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Effects of Unsaturated Fatty Acid Deprivation on Neutral Lipid Synthesis in *Saccharomyces cerevisiae*

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The effects of unsaturated fatty acid deprivation on lipid synthesis in *Saccharomyces cerevisiae* strain GL7 were determined by following the incorporation of [^{14}C]acetate. Compared to yeast cells grown with oleic acid, unsaturated fatty acid-deprived cells contained 200 times as much ^{14}C label in squalene, with correspondingly less label in 2,3-oxidosqualene and 2,3;22,23-dioxidosqualene. Cells deprived of either methionine or cholesterol did not accumulate squalene, demonstrating that the effect of unsaturated fatty acid starvation on squalene oxidation was not due to an inhibition of cell growth. Cells deprived of olefinic supplements displayed additional changes in lipid metabolism: (i) an increase in ^{14}C-labeled diacylglycerides, (ii) a decrease in ^{14}C-labeled triacylglycerides, and (iii) increased levels of ^{14}C-labeled decanoic and dodecanoic fatty acids. The changes in squalene oxidation and acylglyceride metabolism in unsaturated fatty acid-deprived cells were readily reversed by adding oleic acid. Pulse-chase studies demonstrated that the [^{14}C]squalene and ^{14}C-labeled diacylglycerides which accumulated during starvation were further metabolized when cells were resupplemented with oleic acid. These results demonstrate that unsaturated fatty acids are essential for normal lipid metabolism in yeasts.

Saccharomyces cerevisiae displays an absolute requirement for unsaturated fatty acids during growth. The necessity for olefinic acids was first demonstrated by using anaerobically-grown cells (1), but subsequent studies with mutants deficient in either fatty acid (16, 30, 31) or heme (5, 22) biosynthesis have shown that *S. cerevisiae* requires unsaturated fatty acids for aerobic growth as well. In fact, the amount of unsaturated fatty acid required by yeasts is fourfold higher during respirative growth than during fermentative growth (25). This enhanced requirement during respiration has led numerous workers to examine the role of olefinic acids in mitochondrial development and function in considerable detail (12, 14, 15, 22, 25, 28, 29), and it is now apparent that unsaturated fatty acids are obligatory for coupling oxidative phosphorylation to ATP synthesis (14, 15).

In contrast to the role of olefinic acids in respirative growth, considerably less is known about the function of these lipids during fermentative growth. Although only 5% of the cellular fatty acids need to be unsaturated to support fermentation (5, 25), the necessity for olefinic acids is nevertheless absolute; medium-length saturated fatty acids cannot completely satisfy the requirement (5, 21, 22, 24). Since yeasts may not contain mitochondria under anaerobic conditions (29), the small amount of unsaturated fatty acid required for fermentative growth might perform additional necessary functions. Exactly what these specific functions are, however, has yet to be determined.

In recent studies (2, 4) an ergosterol-deficient mutant, *S. cerevisiae* strain GL7 (11) has been used to further define the sterol requirements of fermentatively growing yeasts. Due to a genetic lesion affecting heme biosynthesis, strain GL7 also requires exogenous unsaturated fatty acids (11) and thus constitutes an ideal system for delineating the role of olefinic acids in yeasts. It has been shown previously that in the presence of ergosterol, strain GL7 can grow with an unsaturated fatty acid content of less than 10% (5). Furthermore, when strain GL7 is deprived of exogenous unsaturated supplements, the mutant incorporates most of its olefinic residues into phosphatidylethanolamine (5). This suggests a specific requirement for phosphatidylethanolamine, possibly as a membrane fusogen (9). Previous studies by Graff and Lands (13) and Holub and Lands (17) have correlated changes in the lipid composition of yeast with the availability of *cis* unsaturated fatty acids. To further characterize the functions of olefinic fatty acids in *S. cerevisae*, we have now examined the effects of unsaturated fatty acid starvation on lipid biosynthesis in strain GL7. Our results demonstrate that unsaturated fatty acids are

essential for normal lipid metabolism and, in particular, for the oxidation of squalene.

MATERIALS AND METHODS

Culture conditions. *S. cerevisiae* strain GL7 (*erg12 heme3* [11]) was grown in a synthetic medium supplemented with methionine and cholesterol (4). Unsaturated fatty acids, when added, were present at a final concentration of 100 μg/ml. Cultures were grown at 30°C with shaking, and growth was monitored by measuring the absorbance at 540 nm or by dry weight determinations. Deprivation experiments were performed by collecting yeast cells from the log phase of growth, washing twice with media, and suspending the cells to the desired density either in complete media or in media lacking unsaturated fatty acids, cholesterol, or methionine. Reversibility studies were performed by growing the mutant in the absence of unsaturated fatty acid supplements for 12 to 14 h. At the start of the experiment, one-half of the culture was supplemented with oleic acid (100 μg/ml); the other half of the culture served as a control.

Lipid synthesis was followed by measuring the incorporation of [1-^{14}C]acetate. At various times, 50-ml samples were removed from larger cultures, and the yeast cells were pulsed with 10 μCi of [^{14}C]acetate for 2 to 4 h before harvesting. Pulse-chase experiments were performed with cells grown in the absence of exogenous unsaturated fatty acids for at least 12 h. The starved cells were subsequently incubated with [^{14}C]acetate for an additional 3 to 4 h before harvesting. Labeled cells were washed twice with media containing 100 μM sodium acetate (unlabeled) and suspended in the same medium. One-half of the culture was supplemented with oleic acid and the other half of the culture served as a control. At various times after the pulse, 50-ml samples were removed from each flask for lipid determinations.

Lipid analyses. Total ^{14}C-labeled lipids were extracted from lyophilized yeast cells (4) and separated by thin-layer chromatography (TLC) on silica gel plates (250 μM; E. Merck) developed with petroleum ether-diethyl ether-acetic acid (85:15:1, vol/vol/vol). The ability of this solvent system to completely resolve the labeled neutral lipids was confirmed by chromatographing the total lipids in a two-dimensional system employing petroleum ether-diethyl ether-acetic acid (70:30:2, vol/vol/vol) in the first direction, and hexane-diethyl ether (90:10, vol/vol) in the second direction. Lipids were visualized with I_2 or located by autoradiography. Yeast lipids were identified by cochromatography with authentic standards in both of the above TLC systems. Saponification of the separated lipids showed that >95% of the label associated with putative acylated lipids (phospholipid, diacylglyceride, free fatty acids, triacylglycerides, and cholesteryl esters) was extracted into the saponifiable fraction. In the case of the separated sterol intermediates, greater than 90% of the initial radioactivity was recovered in the nonsaponifiable fraction. Fatty acid compositions of the individual acylated lipids were determined by transesterifying the fatty acids directly from the gel (4), followed by reversed-phase, and in some cases, argentation TLC with impregnated silica gel G plates (250 μM; Analtech [3]). ^{14}C-labeled lipids were quantified by scraping appropriate regions of the gel into vials followed by liquid scintillation counting. All experiments were performed at least twice, and in many cases three times. The data presented are from representative experiments and are usually expressed as picomoles of [^{14}C]acetate incorporated per 50 milliliters of culture.

Isolation and identification of 2,3;22,23-dioxidosqualene. Due to the instability of dioxidosqualene, we were not able to recover this lipid intact after TLC. Therefore, nonsaponifiable lipids were extracted from ergosterol-grown yeast (ergosterol was more easily separated from dioxidosqualene than cholesterol) and fractionated by high-performance liquid chromatography (HPLC) with a Perkin-Elmer series 3 liquid chromatograph containing a Silica A column (0.26 by 25 cm; Perkin-Elmer). Lipids were eluted with hexane-ethyl acetate (98:2, vol/vol) at a flow rate of 2 ml/min and fractions were collected for subsequent analysis by TLC and gas-liquid chromatography (GLC). The lipid eluting with a retention volume of 11 ml (0.52 relative to ergosterol) was characterized as dioxidosqualene on the basis of its mobility on TLC (6), as well as by its anomalous behavior on GLC (10). Gas chromatography was performed with a Perkin-Elmer Sigma 3B gas chromatograph equipped with a 6 ft (ca. 180 cm) column of 3% SP2250 on 100/120 Supelcoport (Supelco). Analyses were run isothermally at 280°C; dioxidosqualene displayed a retention time of 8.9 min (0.7 relative to ergosterol). Positive identification of the lipid as dioxidosqualene was obtained with the aid of a Finnigan gas chromatograph-mass spectrometer. The leading edge of the dioxidosqualene peak gave the characteristic ion peaks at 443 (M^+), 153, and 135 (10).

Reagents. Sterols, fatty acids, and lipid standards were obtained from Sigma Chemical Co. [1-^{14}C]acetic acid (56 mCi/mmol) was a product of New England Nuclear Corp.

RESULTS

Lipid synthesis in *S. cerevisiae* strain GL7. When grown in the presence of cholesterol and oleic acid, *S. cerevisiae* strain GL7 incorporated [^{14}C]acetate into six different neutral lipids (Fig. 1, lane A). In addition, a considerable amount of radioactivity was associated with the polar lipids remaining at the origin. This region is primarily comprised of phospholipids, but small amounts of glycolipids may also be present (26). All cellular lipids were labeled within 3 h, although longer labeling periods (18 h) led to an increased proportion of label in dioxido-squalene (Table 1).

Growing strain GL7 in the absence of unsaturated fatty acids resulted in several changes in lipid biosynthesis (Fig. 1, lane B; Table 1). The most striking effect was an accumulation of squalene with a slight decrease in oxidosqualene and the complete loss of dioxidosqualene. By contrast, squalene was barely detectable in control cells. These results suggest that cells starved for unsaturated fatty acids display a reduced capacity for oxidizing squalene. Starved cells also displayed changes in their syntheses of

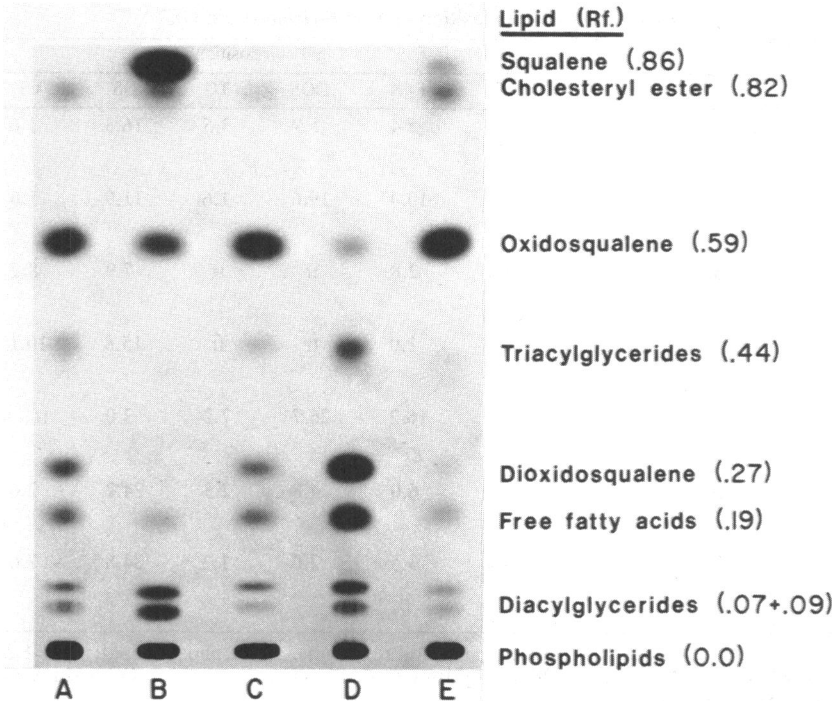

FIG. 1. Autoradiogram of TLC separation of ^{14}C-labeled lipids recovered from yeast grown under varying conditions. Strain GL7 was grown in a defined medium supplemented with: A, methionine, cholesterol, and oleic acid; B, methionine and cholesterol; C, cholesterol and oleic acid; D, methionine and oleic acid; and E, methionine, cholesterol, and petroselenic acid. The R_f values are shown in parentheses.

acylated lipids. The amount of radioactivity associated with phospholipids (percent of total) was found to decline after starvation for unsaturated fatty acids, a finding consistent with a reduced rate of phospholipid synthesis (5).In addition, unsaturated fatty acid-deprived cells displayed a loss of triacylglycerides as well as a twofold increase in diacylglycerides.

Starvation for unsaturated fatty acids leads to a reduced growth rate as well as a lower final cell yield (5). To determine whether the lipid changes observed in unsaturated fatty acid-starved cells simply reflected an inhibition of growth, lipid synthesis was examined under three additional growth conditions: in the absence of methionine (9 h), in the absence of cholesterol (12 h), or in the presence of cholesterol plus petroselenic acid (12 h). In each case, [^{14}C]acetate was added during the final 3 h of incubation. All three conditions led to a marked inhibition of growth as well as a reduced uptake of [^{14}C]acetate (not shown). The lipid composition of strain GL7 was unaffected by methionine starvation (Fig. 1, lane C), and cholesterol deprivation actually led to an increase in dioxidosqualene (Fig. 1, lane D). Although cells supplemented with cholesterol plus petroselenic acid displayed a slight increase in both squalene and oxidosqualene (Fig. 1, lane E), it is obvious that none of these three conditions induced lipid changes of the type and magnitude induced by unsaturated fatty acid deprivation (Table 1).

To characterize the effects of unsaturated fatty acid deprivation on lipid biosynthesis in greater detail, we next measured lipid synthesis in strain GL7 as a function of time. In oleate-supplemented cells, the amount of [^{14}C]acetate incorporated into individual lipids increased logarithmically (Fig. 2 and 3) as would be expected for exponentially growing cells. Furthermore the relative amounts of the various lipids remained constant, indicative of balanced growth. By contrast, noticeable changes in lipid synthesis were observed within 3 h after depriving strain GL7 of exogenous unsaturated fatty acids. The most immediate effects were an increased incorporation of [^{14}C]acetate into cholesteryl esters and a decreased incorporation into triacylglycerides and dioxidosqualene (Fig. 2 and 3). The accumulation of radiolabel in squalene was first detectable between 3 and 6 h after starvation, and by 15 h the amount of label in squalene was 200-fold higher than in supplemented cells (Fig. 3). This accumulation was primarily due to an inhibition of squalene oxidation as demonstrated by the lower amounts of [^{14}C]acetate incorporated into

TABLE 1. Lipid composition of *S. cerevisiae* strain GL7

Growth conditions	Labeling period (h)	Lipid composition[a]							
		PPL	DG	FFA	DOS	TG	OS	CE	SQ
Complete medium	3	59.4	5.7	5.4	5.9	3.5	16.3	3.6	tr[b]
Complete medium	18	44.6	6.1	10.4	19.6	1.6	11.9	5.6	tr
Minus oleic acid	3	38.4	13.1	2.8	tr	tr	7.9	8.2	28.8
Minus oleic acid	18	31.3	10.8	1.0	tr	tr	15.8	10.1	30.5
Minus cholesterol	3	36.7	10.1	16.2	26.7	7.2	3.0	tr	tr
Minus methionine	3	54.9	4.8	6.0	4.8	2.3	24.2	2.6	tr
Plus petroselenic acid	3	42.2	4.4	3.5	2.0	1.2	34.8	7.6	4.2

[a] Expressed as percent of total ^{14}C-labeled lipids. Abbreviations: PPL, phospholipid; DG, 1,2- and 1,3-diacylglycerides; FFA, free fatty acids; DOS, dioxidosqualene; TG, triacylglycerides; OS, oxidosqualene; CE, cholesteryl esters; SQ, squalene.

[b] Less than 1%.

oxidosqualene and dioxidosqualene. Unsaturated fatty acid-deprived cells also incorporated more [^{14}C]acetate into total nonsaponifiable lipids than did supplemented cells (Fig. 3), but it is not clear whether this reflects a stimulation of squalene synthesis or simply an enhanced specific activity of intracellular acetate pools.

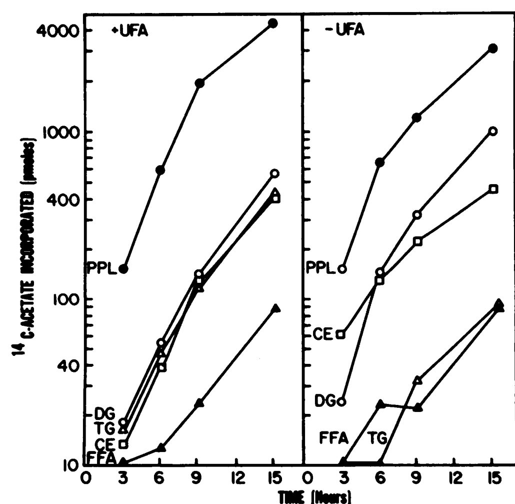

FIG. 2. Time course of [^{14}C]acetate incorporation into the acylated lipids of strain GL7 during growth in the presence or absence of oleic acid. Abbreviations: UFA, unsaturated fatty acid; PPL, phospholipid (●); DG, diacylglyceride (○); TG, triacylglyceride (△); CE, cholesteryl esters (□); and FFA, free fatty acids (▲).

Reversibility of lipid changes induced during starvation for unsaturated fatty acids. Our results clearly demonstrate that starvation for unsaturated fatty acids affects two steps in lipid biosynthesis: squalene oxidation and diacylglyceride metabolism. To determine whether these lipid changes are reversible, we added oleic acid to cells which had been deprived of unsaturated fatty acids for approximately 12 h. At various times after resupplementing the culture, samples were removed and lipid biosynthesis was measured by using [^{14}C]acetate. Cells deprived of unsaturated fatty acids for 12 h incorporated 50 times as much [^{14}C]acetate into diacylglycerides as triacylglycerides during the entire 6-h experiment (Fig. 4). However, within 2 h of adding back oleic acid, strain GL7 decreased the amount of [^{14}C]acetate incorporated into diacylglycerides by 80% while doubling the amount of radiolabel incorporated into triacylglycerides. This resulted in a decrease in the ratio of diacylglycerides to triacylglycerides from 50 to 4.6. During the same time interval (0 to 2 h) the amount of radiolabel incorporated into phospholipids and free fatty acids declined by 25 and 35%, respectively (not shown). Between 2 and 6 h, the synthesis of diacylglycerides paralleled the synthesis of triacylglycerides (Fig. 4), as did the synthesis of phospholipids, free fatty acids, and cholesteryl esters (not shown). Thus, the

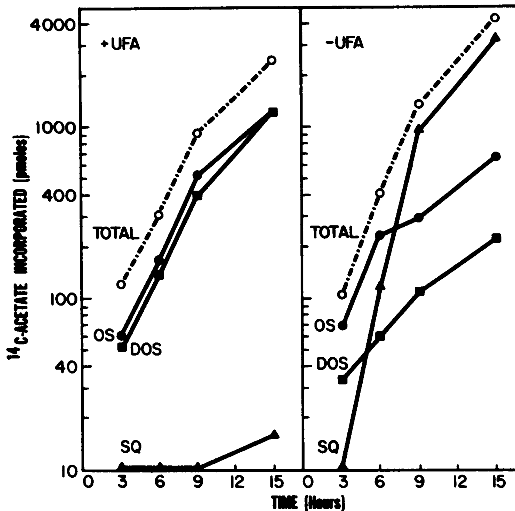

FIG. 3. Time course of [^{14}C]acetate incorporation into the nonsaponifiable lipids of strain GL7 during growth in the presence or absence of oleic acid. Abbreviations: UFA, unsaturated fatty acid; OS, 2,3-oxidosqualene (●); DOS, 2,3;22,23-dioxidosqualene (■); and SQ, squalene (▲).

effects of unsaturated fatty acid starvation on the syntheses of diacylglycerides and triacylglycerides were reversible.

Fig. 5 demonstrates that the effects of unsaturated fatty acid starvation on the synthesis of nonsaponifiable lipids were also reversible. Growth of strain GL7 in the absence of oleic acid resulted in 90% of the radiolabel being incorporated into squalene, with oxidosqualene and dioxidosqualene accounting for 9 and 1% respectively. During the entire 6-h experiment, this composition remained nearly unchanged. However, 2 h after adding oleic acid, the amount of label incorporated into squalene decreased by 50%, with a concomitant sevenfold increase in the incorporation of radiolabel into oxidosqualene. Between 2 and 6 h there was a further decline in the incorporation of [^{14}C]acetate into squalene, with corresponding increases in oxidosqualene and dioxidosqualene. Thus, the inhibition of squalene oxidation observed in unsaturated fatty acid-deprived cells was completely reversed within 6 h after the addition of oleic acid.

Metabolic fate of ^{14}C-labeled lipids accumulated during unsaturated fatty acid starvation. Our studies demonstrate that the accumulation of diacylglycerides and squalene which occurs in strain GL7 during starvation for unsaturated fatty acids is curtailed upon readdition of oleic acid. To determine the metabolic fate of the accumulated lipids, yeast cells were pulsed for 3 to 4 h with [^{14}C]acetate and subsequently incubated in the presence of 100 μM sodium acetate (chase) with or without unsaturated fatty acid supplements. Phospholipids and diacylglycerides turned over in starved cells with a half-life ($T_{1/2}$) of about 9 h. Cholesteryl esters and triacylglycerides were considerably more stable, each having a $T_{1/2}$ greater than 24 h. Supplementation with oleic acid led to an enhanced turnover of both phospholipids ($T_{1/2}$, 4 h) and diacylglycerides ($T_{1/2}$, 1.5 h between 0 and 4 h). By contrast, the levels of ^{14}C-labeled cholesteryl esters and triacylglycerides actually increased during the first 4 h after supplementation and then subsequently declined. These results imply a transfer of fatty acids from phospholipids and diacylglycerides to cholesteryl esters and triacylglycerides. Unfortunately, our data do not allow us to determine whether the increase in triacylglycerides occurred at the expense of diacylglycerides, phospholipids, or both. It is worth noting that during the 8-h chase, considerable amounts of radiolabel were lost to the medium by starved cells (20%) as well as resupplemented cells (60%). Extraction and subsequent TLC analyses demonstrated that the extracellular ^{14}C-lipids consisted entirely of free fatty acids (not shown).

Figure 7 shows the effect of adding back oleic

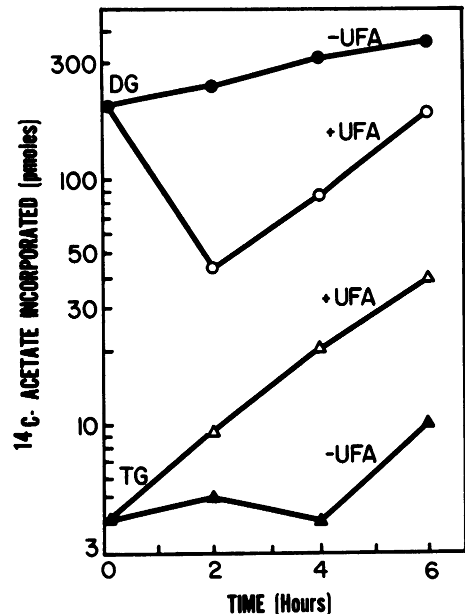

FIG. 4. Reversibility of the effects of unsaturated fatty acid deprivation on acylglyceride metabolism in strain GL7. Cells were deprived of olefinic acids for 12 h, after which time one-half of the culture was supplemented with oleic acid (+UFA). At various times, samples were removed and incubated with [^{14}C]acetate as described in the text. Abbreviations: DG, diacylglyceride (O, ●); and TG, triacylglyceride (Δ, ▲).

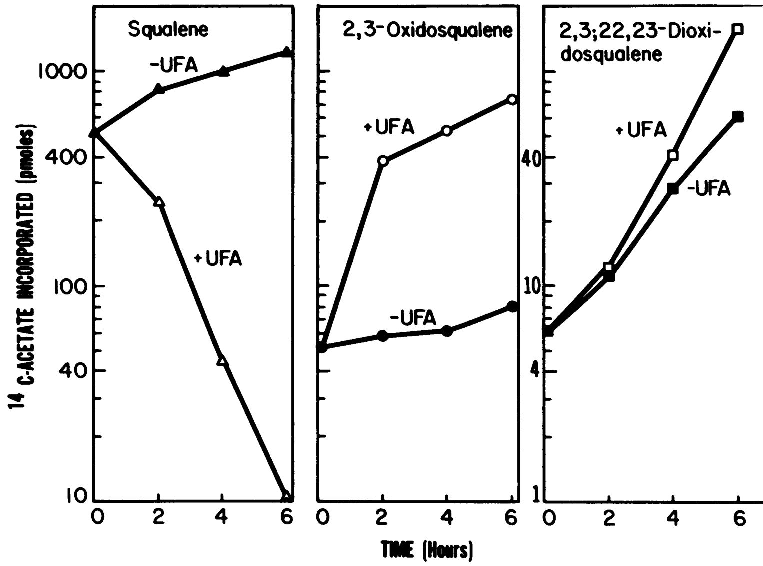

FIG. 5. Reversibility of the effects of unsaturated fatty acid deprivation on squalene metabolism in strain GL7. Protocol is the same as that described in the legend to Fig. 4. Note the difference in scale for dioxidosqualene.

acid on the metabolism of ^{14}C-nonsaponifiable lipids. After a 12-h growth period in the absence of unsaturated fatty acids, strain GL7 incorporated [^{14}C]acetate primarily into squalene (71%) and lesser amounts into oxidosqualene (27%) and dioxidosqualene (2%). During the 8-h chase, cells deprived of unsaturated fatty acids converted about 15% of the squalene to oxidosqualene, whereas the level of dioxidosqualene remained unchanged. Supplementation with oleic acid led to a rapid turnover of squalene ($T_{1/2}$, 1.5 h), with the label being chased into oxidosqualene and subsequently into dioxidosqualene. After the 8-h chase, squalene accounted for only 3% of the label, whereas oxidosqualene and dioxidosqualene accounted for 77 and 20%, respectively. The results of the pulse-chase experiments clearly demonstrate that after the addition of exogenous unsaturated fatty acids, the diacylglycerides and squalene which accumulated during starvation are further metabolized at a relatively rapid rate ($T_{1/2}$, 1.5 h). It is interesting to note that in contrast to acylated lipids (Fig. 6), ^{14}C-labeled nonsaponifiable lipids were not lost to the medium during the 8-h chase (Fig. 7).

Effects of unsaturated fatty acid deprivation on

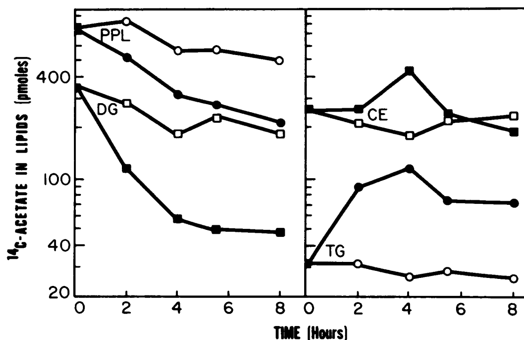

FIG. 6. Pulse-chase studies to determine the fate of ^{14}C-labeled acylated lipids in yeast cells continually deprived of unsaturated fatty acids (○, □) or resupplemented with oleic acid (●, ■). Cells were grown and labeled with [^{14}C]acetate as described in the text. Abbreviations are the same as those given in Fig. 2.

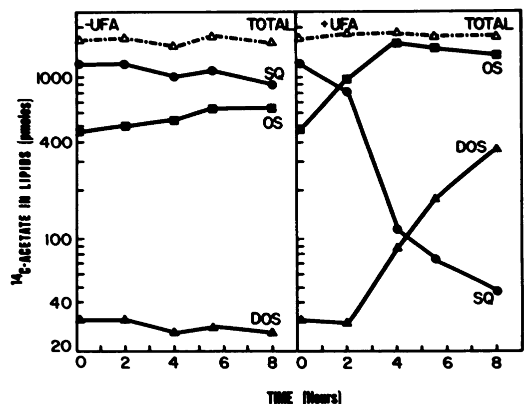

FIG. 7. Pulse-chase studies to determine the fate of ^{14}C-labeled nonsaponifiable lipids in yeast cells continually deprived of unsaturated fatty acids (−UFA) or resupplemented with oleic acid (+UFA). Abbreviations are the same as those given in Fig. 3.

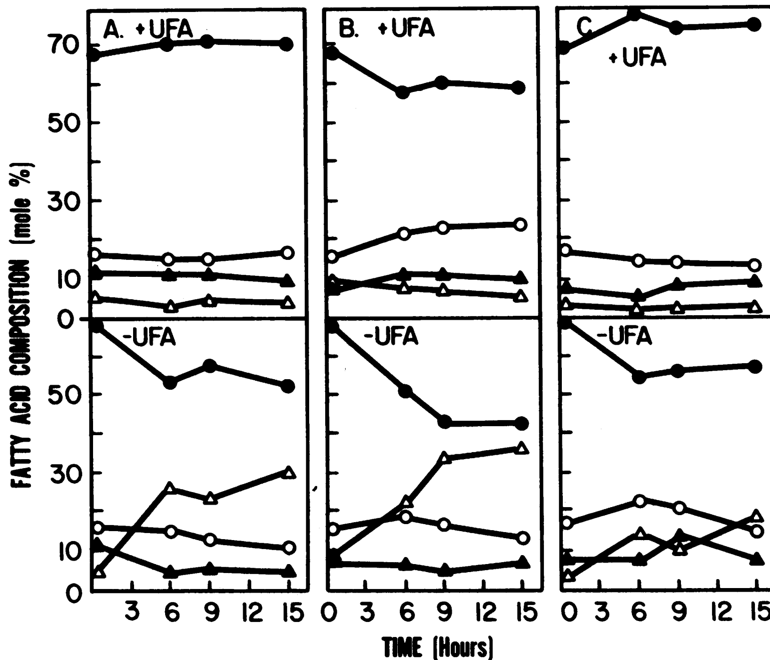

FIG. 8. Time course of [^{14}C]acetate incorporation into the fatty acids of: A, phospholipids; B, diacylglycerides; and C, free fatty acids isolated from cells grown in the presence (+UFA) or absence (−UFA) of oleic acid. The procedures for extracting and separating ^{14}C-labeled fatty acids are given in the text. Symbols: ●, 16:0; ○, 14:0; ▲, 18:0; and △, 10:0 plus 12:0.

the fatty acid composition of strain GL7. It has been shown previously that strain GL7 synthesizes large amounts of decanoic, dodecanoic, and tetradecanoic fatty acids (5) during growth in the absence of unsaturated fatty acids supplements. By analyzing the fatty acid compositions of the various acylated lipids, it was possible to determine whether the fatty acid changes observed previously in phospholipids (5) occurred to an equal extent in neutral lipids. ^{14}C-labeled fatty acids were recovered from the separated lipid fractions and separated by TLC on the basis of chain length and degree of unsaturation. Greater than 95% of the fatty acids were saturated, demonstrating that strain GL7 was synthesizing little, if any, unsaturated fatty acids. In the presence of unsaturated supplements, the mutant incorporated primarily hexadecanoic acid with lesser amounts of octadecanoic and tetradecanoic fatty acids into phospholipids, diacylglycerides, free fatty acids (Fig. 8), and triacylglycerides (not shown). Decanoic and dodecanoic fatty acids accounted for only 5 to 7% of the total fatty acids present in these cells. In agreement with previous results (5), depriving strain GL7 of unsaturated fatty acids led to a decrease in hexadecanoic acid with corresponding increases in decanoic and dodecanoic acids. The shift towards medium-length fatty acids occurred within 6 h and was evident in phospholipids, diacylglycerides, free fatty acids (Fig. 8), and triacylglycerides (not shown). The change in fatty acid synthesis was reflected by a decrease in the average chain length of newly synthesized fatty acids from 15.6 carbons in oleate-supplemented cells to 14.6 carbons in the starved cells.

As mentioned above, during the pulse-chase experiments we found that strain GL7 leaked ^{14}C-labeled free fatty acids to the medium. To determine if decanoic and dodecanoic acids were preferentially lost by the oleate-supplemented cells, the extracellular fatty acids were recovered from starved and supplemented cultures and analyzed by TLC. The profile of ^{14}C-labeled fatty acids released by both cultures was identical (not shown), demonstrating that oleate supplementation did not lead to a preferential loss of medium-length fatty acids.

DISCUSSION

Depriving *S. cerevisiae* strain GL7 of unsaturated fatty acids led to complex changes in neutral lipid metabolism. Of considerable interest was the finding that cells deprived of olefinic supplements accumulated squalene at the expense of the squalene oxides. These results imply that unsaturated fatty acids are essential for the conversion of squalene to ergosterol in yeast. Although the function of olefinic fatty

acids in squalene oxidation is not known, several possibilities can be considered. First, the epoxidase enzyme may have a specific requirement for unsaturated acyl chains, either as free fatty acids or in the form of phospholipids. Alternatively, the epoxidase enzyme may require a greater degree of bilayer fluidity than can be achieved with medium-length saturated fatty acids. Both possibilities assume that the epoxidase enzymes continue to be synthesized during oleic acid starvation but are inactive in the endoplasmic reticulum. Supplementing starved cells with oleic acid, which readily reverses the inhibition of squalene oxidation, would then lead to either the activation of preexisting epoxidase enzymes or the de novo synthesis of active epoxidase enzymes. A third possibility is that squalene oxidation involves a mitochondrial activity which is lacking in cells depleted of unsaturated fatty acids (25). Although cytochromes do not seem to be required (11), the mechanism by which oxygen is made available to the epoxidase enzyme is unknown and it is conceivable that mitochondria are somehow involved.

Unsaturated fatty acid deprivation also had a marked affect on the synthesis of acylglycerides. In the absence of olefinic acids, strain GL7 incorporated twice as much [^{14}C]acetate into diacylglycerides as control cells but only one-fifth as much into triacylglycerides. The change in acylglyceride metabolism could result from an inhibition of the diacylglyceride acylase (7, 8) or from an enhanced triacylglyceride lipase activity (27). Either mechanism would lead to an accumulation of diacylglycerides. Unfortunately, our pulse-chase studies do not distinguish between these two possibilities. Strain GL7 displays considerable phospholipid turnover, and previous workers have shown that phospholipid turnover can lead to a flux of acyl chains from phospholipids to triacylglycerides (24, 27). Therefore, we could not determine whether the ^{14}C-labeled fatty acids which were incorporated into triacylglycerides during the chase originated from phospholipids or the accumulated diacylglycerides. Nevertheless, *S. cerevisiae* frequently accumulates triacylglycerides during suboptimal growth (13, 27), an action which would be more likely to involve a diacylglyceride acylase rather than a triacylglyceride lipase. It is worth noting that strain GL7 shows a twofold increase in phosphatidic acid content during starvation for unsaturated fatty acids (5), which may result from the activity of a diacylglyceride kinase (26) on the accumulated diacylglycerides.

A third effect of unsaturated fatty acid deprivation was an increase in the amounts of decanoic and dodecanoic fatty acids. The shift towards shorter acyl chains in response to growth with limited unsaturated fatty acids has been observed previously in strain GL7 (5) and in yeasts in general (21–24). In strain GL7, the change in fatty acid composition was complete within 6 h of oleate deprivation and occurred to an equal extent in all of the acylated lipids examined. The rise in decanoic and dodecanoic fatty acids decreases the average chain length of newly synthesized fatty acids from 15.6 carbons in oleate-supplemented cells to 14.6 carbons in cells deprived of unsaturated fatty acid supplements. The mechanisms by which *S. cerevisiae* regulates fatty acid chain length are not known, but studies with strain GL7 have shown that cell-free extracts prepared from both oleate-supplemented and oleate-deprived cells synthesize primarily hexadecanoic acid (A. L. Pyle, unpublished data).

The results of our pulse-chase studies demonstrated that adding back oleic acid to deprived cells led to the loss of considerable amounts of ^{14}C-labeled fatty acids to the medium. It therefore seemed possible that oleic acid would be used to preferentially replace esterified decanoic and dodecanoic acids; and as a result, the medium-length saturated fatty acids would constitute a major portion of the labeled fatty acids recovered from the medium. This was, however, not the case, and the average chain lengths of fatty acids released by starved cells and resupplemented cells were the same. Thus, strain GL7 does not regulate its phospholipid-fatty acid composition by selective deacylation and reacylation, a finding which agrees with a previous study (27).

Although strain GL7 is unable to synthesize unsaturated fatty acids, the mutant can continue growing for 18 to 20 h after the removal of olefinic supplements. The ability of strain GL7 to tolerate extended periods of unsaturated fatty acid deprivation makes this organism attractive for defining the role of unsaturated fatty acids in yeast. In many respects strain GL7 is similar to the *ole1* mutant described by Henry (16). Starvation for unsaturated fatty acids does not significantly reduce the viability of either strain, and like *ole1* (16), strain GL7 becomes "clumpy" after growth without olefinic supplements. Since *ole1* is wild type for sterol biosynthesis, it seems unlikely that the extended survivability of strain GL7 in the absence of unsaturated fatty acids is due to its additional defect in ergosterol synthesis. One possibility is that yeasts have evolved mechanisms for surviving with the lower amounts of unsaturated fatty acids which they can synthesize under the reduced-oxygen conditions of fermentation. The continued production of squalene under oxygen-limiting conditions (19) may actually represent one type of adaptive mechanism. By intercalating between adjacent acyl chains and increasing bilayer disorder (20),

squalene may compensate for the inability of medium-length saturated acyl chains to fluidize the membrane as effectively as olefinic fatty acids. Alternatively, the hydrocarbon may be preserving bilayer thickness (18). If we consider the contribution of oleic acid to the average fatty acid chain length of supplemented cells, then one effect of unsaturated fatty acid deprivation is to decrease the average chain length of phospholipid-fatty acids from 16.8 carbons to 14.6 (5). The loss of two carbons from each half of the bilayer would either create a "pocket" within the middle of the bilayer or reduce the thickness of the membrane by about 5Å. Sequestering the squalene within the proposed "pocket" could provide one mechanism for maintaining membrane thickness (18). Future studies with yeast mutants which require olefinic supplements but are capable of synthesizing ergosterol are necessary to determine whether the accumulation of squalene in strain GL7 is, in fact, an adaptive response characteristic of yeasts in general. Nevertheless, our finding that squalene and squalene oxides are not released from strain GL7 whereas free fatty acids are demonstrates that *S. cerevisiae* can retain considerable amounts of these hydrocarbons within its membranes. This ability distinguishes yeast from animal cells, which have been shown to excrete excess squalene oxides (6).

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