Saccharomyces kluveri FAD3 encodes an \( \omega3 \) fatty acid desaturase

Takahiro Oura and Susumu Kajiwara

Fungi, like plants, are capable of producing the 18-carbon polyunsaturated fatty acids linoleic acid and \( \omega3 \)-linolenic acid. These fatty acids are synthesized by catalytic reactions of \( \Delta12 \) and \( \omega3 \) fatty acid desaturases. This paper describes the first cloning and functional characterization of a yeast \( \omega3 \) fatty acid desaturase gene. The deduced protein encoded by the Saccharomyces kluveri FAD3 gene (Sk-FAD3) consists of 419 amino acids, and shows 30–60% identity with \( \Delta12 \) fatty acid desaturases of several eukaryotic organisms and 29–31% identity with \( \omega3 \) fatty acid desaturases of animals and plants. During Sk-FAD3 expression in Saccharomyces cerevisiae, \( \omega3 \)-linolenic acid accumulated only when linoleic acid was added to the culture medium. The disruption of Sk-FAD3 led to the disappearance of \( \omega3 \)-linolenic acid in S. kluveri. These findings suggest that Sk-FAD3 is the only \( \omega3 \) fatty acid desaturase gene in this yeast. Furthermore, transcriptional expression of Sk-FAD3 appears to be regulated by low-temperature stress in a manner different from the other fatty acid desaturase genes in S. kluveri.

INTRODUCTION

Long-chain unsaturated fatty acids are essential components of cellular membranes and play important roles in maintaining membrane fluidity for many organisms. These fatty acids, particularly polyunsaturated fatty acids (PUFAs), are also precursors to signalling molecules in higher organisms (reviewed by Wallis et al., 2002). Many organisms are capable of producing long-chain PUFAs such as linoleic acid (C18:2) and \( \omega3 \)-linolenic acid (\( \omega3 \)-C18:3) as well as monounsaturated fatty acids, e.g. oleic acid (C18:1) and palmitoleic acid (C16:1). However, vertebrates are unable to produce C18:2 and \( \omega3 \)-C18:3, and must therefore obtain these fatty acids from other organisms. These animals synthesize PUFAs such as arachidonic acid and eicosapentaenoic acid using other PUFAs obtained from the diet. Thus, understanding the mechanism of PUFA biosynthesis in all biological systems is of interest.

A major long-chain PUFA contained in membrane lipids, C18:2, is synthesized from C18:1 by the reaction catalysed by \( \Delta12 \) fatty acid desaturase. Subsequently, an \( \omega3 \) fatty acid desaturase introduces the third double bond into the \( \Delta15 \) position of C18:2 to produce \( \omega3 \)-C18:3. In higher plants, these reactions occur in both the plastid and endoplasmic reticulum (ER) (Browse & Somerville, 1991) and the corresponding desaturase genes have been cloned from several plant species (reviewed by Tocher et al., 1998). The plastid \( \Delta12 \) fatty acid desaturase (FAD6) and \( \omega3 \) fatty acid desaturases (FAD7 and FAD8) utilize acyl groups bound to glycosylglycerolipids and phosphatidyglycerol as substrates for the reactions (Browse et al., 1986, 1989; McConn et al., 1994). Plastid-specific desaturase gene homologues (desA and desB) were also cloned from cyanobacteria (Wada et al., 1990; Sakamoto et al., 1994). On the other hand, the products of the ER-specific \( \Delta12 \) desaturase gene (FAD2) and \( \omega3 \) desaturase gene (FAD3) in plants desaturate acyl groups bound to phospholipids (mainly phosphatidylcholine and phosphatidylethanolamine) as substrates (Miquel & Browse, 1992; Browse et al., 1993). Recently, \( \Delta12 \) and \( \omega3 \) fatty acid desaturase genes (FAT-2 and FAT-1, respectively), whose products are probably localized in the ER, were cloned from the nematode Caenorhabditis elegans (Spychalla et al., 1997; Peyou-Ndi et al., 2000).

The cloning of a \( \Delta12 \) fatty acid desaturase gene has been reported for three filamentous fungi (Passorn et al., 1999; Sakuradani et al., 1999; Calvo et al., 2001), and we also isolated this gene from the yeast Saccharomyces kluveri (Watanabe et al., 2004). The Aspergillus nidulans odeA and S. kluveri Sk-FAD2 genes are induced by low-temperature stress and the former gene is also induced by the addition of oleic acid, although the latter did not respond to unsaturated fatty acids (Calvo et al., 2001; Watanabe et al., 2004). With regard to \( \omega3 \) fatty acid desaturase, more recently, a 20-carbon PUFA-specific \( \omega3 \) desaturase gene (SDD17), whose product converts arachidonic acid (C20:4) to eicosapentaenoic acid (C20:5), was cloned from the fungus Saprolegnia diclina (Pereira et al., 2004). However, no fungal
18-carbon PUFA-specific ω3 desaturase gene has been cloned, although many fungal species can produce ω-C18:3.

In the present study we describe the cloning of an 18-carbon PUFA-specific ω3 desaturase gene (Sk-FAD3) from an ω-C18:3-producing yeast, S. kluveri, and the expression of this gene in a PUFA-nonproducing yeast, Saccharomyces cerevisiae. A transformed S. cerevisiae strain growing in medium supplemented with C18:2 was capable of producing ω-C18:3. Moreover, an Sk-fad3 disruptant strain of S. kluveri was unable to produce ω-C18:3.

METHODS

Strains, plasmids and media. Escherichia coli strain DH5α and plasmid pBluescript II SK(+) (Stratagene) were used for the manipulation of plasmid DNA and construction of a DNA library for S. kluveri. Yeast strains used are listed in Table 1. Plasmid pJJ242 (Jones & Prakash, 1990), which includes the S. cerevisiae URA3 gene, was used for gene disruption in S. kluveri. The gene expression vector for S. cerevisiae, pYES2, was purchased from Invitrogen. Yeast cells were grown in YPD medium (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose) and complete minimal (CM) medium (Kajiwara et al., 1996) without uracil. For induction of gene expression in S. cerevisiae, yeast cells were cultivated in CM (raffinose) medium without uracil (Kajiwara et al., 1996) and then 1/100 vol. 20% galactose solution and C18:2 to a final concentration of 0.5 mM were added. CM medium supplemented with 1 mM C18:1, C18:2 or ω-C18:3 was used for the analysis of transcriptional expression of the Sk-FAD3 gene.

Construction of genomic DNA library of S. kluveri. DNA manipulation of E. coli and yeast followed standard procedures (Sambrook et al., 1989; Ausubel et al., 1992; Burke et al., 2000). Genomic DNA isolated from S. kluveri was digested by HindIII and then size-fractionated on a 1% agarose gel. The HindIII-digested DNA fragments from 5 kb to 6.5 kb were inserted into the HindIII site of pBluescript II SK(+) to construct the genomic DNA library of S. kluveri.

Construction of plasmid for gene expression. DNA fragments of the Sk-FAD3 ORF were amplified by PCR using the primers Sk-FAD3-1 (5’-CCAAGCTTATGCTATATTGAAACAGCTGGAATCTCGTC-3’) and Sk-FAD3-2 (5’-GGGATCTCAATTGACGGAACCATCCTCCGGCGTTG-3’). The PCR-amplified DNA fragment of the Sk-FAD3 ORF (1.3 kb) was digested by HindIII and BamHI and then ligated between the HindIII and BamHI sites on the yeast vector pYES2. The plasmid obtained was named pYSK-FAD3.

Gene disruption of S. kluveri. The strategy for constructing the fad3 mutant of S. kluveri was as follows. A 1.2 kb Xhol-EcoRI fragment was isolated from the 6.0 kb HindIII fragment containing the Sk-FAD3 gene and inserted between the Xhol and EcoRI sites on pBluescript II SK(+) (pJJ242). The plasmid obtained was digested by HindIII, and then both ends of the larger DNA fragment containing parts of the Sk-FAD3 DNA and pBluescript II SK(+) were ligated with HindIII linkers. This plasmid was named pUC-AFAD3. The 1.2 kb HindIII URA3 DNA fragment was isolated from plasmid pJJ242 (Johns & Prakash, 1990) and inserted into the HindIII site of pUC-AFAD3. The 1.9 kb DNA fragment in the plasmid obtained (named pafad3-URA3) was introduced into the S. kluveri ura3 mutant YKC165 (Watanabe et al., 2004). Disruption of the Sk-FAD3 gene was confirmed by Southern blot analysis using Sk-FAD3 as a probe. The fad3-disruptant obtained was named YKC167.

Hybridization and DNA sequencing. Colony hybridization was performed in a solution containing 5 × SSC/0.1% Denhardt’s solution/0.1 mg herring sperm DNA per ml at 58°C according to the methods of Sambrook et al. (1989). Southern blot analysis was performed at 65°C in the same solution as used for colony hybridization. Isolation of yeast RNA and Northern blot analysis were carried out using a method described previously (Kajiwara et al., 2000b). The intensity of the bands was measured with a bioimaging analyser (Fuji model BAS2000, Japan). For DNA sequencing, the DNA fragments containing the genomic DNA were cloned into pBluescript II SK(+) (pJJ242). Both strands were sequenced using a Thermo Sequenase cycle sequencing kit (Amersham Biosciences) on a LI-COR DNA sequencer (Aloka).

Fatty acid analysis. Total lipids were extracted from exponentially growing cells (OD600 1.0–1.5) with chloroform/methanol (1:2, v/v) and were then methylated using 1% sulfuric acid in methanol at 90°C for 2 h as described previously (Kajiwara et al., 2000a). The resulting fatty acid methyl esters (FAMEs) were extracted in hexane. FAMEs were analysed by GC (GC-18A, Shimadzu) with a 0.25 mm × 25 m HR-SS-10 capillary column (Shinwa Chemical Industries). The initial column temperature of 150°C was held for 2 min, then raised to 2 °C min⁻¹ to 180°C and finally held for 2 min. A FAME mixture used as a lipid standard was purchased from Sigma. Fatty acid 4,4-dimethylxazoline (DMOX) derivatives were prepared from the FAMEs by evaporating the hexane phase, resuspending the residue in 0.5 ml 2-aminomethyl-2-methylpropanol, and heating overnight at 180°C (Fay & Richl, 1991). After cooling, the DMOX derivatives were dissolved in 4 ml dichloromethane and washed twice with 1.5 ml distilled water. The dichloromethane solution was evaporated under a stream of nitrogen and the residue was dissolved in hexane for injection. Analysis of DMOX derivatives was conducted using the same equipment as for GC analysis and GC-MS (QP5000, Shimadzu) operating at an ionization voltage of 70 eV, with a scan range of 40–400 kDa. The initial column temperature of 150°C was held for 2 min, then raised at 10°C min⁻¹ to 190°C and at 2 °C min⁻¹ to 210°C, and finally held for 2 min. The mass spectrum of any new peak obtained was interpreted by comparison to that of the DMOX derivative of ω-C18:3 prepared from ω-C18:3 methyl ester (Sigma) and the published value (reviewed by Spitzer, 1997).

RESULTS

Cloning and sequencing of the Sk-FAD3 gene

The amino acid sequences of ω3 fatty acid desaturases show high similarities to those of Δ12 fatty acid desaturases.

Table 1. Yeast strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<tbody>
<tr>
<td>S. cerevisae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFO10150</td>
<td>MATa ste-VC9 ura3-52 trpl-289 his3Δ1 leu2-3,112</td>
<td>IFO*</td>
</tr>
<tr>
<td>S. kluveri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFO1893</td>
<td>Haploid wild-type ura3 derivative of IFO1893</td>
<td>IFO*</td>
</tr>
<tr>
<td>YKC165</td>
<td>ura3 derivative of IFO1893 Watanabe et al. (2004)</td>
<td>This study</td>
</tr>
<tr>
<td>YKC167</td>
<td>ura3 fad3:: URA3 disruption transformant of YKC165</td>
<td></td>
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*IFO, Institute for Fermentation Osaka, Japan.
Therefore, we attempted to clone the ω3 fatty acid desaturase gene of S. kluyveri using the Δ12 fatty acid desaturase gene (Sk-FAD2) of this yeast as a probe. The genomic DNA isolated from S. kluyveri IFO1893 was digested by HindIII and then Southern hybridization was performed using the 32P-labelled 0·8 kb EcoRI–SphI fragment of the Sk-FAD2 gene as a probe. As a result, in addition to a strong positive signal derived from the Sk-FAD2 gene, a weak signal with a size estimated at 6·0 kb was detected (data not shown). Then a 5·0–6·5 kb HindIII-digested genomic DNA library of S. kluyveri was constructed and approximately 6000 DNA clones were screened using the 0·8 kb EcoRI–SphI Sk-FAD2 fragment. Three positive clones containing an identical 6·0 kb HindIII DNA fragment were identified (data not shown). A physical map and Southern blot analysis showed that they possessed a region of extensive homology to the 0·8 kb Sk-FAD2 EcoRI–SphI fragment. This 2·3 kb region, consisting of two Clal DNA fragments (1·9 kb and 0·4 kb) in the 6·0 kb HindIII fragment, was sequenced (GenBank/DDBJ accession number AB118663).

This result revealed that the 2·3 kb region included 1260 bp of a complete ORF encoding a putative protein of 419 amino acids.

Comparisons of amino acid sequences using the FASTA algorithm showed that the 419 amino acid sequence has highest identity with the amino acid sequence of Sk-FAD2 protein (60 %) (Watanabe et al., 2004; Fig. 1); it has 52·5 %, 38·6 %, 38·1 % and 29·7 % identities with the sequences of the Δ12 fatty acid desaturases from Aspergillus nidulans (Calvo et al., 2001), Mortierella alpina (Sakuradani et al., 1999), Arabidopsis thaliana (Okuley et al., 1994) and C. elegans (Peyou-Ndi et al., 2000), respectively. On the other hand, the comparison with ω3 fatty acid desaturases from Arabidopsis thaliana (Yadav et al., 1993) and C. elegans (Spychalla et al., 1997) revealed identities of 30·5 % and 28·8 %, respectively (Fig. 1). These results suggested that the gene obtained might encode another Δ12 fatty acid desaturase, rather than an ω3 fatty acid desaturase, in S. kluyveri. The gene was named Sk-FAD3.

**Fig. 1.** Comparison of the deduced amino acid sequence of Sk-FAD3 protein and closely related fatty acid desaturases. Sk-FAD2, S. kluyveri Δ12 fatty acid desaturase (Watanabe et al., 2004); An-ODEA, Aspergillus nidulans Δ12 fatty acid desaturase (Calvo et al., 2001); At-FAD3, Arabidopsis thaliana ω3 fatty acid desaturase (Yadav et al., 1993). Sequences were aligned using the FASTA algorithm. Amino acid identities are shaded black. The positions of amino acid residues relative to the start codon are given on the right-hand side. Underlines indicate the histidine cluster motifs.
Fig. 2. GLC analysis of fatty acid patterns in total lipids from S. cerevisiae transformants. The IFO10150 strain harbouring pYSk-FAD3 (A) or pYES2 vector (B) was cultured in CM (raffinose) medium supplemented with 0.2% galactose and 0.5 mM C18:2. These cells were grown at 30°C for four generations, and then total lipids were extracted and subjected to GLC analysis (Fig. 2, Table 2). Total lipids from IFO10150 harbouring pYES2 were also analysed as a control. One unique major peak was observed in the transformant carrying pYSk-FAD3 (Fig. 2A) and the retention time of this peak was the same as that of α-C18:3 desaturated at the Δ15 position, not γ-linolenic acid (γ-C18:3) desaturated at the Δ6 position (Fig. 2C). This new desaturation product was confirmed by GC-MS analysis of DMOX derivatives. The mass spectrum of the fatty acid DMOX derivative exhibited a peak at m/z = 331 expected for the molecular ion of the DMOX derivative and peaks at m/z = 196, 208, 236, 248, 276 and 288 consistent with double bonds at the Δ9, Δ12 and Δ15 positions of the fatty acid (Fig. 3). From these analyses, the new peak observed in the S. cerevisiae strain carrying pYSk-FAD3 was identified as α-C18:3. This transformant accumulated α-C18:3 to 7.8 mol% of total yeast fatty acids, whereas when it was cultured in the medium without C18:2, no production of PUFAs such as Δ9,Δ15-octadecadienoic acid was observed (data not shown). These results demonstrate that the Sk-FAD3 gene does not encode another Δ12 fatty acid desaturase, but rather an α3 fatty acid desaturase, in S. kluyveri. They also show that the Sk-FAD3 protein is able to function in S. cerevisiae to synthesize α-C18:3.

Disruption of the Sk-FAD3 gene in S. kluyveri

To clarify whether the Sk-FAD3 protein is the only α3 fatty acid desaturase in S. kluyveri, we constructed a fad3 mutant of this yeast, as described in Methods. S. kluyveri strain YKC165 (Watanabe et al., 2004), a ura3 mutant, was transformed with the 1.9 kb DNA fragment isolated from the plasmid pfad3-URA3 and spread on CM medium without uracil. The transformant obtained, YKC167, was disrupted at the FAD3 locus and its growth rate in YPD medium was identical to that of the parental strain YKC165 (data not shown). Fatty acid analyses of YKC167 and YKC165 showed that YKC167 is not capable of producing

<table>
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<th>Transforming plasmid</th>
<th>Fatty acid composition (mol%)*</th>
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<tr>
<td></td>
<td>C16:0</td>
</tr>
<tr>
<td>pYSk-FAD3</td>
<td>23.9 ± 1.4</td>
</tr>
<tr>
<td>pYES2</td>
<td>24.4 ± 0.3</td>
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*Results were determined from the peak areas of methyl esters. Values are means of four replicate analyses ± SD.

Functional expression of the Sk-FAD3 gene in S. cerevisiae

To investigate whether the Sk-FAD3 gene encodes a Δ12 or an α3 fatty acid desaturase, it was expressed in S. cerevisiae. Plasmid pYSk-FAD3, carrying the DNA fragment of Sk-FAD3 ORF, the S. cerevisiae GAL1 promoter and the S. cerevisiae CYC terminator, was constructed as described in Methods. Because this gene was inserted downstream of the GAL1 promoter, gene expression could be induced by addition of galactose. pYSk-FAD3 was introduced into the S. cerevisiae strain IFO10150. The IFO10150 transformant obtained was cultured in CM (raffinose) medium supplemented with 0.2% galactose and 0.5 mM C18:2. These cells were grown at 30°C for four generations, and then total lipids were extracted and subjected to GLC analysis (Fig. 2, Table 2). Total lipids from IFO10150 harbouring pYES2 were also analysed as a control. One unique major peak was observed in the transformant carrying pYSk-FAD3 (Fig. 2A) and the retention time of this peak was the same as that of α-C18:3 desaturated at the Δ15 position, not γ-linolenic acid (γ-C18:3) desaturated at the Δ6 position (Fig. 2C). This new desaturation product was confirmed by GC-MS analysis of DMOX derivatives. The mass spectrum of the fatty acid DMOX derivative exhibited a peak at m/z = 331 expected for the molecular ion of the DMOX derivative and peaks at m/z = 196, 208, 236, 248, 276 and 288 consistent with double bonds at the Δ9, Δ12 and Δ15 positions of the fatty acid (Fig. 3). From these analyses, the new peak observed in the S. cerevisiae strain carrying pYSk-FAD3 was identified as α-C18:3. This transformant accumulated α-C18:3 to 7.8 mol% of total yeast fatty acids, whereas when it was cultured in the medium without C18:2, no production of PUFAs such as Δ9,Δ15-octadecadienoic acid was observed (data not shown). These results demonstrate that the Sk-FAD3 gene does not encode another Δ12 fatty acid desaturase, but rather an α3 fatty acid desaturase, in S. kluyveri. They also show that the Sk-FAD3 protein is able to function in S. cerevisiae to synthesize α-C18:3.
α-C18:3 (Fig. 4). This result suggests that Sk-FAD3 is the only α3 fatty acid desaturase gene in *S. kluyveri*.

**Transcription of the Sk-FAD3 gene in *S. kluyveri***

Previously, we observed that the transcriptional expression of Sk-FAD2 was induced by low-temperature stress and this transcript reached the highest level 2 h after a temperature downshift (Watanabe *et al.*, 2004). The influence of low-temperature stress on Sk-FAD3 mRNA was analysed (Fig. 5A). After culturing at 30 °C, *S. kluyveri*IFO1893 was transferred to 10 °C for 0, 1, 2, 4, 6, 8, 12 or 24 h. Northern blot analysis was performed using the Sk-FAD3 gene as a probe. After a temperature downshift to 10 °C, the level of Sk-FAD3 mRNA increased, and reached a maximum (2-7-fold increase) at 4–6 h. Thereafter, the levels gradually decreased. At 24 h after the temperature downshift, the mRNA level was 1-5-fold higher than that at 0 h. This response was different from that of the Sk-FAD2 gene in *S. kluyveri*, suggesting that the Sk-FAD3 gene is a low-temperature-inducible gene but that the regulation of its expression is different from that of Sk-FAD2 gene in *S. kluyveri*.

In several yeasts, the addition of unsaturated fatty acid causes a reduction in mRNA levels of the Δ9 fatty acid desaturase gene (McDonough *et al.*, 1992; Anamnart *et al.*, 1997). However, in *S. kluyveri*, levels of Sk-OLE1 and Sk-FAD2 transcripts were unaffected by unsaturated fatty acids (Kajiwara, 2002; Watanabe *et al.*, 2004). The effects of unsaturated fatty acids on the levels of Sk-FAD3 mRNA in *S. kluyveri* were thus investigated (Fig. 5B). Total RNA was isolated from *S. kluyveri*IFO1893 cultured in medium supplemented with C18:1, C18:2 or α-C18:3. Like the Sk-OLE1 and Sk-FAD2 genes, expression of Sk-FAD3 was not influenced by the presence of unsaturated fatty acids in the growth medium.

**DISCUSSION**

α-Linolenic acid (α-C18:3) plays important roles not only as a structural component of membrane lipids but also as a precursor to signalling molecules in organisms (for reviews see Kinsella *et al.*, 1990; Wallis & Browse, 2002). Many yeast species are known to be able to produce α-C18:3 (Ratledge
suggest that the sequence homologies between
then cultured at 10°C.

Total cellular RNA isolated from the cells at 0, 1, 2, 4, 6, 8, 12 and 24 h after the temperature shift and was probed using a 32P-labelled Sk-FAD3 probe. The 26S and 18S rRNAs were used as internal controls for S. kluveyri in both (A) and (B).

Fig. 5. Northern blot analysis of total RNA from S. kluveyri strain IFO1893. (A) The IFO1893 strain was cultured at 30°C in YPD medium to mid-exponential phase (OD600 1-0) and was then cultured at 10°C. Total RNA was isolated from the cells at 0, 1, 2, 4, 6, 8, 12 and 24 h after the temperature shift and was probed using a 32P-labelled Sk-FAD3 probe. (B) An overnight culture of the IFO1893 strain was inoculated (10% by volume) into CM medium and shaken at 30°C for 6 h. A 1 mM aliquot of each unsaturated fatty acid (C18 : 1, C18 : 2 or C18 : 3) was added to the medium. Total cellular RNA isolated from these cultures was probed using a 32P-labelled Sk-FAD3 probe.

We succeeded in expression of the Sk-FAD3 gene and production of α-C18 : 3 in S. cerevisiae in the presence of C18 : 2. Recently, an α3 fatty acid desaturase gene (sdd17) whose product converts C20 : 4 to C20 : 5 was cloned from the filamentous fungus Saprolagena diclina (Pereira et al., 2004). However, this fungal desaturase was not capable of converting C18 : 2 to α-C18 : 3. Thus, Sk-FAD3 appears to be the first cloned 18-carbon fatty acid-specific α3 desaturase gene in the fungal kingdom. Furthermore, the α-C18 : 3 in the S. cerevisiae transformant expressing the Sk-FAD3 gene accounted for 7-8 mol% of total fatty acids, a level higher than those in S. cerevisiae expressing Brassica napus FAD3 or C. elegans FAT-1 (Meesapyodsuk et al., 2000).

An Sk-fad3 disruptant of S. kluveyri was not capable of producing α-C18 : 3, suggesting that Sk-FAD3 is the only α3 fatty acid desaturase gene in S. kluveyri. The vegetative growth rate of the Sk-fad3 disruptant was identical to that of the control strain YKC165. This result suggests that S. kluveyri does not need α-C18 : 3 for vegetative growth; this is in agreement with the observation concerning the growth of the Sk-FAD2 disruptant, which does not produce C16 : 2 and C18 : 2 or α-C18 : 3 (Watanabe et al., 2004). However, in higher organisms, derivatives of α-C18 : 3 have been reported to function as inter- and intracellular signals for cell differentiation and physiological phenomena (for reviews see Kinsella et al., 1990; Wallis & Browse, 2002). Thus, further study will be needed to elucidate the relationship between the synthesis of α-C18 : 3 and the morphology of S. kluveyri.

Transcriptional expression of fatty acid desaturase genes of several fungi is induced by low-temperature stress (Laoteng et al., 1999; Calvo et al., 2001; Nakagawa et al., 2002; Sakai & Kajiwara, 2003). The expression of the Sk-FAD3 gene was also induced by low temperature. However, the pattern of transcriptional expression of the Sk-FAD3 gene after a temperature downshift to 10°C was different from that of other desaturase genes in S. kluveyri (SK-OLE1 and Sk-FAD2). The transcripts of Sk-FAD3 reached the highest level 4-6 h after temperature downshift, whereas the transcripts of both Sk-OLE1 and Sk-FAD2 reached the highest levels after only 2 h. Moreover, unlike the Sk-OLE1 and Sk-FAD2 genes, the levels of Sk-FAD3 mRNA at and after 8 h were maintained at higher level than that before the temperature downshift. Therefore, the regulatory mechanism for the Sk-FAD3 gene

protein as well as C. elegans FAT-1 protein were observed to be somewhat different from the conserved sequences for histidine clusters in higher-plant α3 desaturases (HxCGH, HxxxxxHRTHH and HxHxxxHVIIH), which are thought to be the residues that coordinate the diiron-oxo structure at the active site of these enzymes (Los & Murata, 1998). These data suggest that the conserved histidine clusters in α3 fatty acid desaturases are more ambiguous than indicated previously and are more similar to those of ΑΔ2 desaturases (HxCGH, ExxxxxHxxHH and HxxHH) (Los & Murata, 1998). Therefore, the functional differences between α3 and ΑΔ2 fatty acid desaturases are not predictable from the primary structures of the active site in these enzymes.

& Evans, 1991); in this study we investigated a yeast α3 fatty acid desaturase gene.

From the comparison of the amino acid sequence of α3 fatty acid desaturases with those of ΑΔ2 fatty acid desaturases in the same organism, the C. elegans α3 fatty acid desaturase, FAT-1 protein, showed 54·9% identity with the ΑΔ2 fatty acid desaturase (FAT-2) from this animal; in the cress Arabidopsis thaliana, the microsomal α3 fatty acid desaturase, FAD3 protein, showed 41% identity with the microsomal ΑΔ2 fatty acid desaturase, FAD2 protein. These results suggest that the sequence homologies between α3 and ΑΔ2 fatty acid desaturases possessed by an organism are high and thus the α3 fatty acid desaturase gene of S. kluveyri was cloned by a heterologous hybridization approach using the ΑΔ2 fatty acid desaturase gene, Sk-FAD2, as a probe. The amino acid sequence of Sk-FAD3 protein has the highest identity with that of Sk-FAD2 protein (60%). The Sk-FAD3 protein exhibited higher sequence similarities to other ΑΔ2 fatty acid desaturases than to other α3 fatty acid desaturases. Moreover, the three histidine-rich sequences of Sk-FAD3
may be different from those of other fatty acid desaturase genes in S. kluuyeri. The analysis of promoters of these desaturase genes will clarify the overall regulatory mechanism of fatty acid desaturation in S. kluuyeri.

ACKNOWLEDGEMENTS

This work was financed by Grants for Young Scientists (nos 10760045 and 13760061) from the Japan Society for the Promotion of Science.

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