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A downstream regulatory element located within the coding sequence mediates autoregulated expression of the yeast fatty acid synthase gene *FAS2* by the *FAS1* gene product

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ABSTRACT

The fatty acid synthase genes *FAS1* and *FAS2* of the yeast *Saccharomyces cerevisiae* are transcriptionally co-regulated by general transcription factors (such as Reb1, Rap1 and Abf1) and by the phospholipid-specific heterodimeric activator Ino2/Ino4, acting via their corresponding upstream binding sites. Here we provide evidence for a positive autoregulatory influence of *FAS1* on *FAS2* expression. Even with a constant *FAS2* copy number, a 10-fold increase of *FAS2* transcript amount was observed in the presence of *FAS1* in multi-copy, compared to a *fas1* null mutant. Surprisingly, the first 66 nt of the *FAS2* coding region turned out as necessary and sufficient for *FAS1*-dependent gene expression. *FAS2–lacZ* fusion constructs deleted for this region showed high reporter gene expression even in the absence of *FAS1*, arguing for a negatively-acting downstream repression site (DRS) responsible for *FAS1*-dependent expression of *FAS2*. Our data suggest that the *FAS1* gene product, in addition to its catalytic function, is also required for the coordinate biosynthetic control of the yeast FAS complex. An excess of uncomplexed Fas1 may be responsible for the deactivation of an *FAS2*-specific repressor, acting via the DRS.

INTRODUCTION

The biosynthesis of multiprotein complexes with a defined stoichiometry of subunits requires coordinate expression of the corresponding structural genes. In eukaryotes, gene activation by related upstream sequences is a general mechanism to ensure balanced production of the respective polypeptides in response to regulatory signals (1). In previous work, we studied the genetic control of structural genes encoding subunits of the fatty acid synthase (FAS) complex in the yeast *Saccharomyces cerevisiae*. *FAS1* and *FAS2* are genetically unlinked and encode the multifunctional subunits β and α, respectively (2–5), which finally constitute the $\alpha_6\beta_6$ heteromultimeric complex (6).

Deletion analysis of *FAS1* and *FAS2* promoters revealed the existence of a common type of upstream activation site (UAS), designated ICRE (inositol/choline responsive element; 7), which could be also identified in the control regions of several structural genes involved in phospholipid metabolism (such as *INO1*, *CHO1*, *ACC1* and *ACS2*; 8–11, respectively). Importantly, transcriptional activation of target genes by ICRE motifs (= UAS_{INO}; consensus sequence, WYTTCAYRTG; 12) is regulated by phospholipid precursors inositol and choline and requires the positive factors Ino2 and Ino4 (13–16). Ino2 and Ino4 both contain a basic helix–loop–helix structural motif and bind to the ICRE as a heterodimer (16). Negative regulation of ICRE-containing genes by phospholipid precursors is mediated by the Opi1 repressor (17,18), which contacts Ino2 as well as the pleiotropic repressor Sin3 (19). In addition to UAS elements influenced by phospholipid precursors, *FAS* promoters are also activated by binding sites of the essential transcription factors Rap1, Abf1 and Reb1 (20). These proteins are also required for the expression of glycolytic as well as ribosomal protein genes (21,22). Since *FAS* genes fulfill a housekeeping function in cellular biochemistry, constitutively activating motifs ensure fatty acid biosynthesis even under conditions of inositol/choline repression. However, neither ICRE motifs nor constitutive elements of transcription appear suitable to ensure a defined stoichiometry of *FAS1* and *FAS2* gene products within the cell.

The construction and subsequent characterization of *fas1* and *fas2* null mutants provided evidence for additional mechanisms leading to a balanced ratio of FAS subunits α and β. While the substantially increased sensitivity of individual FAS subunits against vacuolar or proteosomal proteinases (23) may be a trivial consequence of unsuccessful complex formation, the influence of a *fas1* deletion on the amount of the *FAS2* transcript suggested a biosynthetic cross-talk among both genes (24). In the absence of *FAS1* (but with an intact *FAS2* gene), a decrease of *FAS2* mRNA to ~35% of the wild-type level was found. In contrast, the concentration of the *FAS1* transcript was not affected by the allelic status of *FAS2*. In this work, we

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investigated the influence of *FAS1* on *FAS2*. To our surprise, the *FAS2* upstream region did not respond to a variation of *FAS1* gene dosage. Instead, we identified a region within its reading frame that was necessary and sufficient for mediating *FAS1*-dependent expression of *FAS2*. The corresponding *FAS2* coding sequence turned out to be a negatively-acting element, requiring an excess of individual Fas1 to overcome its inhibitory influence. This finding supports the existence of a new mechanism contributing to a coordinate biosynthesis of proteins within multi-subunit complexes.

MATERIALS AND METHODS

Strains and media

The strains of *S.cerevisiae* used for this study are isogenic to the regulatory wild-type JS91.15-23 (*MAT*α *ura3 leu2 his3 trp1 can1*) and were obtained by introduction of the mutant alleles indicated (IKY1, *pra1::HIS3*; IKY2, Δ*fas1::LEU2*; IKY3, *pra1::HIS3* Δ*fas1::LEU2*; IKY4, Δ*fas2::LEU2*; PWY12, Δ*fas1::HIS3* Δ*fas2::LEU2*). Disruption plasmids that were used for strain construction have been described [pBF3, Δ*fas1::HIS3*; pBF4, Δ*fas2::LEU2*; pBF5, Δ*fas1::LEU2* (24); *pra1::HIS3* (23)]. Strain WCG4-11/22A (*MATa ura3 leu2 his3 pre1 pre2*) (25) containing two defective subunits of the proteasome represents a different strain background. Transformants were grown in synthetic complete media selecting for the introduced genetic markers (e.g. SCDFA-Ura-Trp). To allow growth of *fas* mutants, all media were supplemented with 1% Tween-40 and 0.03% hydrolyzed butter.

Plasmid construction

Reporter plasmid pSAK1 was derived from YIplac211 (integrating *LEU2* vector) (26) by transfer of a *FAS2–lacZ* fusion from pJS203 (7), containing an ~1 kb upstream region together with 921 bp of the coding sequence. Similarly, pBF16 was obtained by insertion of the same fusion gene into YCplac22 (*ARS CEN TRP1* vector). *FAS* gene dosage variation was achieved with effector plasmids pJS222 and pJS225 (6.9 kb *Bam*HI/*Sal*I fragment with *FAS2*, inserted into 2 μm *LEU2* vector YEp351 and 2 μm *URA3* vector YEp352, respectively), and pJS229 (9.9 kb *Sac*I/*Sph*I fragment with *FAS1*, inserted into YEp352) (27).

To obtain *FAS2–lacZ* reporter plasmids with a varying portion of the coding sequence, fragments were amplified by PCR, using a constant upstream primer in combination with downstream primers scanning the *FAS2* reading frame. The resulting single-copy plasmids [*ARS CEN TRP1 FAS2*(1/x)–*lacZ*; otherwise identical to pBF16] contain 1008 bp of the *FAS2* control region together with 3, 45, 66, 75, 84, 93, 99, 150 or 228 bp of the coding sequence. To further define the *FAS2* regulatory element necessary and sufficient for *FAS1*-mediated autoregulation, a *lacZ* variant lacking its start codon was inserted into expression plasmid p414-MET25 (28). Finally, *MET25–FAS2*(x/y)–*lacZ* reporter plasmids were constructed by insertion of PCR fragments containing varying sequences of the *FAS2* reading frame. To avoid a different translational efficiency, all constructs contain the natural (–6/+6) sequence of *FAS2*.

Miscellaneous procedures

For northern blot hybridization, 20 μg of total RNA from yeast transformants was separated by gel electrophoresis under denaturing conditions (1% agarose with 2.2 M formaldehyde). Following membrane transfer, hybridization was done under standard conditions, using ^{32}P-labeled *FAS2* and *ACT1* (internal loading control) probes. Phosphoimager quantification of signal intensities was performed with Fujifilm Bio-Imaging Analyser BAS-1500. Yeast transformation was done by a simplified lithium acetate procedure (29). Activity of FAS was determined by assaying β-ketoacyl reductase-dependent oxidation of NADPH in the presence of acetyl-CoA and malonyl-CoA (30). The β-galactosidase assay has been described (7).

RESULTS

Regulation of *FAS2* mRNA level by the *FAS1* gene dosage

Our previous characterization of *fas1* and *fas2* deletion mutants by northern blot hybridization provided evidence for influence of *FAS1* on *FAS2* gene expression. Even with an intact *FAS2* gene, the amount of *FAS2* mRNA decreased to ~35% of the wild-type level in the presence of a *fas1* mutation (24). We now ask whether an increase of *FAS1* gene dosage also affects the steady-state concentration of *FAS2* mRNA. Isogenic strains carrying a *fas1* null mutation (IKY3) or the chromosomal *FAS1* wild-type allele (IKY1) were transformed with an episomal multi-copy plasmid containing *FAS1* under natural promoter control (pJS229). The transformants obtained contain a single chromosomal *FAS2* gene but differ with respect to the amount of *FAS1* encoded β-subunit (verified by immunoblot analysis, using β-specific antibodies; not shown). As depicted in Figure 1 (lanes 1 and 2), elevating the *FAS1* copy number from 0 to *n* (copy number of a plasmid containing the 2 μm origin) led to a strong increase of *FAS2* mRNA (~10-fold, according to phosphoimager quantification). With a *FAS1* wild-type strain, a further 2.5-fold increase of the *FAS2* transcript amount was detected in the presence of additional *FAS1* copies (lanes 3 and 4). Thus, in addition to its catalytic function, *FAS1* must be considered as a regulatory factor of *FAS2* gene expression.

We also investigated whether *FAS1* dosage variation similarly affects FAS enzyme activity. For a functional FAS, both α and β subunits are required (31). Individual FAS subunits were sensitive against proteolytic attack, with vacuolar proteinases yscA and yscB being required for degradation of β (24) while the proteasome (yscE) is involved in cleavage of α (23). Thus, overexpression of a single *FAS* gene should not lead to an increase in FAS activity. A *FAS* wild-type strain lacking the vacuolar proteinase yscA was transformed with *FAS1* or *FAS2* containing multi-copy plasmids, and transformants were subsequently assayed for specific FAS activity. As is apparent from Figure 2, multiple copies of *FAS2* only did not lead to an increased FAS activity in transformants. In contrast, a 2.8-fold increase was assayed as a result of overexpressing only *FAS1*. This finding agrees with the idea of a dual role for *FAS1*: overexpression not only results in increased production of β but also allows stimulation of α biosynthesis. Due to its positive regulatory function, *FAS1* overexpression is sufficient for a

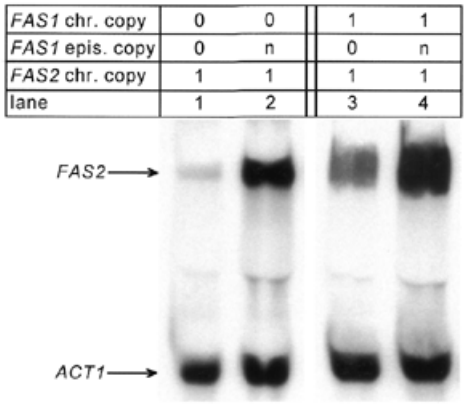


Figure 1. *FAS1*-dependent steady-state concentration of the *FAS2* mRNA. For the northern blot hybridization shown, total RNA was isolated from strains with identical *FAS2* copy number (single chromosomal copy, chr. copy) but varying *FAS1* gene dosage. Strains IKY1 (*ura3 pra1 FAS1 FAS2*, lanes 3 and 4) and IKY3 (*ura3 pra1* Δ*fas1 FAS2*, lanes 1 and 2) were transformed with plasmids YEp352 (vector control, 2 µm *URA3*, lanes 1 and 3) or pJS229 (copy number *n*; 2 µm *URA3 FAS1*, lanes 2 and 4) and subsequently grown in SCD-Ura, supplemented with fatty acids. The filter was simultaneously hybridized against *FAS2* and *ACT1* (internal control) DNA probes. Quantification of signal intensities was done by phosphoimager analysis. After background subtraction, the ratio of PSL values (photo stimulated luminescence) for *FAS2* and *ACT1* signals was calculated for each lane (PSL_{FAS2}/PSL_{ACT1}; lane 1, 0.15; lane 2, 1.39; lane 3, 0.76; lane 4, 1.9).

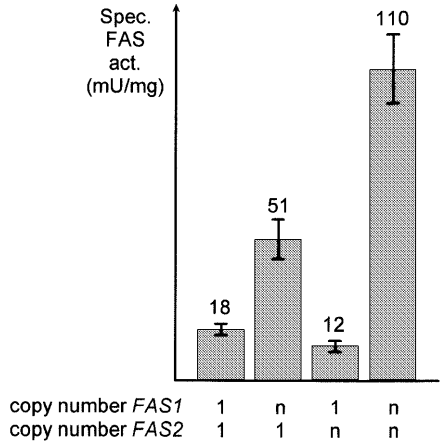


Figure 2. Specific FAS activity in transformants with varying *FAS1* and *FAS2* gene dosage. Strain IKY1 (*ura3 leu2 pra1 FAS1 FAS2*) was transformed in pairs with combinations of empty vectors (YEp352, 2 µm *URA3*; YEp351, 2 µm *LEU2*; total *FAS* copy numbers 1), or multi-copy plasmids (pJS229, 2 µm *URA3 FAS1*; pJS225, 2 µm *LEU2 FAS2*; total *FAS* copy numbers *n*). Transformants were grown in selective medium (SCD-Ura-Leu). Specific FAS activity is given in nanomoles NADPH oxidized per minute per milligram of protein (mU/mg). Standard deviation of the mean value is indicated by bars.

substantially enhanced FAS activity. As expected, a further increase of FAS activity could be assayed when both *FAS* genes were introduced in multi-copy. With a *pre1 pre2* double mutant allowing stable overproduction of α, almost identical results were obtained (not shown).

Table 1. Influence of *FAS1* gene dosage variation on *FAS2*(1/921)–*lacZ* reporter gene expression

Genetic background	Specific β-galactosidase activity (U/mg) with		
	Δ*fas1*	*FAS1*	$(FAS1)_n$
FAS2–lacZ (integrated) in *PRA1* strain	25	105	280
FAS2–lacZ (ARS CEN) in *PRA1* strain	20	85	250
FAS2–lacZ (ARS CEN) in *pra1* strain	40	150	735

Reporter plasmids pSAK1 (*FAS2–lacZ LEU2*, integrative) and pBF16 (*FAS2–lacZ ARS CEN TRP1*) were transformed into isogenic strains JS91.15-23 (*PRA1*) and IKY1 (*pra1*), respectively. *FAS1* dosage variation was achieved by co-transformation with empty vector YEp352 (2 µm *URA3*) or pJS229 (2 µm *URA3 FAS1*). Transformants were grown in selective medium (SCD-Ura-Leu or SCD-Ura-Trp), supplemented with fatty acids. Specific β-galactosidase activities are given in nanomoles ONPG hydrolyzed per minute per milligram of protein. Standard deviation was ≤25% of the mean value.

Influence of *FAS1* and *FAS2* on *FAS2–lacZ* reporter gene expression

For the identification of the *cis*-acting element mediating the positive influence of *FAS1*, we first tested whether the autoregulation among *FAS* genes can be also detected with a *FAS2–lacZ* reporter gene. An *FAS2–lacZ* fusion containing 1008 bp of the upstream region together with 921 bp of the coding sequence was integrated at the *LEU2* locus of a regulatory wild-type strain. Subsequently, we introduced either a Δ*fas1::HIS3* mutant allele or additional *FAS1* copies by transformation with pJS229, allowing a comparison of the influence of *FAS1* copy numbers 0, 1 and *n* on *FAS2–lacZ* expression. As is apparent from Table 1, deletion of *FAS1* reduced *FAS2–lacZ* expression to ~24% of the wild-type level. In contrast, a 2.6-fold increase was assayed in the presence of additional *FAS1* copies. Similar results were obtained with an identical *FAS2–lacZ* fusion transferred to a single-copy *ARS CEN* plasmid. Importantly, with a strain lacking the vacuolar proteinase yscA (*pra1* mutant), elevating the *FAS1* copy number had an even greater effect on *FAS2–lacZ* expression. A 4.9-fold increase of specific β-galactosidase activity was assayed with multiple copies of *FAS1* in the *pra1* mutant (compared with the chromosomal *FAS1* single-copy situation) while a 2.9-fold increase could be detected in the isogenic *PRA1* background. Since the *FAS1* gene product is more stable in the absence of yscA, *FAS2–lacZ* expression may indeed be stimulated by the FAS β-subunit.

These experiments were extended by a systematic comparison of *FAS2–lacZ* expression in strains containing *FAS1 FAS2*, *fas1 FAS2*, *FAS1 fas2* and *fas1 fas2* alleles, transformed with empty vector or either *FAS1* or *FAS2* in multi-copy (data shown in Table 2). Interestingly, introduction of a *fas2* deletion led to an increase of *FAS2–lacZ* expression by a factor of 2.4. The *FAS2* gene product may act as a repressor of its own expression. Consequently, the stimulating effect of *FAS1* might be the indirect result of removal of individual α-subunits, becoming assembled into the FAS complex. Assuming such a

Table 2. Expression of the *FAS2–lacZ* reporter gene with varying *FAS* gene dosage

Relevant genotype	Specific β-galactosidase activity with effector plasmids		
	YEp352 (vector control)	pJS229 $(FAS1)_n$	pJS222 $(FAS2)_n$
FAS1 FAS2	85	310	60
Δ*fas1 FAS2*	20	250	30
FAS1 Δ*fas2*	200	370	70
Δ*fas1* Δ*fas2*	25	480	40

Reporter plasmid pBF16 (*FAS2–lacZ ARS CEN TRP1*) was transformed into isogenic strains JS91.15-23 (*FAS1 FAS2*), IKY2 (Δ*fas1 FAS2*), IKY4 (*FAS1* Δ*fas2*) and PWY12 (Δ*fas1* Δ*fas2*), respectively. *FAS1* or *FAS2* dosage variation was achieved by co-transformation with empty vector YEp352 (2 µm *URA3*), pJS229 (2 µm *URA3 FAS1*) or pJS222 (2 µm *URA3 FAS2*). Transformants were grown in selective medium (SCD-Ura-Trp), supplemented with fatty acids. Specific β-galactosidase activities are given in nanomoles ONPG hydrolyzed per minute per milligram of protein. Standard deviation was ≤25% of the mean value.

mechanism, deletion of both *FAS* effector genes should allow high expression of *FAS2–lacZ*. However, the idea of *FAS2* encoding its own repressor appears unlikely, since a *fas1 fas2* double deletion mutant shows low expression of *FAS2–lacZ*, similarly to the *fas1* single mutant. Nevertheless, *FAS2* indirectly influences its own expression by controlling the level of individual β-subunits. Overexpression of *FAS2* in the *fas2* single mutant led to a 2.9-fold decrease of *FAS2–lacZ* expression, compared with the vector control. In contrast, with all genetic situations tested, *FAS1* overexpression stimulated *FAS2–lacZ* expression. We conclude that *FAS1* does not simply counteract repression by the *FAS2* encoded α-subunit.

In contrast with what was found for the *FAS2–lacZ* fusion, no influence of either *FAS1* or *FAS2* on the expression of an *FAS1–lacZ* reporter gene could be detected (not shown). These findings agree with northern blot hybridizations described previously (24). To complete these experiments on the mutual influence of genes involved in fatty acid biosynthesis, we also considered the essential acetyl-CoA carboxylase gene *ACC1/FAS3*. However, gene dosage variation of either *FAS1* or *FAS2* did not affect expression of an *ACC1–lacZ* fusion. Similarly, *FAS1–lacZ* and *FAS2–lacZ* fusions were not influenced by overexpression of the *ACC1* structural gene (not shown). Thus, stimulation of *FAS2* expression by *FAS1* is the sole autoregulatory influence among structural genes *ACC1*, *FAS1* and *FAS2*, which are specifically required for fatty acid synthesis in yeast.

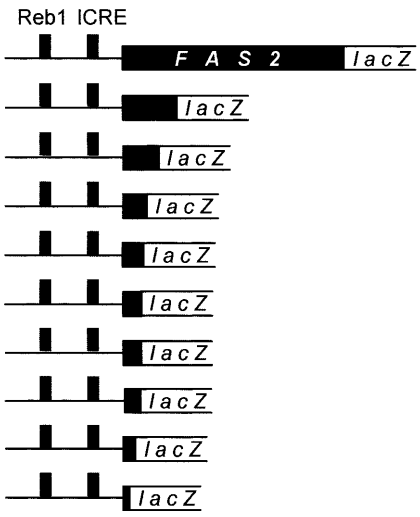
Negative regulation of *FAS2* by a downstream element

Initially, we expected to map the *cis*-acting element(s) responsible for *FAS1*-mediated autoregulation of *FAS2* in the upstream region of the gene. However, *FAS2–lacZ* fusions devoid of *FAS2* coding sequences did not respond to variations of *FAS1* copy number. With the *FAS2*(1/3)*–lacZ* fusion containing only the natural ATG codon, a high β-galactosidase activity was measured even in the absence of *FAS1* (Fig. 3). This finding argues for the existence of a negatively-acting element leading to low reporter gene expression when fusion constructs contain a considerable portion of the *FAS2* coding region. Thus, the corresponding *cis*-acting element within the *FAS2* reading frame must be considered as a downstream repression site (DRS). For a precise localization of the DRS, we shortened the coding region of *FAS2* which, together with an upstream region of constant length, was then inserted 5′ to the *lacZ* reporter gene. As is apparent from Figure 3, a *FAS2*(1/66)*–lacZ* fusion still showed *FAS1*-dependent expression while *FAS2*(1/45)*–lacZ* was constantly activated at a high level. We conclude that autoregulation among *FAS* genes requires at least 66 nt of the *FAS2* coding region. It remained undetermined whether *FAS2*-specific upstream promoter elements or sequences of the 5′-untranslated part of the mRNA are also necessary.

Thus, *FAS2* reading frame fragments of varying length and position were inserted between the heterologous *MET25* promoter (containing its natural transcription initiation site) and *lacZ* as a reporter gene. The *MET25–FAS2*(1/66)*–lacZ* construct clearly showed *FAS1*-dependent expression (cf. Fig. 4), supporting the view that 66 nt of the *FAS2* reading frame are necessary and sufficient for autoregulation. A weakened but still significant influence of *FAS1* copy number was also observed with the *MET25–FAS2*(22/150)*–lacZ* construct. However, a fusion gene lacking the first 39 nt of *FAS2* ORF was constantly expressed at an intermediate level. We conclude that nt 22–66 of the *FAS2* coding region may represent the essential core of the DRS, which is able to negatively regulate gene expression even when separated from its natural context.

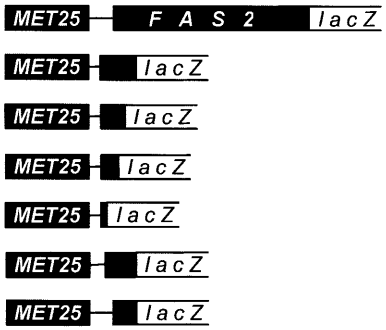
DISCUSSION

Coordinate expression of genes that encode functionally associated proteins is usually achieved by similar upstream promoter elements such as UAS or URS motifs. In this work, we present evidence for the existence of an autoregulatory mechanism depending on a sequence within the coding region of the yeast FAS gene *FAS2*. *FAS* autoregulation comprises genes and gene products of the FAS complex as a whole, involving *FAS1* as a positive factor of *FAS2* expression. In the absence of Fas1 (β subunit), *FAS2* expression is down regulated while, conversely, overexpression of *FAS1* and the subsequent accumulation of individual β subunits may counteract negative regulation. Thus, a temporary excess of Fas1 should derepress *FAS2* expression and subsequently allow synthesis of Fas2 (α subunit), finally ensuring a balanced ratio of both subunits of the FAS complex. In contrast with the positive influence of the *trans*-acting factor Fas1, the autoregulatory *cis*-acting sequence (DRS) was identified as a negative element. Thus, Fas1 may function as an indirect positive factor, mediating deactivation of an unknown repressor that is responsible for a low level of *FAS2* mRNA. Together with previous work, these results argue for the existence of a temporal order of events (summarized in Fig. 5A) leading to a defined stoichiometry of FAS subunits: (i) ICRE-dependent control requiring the heterodimeric activator Ino2/Ino4 coordinates transcription of structural genes involved in phospholipid biosynthesis (7,14,16); (ii) pleiotropic transcription factors such as Rap1, Abf1 and Reb1 are needed to fulfill the housekeeping function of fatty acids even under conditions of inositol/choline repression (20); (iii) autoregulation of *FAS*



nt *FAS2* ORF	Spec. β-gal. act. (U/mg) copy number *FAS1* 0	n	Af
(1 / 921)	40	735	18,4
(1 / 228)	50	810	16,2
(1 / 150)	45	880	19,6
(1 / 99)	40	760	19,0
(1 / 93)	35	560	16,0
(1 / 84)	35	480	13,7
(1 / 75)	50	580	11,6
(1 / 66)	50	440	8,8
(1 / 45)	400	430	1,1
(1 / 3)	600	550	0,9

Figure 3. Deletion analysis of the *FAS2* coding region responsible for *FAS1*-dependent gene expression. Deletion constructs contain identical upstream sequences (–1008) but differ with respect to the length of the *FAS2* reading frame, fused to *lacZ*. Reporter plasmids [*ARS CEN TRP1 FAS2*(1/x)–*lacZ*; data are given in nucleotide positions] were transformed into *fas1* mutant strain IKY3, containing either the empty vector YEp352 (*FAS1* copy number 0) or *FAS1* multi-copy plasmid pJS229 (copy number *n*). Transformants were grown in selective medium (SCD-Ura-Trp), supplemented with fatty acids. Specific β-galactosidase activities are given in nanomoles ONPG hydrolyzed per minute per milligram of protein. Standard deviation was ≤25% of the mean value. Af, activation factor of specific enzyme activity in the presence of multiple *FAS1* copies, compared with the null mutant.



nt *FAS2* ORF	Spec. β-gal. act. (U/mg) copy number *FAS1* 0	n	Af
(52 / 921)	65	110	1,7
(1 / 150)	8	240	30,0
(1 / 99)	12	250	20,8
(1 / 66)	25	260	10,4
(1 / 6)	280	210	0,8
(22 / 150)	35	120	3,4
(40 / 150)	92	113	1,2

Figure 4. *FAS1*-dependent expression of *FAS2–lacZ* reporter constructs under heterologous promoter control. Various sequences of the *FAS2* coding region (indicated by nucleotide positions) were inserted between the *MET25* promoter and the *lacZ* reporter gene. To ensure similar translational efficiency, reading frame fragments with an artificial start codon contain the natural –6/–1 sequence of *FAS2*. Reporter plasmids [*ARS CEN TRP1 MET25–FAS2*(x/y)–*lacZ*] were transformed into *fas1* mutant strain IKY3, containing either the empty vector YEp352 (*FAS1* copy number 0) or *FAS1* multi-copy plasmid pJS229 (copy number *n*). For a maximal promoter strength, transformants were grown in selective medium without methionine (SCD-Ura-Trp-Met), supplemented with fatty acids. Specific β-galactosidase activities are given in nanomoles ONPG hydrolyzed per minute per milligram of protein. Standard deviation was ≤25% of the mean value. Af, activation factor of specific enzyme activity in the presence of multiple *FAS1* copies, compared to the null mutant.

genes at the transcriptional level as a mechanism of fine-tuning leads to a balanced biosynthesis of α and β subunits (this work); and (iv) degradation of individual subunits by different proteolytic systems may support biosynthetic autoregulation under certain conditions (23,24; not depicted in Fig. 5A).

In contrast with what has been reported for higher eukaryotes, only few examples of intragenic regulatory sites were identified in yeast genes. Downstream activation sites (DASs) have been described for the glycolytic genes *PGK1* (32) and *PYK1* (33) as well as for the glucose-inducible *SRP1* gene (= *TIR1*; 34),

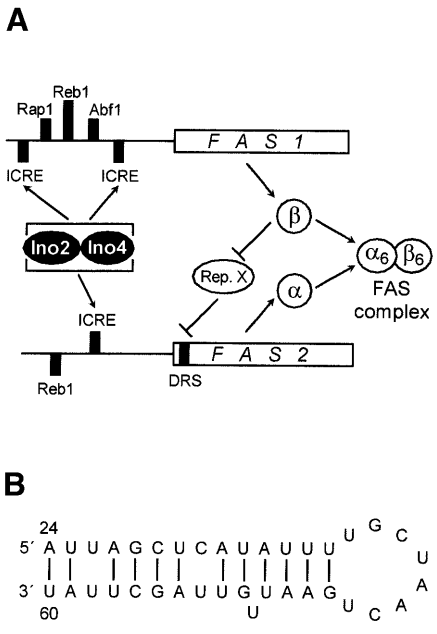


Figure 5. (**A**) Hypothesis on coordinate control of *FAS* genes by Fas1-dependent anti-repression of *FAS2* gene expression. In the absence of non-complexed Fas1 (β-subunit), the *FAS2* control region (including downstream sequences) is substantially weaker than the *FAS1* promoter. An excess of free β-subunit may directly or indirectly deactivate the repressor (Rep. X; acts via the DRS), leading to maximal *FAS2* expression and subsequent synthesis of a balanced amount of α-subunit (*FAS2* gene product), which may then associate with β to form a functional FAS complex ($\alpha_6\beta_6$). Thereby, withdrawal of β again reduces *FAS2* expression. The hypothesis considers *FAS1* expression as the leading and *FAS2* expression as the lagging step of FAS complex formation. It is unknown whether anti-repression acts at the level of transcriptional initiation or elongation. (**B**) Hypothetical stem–loop structure within *FAS2* mRNA. Nucleotide positions refer to the start of the *FAS2* reading frame.

encoding a cell wall protein. While *trans*-acting factors affecting DAS elements of *PGK1* and *PYK1* were not identified, the pleiotropic repressor/activator protein Rap1 binds to a 33 bp intragenic sequence of *SRP1* (34). Transcription of yeast retrotransposons Ty1 and Ty2 is controlled by an activating (35,36) as well as by a repressing site (37), both of which map to the coding region. Similarly, a combination of DAS and DRS elements influences transcription of the lipoamide dehydrogenase gene *LPD1* (38). Two DRSs (DRS1 and DRS2) involved in negative regulation with ethanol as a carbon source were precisely mapped in the coding region of the hexokinase PII gene *HXK2* (39). Interestingly, both DRS elements contain the core sequence (A/C)(A/G)GAAAT, which has been also identified as a UAS of the invertase gene *SUC2* (40). A subunit of the RNA polymerase II mediator complex (Med8, containing a putative leucine zipper motif) could be identified as the corresponding binding factor (41,42). It has been also reported that transcription factor binding sites occurring fortuitously within reading frames may negatively affect expression of the respective gene (shown for a *RPL16–lacZ* fusion containing Leu3 binding sites and *ACC1* containing Gal4 binding sites; 43,44). However, the regulatory significance of such interactions is unknown. While some downstream regulatory sites may also affect gene expression from an upstream position (cf. ref. 38), no such evidence could be obtained for the *FAS2* DRS (data not shown).

Our present results do not yet precisely define the regulatory mechanism responsible for *FAS* autoregulation. In an attempt to map a domain specifically required for its regulatory function and to separate catalytic and regulatory properties of Fas1, we constructed truncation variants of the *FAS1* reading frame. Using specific antibodies, Fas1 variants lacking 216 amino acids of the N-terminus or 336 amino acids of the C-terminus were clearly detectable in crude extracts prepared from the respective transformants. As expected, these truncated genes failed to complement a *fas1* null mutation due to the lack of acetyl transferase and malonyl transferase catalytic domains, respectively. However, both truncation variants lacking distinct functional domains were also unable to stimulate *FAS2–lacZ* expression in a *fas1* mutant background. Thus, these results do not yet allow us to map a regulatory domain within a defined region of Fas1.

Although Fas1 clearly influences the steady-state concentration of the *FAS2* transcript (Fig. 1), biosynthesis of the mRNA or its stability may be affected. However, preliminary data (based on inhibition of *de novo* transcription in the presence of 1,10-phenanthroline) argue against an increased rate of decay of *FAS2* mRNA in the absence of Fas1. Thus, we favor a mechanism based on DRS-affected biosynthesis of *FAS2* mRNA, possibly mediated by a factor interacting with the DRS. In contrast with downstream binding sites described so far, sequence comparisons gave no evidence for an interaction of pleiotropic factors such as Rap1 or Med8 with the *FAS2* DRS. Gel retardation assays with total protein extract from a *fas1* deletion strain and a *FAS1* multi-copy transformant did not reveal a different binding pattern to the *FAS2* DRS (1/150 fragment used as a probe; not shown). We also attempted to relieve DRS-dependent repression of an *FAS2–lacZ* reporter gene by an unproductive increase of DRS copy number (multiple copies of *FAS2* 1/150 fragment on an episomal plasmid). However, competition of binding for a presumed negative factor by an excess of individual DRS elements did not stimulate *FAS2–lacZ* expression (data not shown). Thus, it remains to be shown whether the *FAS2* DRS does in fact act at the DNA level. Alternatively, the DRS could function as a regulator of transcriptional elongation in the initiated *FAS2* mRNA, similar to what has been shown for the tat-responsive element TAR in the HIV mRNA (45). Nuclear run-on assays may provide evidence for such a mechanism. In contrast with TAR, which is a positive element, the *FAS2* DRS may reduce processivity of transcript elongation, possibly mediated by an unknown RNA-binding factor. An excess of Fas1 could increase processivity of elongation by deactivation of the repressor. Sequence modelling with nt 22–66 of the *FAS2* reading frame, which represents the core of the DRS, allowed us to propose a potential stem–loop structure comprising nt 24–60, containing 10 conventional AU and GC base pairs together with 2 GU base pairs and a loop of 8 nt (Fig. 5B). With data supporting regulation of transcriptional elongation, the significance of this secondary structure may be investigated by RNA/protein interaction assays as well as by the introduction of mutations into the DRS that specifically interfere with the stem–loop but maintain its coding potential.

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