ABSTRACT  In *Saccharomyces cerevisiae*, FAS1, FAS2, and FAS3 are the genes involved in saturated fatty acid biosynthesis. The enzymatic activities of both fatty acid synthase (FAS) and acetyl-CoA carboxylase are reduced 2- to 3-fold when yeast cells are grown in the presence of exogenous fatty acids. The mRNA levels of the FAS genes are correspondingly lower under repressive conditions. Expression of the FAS-lacZ reporter gene is also repressed by fatty acids. When a FAS2 multicopy plasmid is present in the cells, expression of both FAS1 and FAS3 increases. Thus, the FAS genes are coordinately regulated. Deletion analyses of FAS2 regulatory sequences. These include the GCCCAAAAC and AGCCCAAGCA sequences that have a common GCCA core sequence and the UASNO upstream activation sequence. Derepression of the FAS genes in the absence of exogenous inositol is not observed when UASNO is mutated, indicating that this cis element is a positive regulator of these genes. The GCCCA elements and UASNO act synergistically for optimal expression of the FAS genes.

In eukaryotes, the synthesis of long-chain fatty acids from acetyl-CoA is catalyzed by two multifunctional enzymes, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (1). In the yeast *Saccharomyces cerevisiae*, the native ACC is a tetramer of a multifunctional protein (Mr, 251,499) encoded by the FAS3 gene. The yeast FAS consists of two multifunctional proteins, α (Mr, 207,683) and β (Mr, 220,077), that are organized in an αββα complex. The subunits α and β are encoded by two unlinked genes, FAS1 and FAS2, respectively. All three genes have been cloned and sequenced (2–8). It is generally assumed from studies of amino acid biosynthesis (9) and ribosomal protein synthesis (10) in yeast that genes involved in common metabolic pathways or coding for subunits of complex enzymes are regulated by coordinated expression. Thus, it seems possible that the three FAS genes may also be coordinately regulated.

Little is known about the regulation of these genes in yeast. Numa and coworkers (11, 12) reported that ACC activity is reduced by ~50% when yeast cells are grown in the presence of fatty acids. They attributed this repression to the presence of fatty acyl-CoA derivatives or some metabolites derived therefrom (12). The regulation of FAS, on the other hand, remains obscure. In this report, I describe experiments performed to elucidate the coordinated regulation and the fatty acid-mediated repression of these genes. Through deletion analysis of the promoter region, common cis-acting elements of both the FAS1 and FAS2 genes were identified.

The materials and methods section discusses the yeast strain used in these experiments (SEY2102 (α, ura3-52, leu2-3, leu2-112, suc2-Δ9, his4-519, gal2)) which was obtained from M. G. Douglas (University of North Carolina Medical School, Chapel Hill). The cells were grown in synthetic dextrose (SD) medium containing appropriate nutritional supplements (13). To study fatty acid-mediated repression, the cells were grown in SD medium supplemented with 2 mM myristic acid and 0.5% Tween 40.

Construction of Plasmids. A CEN4-based reporter gene plasmid was constructed from pLG669-Z-A312 (14) (obtained by S. Hahn, Fred Hutchinson Cancer Research Center, Seattle) and pSE679 (obtained from S. J. Elledge, Baylor College of Medicine). The plasmid pSCFAS1 contains the FAS1 regulatory region and the coding regions of 23 amino acids of the β subunit that are fused in frame with lacZ. Similarly, pSCFAS2 contains the FAS2 regulatory region and the coding region of 113 amino acids fused with lacZ. The deletions were made by using available restriction sites or by using PCR techniques (15).

Determination of Enzymatic Activities. The cells were harvested at room temperature and washed once with water, three times with 10% (vol/vol) ethanol to remove excess fatty acids, and once with 50 mM Hepes buffer (pH 7.5) containing 1 mM EDTA and 1 mM dithiothreitol. The cells were broken with glass beads as described (5), and the extracts were centrifuged. The FAS and ACC activities were determined as described (16, 17). To determine the β-galactosidase activity, three colonies from each transformation plate were resuspended separately in 0.5 ml of SD medium. Each of these cell suspensions, 0.2-ml samples were added to 1 ml of SD medium containing fatty acids or devoid of fatty acids and grown overnight under selective conditions to retain the URA3-based plasmids. The cultures were then diluted 1:10 with the same medium in which they were grown. After growing another 4–5 hr, the cells were harvested and washed as described above, except that Z buffer (50 mM Hepes, pH 7.5/10 mM KCl/1 mM MgSO4/50 mM 2-mercaptoethanol) was used for the final wash (18). The cell pellets were resuspended in 1 ml of Z buffer, and the β-galactosidase activity was determined in permeabilized cells (18, 19).

Miscellaneous Procedures. DNA sequencing (7, 20), protein estimations (21), yeast transformations (22), RNA isolation and Northern analysis (5, 7, 23), labeling of DNA probes by nick translation (5, 7), site-directed mutagenesis (24), and SDS/PAGE (25) were performed as described.

RESULTS  Fatty Acid-Mediated Repression and Coordinated Expression of FAS Genes. Exogenous fatty acids are known to

Abbreviations: FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; SD, synthetic dextrose; UAS, upstream activation sequence.
Table 1. Fatty acid-mediated repression of FAS and ACC

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Culture medium</th>
<th>Specific activity FAS</th>
<th>Specific activity ACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEY 2102</td>
<td>- FA</td>
<td>90</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>+ FA</td>
<td>65</td>
<td>2.5</td>
</tr>
<tr>
<td>SEY 2102/YPFAS2</td>
<td>- FA</td>
<td>184</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td>+ FA</td>
<td>69</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Untransformed cells or cells transformed with YEPFAS2 were grown in the presence (+ FA) or absence (- FA) of exogenous fatty acids. The cell extracts were made and the enzymatic activities were determined. Specific activity was measured for FAS as nmol of NADPH oxidized per min per mg and for ACC as nmol of malonyl-CoA formed per min per mg. Values given are the averages of three experiments.

Repress the activity of ACC by 50% (11, 12). As shown in Table 1, fatty acids repress not only ACC activity but also FAS activity by a factor of 2-3. Thus, both enzymes appear to be coordinately repressed. When whole-cell extracts of yeast were analyzed by SDS/PAGE, the three proteins (ACC and the α and β subunits of FAS) appeared to be of equal amounts (Fig. 1, lane 1). However, when the cells were transformed with a FAS2 multicopy plasmid and the extracts were analyzed, increased expression not only of the FAS α subunit for which FAS2 codes but also of the FAS β subunit and ACC was observed (Fig. 1). The increase in protein in the presence of the FAS2 multicopy plasmid correlated very well with the increase in the activities of FAS (2-fold) and ACC (5-fold) as shown in Table 1. However, when the transformed cells were grown in the presence of fatty acids, the activities of these enzymes were reduced by a factor of 2-3 (Table 1) as in the nontransformed cells. Northern blot analysis showed that the levels of FAS1 and FAS3 mRNAs were lower when cells were grown in the presence of fatty acids (Fig. 2). The increase in FAS1 and FAS3 mRNA levels in cells transformed with the FAS2 multicopy plasmid was readily observed. These results suggest that the multicopy plasmid titrated a limiting factor, or factors, involved in the regulation of FAS gene expression. The effect of a multicopy plasmid containing FASI under the control of the TDH3 promoter was also tested. In cells transformed with this plasmid, only the level of the FAS β subunit increased; there was no coordinated increase in either the level of the FAS α subunit or of ACC (data not shown). Hence, the FAS regulatory region is essential for this coordinated regulation.

Deletion Analysis of the FASI Regulatory Region. To delineate the regulatory regions, a deletion analysis of the 5′ noncoding region of FASI was performed. As shown in Fig. 3, deletion of the sequence between nucleotides −920 and −760 reduced the expression of β-galactosidase by ∼50% (Fig. 3, pSCFAS1-312). In this region, there are putative GCRI (26), RAPI/GRF1 (27-30), ABFI (28), and upstream activation sequence UASINO (31-35) elements (Fig. 4). However, deletion of the region between nucleotides −760 and −670 reduced the expression of lacZ by 95%. In this region (nucleotides −760 to −670), there are two sequences, GCCCAAAAC and AGCCCAAGCA, that have a common GCCAA core sequence (underlined) (Fig. 4). In addition, there is a UASINO located between the GCCAA repeats that has been implicated in coordinated regulation of FASI and FAS2 in yeast (35).

Deletion Analysis of FAS2. The two sequences containing GCCAA are also present in FAS2 between nucleotides −351 and −295 (Fig. 4). As shown in Fig. 5, the β-galactosidase activity was reduced to ∼20% of the control activity when the sequence between nucleotides −363 and −292 (represented

![Fig. 1. Effect of the FAS2 multicopy plasmid on FAS and ACC. Yeast cells were transformed with a multicopy plasmid, a derivative of YEPFAS2 (2, 7). The untransformed cells were grown in SD medium containing histidine, uracil, and leucine (each at 20 µg/ml); the transformed cells were grown in the same medium but without leucine. Cell-free extracts were made using glass beads (5). Equal amounts (200 µg) of protein were analyzed by SDS/PAGE and stained with Coomasie blue. In lane 1, purified yeast FAS (5) was used as a marker; the top two bands are the α and β subunits of FAS, and the other bands are degradation products. In lanes 2 and 3, the extracts of untransformed and transformed cells, respectively, were analyzed.](image-url)

![Fig. 2. Effects of the FAS2 multicopy plasmid and fatty acids on the levels of FAS1 and FAS3 mRNAs. Cells were grown in the SD medium as described in Fig. 1, but with or without fatty acids. Total yeast RNA was treated with glyoxal and fractionated on 1% agarose gel. Lanes: 1 and 2, total RNA from untransformed yeast; 3 and 4, RNA from cells transformed with the FAS2 multicopy plasmid; 1 and 3, RNA from cells grown in the absence of fatty acids; 2 and 4, RNA from cells grown in the presence of fatty acids. The RNA was transferred to a nitrocellulose sheet by blotting and probed with nick-translated 2.8- and 2.1-kilobase-pair HindIII fragments from FASI (5) and an EcoRI fragment of ACT1 DNA (A). (B) Same blot was reprobed with a 6.6-kilobase-pair SstI fragment from FAS3 (4). Densitometric analysis was performed on an autoradiogram exposed for a shorter time, and the values were normalized with densities of actin mRNAs. If the levels of FAS and ACC mRNAs in the untransformed cells grown in the absence of fatty acids equal 1, the levels of these mRNAs were 0.5 for untransformed cells under repressing conditions, 2 for transformed cells under nonrepressing conditions, and 0.4 for transformed cells under repressing conditions.](image-url)
The results are expressed as the percentage of pSCFAS1-Eco, the longest insert (100%); -FA, absence of fatty acids in culture medium; +FA, presence of fatty acids. The extent of fatty acid-mediated repression can be judged from the -FA and +FA values. B. BamHI; BstUI; C. ClaI; E. EcoRI; H. HindIII; K. KpnI; S. Sau3AI. The deletions generated using PCR methods are indicated by an asterisk.

Substitution Analysis of the Regulatory Regions to Delineate the Function of the GCCAA Repeats and UASINO. As shown by 5' deletion analysis, the regions between nucleotides -760 and -669 in FASI and nucleotides -363 and -292 in FAS2 apparently are important for the expression of these genes. The only common sequences between these two regions in FASI and FAS2 are the two GCCAA repeat-containing sequences. The results of deletion analyses of FAS2 indicated that the UASINO by itself does not support expression of the lacZ reporter gene (Fig. 5, pSCFAS2-Bst). To confirm these observations, specific regions were cloned in deletion constructs of both FASI and FAS2 that express background levels of lacZ. As shown in Fig. 6, pSCFAS2-Bst, which expresses background levels of lacZ even though it contains the UASINO, can be made to express substantially increased levels of lacZ by the region of FAS2 that contains only the GCCAA repeats, nucleotides -363 to -292. Similarly, the basal level expression of pSCFAS1-Sau 3A, which has no known cis element, can be improved by adding the GCCAA elements of FAS2 (nucleotides -363 to -233) or made even better by adding to the construct the FASI region containing both GCCAA repeats and the UASINO (nucleotides -760 to -675; Fig. 6). Thus, it appears that both UASINO and the GCCAA repeats are required to express efficiently FASI and FAS2.

**Mutation Analysis of UASINO.** The FASI regulatory region has two UASINO sequences (Fig. 4). Using site-directed mutagenesis, the UASINO between nucleotides -709 and -699 in FASI was converted from ACTTTCACTTGC to ACTTC-CCGGGC in pSCFAS1-152 and generated pSCFAS1-152M, which has only one UASINO (Fig. 6). By performing a PCR-mediated deletion on pSCFAS1-152M, pSCFAS1-312M, which has no UASINO, was generated. As shown in Fig. 6, the UASINO is essential for optimal expression of FASI. However, there is measurable lacZ expression even when there is no functional UASINO. In fact, the level of expression of pSCFAS1-312M is similar to that of pSCFAS1-Sau 3A, which has a substituted FAS2 region (nucleotides -233 to -363) that does not contain any UASINO (Fig. 6). FAS2 has only one UASINO. When the conserved CACATG sequence of UASINO was mutated to CGCTAG, lacZ expression decreased only 50% (Fig. 6). As shown in Figs. 4 and 6, the BstUI deletion of FAS2, which still contains the UASINO, does not suppress lacZ expression. These observations suggest that UASINO is not critical for expression of FAS2.

**Inositol-Mediated Repression of FAS Genes.** When culture medium lacked inositol, expression of the FAS-linked reporter gene was enhanced ~3-fold (Table 2). Even the activities of FAS and ACC decreased when inositol (11 μM) was present in the medium (Table 2); this concentration of inositol is the same as that present in SD medium prepared from yeast nitrogen base. Thus, the UASINO apparently mediates coordinated regulation of the biosynthesis of phospholipids and saturated fatty acids. Interestingly, derepression in the absence of inositol occurred only when the UASINO was not mutated (Table 2), suggesting that it is a positive regulator of these genes.

**Gel-Exclusion Assay for GCCAA Repeat Elements.** From the deletion, substitution, and mutagenesis analyses described above, the GCCAA repeats apparently play a role in enhancing the transcription of FASI and FAS2. To determine whether the DNA containing these repeats can bind transcription factors, an electrophoretic mobility shift assay using whole-cell extracts was performed according to the method of Buchanan et al. (28). As shown in Fig. 7, these sequences can bind proteins. Specific fragments and plasmids containing the GCCAA repeats act as competitors in this binding assay.

**DISCUSSION**

Numa and coworkers (11, 12) have shown that exogenous fatty acids reduce the activity of yeast ACC by ~50%. Based

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>5' Endpoint</th>
<th>% lacZ Act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSCFAS1Eco</td>
<td>-2800</td>
<td>100 50</td>
</tr>
<tr>
<td>pSCFAS1</td>
<td>-1482</td>
<td>90 40</td>
</tr>
<tr>
<td>pSCFAS1-152</td>
<td>-620</td>
<td>95 58</td>
</tr>
<tr>
<td>pSCFAS1-312</td>
<td>-760</td>
<td>62 36</td>
</tr>
<tr>
<td>pSCFAS1-Sau 3A</td>
<td>-670</td>
<td>3 3</td>
</tr>
<tr>
<td>pSCFAS1-BstUI</td>
<td>-617</td>
<td>3 3</td>
</tr>
<tr>
<td>pSCFAS1-Cla</td>
<td>-475</td>
<td>3 4</td>
</tr>
</tbody>
</table>

*Fig. 3.* Deletion analysis of FASI. The 5' deletions were made using either available restriction sites or by using PCR methods. The lacZ activity is expressed as the percentage of pSCFAS1-Eco, the longest insert (100%); -FA, absence of fatty acids in culture medium; +FA, presence of fatty acids. The extent of fatty acid-mediated repression can be judged from the -FA and +FA values. B. BamHI; BstUI; C. ClaI; E. EcoRI; H. HindIII; K. KpnI; S. Sau3AI. The deletions generated using PCR methods are indicated by an asterisk.

**Fig. 4.** Comparison of the FASI and FAS2 regulatory regions. The GCCAA I and II repeats containing deameter and nonamer sequences and the putative GCR1 and RAP1/GRF1 sequences that are common to both genes are indicated by boldfaced type. The ABP1 sequence present only in FASI is also indicated by boldfaced type. The UASINO sequences are italicized and underscored with a thick line. The sequences underscored with a thin line are either the restriction sites or the oligonucleotides used for making PCR-mediated deletions.
on an earlier report (36), it was generally presumed that FAS is not subjected to this repression. Here, I have shown that FAS, like ACC, is also subject to fatty acid-mediated repression in yeast grown for several generations in synthetic medium containing fatty acids. The three genes FAS1, FAS2, and FAS3 appear to be coordinately regulated. This conclusion is based on the following observations: (i) fatty acids repress all three genes to the same extent; (ii) mRNA levels of FAS1 and FAS3 increase when yeast cells are transformed with a FAS2 multicopy plasmid; and (iii) there are common cis-acting elements in both FAS1 and FAS2. It is conceivable that FAS3 also has similar cis elements.

The conserved GCCAA repeats specifically enhance the transcription of FAS genes. This conclusion is based on the following observations. The deletion of the region containing only these repeat sequences severely reduced the expression of lacZ fusion. In addition, the region containing the GCCAA repeats alone stimulated transcription (Fig. 6), and these repeat sequences appear to bind some factors (Fig. 7). Given these results, the GCCAA repeats apparently have the characteristics of a UASFAS. As shown in Figs. 3, 5, and 6, fatty acid-mediated repression probably is caused by more than one element.

The UASINO has been identified as a nonamer sequence common to genes involved in phospholipid biosynthesis (31–34). Recently, Schuller et al. (35) concluded that this sequence is UASFAS and is responsible for coordinated regulation of the FAS genes. However, mutation and deletion analyses of the FAS1 and FAS2 regulatory regions (Figs. 3–6 and Table 2) suggest that UASINO is not the only sequence required for efficient transcription of FAS genes. The inositol-mediated repression of FAS genes is an interesting finding (Fig. 6 and Table 2). When the UASINO was mutated, it was expected that the expression of FAS-linked reporter genes would be constitutively derepressed. However, it was found that a functional UASINO is required for efficient expression of these genes (Fig. 6). Hence, it appears that UASINO is a positive regulator of the genes. The mutation analysis further demonstrated that the function of UASINO is lost when the conserved CACATG sequence is mutated.

Table 2. Inositol-mediated and fatty acid-mediated repression of FAS and ACC

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Culture medium</th>
<th>Specific activity</th>
<th>FAS</th>
<th>ACC</th>
<th>ρ-Gal</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSCFAS2</td>
<td>−INO, −FA</td>
<td>−INO, −FA</td>
<td>21.9</td>
<td>13.8</td>
<td>6.02</td>
</tr>
<tr>
<td></td>
<td>+INO, −FA</td>
<td>+INO, −FA</td>
<td>9.4</td>
<td>5.5</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>+INO, +FA</td>
<td>+INO, +FA</td>
<td>5.5</td>
<td>3.3</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>+YNB, −FA</td>
<td>+YNB, −FA</td>
<td>6.4</td>
<td>4.09</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>+YNB, +FA</td>
<td>+YNB, +FA</td>
<td>4.09</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>pSCFAS1-152ino</td>
<td>−INO, −FA</td>
<td>−INO, −FA</td>
<td>6.4</td>
<td>4.09</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>+INO, −FA</td>
<td>+INO, −FA</td>
<td>4.09</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>+INO, +FA</td>
<td>+INO, +FA</td>
<td>2.5</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>pSCFAS1-152ino</td>
<td>−INO, −FA</td>
<td>−INO, −FA</td>
<td>13.8</td>
<td>5.5</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>+INO, −FA</td>
<td>+INO, −FA</td>
<td>5.5</td>
<td>3.3</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>+INO, +FA</td>
<td>+INO, +FA</td>
<td>3.3</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>(cell-free extract)</td>
<td>−INO, −FA</td>
<td>−INO, −FA</td>
<td>13.6</td>
<td>5.5</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>+INO, −FA</td>
<td>+INO, −FA</td>
<td>5.5</td>
<td>3.3</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>+INO, +FA</td>
<td>+INO, +FA</td>
<td>3.3</td>
<td>2.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Enzymatic activities were measured as described in Table 1. β-Galactosidase (β-Gal) activity in permeabilized cells was measured as described in Fig. 1. pSCFAS1-152ino refers to the mutated UASINO sequence in pSCFAS1-152. −INO, without inositol; +INO, with inositol; −FA, without fatty acids; +FA, with fatty acids; YNB, yeast nitrogen base.
The function of the GCRI and RAPI1/GRF1 motifs was deduced only from deletion analyses of the promoters of the FAS1 and FAS2 genes. The product of GCRI is considered to be the regulator of glycolysis (26, 37–40) and binds to the sequence CTCCTC. This sequence is located close to and influences the RAPI1/GRF1 sequence in the expression of several genes involved in glycolysis (26, 29, 37–42). It is interesting that the putative RAPI1/GRF1 sequence present in both FAS1 and FAS2 lies in close proximity to the CTCCTC sequence. Similar to the RAPI1/GRF1 and CTCCTC sequences that work together to express the glycolytic genes, the GCCAA repeats and the UASNO sequences may influence each other in regulating the FAS genes. However, in FAS2, the UASNO plays a trivial role in regulating gene expression. Hence, I cannot suggest that this element is UASFAS.

I thank Q. Zhong and Dr. W.-Z. Huang for their technical assistance; Dr. W. Al-Feel, Dr. L. Abu-Elheiga, and M.-H. Tai for their valuable discussions; Dr. R. Reddy for his critical reading of the manuscript; and P. P. Powell for her editorial review. I am also grateful to Dr. S. J. Waki for his helpful discussions, encouragement, and support. This work was supported in part by Grant DK-41872 from the National Institutes of Health.