cat8 double mutant, almost no
Table 1. Influence of regulatory mutations on UAS2 (= CSRE<sub>ADH2</sub>)-dependent gene activation

Isogenic strains differing with respect to the regulatory genes indicated were transformed with pKW7NNR [episomal plasmid containing a (UAS2)<sub>ICL1-lacZ</sub> reporter gene] and grown under repressing (2% glucose) and derepressing conditions (0-2% glucose + 2% ethanol). Specific β-galactosidase activities are given in nmol ONPG hydrolysed min<sup>−1</sup> (mg protein)<sup>−1</sup>. SD, Standard deviation of the mean value.

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Specific β-galactosidase activity of pKW7NNR transformants</th>
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<tbody>
<tr>
<td></td>
<td>Repressed (SD)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>11 (2)</td>
</tr>
<tr>
<td>cat8</td>
<td>11 (4)</td>
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<tr>
<td>sip4</td>
<td>16 (5)</td>
</tr>
<tr>
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<td>8 (2)</td>
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<tr>
<td>adr1</td>
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</tr>
<tr>
<td>cat1</td>
<td>8 (1)</td>
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Table 2. Influence of deregulated variants of CAT8 and SIP4 on CSRE<sub>ADH2</sub>-dependent gene expression

Double mutant strain SRH24 (cat8 sip4) was transformed with episomal plasmids pAR33 (MET25-CAT8-INO2<sub>TAD</sub>) or pMH33 (MET25-SIP4-VP16) and subsequently grown under repressing (2% glucose) and derepressing conditions (0-2% glucose + 2% ethanol). For comparison, the isogenic regulatory wild-type strain JS91.15-23 (CAT8 SIP4) was transformed with empty vectors. Both strains also contained plasmid pKW7NNR [(UAS2)<sub>ICL1-lacZ</sub> reporter gene]. Specific β-galactosidase activities are given in nmol ONPG hydrolysed min<sup>−1</sup> (mg protein)<sup>−1</sup>. SD, Standard deviation of the mean value; -fold wt, increase with respect to wild-type.

<table>
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<tr>
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<td>CAT8 SIP4</td>
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<td>cat8 sip4</td>
<td>8 (2)</td>
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<tr>
<td>MET25-CAT8-INO2&lt;sub&gt;TAD&lt;/sub&gt;</td>
<td>590 (130)</td>
</tr>
<tr>
<td>MET25-SIP4-VP16</td>
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Fig. 2. Influence of Adr1 and Cat8 on wild-type and mutant ADH2 control regions. Strains JS91.15-23 (wild-type), JS95.14-1 (adr1), EMY1 (cat8) and JS95.16-1 (cat8 adr1) were transformed with the episomal reporter plasmids shown. Plasmids pKW13 and pKW14 differ with respect to 4 nt of the CSRE core sequence within UAS2 (underlined). Transformants were grown under repressing (rep.; 2% glucose) and derepressing conditions (der.; 0.2% glucose + 2% ethanol). Specific β-galactosidase activities are given in nmol ONPG hydrolysed min<sup>−1</sup> (mg protein)<sup>−1</sup>. SD, Standard deviation of the mean value; T, TATA box.

derepression occurred. The same strains were also transformed with an ADH2-lacZ fusion, deviating at four UAS2 nucleotide positions which are critical for CSRE function. With this reporter construct (pKW14), gene expression in the wild-type strain decreased to 50% of the activity found for the natural control region.
In the absence of Adr1, mutation of \( \text{ADH2} \) additional Cat8-dependent UAS element in the cat8 mutant, arguing for the existence of an additional Cat8-dependent UAS element in the \( \text{ADH2} \) promoter. Importantly, a further decrease (to 14.5%) was observed in the cat8 mutant, arguing for the existence of an additional Cat8-dependent UAS element in the \( \text{ADH2} \) promoter. In the absence of Adr1, mutation of \( \text{CSRE}_{\text{ADH2}} \) led to a reduction of derepressed activity by a factor of eight. Again, derepression was almost completely defective in the \( \text{adr1 cat8} \) double mutant. These findings show that Adr1 and Cat8 together mediate >98% of total \( \text{ADH2} \) gene derepression. Since the defects observed with \( \text{adr1} \) and \( \text{cat8} \) single mutants are more than additive, a synergistic derepression mediated by both activators is suggested.

**In vitro interaction of Cat8 with \( \text{CSRE}_{\text{ADH2}} \)**

Previous work demonstrated binding of Sip4 and Cat8 to CSRE sequence variants (Vincent & Carlson, 1998; Rahner et al., 1999; Roth & Schüller, 2001; Hiesinger et al., 2001). To confirm the direct influence of Cat8 on UAS2, gel retardation experiments with protein extracts prepared from \( E. \text{coli} \) lysates, or yeast transformants were performed. GST fusion proteins containing the zinc cluster domains of Gal4 (negative control), Sip4 and Cat8 were affinity-purified and subsequently incubated with a \( \text{CSRE}_{\text{ADH2}} \)-containing probe. While strong retardation signals were observed with GST-Cat8 (Fig. 3, lane 4), only a weak signal could be obtained with an identical amount of GST-Sip4 (lane 3). In contrast, signals of similar intensity for both binding factors were previously found with \( \text{CSRE}_{\text{ADH2}} \) probes (Roth & Schüller, 2001). Obviously, Cat8 and Sip4 bind to CSRE sequence variants with different affinities.

In addition to bacterially synthesized binding factor, gel retardation experiments were also done with yeast protein extract containing an epitope-tagged Cat8-Ino2-FLAG variant (Fig. 4). Extracts were prepared from a \( \text{cat8 sip4} \) double mutant (SRH24), transformed with the corresponding fusion gene (on plasmid pAR34). Compared to the vector control, a strong retardation signal could be observed (lane 3) which was further shifted in the presence of specific anti-FLAG antibody (lane 4). Competition by an excess of unlabelled DNA fragment OAS12 containing \( \text{CSRE}_{\text{ICL1}} \) an unspecific protein/DNA complex (still existent with an excess of competitor) migrates close to the position of Cat8-Ino2-FLAG. C8-I2-FLAG, Cat8-Ino2-FLAG.

**Fig. 3.** Binding of bacterially synthesized GST-Cat8 to \( \text{CSRE}_{\text{ADH2}} \). For the gel retardation experiments shown, about 0.5 \( \mu \text{g} \) GST fusion proteins, affinity-purified from \( E. \text{coli} \) lysates, were incubated with 5000 c.p.m. of the \( ^{32} \text{P} \)-labelled synthetic DNA fragment ADH2-UA52 containing the \( \text{CSRE}_{\text{ADH2}} \) motif. Multiple retardation signals may result from truncated fusion proteins. For competition studies, a 100-fold molar excess of unlabelled fragments ADH2-UA52 (lane 5) and OAS12 (lane 6, \( \text{CSRE}_{\text{ICL1}} \)) was used. G4, GST-Gal4 (negative control, lane 2); S4, GST-Sip4 (lane 3); C8, GST-Cat8 (lanes 4–6).

![Fig. 3](image1.png)

**Fig. 4.** Interaction of epitope-tagged Cat8-FLAG from yeast transformants with \( \text{CSRE}_{\text{ADH2}} \). About 40 \( \mu \text{g} \) total protein from transformants of strain SRH24 (\( \text{cat8 sip4} \) double mutant) was incubated with 5000 c.p.m. of a \( ^{32} \text{P} \)-labelled synthetic DNA fragment ADH2-UA52. SRH24 was transformed with plasmid pAR34, containing a MET25-CAT8-INO2-FLAG fusion gene (lanes 3–5) or a corresponding empty vector (lane 2). Transformants were grown under derepressing conditions (0.2% glucose + 2% ethanol). For the supershift experiments, 200 ng monoclonal anti-FLAG antibody M2 (Kodak/IBI) was used (lanes 4, 5). Competition (lane 5) was achieved by a 100-fold excess of unlabelled DNA fragment OAS12 containing \( \text{CSRE}_{\text{ICL1}} \). An unspecific protein/DNA complex (still existent with an excess of competitor) migrates close to the position of Cat8-Ino2-FLAG/\( \text{CSRE}_{\text{ADH2}} \). C8-I2-FLAG, Cat8-Ino2-FLAG.

![Fig. 4](image2.png)
shift assays show that CSRE\textsubscript{ADH2} specifically interacts with Cat8, but not with the related transcriptional activator Sip4.

**DISCUSSION**

For the utilization of ethanol, alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALD) and acetyl-CoA synthetase (ACS) are required to finally produce acetyl-CoA which is subsequently used for mitochondrial oxidation and cytoplasmic gluconeogenesis. We have previously shown that ACS\textsubscript{1} of *S. cerevisiae* is activated additively by Cat8 and Adr1, together being responsible for about 80% of its promoter strength (Kratzer & Schüller, 1997). This result prompted us to look for a similar genetic control of ADH2 which encodes the first enzyme of ethanol oxidation. In addition to Adr1 and its corresponding binding site in the ADH2 control region (UAS1), the existence of an additional activation sequence (UAS2) is well known (Yu et al., 1989; Thukral et al., 1991). However, the nature of the transcriptional regulator responsible for UAS2-dependent derepression has remained unclear. In this work, we identified a functional CSRE variant within UAS2 which specifically interacts in vitro with the Cat8 (but not the Sip4) activator and contributes to transcriptional derepression of ADH2 synergistically with the Adr1-dependent UAS1 element.

Although both almost completely overlapping CSRE motifs within UAS2 deviate from the previously defined consensus sequence, a regulated, 80-fold activation of basal gene expression was found in the presence of at least two CSRE\textsubscript{ADH2} copies (Fig. 1). Recent data derived from the saturation mutagenesis of a strong CSRE suggest that the sequence CCGGTGTTCCG is closer to the modified consensus than the CCGGAAACCG motif on the complementary strand (S. Roth & H.-J. Schüller; unpublished). Gel retardation experiments showed that Cat8 and the related zinc cluster protein Sip4 differ with respect to binding affinity to CSRE sequence variants. Thus, Cat8 must be considered as the dominating regulator acting via UAS2. This conclusion is in agreement with a recently published microarray analysis, comparing mRNA levels from wild-type and *cat8* mutant strains on a genome-wide scale (Haurie et al., 2001). A minor role, even of Adr1, can be derived from the reduction of UAS2-dependent gene expression in an *adr1* mutant (Table 1) as well as from the previous demonstration of low-affinity binding of Adr1 to UAS2 (Donoviel et al., 1995). Synergistic gene activation is not only observed in the presence of multiple UAS2 copies in a synthetic promoter (Fig. 1) but also with UAS1 and UAS2 in the natural ADH2 control region (Yu et al., 1989). Presumably, Cat8 and the UAS1-binding factor Adr1 cooperate in maximal derepression of an ADH2-lacZ reporter gene. This conclusion derived from the analysis of *cat8* and *adr1* single mutants is in agreement with previous results, comparing UAS2 promoter mutants in the absence or presence of Adr1 (Donoviel et al., 1995). Synergistic activation of ADH2 by Cat8 and Adr1 contrasts to the additive contribution of these regulators to derepression of ACS\textsubscript{1} (Kratzer & Schüller, 1997). This may simply result from varying distances of Cat8- and Adr1-binding sites upstream of ACS\textsubscript{1} (separated by 200 bp) and ADH2 (3 bp apart). Synergistic derepression of ADH2 by Cat8 and Adr1 could be the result of recruiting a common coactivator/mediator of transcriptional activation. Possible candidates may be Ada2 and the histone acetyltransferase Gcn5, as well as basal transcription factors TFIIB and components of TFIID (TPB, Taf90 and Taf130/145) for which direct contacts to activation domains within Adr1 have been demonstrated (Chiang et al., 1996; Komarnitsky et al., 1998).

The influence of Cat8 on UAS2-dependent gene activation is further confirmed by the obvious deregulation of a reporter gene by the MET25-CAT8-INO2\textsubscript{TAD} variant, encoding a protein with a constitutive activation domain (INO2\textsubscript{TAD}) driven by a carbon source-independent promoter (MET25). Compared to a wild-type control, gene expression was clearly increased under repressing, but not under derepressing conditions (Table 2). An increased expression of UAS2-dependent ADH2 was previously shown in the presence of multicopy plasmids containing SPT6 and MEU1 genes (Donoviel & Young, 1996). While the essential SPT6 gene may be involved in chromatin modification during transcriptional elongation (Hartzog et al., 1998), the function of MEU1 (multicopy enhancer of UAS2) remained unclear. A high copy number of MEU1 increased ADH2 expression about three- to fivefold under either condition, while its deletion led to a twofold reduction (Donoviel & Young, 1996). Our data on the contribution of Cat8 (which are in agreement with the findings of Haurie et al., 2001) completely differ from results reported by these authors. In contrast to the complete loss of UAS2-driven gene expression documented in this work (Table 1), Donoviel & Young (1996) merely describe a slight reduction by a factor of two. The reason for this discrepancy remains unclear.

Assuming UAS2 is the sole Cat8-dependent activating element, equivalent and non-additive effects of *cis* and *trans* mutations (CSRE\textsubscript{ADH2} and *cat8*) on ADH2 gene expression should be expected. However, in the absence of Cat8, gene expression was clearly more affected than with a mutant UAS2 (Fig. 2). Possibly, the site-directed mutagenesis did not abolish all UAS2 activity. This appears unlikely since positions critical for a functional CSRE had been altered. Instead, we suggest the existence of (at least) one additional CSRE motif in the ADH2 upstream region. We were able to identify the sequence motif CCGTCTCTCCG (−478/−468) which fits the weakly stringent consensus CCN\textsubscript{5}CCG typical of a subfamily among zinc cluster proteins. This element may be considered as a weak CSRE, being responsible for Cat8-dependent ADH2 derepression in the absence of UAS2.

We finally asked whether at least one among five aldehyde dehydrogenase genes (ALD2, ALD3, ALD4/
ALD7, ALD5 and ALD6 in *S. cerevisiae* may also require transcriptional activation by Cat8 and Adr1. Mutant phenotype (no growth with ethanol as sole carbon source; Meaden *et al.*, 1997), as well as expression pattern (Haurie *et al.*, 2001), indicates that the ALD6 gene encoding a cytoplasmic, NADP-dependent aldehyde dehydrogenase is a possible candidate. *In silico* analysis of its upstream region revealed the existence of two CSRE-related motifs (CCTTCTGGCCCG, -615/ -605 and CCTTATTCCCG, -484/-474) and a partially palindromic sequence similar to an Adr1-binding site (TCTCCN₁₀GGTGA; according to the consensus as defined by Cheng *et al.*, 1994). Thus, the metabolic pathway responsible for the cytoplasmic synthesis of acetyl-CoA from ethanol involves structural genes ADH2, ALD6 and ACS1, which may be transcriptionally coregulated by Adr1 and Cat8 (summarized in Fig. 5).

**ACKNOWLEDGEMENTS**

This work was supported by the Deutsche Forschungsgemeinschaft. We thank Stephanie Roth and Margit Hiesinger for providing strains and plasmids.

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Received 8 March 2001; accepted 30 April 2001.