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Storage Lipid Synthesis Is Non-essential in Yeast*

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Steryl esters and triacylglycerol (TAG) are the main storage lipids in eukaryotic cells. In the yeast *Saccharomyces cerevisiae*, these storage lipids accumulate during stationary growth phase within organelles known as lipid bodies. We have used single and multiple gene disruptions to study storage lipid synthesis in yeast. Four genes, *ARE1*, *ARE2*, *DGA1*, and *LRO1*, were found to contribute to TAG synthesis. The most significant contribution is made by *DGA1*, which encodes a novel acyl-CoA:diacylglycerol acyltransferase. Two of the genes, *ARE1* and *ARE2*, are also involved in steryl ester synthesis. A yeast strain that lacks all four genes is viable and has no apparent growth defects under standard conditions. The strain is devoid of both TAG and steryl esters, and fluorescence microscopy revealed that it also lacks lipid bodies. We conclude that neither storage lipids nor lipid bodies are essential for growth in yeast.

Lipids are important storage compounds in plants, animals, and fungi. The main storage lipids in eukaryotes are triacylglycerol (TAG)[1] and steryl esters (1). Storage lipids are usually found within special organelles known as lipid particles or lipid bodies (2). In yeast, these lipid bodies accumulate during stationary phase, and they can constitute up to 70% of the total lipid content of the cell (3–5). Several lipid-metabolizing enzymes are preferentially localized to the lipid bodies in yeast, and it has therefore been proposed that they do not solely serve as a depot for lipids but instead may have a more complex role in lipid biosynthesis, metabolism, degradation, and trafficking (6).

Steryl esters, the esterified form of sterols linked to a long chain fatty acid, are synthesized by the enzyme acyl-CoA:sterol acyltransferase (ASAT) which is encoded by the duplicated *ARE1* and *ARE2* genes in the yeast *Saccharomyces cerevisiae* (7–9). Related genes encoding enzymes with ASAT activity exist also in higher eukaryotes (10, 11). It has been shown that the other major storage lipid, TAG, can be synthesized in at least two different ways. One is an acyl-CoA-dependent reaction that is catalyzed by acyl-CoA:diacylglycerol acyltransferase (DGAT). Two different DGATs are known to exist in eukaryotes. One DGAT, also known as DGAT1, was first described in mammals but is also found in plants and is related to ASAT (12, 13). In addition, a second DGAT, DGAT2, was recently identified in the fungus *Mortiella ramanniana* (14). DGAT2 is not related to any previously known enzyme, but genes encoding homologues of DGAT2 exist in other eukaryotes (14, 15). One such gene is the *DGA1* gene in yeast (open reading frame YOR245c). In addition to the reaction catalyzed by the two types of DGATs, an acyl-CoA-independent pathway for TAG synthesis was also recently discovered in plants and yeast (16). This pathway involves the novel enzyme phospholipid:diacylglycerol acyltransferase (PDAT), which can synthesize TAG from phospholipids and diacylglycerol (16). PDAT is distantly related to the mammalian enzyme lecithin:cholesterol acyltransferase and is encoded by the *LRO1* gene in yeast (17).

We used single and multiple gene disruptions to study the relative contributions of the four genes, *ARE1*, *ARE2*, *DGA1* and *LRO1*, to storage lipid synthesis in yeast. We find that the DGAT2 encoded by the *DGA1* gene is the most important yeast enzyme involved in TAG synthesis. The *LRO1* gene, which encodes PDAT, also contributes significantly to TAG accumulation, whereas the products of the *ARE1* and *ARE2* genes both make small but detectable contributions. Steryl ester synthesis is exclusively dependent on *ARE1* and *ARE2*. Significantly, a strain that lacks all four genes contains neither storage lipids nor lipid bodies but is still viable. We conclude that storage lipid synthesis is non-essential in yeast.

EXPERIMENTAL PROCEDURES

Yeast Strains—All strains used (Table I) are congenic to W303-1A (18) and therefore also carry the *ade2-1 can 1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1* markers except where otherwise noted. Strains SCY60, SCY61, and SCY62 (the wild type control) were obtained from Stephen Sturley. Strains H1111 and H1112 were made by crossing SCY60 to SCY61 and dissecting tetrads. Other strains were made either by crosses or by successive one-step gene disruptions. The *lro1*-Δ::*URA3* and *lro1*-Δ::*TRP1* disruptions were made by cloning the *URA3 Hin*dIII fragment or the *TRP1 Eco*RI fragment, respectively, between the *Bbs*I and *Mun*I sites of *LRO1*. To disrupt *DGA1*, an *Sma*I-*Sac*I fragment carrying the *KanMX4* marker was cloned between the *Cla*I and *Stu*I sites in *DGA1*.

Cell Growth and Lipid Analysis—Yeast cells were grown in YNB complete medium, which is yeast nitrogen base without amino acids (Sigma), complete supplement mixture (Bio 101, Inc.), and 2% glucose. The cells were pre-cultivated on YNB complete plates for 1–2 days and then transferred to a preculture in liquid YNB. Stationary phase cells were grown for 52 h prior to being harvested. The total lipid content and lipid composition of the cells were determined as described previously (16).

Enzyme Assays—In vitro DGAT activity was determined in crude extract of yeast cells in assay buffer (50 mM HEPES, pH 7.2, 5 mM $MgCl_2$, 1 mg/ml bovine serum albumin). Yeast cells were homogenized in 2-ml Eppendorf tubes containing 0.2 ml of glass beads (0.45–0.5 mm

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[1] The abbreviations used are: TAG, triacylglycerol; ASAT, acyl-CoA:sterol acyltransferase; DGAT, diacylglycerol acyltransferase; PDAT, phospholipid:diacylglycerol acyltransferase.

TABLE I
Yeast strains

Strain	Relevant genotype
SCY60	*MATα are1-Δ::HIS3*
SCY61	*MAT***a** *are2-Δ::LEU2 ADE2*
SCY62	*MAT***a** *ADE2*
H1111	*MATα are1-Δ::HIS3 ADE2*
H1112	*MATα are1-Δ::HIS3 are2-Δ::LEU2 ADE2*
H1228	*MAT***a** *dga1-Δ::KanMX4 ADE2*
H1025	*MAT***a** *lro1-Δ::URA3 ADE2*
H1226	*MAT***a** *lro1-Δ::URA3 dga1-Δ::KanMX4 ADE2*
H1262	*MATα are1-Δ::HIS3 are2-Δ::LEU2 lro1-Δ::URA3 ADE2*
H1237	*MATα are1-Δ::HIS3 are2-Δ::LEU2 dga1-Δ::KanMX4 ADE2*
H1236	*MATα are1-Δ::HIS3 dga1-Δ::KanMX4 lro1-Δ::TRP1 ADE2*
H1266	*MAT***a** *are2-Δ::LEU2 dga1-Δ::KanMX4 lro1-Δ::TRP1 ADE2*
H1246	*MATα are1-Δ::HIS3 are2-Δ::LEU2 dga1-Δ::KanMX4 lro1-Δ::TRP1 ADE2*

in diameter) using a Mini Beadbeater-8 (Biospec Products) for three cycles of 60 s. Aliquots corresponding to 200 μg of protein were diluted to a total volume of 90 μl. To each aliquot, 10 μl of [^{14}C]palmitoyl-CoA (20 nmol, 5000 dpm/nmol) and di-6:0 diacylglycerol (5 nmol) were added. The suspension was thoroughly mixed and incubated at 30 °C for 15 min. Lipids were extracted into chloroform (19) and separated by TLC on Silica Gel 60 plates (Merck). The plate was first developed in chloroform/methanol/acetic acid/water (85:15:10:3.5) for 80 mm. The dried plate was then developed in hexane/diethyl ether/acetic acid (70:30:1.5) for 180 mm using an automatic developing chamber (Camag). The radioactive lipids were quantified by electronic autoradiography (Instant Imager, Packard Instrument Co.).

Microscopy—Aliquots of stationary phase cells (400 μl) were pelleted, washed twice in water, dissolved in 25 μl of water, and then stained with Nile Red (20). The stained cells were incubated in the dark for 10 min, after which they were washed in water and then diluted into 100 μl of water. Prior to microscopy, the cells were mixed with ProLong Anti-fade reagent (Anti-fade kit, P-7481, Molecular Probes, Leiden, The Netherlands). Images were obtained using a Zeiss Axioscop 2 MOT (Carl Zeiss, Göttingen) equipped with an HBO 100 arc lamp system and a 63× plan apochromat objective with a fluorescein isothiocyanate fluorescence filter, using an excitation wavelength of 480–520 nm and emission at 510 nm. For the interference contrast images, a Nomarski prism was used instead of the fluorescence filter.

RESULTS

Four Genes Contribute to Storage Lipid Synthesis in Yeast—We wanted to determine whether *DGA1*, *LRO1*, *ARE1,* and *ARE2* are the only genes that contribute to storage lipid synthesis in yeast or whether other lipid-synthesizing pathways remain to be discovered. We additionally wanted to estimate the relative contribution of each gene to TAG and steryl ester synthesis. To this end, we made two sets of yeast strains (Table I). In the first set, each strain was disrupted for one of the four genes under study. A comparison of these single disrupted strains to the wild type allowed us to determine to what extent removal of one of the genes will reduce steryl ester or TAG accumulation. However, it is possible that up-regulation of one enzyme may compensate for the loss of another enzyme, particularly in the case of duplicated genes such as *ARE1* and *ARE2*. We therefore also made a set of triple disrupted strains in which each strain expresses only one of the four genes. These strains allow us to determine the contribution to storage lipid synthesis of each enzyme in isolation. Finally, we also made a quadruple disrupted strain that lacks all four genes.

We found that all seven strains are viable with normal growth rates. However, the final density of the quadruple disrupted strain in stationary phase is 75% of the wild type. Cell counting showed that this reflects a reduced cell number. The final densities of the other six strains all fell in the range between the wild type and the quadruple disrupted strain. Because storage lipids accumulate mainly during late exponential and stationary phase, we proceeded to analyze the lipid content of each strain after entry into stationary phase. We found that the lipid composition is strongly affected in several of the strains. Most significantly, the quadruple disrupted strain lacked storage lipids (Fig. 1). Because storage lipids constitute a major part of the lipids in stationary phase cells, the total lipid content is therefore reduced to 29% of the wild type strain (Fig. 1). From this we conclude that the four genes under study are the only genes that contribute to storage lipid synthesis in yeast, and also that storage lipids are not essential for growth. It should be noted, however, that the quadruple disrupted strain shows a reduced survival after nitrogen starvation (data not shown), indicating that the ability to accumulate storage lipids is important for long term survival in stationary phase.

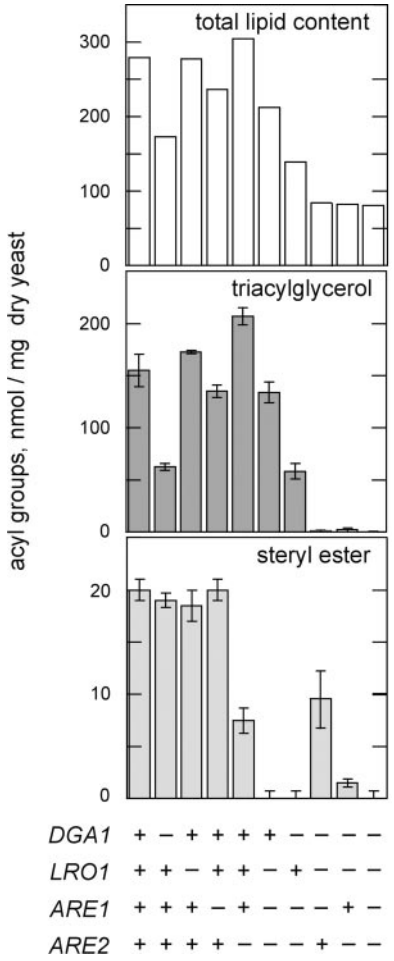

FIG. 1. **Lipid composition of wild type, single, triple, and quadruple disrupted yeast strains in stationary growth phase.** The yeast strains used are listed in Table I. The presence or absence of specific genes in each strain is indicated at the *bottom* of the figure. *Open bars* show the total amount of lipid, *dark gray bars* the amount of TAG, and *light gray bars* the amount of steryl esters, all expressed in nanomoles of acyl group per mg of dry yeast. The data for total amount of lipid are mean values from two independent experiments, and the data for triacylglycerol and steryl esters are the mean ± S.E. of four experiments.

To determine to what extent the absence of storage lipid synthesis in the quadruple disrupted strain affects the lipid composition in general, we also measured the amounts of fatty acid, diacylglycerol, and polar lipid in both exponential and stationary phase cells. As shown in Table II, there are only minor differences between the quadruple disrupted strain and the wild type in exponential phase, where the amount of storage lipids is low. In stationary phase, the quadruple disrupted strain has a 2.5-fold increase in fatty acids and a 3.7-fold decrease in diacylglycerol, as compared with the wild type

TABLE II
Lipid composition of wild type and quadruple disrupted yeast strains

The numbers shown are nanomoles of acyl groups per mg dry weight yeast. Values are given as the mean ± S.E. ($n = 4$).

	Exponential		Stationary	
	Wild type	H1246	Wild type	H1246
Steryl ester	0.2 ± 0.2	0.0 ± 0.0	16.5 ± 0.5	0.2 ± 0.1
Triacylglycerol	17.7 ± 1.0	0.2 ± 0.0	160.2 ± 2.2	0.3 ± 0.3[a]
Fatty acid	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	2.5 ± 0.1
Diacylglycerol	5.3 ± 0.2	4.4 ± 0.1	15.6 ± 1.1	4.2 ± 0.2
Polar lipids	93.5 ± 1.0	88.4 ± 0.1	84.9 ± 0.8	100.6 ± 0.7

[a] S.D. ($n = 3$).

TABLE III
Incorporation of palmitoyl-CoA into TAG in yeast extracts

The incorporation of [^{14}C]palmitoyl-CoA into TAG was assayed *in vitro* as described under "Experimental Procedures." The data shown is the mean ± S.E. ($n = 4$).

Strain	Expressed gene	Labeled TAG
		% wild type
H1262	*DGA1*	112.2 ± 6.3
H1237	*LRO1*	3.3 ± 0.3
H1236	*ARE2*	1.0 ± 0.1
H1266	*ARE1*	1.7 ± 0.1
H1246	*None*	0.4 ± 0.1

(Table II). These changes are significant but should be interpreted with some caution, particularly in the case of fatty acids that are easily generated by lipid hydrolysis during the extraction procedure (5).

Contribution of Each Gene to TAG and Steryl Ester Synthesis in Vivo—It is evident that all four genes contribute to TAG synthesis *in vivo*, because only the quadruple disrupted strain is completely devoid of TAG (Fig. 1). However, the contribution of each gene varies significantly. Thus, the triple disrupted strains carrying only the *DGA1* or *LRO1* gene synthesizes TAG at 87 and 38% of the wild type level, respectively (Fig. 1). In contrast, triple disrupted strains carrying *ARE1* or *ARE2* synthesize only minute, but still detectable, amounts of TAG. We conclude that the DGAT2 enzyme encoded by *DGA1* is the major TAG-synthesizing enzyme in yeast. Consistent with this, the *dga1* strain was the only single disrupted strain where the amount of TAG, and also the total amount of fatty acids, was significantly reduced (Fig. 1).

Unlike the case with TAG, and consistent with previous findings (7–9), we found that only two of the genes, *ARE1* and *ARE2*, contribute to steryl ester accumulation. Thus, triple disrupted strains carrying only *DGA1* or *LRO1* lack steryl esters (Fig. 1). We further note that although *ARE1* and *ARE2* are the only two genes involved in steryl ester synthesis, the amount of steryl esters made by the strain carrying only *ARE1* (8% of wild type) or *ARE2* (48% of wild type) does not add up to 100%. However, steryl ester synthesis is significantly higher in the corresponding single disrupted strains, *i.e.* when either *ARE1* or *ARE2* is present together with *DGA1* and *LRO1*.

Contribution of Each Gene to Acyl-CoA-dependent TAG Synthesis in Vitro—We wanted to determine to what extent the observed *in vivo* effects on TAG levels correlate with TAG-synthesizing DGAT activity *in vitro*. We therefore proceeded to assay extracts from different yeast strains for DGAT activity (Table III). We found that although extracts from the strain expressing only *DGA1* have an acyl-CoA-dependent TAG synthesis that is comparable with that of the wild type, the activity is much lower or absent in extracts from the other strains. This suggests that the DGAT2 enzyme encoded by *DGA1* is responsible for most of the DGAT activity in wild type yeast cells. The triple disrupted strains expressing either *ARE1* or *ARE2* also have some DGAT activity, although 60–100-fold lower than the wild type. This is consistent with the notion that the small amount of TAG present in these strains is made in an acyl-CoA-dependent reaction. Extracts from the strain expressing only *LRO1*, which should be devoid of DGAT activity, also incorporate acyl groups from acyl-CoA into TAG. This is most likely due to an acyl-CoA:lysophospholipid acyltransferase that is present in yeast (21). It will generate labeled phospholipid, which can be metabolized into TAG in a reaction catalyzed by PDAT, the enzyme encoded by *LRO1*. Accordingly, the limited TAG formation in this strain would not reflect a DGAT activity of LRO1 but rather its PDAT activity described previously (16, 17). Extracts from the quadruple disrupted strain produced very small amounts of labeled TAG. When analyzed further, we came to the conclusion that these minute amounts of TAG are produced non-enzymatically, as reported previously (22).

Altered Fatty Acid Composition in Yeast Strains Lacking DGA1—The major fatty acids that accumulate in yeast are palmitic, palmitoleic, stearic, and oleic acids. In addition, trace amounts of lauric, myristic, and *cis*-vaccenic acid are also found. To determine whether a reduced or absent storage lipid synthesis would affect the balance between different fatty acids, we proceeded to analyze the fatty acid composition in our wild type, triple disrupted, and quadruple disrupted strains (Table IV). Interestingly, we found that whereas the triple disrupted strain carrying only *DGA1* has a fatty acid composition that is similar to that of the wild type strain, the other triple disrupted strains, which lack *DGA1*, accumulate significant amounts of vaccenic acid. Moreover, the amount of palmitoleic acid is also elevated in these strains, whereas the amount of palmitic acid is reduced as compared with the wild type (Table IV). The relative distribution of the fatty acids was similar in steryl esters, TAG, and total lipids (data not shown). We conclude that the fatty acid composition of the cell is altered in yeast strains that lack the DGAT2 enzyme encoded by *DGA1*, perhaps as a consequence of the reduced TAG synthesis in these strains.

Lipid Bodies Are Absent in the Quadruple Disrupted Yeast Strain—Because the main content of the lipid bodies that accumulate during stationary phase are TAG and steryl esters, we wanted to determine whether they could be formed in the absence of either of these lipids. The lipid bodies can be visualized using the fluorescent dye Nile Red (20). We therefore stained yeast cells that had been grown to late stationary phase with this dye. As shown in Fig. 2, we found that whereas lipid bodies are abundantly present in the wild type (Fig. 2*A*), they are completely absent in the quadruple disrupted strain (Fig. 2*F*). We next examined the role of each gene in lipid body formation, using the triple disrupted strains. We found that all four strains contain lipid bodies (Fig. 2, *B–E*), whose prominence appears to correlate with the storage lipid content (Fig. 1). The fact that the strains expressing only *DGA1* or *LRO1*, which contain only TAG but no steryl esters, are able to form lipid bodies shows that steryl esters are not required for their formation. We were not able to test whether TAG is required for their formation in the presence of steryl esters, because the strains expressing only *ARE1* or *ARE2* still make small amounts of TAG (Fig. 1). From these experiments we conclude that lipid bodies fail to form in the absence of both storage lipids and also that these organelles are not essential for growth under normal conditions.

DISCUSSION

In the present investigation, we show that four yeast genes, *ARE1*, *ARE2*, *LRO1*, and *DGA1*, contribute to TAG and steryl ester accumulation, and that no other pathways exist for the synthesis of these storage lipids in yeast (Fig. 1). We further

TABLE IV
Fatty acid composition of total lipid in stationary phase yeast cells

Results are expressed as percent of the total fatty acid in each strain and represent the mean ± S.E. (H1262, H1266, and H1236, $n = 10$; wild type, $n = 9$; H1246, $n = 8$; H1237, $n = 7$).

	Wild type,[a] all four	H1262, *DGA1*	H1237, *LRO1*	H1236, *ARE2*	H1266, *ARE1*	H1246, None
Palmitic acid (16:0)	15.4 ± 0.1	10.9 ± 0.1	7.7 ± 0.1	10.8 ± 0.1	8.6 ± 0.1	8.1 ± 0.2
Palmitoleic acid (16:1 Δ^9)	29.8 ± 0.3	31.7 ± 0.2	37.0 ± 0.4	37.8 ± 0.3	33.3 ± 0.2	32.5 ± 0.1
Stearic acid (18:0)	9.9 ± 0.2	9.7 ± 0.1	7.9 ± 0.1	7.7 ± 0.1	9.5 ± 0.1	9.5 ± 0.1
Oleic acid (18:1 Δ^9)	44.6 ± 0.3	47.6 ± 0.3	44.1 ± 0.4	40.9 ± 0.3	40.9 ± 0.1	42.7 ± 0.3
Vaccenic acid (18:1 Δ^{11})	0.1 ± 0.1	0.1 ± 0.1	3.3 ± 0.1	3.4 ± 0.2	7.6 ± 0.1	7.3 ± 0.1

[a] Strains are indicated with the expressed gene.

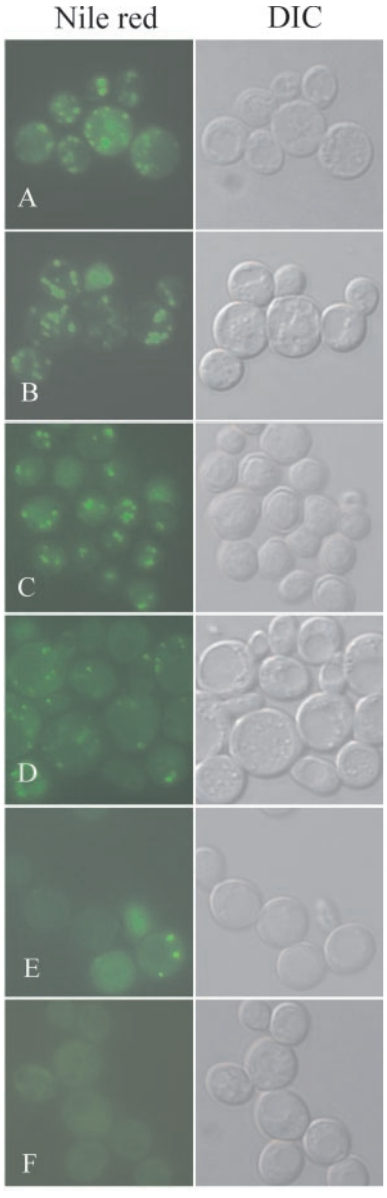

FIG. 2. **Nile Red staining (*left*) and interference contrast images (*right*) of wild type, triple, and quadruple disrupted yeast cells in stationary growth phase.** *A*, wild type cells. *B–E*, triple disrupted strains expressing *DGA1* (*B*), *LRO1* (*C*), *ARE2* (*D*), and *ARE1* (*E*), respectively. *F*, the quadruple disrupted strain.

show that storage lipid synthesis is required for lipid body formation (Fig. 2) and that neither storage lipids nor lipid bodies are essential for growth. The ability to accumulate storage lipids does, however, influence long term survival of stationary phase cells, as evidenced by a reduced survival of the quadruple disrupted strain during nitrogen starvation.

The DGAT2 encoded by *DGA1* (14) seems to be the most important enzyme for TAG synthesis in yeast. Thus, the strain expressing only *DGA1* has 87% of the wild type TAG level (Fig. 1). Conversely, the amount of TAG (and also total lipids) is significantly reduced in the single disrupted strain lacking *DGA1*, which shows that none of the other genes can fully substitute for its role in TAG synthesis. The second most important gene involved in TAG synthesis clearly is *LRO1*, which encodes the PDAT enzyme (16, 17). Thus, the strain expressing only *LRO1* has 38% of the wild type TAG level, and the TAG content is somewhat reduced in the strain lacking *LRO1* (Fig. 1). Interestingly, we further found that both *ARE1* and *ARE2* contribute to TAG synthesis, although at a much lower level. This is evident from the strains expressing only *ARE1* or *ARE2*, both of which contain small but still detectable amounts of TAG. Because some plant and animal homologues of *ARE1* and *ARE2* possess DGAT activity (12, 13), it is likely that this reflects a weak inherent DGAT activity in the two yeast enzymes.

Consistent with previous observations (7–9), we found that steryl ester accumulation is dependent only on *ARE1* and *ARE2*. The amounts of steryl esters made in strains carrying only *ARE1* or *ARE2* do not add up to wild type levels, but steryl ester accumulation is much higher in the corresponding single disrupted strains carrying *DGA1* and *LRO1* together with either *ARE1* or *ARE2* (Fig. 1). This suggests that although *DGA1* and *LRO1* do not themselves contribute to steryl ester synthesis, their presence does permit a more efficient accumulation of steryl esters. It is conceivable that this reflects a more efficient lipid body formation in the presence of normal amounts of TAG. We further note while *ARE2* is the most important gene for steryl ester synthesis, *ARE1* makes a comparatively larger contribution to TAG accumulation. It is conceivable that this reflects the same kind of functional differentiation that produced two distinct but related enzymes, DGAT1 and ASAT, in higher eukaryotes (10–13). It should be noted, however, that the *ARE1*- and *ARE2*-encoded proteins are much more similar to each other than either of them is to the ASATs and DGAT1s in higher eukaryotes. We conclude that *ARE1* and *ARE2* are not direct homologues of the latter, but rather the products of a more recent gene duplication in yeast.

Besides the absence of triacylglycerol and steryl ester, the quadruple disrupted strain also fails to accumulate diacylglycerol in stationary phase (Table II). This is in contrast to the wild type, which has 3 times more diacylglycerol in stationary phase than during exponential growth. Diacylglycerol is still present in the quadruple disrupted strain but only at the lower level seen in exponential phase. This finding suggests that the increased diacylglycerol level in wild type stationary phase cells is causally related to the increased synthesis of storage lipids, notably triacylglycerol. We further found that the fatty acid composition is altered in yeast cells that lack the TAG-synthesizing DGAT2 encoded by *DGA1* (Table IV). Thus, there is an increase in both vaccenic and palmitoleic acid, while the amount of palmitic acid is reduced. The reason for these changes is not known, but we note that palmitoyl-CoA is converted to palmitoleoyl-CoA and then to vaccenoyl-CoA in two consecutive reactions catalyzed by a desaturase and an elon-

gase, respectively. Reducing or eliminating the acyl-CoA-dependent TAG synthesis is likely to affect both the size and the composition of the acyl-CoA pool, with resulting effects also on fatty acid composition.

In conclusion, we have shown that *DGA1*, *LRO1*, *ARE1,* and *ARE2* are the only yeast genes involved in storage lipid synthesis, and also that the DGAT2 encoded by *DGA1* is the most important enzyme for TAG synthesis. Finally, we have shown that neither storage lipid synthesis nor lipid body formation is essential for growth in yeast. It has been proposed that the latter do not simply serve as inert lipid stores but also play an important role in the biosynthesis, mobilization, and trafficking of intracellular lipids (1, 6). It is conceivable that lipid bodies are important for some of these functions; however, our results suggest that a process that is exclusively localized to the lipid bodies or in some other way dependent on them cannot be essential for growth.

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REFERENCES

1. Murphy, D. J., and Vance, J. (1999) *Trends Biochem. Sci.* **24,** 109–115
2. Zweytick, D., Athenstaedt, K., Daum, G. (2000) *Biochim. Biophys. Acta* **1469,** 101–120
3. Clausen, M. K., Christiansen, K., Jensen, P. K., and Benke, O. (1974) *FEBS Lett.* **43,** 176–179
4. Leber, R., Zinser, E., Zellnig, G., Paltauf, F., and Daum, G. (1994) *Yeast* **10,** 1421–1428
5. Rattray, J. B. M. (1988) in *Microbial Lipids* (Ratledge, C. I., and Wilkinson, S. G., eds) Vol. 1, pp. 555–697, Academic Press, London
6. Leber, R., Landl, K., Zinser, E., Ahorn, H., Spök, A., Kohlwein, S. D., Turnowsky, F., and Daum, G. (1998) *Mol. Biol. Cell* **9,** 375–386
7. Yang, H., Bard, M., Bruner, D. A., Gleeson, A., Deckelbaum, R. J., Aljinovic, G., Pohl, T. M., Rothstein, R., and Sturley, S. L. (1996) *Science* **272,** 1353–1356
8. Yu, C., Kennedy, N. J., Chang, C. C., and Rothblatt, J. A. (1996) *J. Biol. Chem.* **271,** 24157–24163
9. Zweytick, D., Leitner, E., Kohlwein, S. D., Yu, C., Rothblatt, J., and Daum, G. (2000) *Eur. J. Biochem.* **267,** 1075–1082
10. Chang, C. C. Y., Huh, H. Y., Cadigan, K. M., and Chang, T. Y. (1993) *J. Biol. Chem.* **268,** 20747–20755
11. Meiner, V. L., Cases, S., Myers, H. M., Sande, E. H., Bellosta, S., Schambelan, M., Pitas, R. E., McGuire, J., Herz, J., and Farese, R. V., Jr. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 14041–14046
12. Cases, S., Smith, S. J., Zheng, Y. W., Myers, H. M., Lear, S. R., Sande, E., Novak, S., Collins, C., Welch, C. B., Lusis, A. J., Erickson, S. K., and Farese, R. V., Jr. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 13018–13023
13. Routaboul, J. M., Benning, C., Bechtold, N., Caboche, M., and Lepiniec, L. (1999) *Plant Physiol. Biochem.* **37,** 831–840
14. Lardizabal, K. D., Mai, J. T., Wagner, N. W., Wyrick, A., Voelker, T., and Hawkins, D. J. (2001) *J. Biol. Chem.* 276, 38862–38869
15. Cases, S., Stone, S., Zhou, P., Yen, E., Tow, B., Lardizabal, K. D., Voelker, T., and Farese, R. V., Jr. (2001) *J. Biol. Chem.* **276,** 38870–38876
16. Dahlqvist, A., Ståhl, U., Lenman, M., Banas, A., Lee, M., Sandager, L., Ronne, H., and Stymne, S. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 6487–6492
17. Oelkers, P., Tinkelenberg, A., Erdeniz, N., Cromley, D., Billheimer, J. T., and Sturley, S. L. (2000) *J. Biol. Chem.* **275,** 15609–15612
18. Thomas, B. J., and Rothstein, R. (1989) *Cell* **56,** 619–630
19. Bligh, E. G., and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37,** 911–917
20. Greenspan, P., Mayer, E. P., and Fowler, S. D. (1985) *J. Cell Biol.* **100,** 965–973
21. Richard, M. G., and McMaster, C. R. (1998) *Lipids* **33,** 1229–1234
22. Stobart, K., Mancha, M., Lenman, M., Dahlqvist, A., and Stymne, S. (1997) *Planta* **203,** 58–66

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