

## Chilling-Sensitive, Post-Transcriptional Regulation of a Plant Fatty Acid Desaturase Expressed in Yeast

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Plants respond to chilling exposure by increasing the relative proportion of polyunsaturated fatty acids in their lipids. However, unlike the response in many other organisms, plant fatty acid desaturase genes are typically not upregulated during this process. We expressed the *Brassica napus* *FAD3* gene, which encodes an enzyme for synthesis of linolenic acid, in *Saccharomyces cerevisiae* and observed a temperature-dependent increase in linolenic acid production at cooler growth temperatures. Untransformed yeast cells, however, responded to cooler temperatures primarily by shortening fatty acid chains, even when polyunsaturated fatty acids were supplied in the growth media. Measurement of the steady-state levels of *Fad3* protein in transformed yeast revealed an 8.5-fold increase in steady-state amount of desaturase enzyme when cells were cultivated at cooler temperatures. The increase was not due to changes in transcriptional activity, since Northern hybridization revealed no appreciable changes in abundance of *FAD3* transcripts at cooler temperatures. Taken together, the results suggest that the increase in linolenic acid content in cells containing *Fad3* was not due to enhanced physiological demand for polyunsaturated fatty acids by yeast, but rather a cold-inducible, post-transcriptional increase in steady-state amount of plant desaturase enzyme. Implications for plant adaptation to chilling are discussed. © 2001 Academic Press

**Key Words:** fatty acid desaturase; chilling; yeast; lipid; epitope tagging.

Poikilothermic organisms such as yeast, bacteria, plants, and fish, must adapt to changes in environmental temperature in order to survive. One of the common

Abbreviations used: ER, endoplasmic reticulum; FAD, fatty acid desaturase; FAME, fatty acid methyl esters; GC, gas chromatography; HA, hemagglutinin; OD, optical density; ORF, open reading frame; PUFA, polyunsaturated fatty acids; SCD, stearoyl-CoA desaturase; TCA, trichloroacetic acid; WT, wild-type.

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responses to chilling temperatures is an alteration in fatty acid composition of biological membranes (1). Fatty acids exhibit a wide degree of structural variation that in turn imparts different fluidic characteristics to membranes. Maintenance of appropriate membrane fluidity, also referred to as homeoviscous adaptation, is thought to be crucial for proper membrane function, integrity, and recovery from low temperature exposure (2, 3). The most common changes in fatty acid composition at low temperatures are increased amounts of unsaturated fatty acids, increased ratio of short chain to long chain fatty acids, and/or alterations in branched chain fatty acid content (4).

Molecular aspects of environmental temperature sensing are beginning to emerge. Information about temperature is likely perceived at the level of the biological membrane itself (5, 6), with changes in the physical status of the membrane activating a signal transduction pathway leading to increased transcription of fatty acid modifying genes. These components are most clearly defined for the cyanobacterium *Synechocystis* and include a putative membrane bound histidine kinase sensor, soluble kinase, and transcription factor (7). Although plants exhibit a significant increase in degree of unsaturation at lower temperature, there is no apparent increase in rate of transcription or stability of fatty acid desaturase mRNA at lower temperature (8–10), with exception of the *Arabidopsis* *FAD8* gene (11). These results suggest that plant fatty acid desaturases are regulated at the post-transcriptional level.

In recent years, many of the genes involved in synthesis of polyunsaturated fatty acids (PUFA) in plants have been identified and sequenced. The fatty acid desaturase (*FAD*) genes are well conserved and encode membrane-bound enzymes localized in either the plastid or endoplasmic reticulum (ER) (12). Expression of plant desaturases in the yeast *Saccharomyces cerevisiae* has offered a rapid method to verify enzymatic activity of the desaturases as well as characterize their substrate/product relationships (13, 14). *S. cerevisiae* has a very simple fatty acid composition dominated by palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0),

and oleic (C18:1) acids, which allows detection of minute amounts of unusual fatty acids in yeast lipids. This is a crucial aspect of the heterologous expression system, since the products of the plant desaturases usually account for a very small percentage of total yeast lipids. In several cases, growth of yeast at cooler temperatures resulted in accumulation of higher amounts of PUFA synthesized by transgenically expressed plant desaturases (15, 16). However, it was unknown whether the increase in PUFA was a result of increased physiological demand for unsaturated lipids at lower temperatures, or whether lower temperatures had some other effect on the plant enzyme.

In the current study, we expressed the *Brassica napus* *FAD3* gene, which encodes an enzyme for synthesis of linolenic acid (C18:3) (17) in *S. cerevisiae* and observed a temperature dependent increase in amount of linolenic acid at cooler growth temperatures. Investigation of steady-state levels of *FAD3* mRNA and protein by Northern and Western blotting, respectively, revealed cold-inducible, post-transcriptional changes in amount of plant desaturase enzyme that correlated with amount of linolenic acid synthesis. Implications for plant adaptation to chilling are discussed.

## MATERIALS AND METHODS

**Materials.** DIFCO brand yeast media were purchased from Fisher Scientific (Pittsburgh, PA) except for synthetic complete amino acid supplements, which were from Bufferad (Lake Bluff, IL). Fatty acids were obtained from Aldrich (Milwaukee, WI). Zymolyase-20T was purchased from ICN Biomedicals (Costa Mesa, CA). Methanolic-HCl kits were purchased from Alltech Associates, Inc. (Deerfield, IL). Restriction enzymes and DNA modifying enzymes were purchased from New England Biolabs (Beverly, MA) unless indicated otherwise. All other general chemicals were purchased from Sigma (St. Louis, MO).

**Vector construction.** The *Brassica napus* *FAD3* gene was obtained from the Arabidopsis Biological Resource Center (plasmid pBNDES3). The open reading frame (ORF) was amplified using PCR with primers BF3top (5'-GTG AAT TCG GAT CCG CTA GCA TGG TTG TTG CTA TGG ACC A-3') and BF3bottom (5'-GCC TGC AGG GAT CCT TAA TTG ATT TTA GAT TTG TCA GAA G-3'). These primers introduced *EcoRI*, *BamHI*, and *NheI* sites just before the ATG start codon and *BamHI* and *PstI* sites just after the stop codon. These sites were created for subsequent subcloning into a variety of expression vectors. PCR was conducted using *Pfu* polymerase (Stratagene, La Jolla, CA) and the product was subcloned into pCRScript-CAM (Stratagene) to generate vector pCRsc-B1. The *FAD3* ORF was transferred from this vector to yeast expression vector pYES2 (Invitrogen, Carlsbad, CA) as a *BamHI* fragment to give pYES2-B1, and orientation was mapped by digestion with *NheI*.

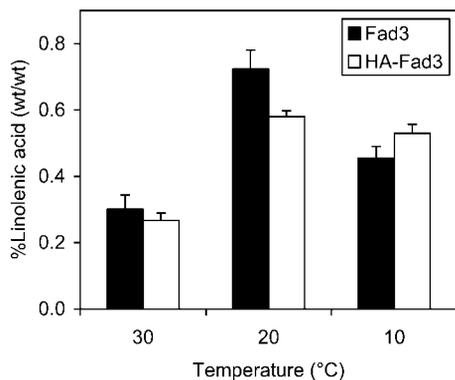
A plasmid was constructed for fusing a single copy of the hemagglutinin (HA) epitope tag to the N-terminus of passenger proteins. Synthetic oligos HAtop (5'-AGC TTG AAT TCC CAT GGC TTA CCC ATA CGA TGT TCC AGA TTA CGC TAG CGG TCT GCA-3') and HABottom (5'-GAC CGC TAG CGT AAT CTG GAA CAT CGT ATG GGT AAG CCA TGG GAA TT CA-3') were annealed and ligated into the *HindIII/PstI* sites of pBluescript KS- to generate vector pKS-HAnt. These oligos, from 5' to 3', encoded *HindIII*, *EcoRI*, and *NcoI* sites, an initiator methionine codon, an alanine spacer codon, the HA peptide epitope sequence (YPYDVPDYA), and *NheI* site for fusion to the 5' end of the passenger protein. The *FAD3* ORF was fused

inframe to the HA epitope tag sequence by transferring a *NheI/PstI* fragment from pCRsc-B1 to the *NheI/PstI* sites of pKS-HAnt. The resulting plasmid was called pKS-HAB1. The HA-*FAD3* fusion sequence was transferred to yeast expression vector pYES2 as an *EcoRI/XbaI* fragment to generate plasmid pYES2-HAB1. All clones involving PCR or synthetic oligo modifications were sequenced to verify integrity. Plasmids pYES2, pYES2-B1, and pYES2-HAB1 were transfected into yeast using the lithium acetate method of Gietz (18).

**Yeast strains, culturing conditions, and preparation of spheroplasts.** *S. cerevisiae* strain MMYO11 (MAT $\alpha$  ade 2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 *Ole*<sup>+</sup>), derived from parent strain W303 (19), was used throughout these studies. Wild-type (WT) yeast cells were maintained on YPD media (1% w/v yeast extract, 2% w/v peptone, 2% w/v dextrose) solidified with 2% w/v agar. Yeast containing expression plasmids were maintained on SD (2% w/v dextrose, 0.67% w/v yeast nitrogen base without amino acids, 2% w/v agar) plates with appropriate amino acid supplements. Single colonies were inoculated into SD-Ura starter cultures and grown overnight at 30°C, 300 rpm. Cell growth was determined by measuring optical density (OD) at 600 nm using a spectrophotometer. In experiments to determine effects of polyunsaturated fatty acids on response of yeast to cooler temperatures, yeast cells containing the empty expression vector pYES2 were backdiluted to 0.25 OD/ml in 50 ml of SGal-Ura media (2% galactose instead of dextrose) supplemented with 0.5% (v/v) Tween 40, 0.05% (v/v) linoleic, and 0.05% (v/v) linolenic acids. For all other experiments addressing *Fad3* enzyme activity, steady-state levels of *Fad3* protein and subcellular localization, and steady-state levels of *FAD3* mRNA, cells expressing the *FAD3* gene from the pYES2 expression plasmid were backdiluted to 0.25 OD/ml in 50 ml of SGal-Ura media containing 0.5% (v/v) Tween 40 and 0.1% (v/v) linoleic acid. Cells were harvested at late log-stationary phase and converted to spheroplasts by enzymatic digestion of cell walls using Zymolyase-20T, as described (20). Spheroplasts were harvested by centrifugation then subjected to either immediate lipid extraction or subcellular fractionation.

**Extraction and analysis of lipids.** Spheroplasts were resuspended in 300  $\mu$ l of 1 M sorbitol then osmotically lysed by addition of 400  $\mu$ l 0.15 M acetic acid. Lipids were extracted using the chloroform/methanol procedure of Bligh and Dyer (21). An aliquot of the lipid extract was removed for gravimetric determination of lipid concentration. Fatty acid methyl esters (FAME) were prepared using 1 M methanolic-HCl as described (22). FAME were analyzed on a Hewlett Packard 5890 Series II Gas Chromatography (GC) instrument equipped with an autoinjector and splitless injection, flame ionization detector, and a 30 M 0.53 mm Supelco SP-2380 column, using helium gas as a carrier. FAME were identified by comparison of retention time to standards and % FAME was calculated based on peak area counts. All lipid analysis experiments were performed independently at least three times. Values are reported as average and standard deviation. Statistical significance was determined using ANOVA.

**Subcellular fractionation and preparation of total protein extracts.** Spheroplasts derived from 250 ml of culture were collected by centrifugation and resuspended in 2 ml of 1 M SMIC buffer (1 M sorbitol, 5 mM 2-[N-morpholino]ethanesulfonate] pH 5.5, and protease inhibitor cocktail including 2 mM [4-(2-aminoethyl) benzenesulfonyl]fluoride HCl], 1 mM EDTA, 130  $\mu$ M bestatin, 1.4  $\mu$ M E64, 1.0  $\mu$ M leupeptin, and 0.3  $\mu$ M aprotinin). Spheroplasts were osmotically lysed by adding 6 ml of 0.25 M SMIC buffer (equivalent to 1 M SMIC except the concentration of sorbitol is 0.25 M) and then reequilibrated with 6 ml of 1.75 M SMIC. The lysate was subjected to differential centrifugation as described (23). Total protein extracts were obtained from yeast using the trichloroacetic acid (TCA)-based method of Veenhuis and Goodman (24). Briefly, cells were incubated in 5% TCA for 1 h at 0°C prior to disruption using glass beads. Protein concentrations of whole cell lysates and subcellular fractions



**FIG. 1.** Temperature-dependent synthesis of linolenic acid in yeast cells expressing *FAD3*. Yeast cells expressing the *Brassica napus FAD3* gene or an HA epitope-tagged version of *FAD3* were cultivated at the indicated temperature and in the presence of the substrate linoleic acid. Total lipid extracts were prepared from spheroplasts and fatty acid composition determined using GC. The amount of linolenic acid detected is shown. Linolenic acid was not detected in WT cells lacking the plant enzyme (not shown).

were determined using the Bio-Rad protein assay (Hercules, CA) using BSA as a standard.

**SDS-PAGE and Western blotting.** Twenty five  $\mu\text{g}$  of protein, from either whole cell lysates or subcellular fractions, were separated on 10% SDS-polyacrylamide gels (stacking gel pH 6.8, resolving gel pH 9.2) and either stained with Coomassie R-250 or transferred to nitrocellulose for Western blotting. Antibodies used in these studies included the anti-HA high affinity rat mAb 3F10 (0.1  $\mu\text{g}/\text{ml}$ ) and goat-anti-rat IgG (H + L) horseradish peroxidase conjugate (1:3,000 dilution) from Boehringer Mannheim (Indianapolis, IN), anti-dolichol phosphate mannosyl synthase mouse mAb 5C5-A7 (4  $\mu\text{g}/\text{ml}$ ) (Molecular Probes, Eugene, OR), and goat-anti-mouse Ig's horseradish peroxidase conjugate (1:10,000 dilution) (Biosource International, Camarillo, CA). Enhanced chemiluminescence detection was performed using substrate and Hyperfilm from Amersham Pharmacia (Piscataway, NJ). Gels and films were scanned and analyzed using a Bio Rad Model GS-700 Imaging Densitometer (Hercules, CA).

**Northern hybridization.** Total RNA was isolated as described (25) from yeast cells grown to mid-logarithmic phase. Ten  $\mu\text{g}$  RNA were separated on 1.2% agarose gels containing 6.7% formaldehyde, transferred to nylon membrane, then probed with a  $^{32}\text{P}$ -labeled 960 bp *Bgl*III fragment inclusive of the majority of the *FAD3* open reading frame.

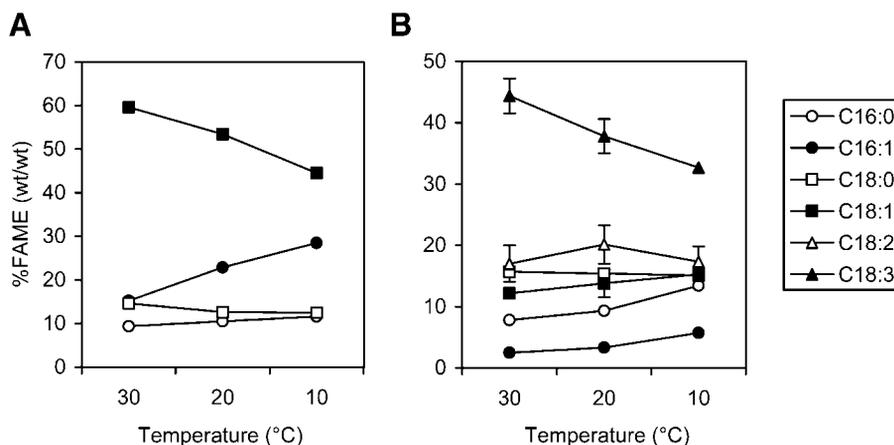
## RESULTS

**Temperature-dependent synthesis of linolenic acid in yeast cells expressing *FAD3* or epitope-tagged *FAD3*.** Expression of the plant *Fad3* enzyme in yeast cells, cultured in the presence of the substrate linoleic acid, resulted in appearance of small amounts of linolenic acid in yeast lipids (Fig. 1). Linolenic acid was not detected in negative control cells lacking the plant enzyme (not shown). Growth of yeast at cooler temperatures led to a significant increase in amount of linolenic acid produced, (30 to 20°C,  $P < 0.0005$ ,  $n = 3$ ; 20 to 10°C,  $P < 0.0005$ ,  $n = 3$ ; 30 to 10°C,  $P < 0.003$ ,  $n = 3$ ), with the highest amount detectable at 20°C.

Yeast cells expressing a hemagglutinin (HA) epitope-tagged version of *Fad3* exhibited a similar pattern of linolenic acid accumulation (Fig. 1), demonstrating that the enzyme retains function despite the presence of the epitope tag. The epitope tag was fused to the N-terminus of *Fad3* rather than C-terminus since the N-terminus does not encode a recognizable ER signal sequence and the C-terminus contains a potential ER retention sequence (17).

**Influence of polyunsaturated fatty acids on the adaptive response of yeast to chilling temperature.** To determine whether the increase in linolenic acid at cooler growth temperatures was due to increased physiological demand for PUFA by yeast, yeast cells lacking the plant *FAD3* gene were cultured in the presence or absence of linoleic and linolenic acids then subjected to the same cooling regime shown in Fig. 1. It was previously shown that the typical response of *S. cerevisiae* to cooler growth temperatures was a shortening of fatty acid chain lengths, rather than increase in degree of unsaturation (26). A similar response is presented in Fig. 2A, in which yeast cells grown in the absence of PUFA exhibited a decrease in C18 fatty acids and an increase in C16 fatty acids. Inclusion of linoleic and linolenic acids in the growth media led to substantial incorporation of these fatty acids into yeast lipids, accounting for 15 and 45% of total yeast fatty acids, respectively, when cells were cultured at 30°C (Fig. 2B). Growth of cells at cooler temperatures led to a significant reduction in linolenic acid content and increase in C16 fatty acid content (Fig. 2B), resulting in a net increase in the ratio of C16/C18 fatty acids, similar to WT. Although the linoleic and linolenic acids were supplied in equal amounts in the culture media in these experiments, linolenic acid accumulated to a greater extent than linoleic acid. A similar result was previously observed by Cahoon *et al.* (13), who cultivated yeast cells in the presence of both fatty acids to characterize substrate/product relationships of a delta-12 oleate desaturase related enzyme expressed in yeast.

To ensure that linoleic and linolenic acid measured in the total lipid extract was not contaminated by free fatty acids adhering to the outside of yeast cells, total lipid extracts obtained from cells grown at each temperature were fractionated into neutral lipid, phospholipid, and free fatty acid fractions. Free fatty acids accounted for less than 5% (wt/wt) of total yeast lipids when cells were grown in the presence or absence of the exogenous fatty acids. Analysis of fatty acid composition of phospholipids and neutral lipids by GC demonstrated that the percentage of linoleic and linolenic acids in each lipid fraction was comparable to percentages reported for the total lipid fraction (not shown), indicating that the majority of linoleic and linolenic acids were esterified into yeast glycerolipids.



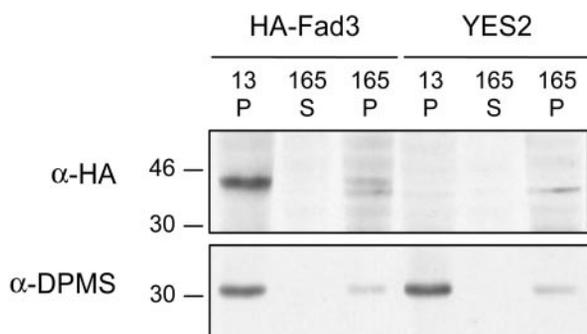
**FIG. 2.** Influence of polyunsaturated fatty acids on the adaptive response of yeast to cooler temperatures. Yeast cells lacking the plant enzyme were cultivated at 30, 20, or 10°C, then lipids extracted and fatty acid composition determined using GC. Panel A represents changes in the lipid profile of WT yeast, and panel B shows changes in lipid content of yeast cells supplemented with linoleic and linolenic acids in the growth media. Abbreviations for fatty acids are palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids. Average and standard deviation of at least three independent experiments are shown. Deviations of less than 1% are not shown for clarity.

**Localization of HA-Fad3 enzyme in the microsomal fraction.** To begin to characterize the Fad3 protein in yeast, we first determined whether the epitope-tagged Fad3 protein could be detected in yeast cells in the expected subcellular fraction (ER). Yeast cells cultured at 20°C were subjected to subcellular fractionation and Western blotting using the anti-HA antibody. A band of approximate expected mass and subcellular location was detected on the blot that was not present in negative controls (Fig. 3). Approximately 90% of HA-Fad3 was found in the 13,000g pellet fraction while 10% was detected in the 165,000g pellet. This distribution matched that of dolichol phosphate mannose synthase,

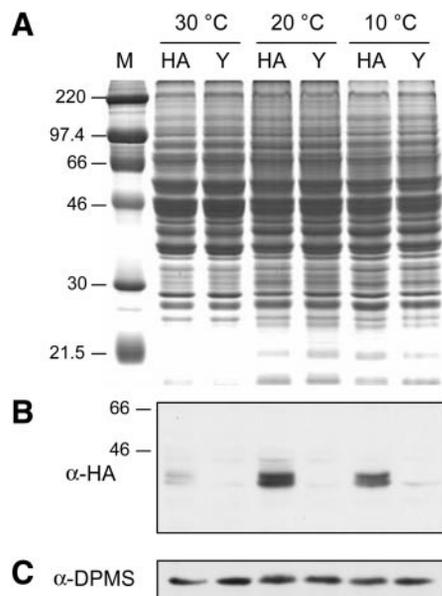
an integral membrane protein located in the yeast ER (23). These results suggested that HA-Fad3 was targeted correctly to the yeast ER.

**Chilling-induced increase in steady-state amount of epitope-tagged Fad3 enzyme expressed in yeast.** To determine whether the increase in linolenic acid content of yeast cells expressing HA-FAD3 might be due to changes in the steady-state level of Fad3 enzyme, whole cell lysates were prepared from yeast strains grown at 30, 20, and 10°C, then subjected to Western blotting (Fig. 4). Results revealed that the amount of HA-Fad3 changed considerably with temperature, with greatest to least observed at 20°C > 10°C > 30°C (Fig. 4B). Notably, HA-Fad3 was detected as a doublet of closely spaced bands on the gel, which may represent modified forms of the protein. The increase in desaturase amount from 30 to 20°C was approximately 8.5-fold. This increase was not a general feature of yeast ER membrane proteins, since no change in amount of the yeast ER membrane protein dolichol phosphate mannose synthase was detected (Fig. 4C). Qualitatively, the changes in amount of HA-Fad3 at lower temperatures correlated with the changes in linolenic acid content presented in Fig. 1.

The differences in banding pattern between HA-Fad3 analyzed in subcellular fractions (single band, Fig. 3) vs whole cell lysates (doublet, Fig. 4) are presently unknown, but may reflect differences in preparation of the protein samples. Whole cell lysates were prepared by rapidly killing cells in TCA, which terminates metabolic activity including proteases, prior to cell lysis using glass bead disruption. Subcellular fractions were prepared by gentle osmotic lysis of yeast spheroplasts followed by differential centrifugation.



**FIG. 3.** Subcellular fractionation of cells expressing HA-Fad3. Yeast cells harboring the HA-Fad3 expression plasmid, or pYES2 negative control plasmid, were cultivated at 20°C, converted to spheroplasts, osmotically lysed, and lysates subjected to differential centrifugation. Twenty-five  $\mu$ g of protein from the 13,000g pellet (13P), 165,000g supernatant (165S), and 165,000g pellet (165P) fractions were separated by SDS-PAGE and blots probed with either anti-HA antibody ( $\alpha$ -HA) or the ER membrane marker protein anti-dolichol phosphate mannose synthase ( $\alpha$ -DPMS). Positions of molecular mass markers (kDa) are shown to the left.



**FIG. 4.** Changes in amount of HA-Fad3 protein as a function of temperature. Yeast cells harboring the HA-Fad3 expression plasmid (HA), or pYES2 negative control plasmid (Y), were cultivated at 30, 20, or 10°C. Cells were rapidly killed in TCA and whole cell lysates prepared by glass bead disruption. Twenty-five  $\mu$ g of protein were separated by SDS-PAGE and total protein stained using Coomassie blue R-250 (A). Equivalently prepared gels were transferred to nitrocellulose and probed with either anti-HA antibody (B) or anti-dolichol phosphate mannose synthase (C). Positions of molecular mass markers (kDa) are shown to the left.

Although protease inhibitors were included during the fractionation, it is likely that a portion of HA-Fad3 was degraded during this process. In support of this hypothesis, it was typically more difficult to detect HA-Fad3 on Western blots of subcellular fractions. To observe bands of similar intensity on blots between whole cell lysates and subcellular fractions, it was necessary to load equal amounts of protein from each subcellular fraction, which significantly increased the relative protein content in pellet fractions.

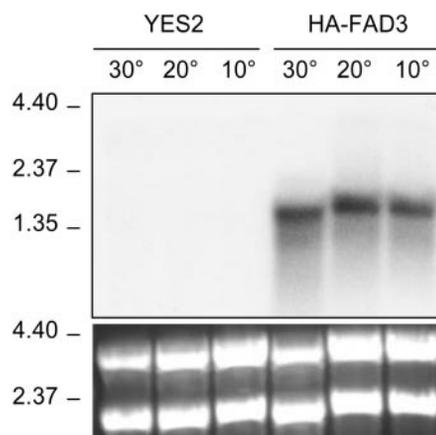
*Expression of FAD3 from the yeast GAL1-10 promoter.* To determine whether the increase in HA-Fad3 protein was due to upregulated transcription of the *FAD3* gene, RNA was extracted from cells cultivated at various temperatures and hybridized with a *FAD3* probe. Although there was no reason *a priori* to suspect that the *GAL1-10* promoter might be differentially regulated by temperature, it was possible that cooler growth temperatures affected some aspect of galactose sensing that might in turn affect transcription of *FAD3* from the *GAL1-10* promoter. The results presented in Fig. 5, however, indicate that there were no significant changes in *FAD3* mRNA levels in response to temperature. Taken together, the data suggest that the increase in linolenic acid synthesized by cells containing Fad3 was due to a cold-inducible, post-

transcriptional increase in steady-state amount of plant desaturase enzyme.

## DISCUSSION

*Post-transcriptional regulation during the adaptive response to chilling.* Many poikilothermic organisms including plants, animals, and microbes utilize post-transcriptional mechanisms in their adaptive response to cooler temperatures (27–31). For example, maize plants exposed to cooler temperatures respond by phosphorylating a minor chlorophyll *a/b* protein rather than synthesizing a new protein from a cold-regulated gene (27). In the Antarctic microbe *Euplote focardii*,  $\beta$ -tubulin, which is rarely phosphorylated, is heavily phosphorylated in this cold-adapted organism (29). Interestingly, there are even examples of homeothermic organisms displaying post-transcriptional regulation in response to cold temperatures. Exposure of rats to 5 h of acute cold resulted in a 6-fold increase in lipoprotein lipase activity, despite only a twofold increase in mRNA level (30).

The most relevant model for understanding plant Fad3 desaturase regulation might be derived from studies of stearoyl-CoA desaturase (SCD) in fish and rat. SCD is a  $\Delta^9$  desaturase responsible for the synthesis of mono-unsaturated fatty acids that plays a central role in adaptation of poikilothermic animals to cooler temperatures. SCD shares a number of features in common with the plant Fad3-type desaturases including protein domain organization, localization in the ER, and dependence on the cytochrome  $b_5$  electron transport chain (32). Plants



**FIG. 5.** Northern blot of HA-FAD3 expression in yeast cultivated at various temperatures. Yeast cells containing plasmid pYES2 (YES2) or pYES2-HAFAD3 (HA-FAD3) were grown at the temperature indicated, harvested at mid-log phase, and total RNA extracted. Ten micrograms RNA were separated on 1.2% agarose gels, transferred to nylon membrane, then probed with a  $^{32}$ P-labeled 960 bp *Bgl*III fragment inclusive of the majority of the *FAD3* open reading frame. The expected size of the HA-FAD3 transcript is approximately 1.4 Kb. Position of RNA markers (Kb) are shown to the left. The ethidium-stained gel is shown at the bottom as a loading control.

contain a soluble form of SCD located in chloroplasts that shares no sequence identity with animal SCD and has apparently arisen independently during evolution (33). Exposure of poikilothermic animals such as carp to cooler temperatures results in upregulation of SCD activity and subsequent increase in PUFA content (28, 31). The regulation of SCD is complex and involves both transcriptional and post-transcriptional controls. Surprisingly, the changes in plant Fad3 expressed in yeast cells described here and changes in SCD of warm-acclimated carp (31) exhibited similar characteristics upon modest cooling. Chilling of yeast cells expressing plant *FAD3* from 30 to 20°C and carp from 30 to 23°C resulted in significant increases in amount of respective desaturase proteins, modest increases in enzyme products, but no apparent changes in transcription of either desaturase gene. Taken together, it is apparent that changes in desaturase transcript amount, protein quantity, and enzyme activity are not necessarily coupled.

How might the enzymes be regulated at the post-transcriptional level? Significant insight to post-transcriptional regulation has been obtained from the studies of Ozols and colleagues on rat liver SCD (34, 35). Rat liver SCD exhibits very rapid turnover, with a half-life of several hours (34). SCD activity is induced in a transcriptional and post-transcriptional manner in animals by a variety of stimuli including dietary fat restriction, insulin, carbohydrates, and peroxisome proliferators (36). Upon withdrawal of the stimuli, SCD is rapidly degraded. The protease activity responsible for degradation has been localized to the ER and is an integral membrane protein (34). Notably, the first 33 amino acids of SCD contain a signal that facilitates proteolytic degradation (35). Removal of this sequence led to a substantial increase in half-life of SCD while attachment of the sequence to the green fluorescent protein resulted in rapid turn over of the fusion protein. The mechanism by which the peptide signal targets the protein for proteolytic degradation is unknown.

It is possible that the plant Fad3-like enzymes, expressed in plants or yeast, are subject to a similar sort of posttranscriptional regulation. In the simplest scenario, the turn over of the desaturase might be influenced by the physical status of the membrane itself, with a reduction of membrane fluidity at lower temperatures promoting stabilization of the enzyme structure and masking of a putative degradation signal. The resultant increase in steady-state amount of enzyme would lead to synthesis of more PUFA, with a subsequent increase in membrane fluidity. The return to fluidity at lower temperatures could then serve as a simple feedback mechanism to once again down-regulate the steady-state amount of the enzyme. In this model, the desaturase could serve as both sensor and effector of the plant homeoviscous response, allowing for rapid response to changes in ambient tempera-

tures. Additional levels of control such as phosphorylation or ubiquitination could be involved in fine-tuning the response.

Very recent results have demonstrated that exposure of wheat tips to cooler temperatures led to a post-transcriptional increase in Fad3 steady-state amount, similar to that reported here. The increase might be due to enhanced translational efficiency, since an increase in poly-ribosomal content of Fad3 mRNA was observed at cooler temperatures. However, changes in post-translational aspects of activity were not ruled out. In light of the results discussed above for SCD, it is likely that additional levels of control are involved in regulating plant desaturase activity. The yeast expression system described here may provide an excellent model system for dissecting the post-transcriptional mechanisms involved in regulation of plant desaturases. Understanding the regulation of plant fatty acid desaturases could have profound effects on our ability to produce engineered plants containing novel fatty acid compositions.

#### ACKNOWLEDGMENTS

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