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Coordinated regulation and inositol-mediated and fatty acid-mediated repression of fatty acid synthase genes in *Saccharomyces cerevisiae*

(acetyl-CoA carboxylase/gene regulation)

SUBRAHMANYAM S. CHIRALA

Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

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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 regulated 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 the regulatory regions of *FAS1* and *FAS2* revealed common regulatory sequences. These include the GGCCAAAAAC and AGCCAAGCA sequences that have a common GCCAA core sequence and the UAS_{INO} (upstream activation sequence). Derepression of the FAS genes in the absence of exogenous inositol is not observed when UAS_{INO} is mutated, indicating that this cis element is a positive regulator of these genes. The GCCAA elements and UAS_{INO} 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 (M_r, 251,499) encoded by the *FAS3* gene. The yeast FAS consists of two multifunctional proteins, α (M_r, 207,683) and β (M_r, 220,077), that are organized in an $\alpha_6\beta_6$ 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.

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MATERIALS AND METHODS

Media and Culture Conditions. The yeast strain used in these experiments SEY2102 (*α, ura3-52, leu2-3, leu2-112, suc2-Δ9, his4-519, gal2*) 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-Δ312 (14) (provided 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 clarified by centrifugation. 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. From 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 $MgSO_4$/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

Strain/plasmid	Culture medium	Specific activity FAS	Specific activity ACC
SEY 2102	− FA	90	3.7
	+ FA	65	2.5
SEY 2102/YEPFAS2	− FA	184	18.5
	+ FA	69	5.5

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

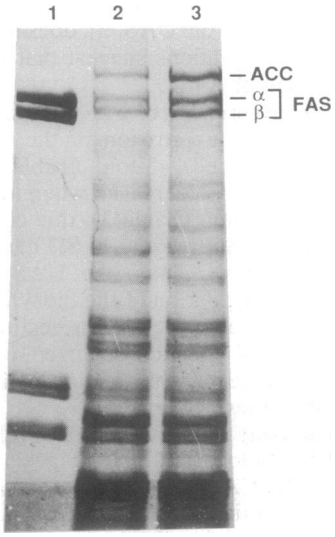


FIG. 1. Effect of the *FAS2* multicopy plasmid on FAS and ACC. Yeast cells were transformed with a multicopy plasmid, a derivative of YEPFAS2 (2, 7). The untransformed cells were grown in SD medium containing histidine, uracil, and leucine (each at 20 μg/ml); the transformed cells were grown in the same 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 Coomassie 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.

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 *FAS1* 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 *FAS1* Regulatory Region. To delineate the regulatory regions, a deletion analysis of the 5′ noncoding region of *FAS1* 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 *GCR1* (26), *RAP1/GRF1* (27–30), *ABF1* (28), and upstream activation sequence UAS_{INO} (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, GGCCAAAAAC and AGCCAAGCA, that have a common GCCAA core sequence (underlined) (Fig. 4). In addition, there is a UAS_{INO} located between the GCCAA repeats that has been implicated in coordinated regulation of *FAS1* 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

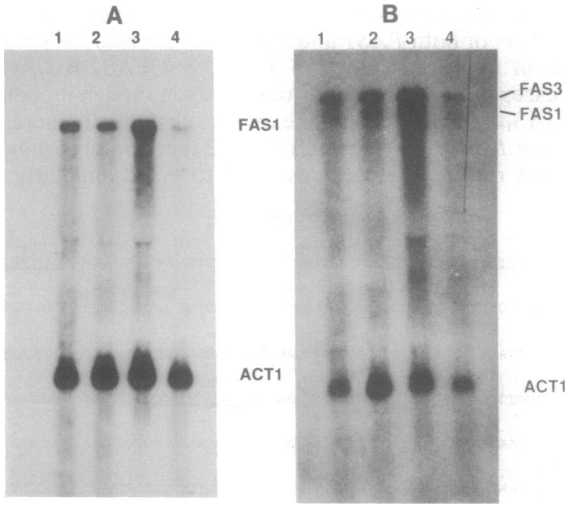


FIG. 2. Effects of the *FAS2* multicopy plasmid and fatty acids on the levels of FAS1 and FAS3 mRNAs. Cells were grown in the SD medium as described in Fig. 1, but with or without fatty acids. Total yeast RNA was treated with glyoxal and fractionated on 1% agarose gel. Lanes: 1 and 2, total RNA from 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 *Hin*dIII fragments from *FAS1* (5) and an *Eco*RI fragment of *ACT1* DNA (*A*). (*B*) Same blot was reprobed with a 6.6-kilobase-pair *Sst* I 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.

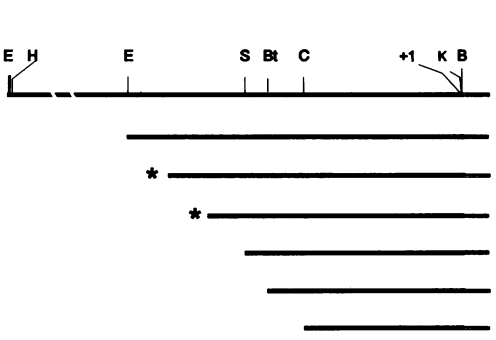


FIG. 3. Deletion analysis of *FAS1*. The 5′ deletions were made using either available restriction sites or by using PCR methods. The *lacZ* activity is expressed as the percentage of pSCFAS1-Eco, the longest insert (100%). −FA, absence of fatty acids in culture medium; +FA, presence of fatty acids. The extent of fatty acid-mediated repression can be judged from the −FA and +FA values. B, *Bam*HI; Bt, *Bst*UI; C, *Cla* I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn* I; S, *Sau*3AI. The deletions generated using PCR methods are indicated by an asterisk.

by pSCFAS2-Ssp) was deleted. Deletion of the putative *GCR1* sequence (nucleotides −278 to −274), represented by pSCFAS2-1104 (Fig. 5), further reduced the expression of *lacZ* to ≈10% of wild type. Deletion of sequences upstream of nucleotides −198 (pSCFAS2-Bst) and −174 (pSCFAS2-1226) reduced the expression of *lacZ* to background (Fig. 5). It is important to note that the *Bst*UI deletion still retained the UAS_{INO} (nucleotides −196 to −186) and that in *FAS2* UAS_{INO} alone did not support transcription.

Substitution Analysis of the Regulatory Regions to Delineate the Function of the GCCAA Repeats and UAS_{INO}. As shown by 5′ deletion analysis, the regions between nucleotides −760 and −669 in *FAS1* 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 *FAS1* and *FAS2* are the two GCCAA repeat-containing sequences. The results of deletion analyses of *FAS2* indicated that the UAS_{INO} by itself does not support expression of the *lacZ* reporter gene (Fig. 5, pSCFAS2-Bst). To confirm these observations, these specific regions were cloned in deletion constructs of both *FAS1* 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 UAS_{INO}, 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

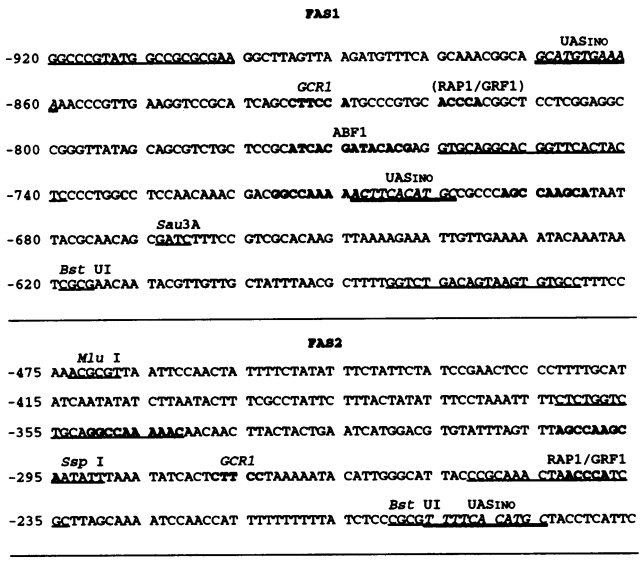


FIG. 4. Comparison of the *FAS1* and *FAS2* regulatory regions. The GCCAA I and II repeats containing decamer and nonamer sequences and the putative *GCR1* and *RAP1/GRF1* sequences that are common to both genes are indicated by boldfaced type. The *ABF1* sequence present only in *FAS1* is also indicated by boldfaced type. The UAS_{INO} 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.

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 *FAS1* region containing both GCCAA repeats and the UAS_{INO} (nucleotides −760 to −675; Fig. 6). Thus, it appears that both UAS_{INO} and the GCCAA repeats are required to express efficiently *FAS1* and *FAS2*.

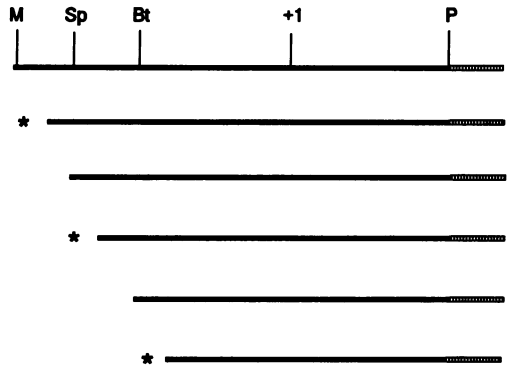
Mutation Analysis of UAS_{INO}. The *FAS1* regulatory region has two UAS_{INO} sequences (Fig. 4). Using site-directed mutagenesis, the UAS_{INO} between nucleotides −709 and −699 in *FAS1* was converted from ACTTCACATGC to ACTTCCCGGGC in pSCFAS1-152 and generated pSCFAS1-152M, which has only one UAS_{INO} (Fig. 6). By performing a PCR-mediated deletion on pSCFAS1-152M, pSCFAS1-312M, which has no UAS_{INO}, was generated. As shown in Fig. 6, the UAS_{INO} is essential for optimal expression of *FAS1*. However, there is measurable *lacZ* expression even when there is no functional UAS_{INO}. 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 UAS_{INO} (Fig. 6). *FAS2* has only one UAS_{INO}. When the conserved CACATG sequence of UAS_{INO} was mutated to CGCTAG, *lacZ* expression decreased only 50% (Fig. 6). As shown in Figs. 4 and 6, the *Bst*UI deletion of *FAS2*, which still contains the UAS_{INO}, does not support *lacZ* expression. These observations suggest that UAS_{INO} 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 UAS_{INO} apparently mediates coordinated regulation of the biosynthesis of phospholipids and saturated fatty acids. Interestingly, derepression in the absence of inositol occurred only when the UAS_{INO} was not mutated (Table 2), suggesting that it is a positive regulator of these genes.

Gel-Retardation 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 *FAS1* and *FAS2*. To determine whether the DNA containing these repeats can bind transacting factors, an electrophoretic mobility shift assay using whole-cell extracts was performed according to the method of Buchman *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



Plasmid	5' Endpoint	% lac Z Act. -FA	% lac Z Act. +FA
pSCFAS2	-473	100	50
pSCFAS2-994	-363	100	45
pSCFAS2-Ssp	-292	20	12
pSCFAS2-1104	-252	10	4
pSCFAS2-Bst	-198	1	0.9
pSCFAS2-1226	-174	1	1

FIG. 5. Deletion analysis of the *FAS2* regulatory region. The plasmid pSCFAS1 (Fig. 3) was cut with *Eco*RI and *Bam*HI, blunt ended, and used as a vector. The *FAS2* regulatory region along with the α-subunit coding region of 113 amino acids was cut with *Mlu* I and *Pvu* II, blunt-ended, and ligated to the vector backbone. This process regenerates both the *Eco*RI and *Bam*HI restriction sites. The deletions were made as described in Fig. 3. The restriction sites indicated are *Mlu* I (M), *Ssp* I (Sp), and *Pvu* II (P). All other details are as described in Fig. 3.

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 UAS_{FAS}. As shown in Figs. 3, 5, and 6, fatty acid-mediated repression probably is caused by more than one element.

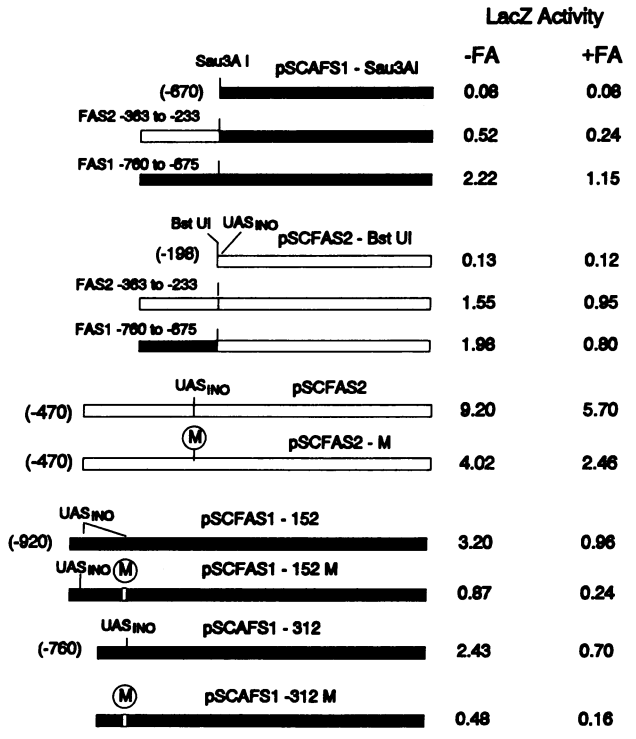


FIG. 6. Role of UAS_{INO} in regulating the reporter gene. The *FAS1* regulatory region containing the GCCAA repeats and UAS_{INO} (nucleotides −760 to −675) and the *FAS2* region containing only the GCCAA repeats (nucleotides −363 to −233) were cloned into pSCFAS1-Sau3A and pSCFAS2-Bst. These plasmids express only the basal level of β-galactosidase activity (Figs. 3 and 5). Also shown is the effect of mutated UAS_{INO} in pSCFAS-152, pSCFAS1-312, and pSCFAS2 in *lacZ* expression. "M" denotes the mutated UAS_{INO}. Numbers in parentheses indicate the 5' endpoints of the various constructs used. The bars are not drawn to scale.

The UAS_{INO} 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 UAS_{FAS} 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 UAS_{INO} 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 UAS_{INO} was mutated, it was expected that the expression of FAS-linked reporter genes would be constitutively derepressed. However, it was found that a functional UAS_{INO} is required for efficient expression of these genes (Fig. 6). Hence, it appears that UAS_{INO} is a positive regulator of the genes. The mutation analysis further demonstrated that the function of UAS_{INO} is lost when the conserved CACATG sequence is mutated.

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

Plasmid	Culture medium	Specific activity FAS	Specific activity ACC	Specific activity β-Gal
pSCFAS2	− INO, − FA	—	—	21.9
	− INO, + FA	—	—	13.8
	+ INO, − FA	—	—	9.4
	+ INO, + FA	—	—	5.5
	YNB, − FA	—	—	10.4
	YNB, + FA	—	—	6.4
pSCFAS1-152ino	YNB, − FA	—	—	0.9
	YNB, + FA	—	—	0.21
pSCFAS1-152	YNB, − FA	—	—	2.5
	YNB, + FA	—	—	1.0
pSCFAS1-152ino	− INO, − FA	—	—	3.3
	− INO, + FA	—	—	1.3
	+ INO, − FA	—	—	2.1
	+ INO, + FA	—	—	0.8
pSCFAS1-152 (cell-free extract)	− INO, − FA	416	13.6	1583
	− INO, + FA	289	6.02	516
	+ INO, − FA	185	4.09	501
	+ INO, + FA	101	3.15	298

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 UAS_{INO1} sequence in pSCFAS1-152. −INO, without inositol; +INO, with inositol; −FA, without fatty acids; +FA, with fatty acids; YNB, yeast nitrogen base.

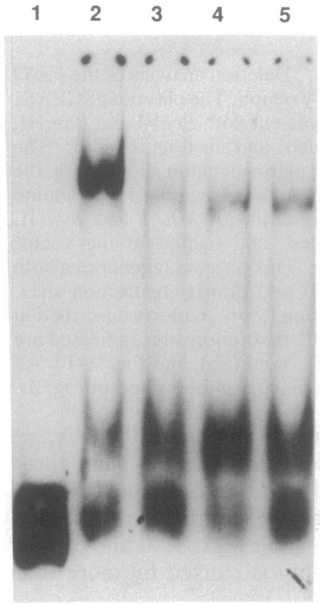


FIG. 7. Gel-retardation assay of GCCAA repeat-containing sequences. The γ-^{32}P-labeled *FAS2* DNA from nucleotides −363 to −234 was used as a probe. The extracts were incubated for 10 min with 1 μg of poly[d(I-C)] and 1 ng of labeled probe, with or without the following competing DNA. Lanes: 1, free probe with no extract; 2, extract with no competitor DNA; 3, 0.1 μg of unlabeled DNA fragment (same as the labeled probe); 4, 1 μg of pSCFAS1 plasmid DNA; 5, 1 μg of pSCFAS2 plasmid DNA.

The function of the *GCR1* and *RAP1/GRF1* motifs was deduced only from deletion analyses of the promoters of the *FAS1* and *FAS2* genes. The product of *GCR1* is considered to be the regulator of glycolysis (26, 37–40) and binds to the sequence CTTCC. This sequence is located close to and influences the *RAP1/GRF1* sequence in the expression of several genes involved in glycolysis (26, 29, 37–42). It is interesting that the putative *RAP1/GRF1* sequence present in both *FAS1* and *FAS2* lies in close proximity to the CTTCC sequence. Similar to the *RAP1/GRF1* and CTTCC sequences that work together to express the glycolytic genes, the GCCAA repeats and the UAS_{INO} sequences may influence each other in regulating the FAS genes. However, in *FAS2*, the UAS_{INO} plays a trivial role in regulating gene expression. Hence, I cannot suggest that this element is UAS_{FAS}.

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1. Wakil, S. J., Stoops, J. K. & Joshi, V. C. (1983) *Annu. Rev. Biochem.* **52**, 537–579.
2. Kuziora, M. A., Chalmers, J. H., Jr., Douglas, M. G., Jr., Hitzerman, R. A., Mattick, J. S. & Wakil, S. J. (1983) *J. Biol. Chem.* **258**, 11648–11653.
3. Schweizer, M., Lebert, C., Holtke, J., Roberts, L. M. & Schweizer, E. (1984) *Mol. Gen. Genet.* **194**, 457–465.
4. Al-Feel, W., Chirala, S. S. & Wakil, S. J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4534–4538.
5. Chirala, S. S., Kuziora, M. A., Spector, D. M. & Wakil, S. J. (1987) *J. Biol. Chem.* **262**, 4231–4240.
6. Schweizer, M., Roberts, L. N., Holtke, H.-J., Takabayashi, K., Hollerer, E., Hoffman, B., Muller, G., Kottig, H. & Schweizer, E. (1986) *Mol. Gen. Genet.* **203**, 479–496.
7. Mohamed, A. H., Chirala, S. S., Mody, N. H., Huang, W.-Y. & Wakil, S. J. (1988) *J. Biol. Chem.* **263**, 12315–12325.
8. Schweizer, E., Muller, G., Roberts, L. M., Schweizer, M., Rosch, J., Weisner, P., Beck, J., Startmann, D. & Zauner, I. (1987) *Fat. Sci. Technol.* **89**, 570–577.
9. Hinnebusch, A. G. (1990) *Prog. Nucleic Acid Res. Mol. Biol.* **38**, 195–240.
10. Mager, W. H. (1988) *Biochim. Biophys. Acta* **949**, 1–15.
11. Numa, S. & Tanabe, T. (1984) in *Fatty Acid Metabolism and Its Regulation*, ed., S. Numa (Elsevier, Amsterdam), pp. 1–27.
12. Kamiryo, T., Parthasarathy, S. & Numa, S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 386–390.
13. Sherman, F., Fink, G. R. & Lawrence, C. W. (1979) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
14. Hahn, S., Pinkham, J., Wei, R., Miller, R. & Guarente, L. (1988) *Mol. Cell. Biol.* **8**, 655–663.
15. Saiki, R. F., Gelfond, D. H., Stoffel, S., Scharf, S. J., Hifguchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
16. Stoops, J. K., Awad, E. S., Arslanian, M. J., Gunsberg, S., Wakil, S. J. & Oliver, R. M. (1978) *J. Biol. Chem.* **253**, 4464–4475.
17. Thampy, K. G. & Wakil, S. J. (1985) *J. Biol. Chem.* **260**, 6318–6323.
18. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
19. Guarente, L. (1983) *Methods Enzymol.* **101**, 181–191.
20. Sanger, R., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
21. Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254.
22. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153**, 163–168.
23. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
24. Pazirandeh, M., Chirala, S. S. & Wakil, S. J. (1991) *J. Biol. Chem.* **266**, 20946–20952.
25. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
26. Baker, H. V. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9443–9447.
27. Shore, D. & Nasmyth, K. (1987) *Cell* **51**, 721–732.
28. Buchman, A., Kimmerley, W. J., Rine, J. & Kornberg, R. D. (1988) *Mol. Cell. Biol.* **8**, 210–225.
29. Buchman, A. R., Lue, N. F. & Kornberg, R. D. (1988) *Mol. Cell. Biol.* **8**, 5086–5099.
30. Moehle, C. M. & Hinnebusch, A. G. (1991) *Mol. Cell. Biol.* **11**, 2723–2735.
31. Carman, G. M. & Henry, S. A. (1989) *Annu. Rev. Biochem.* **58**, 635–669.
32. Nikoloff, D. M. & Henry, S. A. (1991) *Annu. Rev. Genet.* **25**, 559–583.
33. Lopes, J. M. & Henry, S. A. (1991) *Nucleic Acids Res.* **19**, 3987–3994.
34. Lopes, J. M., Hirsch, J. P., Chorgo, P. A., Schluze, K. L. & Henry, S. A. (1991) *Nucleic Acids Res.* **19**, 1687–1693.
35. Schuller, H.-J., Hahn, A., Troster, F., Schutz, A. & Schweizer, E. (1992) *EMBO J.* **11**, 107–114.
36. Meyer, K.-H. & Schweizer, E. (1976) *Eur. J. Biochem.* **65**, 317–324.
37. Clifton, D., Weinstock, S. B. & Fraenkel, D. G. (1978) *Genetics* **88**, 1–11.
38. Clifton, D. & Fraenkel, D. G. (1981) *J. Biol. Chem.* **256**, 13074–13078.
39. Holland, J. P., Brindle, P. K. & Holland, M. J. (1990) *Mol. Cell. Biol.* **10**, 4863–4871.
40. Brindle, P. K., Holland, J. P., Willett, C. E., Innis, M. A. & Holland, M. J. (1990) *Mol. Cell. Biol.* **10**, 4872–4885.
41. Chambers, A., Stanway, C., Kingsman, A. J. & Kingsman, S. M. (1988) *Nucleic Acids Res.* **16**, 8245–8260.
42. Chambers, A., Stanway, C., Tsang, J. S. H., Henry, Y., Kingsman, A. J. & Kingsman, S. M. (1990) *Nucleic Acids Res.* **18**, 5393–5399.