A Lecithin Cholesterol Acyltransferase-like Gene Mediates Diacylglycerol Esterification in Yeast*

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The terminal step in triglyceride biosynthesis is the esterification of diacylglycerol. To study this reaction in the model eukaryote, Saccharomyces cerevisiae, we investigated five candidate genes with sequence conservation to mammalian acyltransferases. Four of these genes are similar to the recently identified acyl-CoA diacylglycerol acyltransferase and, when deleted, resulted in little or no decrease in triglyceride synthesis as measured by incorporation of radiolabeled oleate or glycerol. By contrast, deletion of LRO1, a homolog of human lecithin cholesterol acyltransferase, resulted in a dramatic reduction in triglyceride synthesis, whereas overexpression of LRO1 yielded a significant increase in triglyceride production. In vitro microsomal assays determined that Lro1 mediated the esterification of diacylglycerol using phosphatidylcholine as the acyl donor. The residual triglyceride biosynthesis that persists in the LRO1 deletion strain is mainly acyl-CoA-dependent and mediated by a gene that is structurally distinct from the previously identified mammalian diacylglycerol acyltransferase. These mechanisms may also exist in mammalian cells.

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Triglyceride (TG) biosynthesis is a common method of energy storage and thus has an important role in energy balance. In humans, overaccumulation of TG, either as obesity or elevated serum triglyceride, has been shown to be an independent risk factor for a variety of diseases including diabetes (1) and atherosclerosis (2, 3). In the pathway described by Kennedy (4) for glyceride and glycerophosphate synthesis, a branch point is reached at diacylglycerol (DG) that can serve as a precursor for several phospholipid species and as a substrate for acyl-CoA, diacylglycerol O-acyltransferase (DGAT) (EC 2.3.1.20), which catalyzes the terminal step in TG synthesis. Expression of a recently identified mammalian DGAT cDNA in insect and mammalian cells conferred elevated triglyceride synthesis but did not change incorporation of fatty acids into sterol ester (5–7). The DGAT gene belongs to the acyl-CoA cholesterol acyltransferase (ACAT) gene family that includes two mammalian ACATs (ACAT1, ACAT2) and two yeast ACAT-related enzymes (ARE1, ARE2) that catalyze intracellular sterol esterification (8). Whereas yeast can synthesize TG from oleoyl-CoA and DG, deletion mutants in ARE1 and ARE2 do not reduce [3H]oleate incorporation into TG (9). Therefore, a conspicuous absence from the ACAT gene family is a yeast DGAT.

Further examination of the Saccharomyces cerevisiae genome data base revealed two DGAT-like genes in addition to the ARE genes. We show here that these four genes do not have a major role in TG synthesis in yeast. By contrast, a yeast gene with sequence similarity to mammalian lecithin-cholesterol acyltransferase (LCAT) (EC 2.3.1.43) catalyzes the esterification of DG using phosphatidylcholine as the acyl donor. This novel enzymatic reaction mediates the majority of TG synthesis in the yeast cell during exponential growth.

EXPERIMENTAL PROCEDURES

General—Molecular biology and genetic procedures were performed according to conventional protocols (10). Transformation of yeast was performed with lithium acetate followed by prototrophic selection (11). Yeast extract, yeast nitrogen base, Bacto-peptone, and Bacto-agar were from Difco. D-Glucose, D-galactose, and D-raffinose were from Sigma. Complete (yeast extract, peptone, dextrose;YPD) and synthetic complete (SC) were prepared as described (10). Oleoyl coenzyme A, triolein, and 1,2-dioleoyl-sn-glycerol were from Sigma. Egg phosphatidylcholine was from Avanti Polar Lipids. [2-3H]Glycerol, [9,10-3H(N)]oleic acid, [3H]triolein, and [9,10-3H(N)]palmitic acid were from NEN Life Science Products. [1-14C]Oleoyl-CoA, 1-stearoyl-2-[1-14C]arachidonoyl-sn-glycerol, and 1-palmitoyl-2-[1-14C]oleoyl-1,3-phosphatidylcholine were from Amersham Pharmacia Biotec. Sequence searches were performed using the tblastn program (12), and comparisons were performed using the GAP program (13).

Yeast Strains—Yeast strains with single (SCY060, SCY061) or double (SCY0609) deletions in the ARE genes have been described previously (9). Deletion mutant strains for YGL084c, YPL189w, and LRO1 (YNR008w) were generated in isogenic W303-1B diploids (MATa/MATa ade2-1, can1-1, trpl-1, ura3-1, his3-11, 15, leu2-3, 112) by homologous recombination with PCR products generated using the following oligos, which contained 50–72 bp of gene-specific sequence and 21 bp of Kluyveromyces lactis URA3 sequence (14–16). YGL084C KO oligos were as follows: 8AF, 5’ ATGTCGCTGATCAGCATCCTGTC- TCCCCTAATTACTTCCGAGGGCTTAGACATGGCAATTCCCGGGG- 9O

1 The abbreviations used are: TG, triglyceride; DG, diacylglycerol; DGAT, diacylglycerol O-acyltransferase; ACAT, acyl-CoA cholesterol acyltransferase; ARE, ACAT-related enzymes; LCAT, lecithin-cholesterol acyltransferase; YPD, yeast extract, peptone, dextrose; SC, synthetic complete; PCR, polymerase chain reaction; bp, base pair; log, logarithmic; BSA, bovine serum albumin; PL, phospholipid.
Novel Mechanisms of Triglyceride Synthesis

RESULTS AND DISCUSSION

Deletion of Candidate Yeast DGATs—We screened the S. cerevisiae genome using the human DGAT sequence with the hypothesis that this enzyme would be conserved across kingdoms. As expected, the yeast sterol esterification enzymes, Are1 and Are2 (9), which share 25 and 28% overall identity with DGAT, respectively, were identified. In addition, two uncharacterized open reading frames, YGL084c and YPL189w, were identified with a lower degree of conservation. The identity (26 and 21%, respectively) was restricted to a COOH-terminal region of 146 amino acids where the maximal conservation among Are1, Are2, and DGAT occurs. The YGL084c and YPL189w predicted proteins are 51% identical to each other and were not identified when human ACAT1 was used to screen the yeast genome. To assess the role of these four DGAT-like yeast genes in triglyceride synthesis, we used are1Δ and are2Δ mutants created previously (9) and generated YGL084c and YPL189w deletion mutants. To address the possibility of redundant enzymes, a yeast strain (4XΔ) was created where all four DGAT-like genes were deleted.

Characterization of Deletion Mutants—Growth of the mutants at a variety of temperatures (15, 30, and 37 °C) showed the ygl084cΔ and 4XΔ strains to be temperature-sensitive at 37 °C and to reach mid-log phase about 4 h later than normal strains after dilute inoculation into YPD at 30 °C. The other mutations caused no detectable growth defects. The ability of these strains to synthesize triglyceride was assayed by pulse labeling cells in log phase with either 1-14Coleate or 1-3Holeate, as shown in Tables I and II, respectively. Only the are1Δ mutant showed a reduction in glycerol incorporation into TG. This difference was not observed with oleate labeling or palmitate labeling (data not shown), suggesting Are1 to be, at most, a minor contributor to TG synthesis. By contrast, deletion of ygl084c resulted in an increase in TG synthesis with a concomitant decrease in phospholipid (PL) synthesis. The nature of this biochemical effect is under further investigation.

A Candidate Diacylglycerol Acyltransferase with Identity to Human LCAT—Because abundant TG synthesis remained in the absence of the four yeast DGAT-like enzymes, we sought alternate candidates. Under experimental conditions, porcine LCAT has been shown to esterify diacylglycerol in addition to cholesteryl (26, 27). The yeast genome contains only one gene, LRO1 (LCAT-related open reading frame, YNR008w), with distinct sequence similarity to human LCAT. Aligning the LRO1 predicted protein with human LCAT (28) shows 27% overall identity with conservation of Ser181 and Asp345, two members of the LCAT catalytic triad (29, 30). The third amino acid of the triad, His377, is aligned within four amino acids of a histidine in the Lro1 protein. Lro1, like LCAT, also contains a serine lipase motif (V. IUVGHIS. G) at amino acids 318–326. However, Lro1 is unlikely to be a functional LCAT homolog, a final volume of 200 μl for 15 min. at 23 °C. DGAT activity was assayed as described (22), with the addition of phospholipid liposomes (23). The final concentration of assay components was 150 mM Tris, pH 7.8, 15 μM BSA, 150 μM DAG, 8 μM MgCl2, 150 μM phosphatidylserine/phosphatidylcholine liposomes (1:1 molar ratio), 50 μM [1-14C]oleoyl-CoA (200,000 dpm/nmol), and 80 μg of microsomal protein. The diacylglycerol transacylase assay (24) was the same as for DGAT except oleoyl-CoA, BSA, and MgCl2 were omitted, and radiolabeled DG (1-stearoyl-2-[1-14C]arachidonyl-sn-glycerol, 20,000 dpm/nmol) was included. For the lecinthin/diacylglycerol transferase assay, oleoyl-CoA was omitted, and 7.5 μg of purified nonspecific lipid transfer protein was added to facilitate transfer of phosphatidylcholine (1-palmitoyl-2-[14C]oleoyl-1,2-diacylglycerol) between liposomes and microsomes (25). All reactions were stopped by the addition of chloroform/methanol (2:1). 15 μg of [3H]triolein was added as an internal standard/capillary, and the lipids were separated by TLC in hexane-diethyl ether:acetic acid:water:ether (170:30:1:0).

Isolation of Microsomes—A dilute inoculation of normal or lro1Δ haploids into 500 ml of YPD was grown overnight at 30 °C into log phase. The cells were washed and lysed, and microsomes prepared from a 100,000 × g spin as described previously (20). Protein concentrations were determined as described (21).

In Vitro Assays—All triglyceride synthesis assays were performed in 2 A. Tinkelenberg and S. L. Sturley, manuscript in preparation.
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Analysis of LRO1 by Deletion—LRO1 deletion strains showed no detectable growth defects at 15, 30, and 37 °C. Strains harboring a deletion of LRO1 were grown into log phase and pulse labeled with [3H]oleate as described under “Experimental Procedures.” The method of data expression and statistical significance are as described for Table I. Asterisks denote significant difference (p < 0.01) compared with normal strains.

### Table I

| Genotype   | TG % | DG % | PL % |
|------------|------|------|------|
| Normal     | 13.6 ± 2.9 | 12.9 ± 2.9 | 57.0 ± 8.9 |
| are1Δ      | 13.3 ± 1.0 | 12.2 ± 1.1 | 57.7 ± 2.2 |
| are2Δ      | 17.6 ± 1.7* | 13.2 ± 0.9 | 61.6 ± 2.4 |
| ygl084Δ    | 24.6 ± 2.6* | 20.5 ± 2.7* | 38.6 ± 6.9* |
| ypl189Δ    | 16.1 ± 1.2 | 16.4 ± 1.0* | 49.9 ± 3.6* |
| 4XA        | 26.7 ± 1.0* | 27.9 ± 3.2* | 38.7 ± 3.8* |
| lro1Δ      | 4.5 ± 1.0* | 15.7 ± 3.5 | 62.8 ± 7.3 |
| 5XΔ        | 6.0 ± 1.3* | 28.4 ± 3.7* | 50.8 ± 5.9 |

### Table II

| Genotype   | TG % | DG % | PL % |
|------------|------|------|------|
| Normal     | 26.9 ± 3.3 | 23.2 ± 2.3 | 46.4 ± 5.6 |
| are1Δ      | 21.8 ± 4.1* | 17.7 ± 2.7* | 56.3 ± 8.1* |
| are2Δ      | 25.2 ± 2.5 | 18.7 ± 1.4* | 50.4 ± 4.2 |
| ygl084Δ    | 36.1 ± 4.7* | 24.3 ± 1.5 | 34.9 ± 4.1* |
| ypl189Δ    | 27.5 ± 3.6 | 20.0 ± 2.4 | 50.4 ± 4.6 |
| 4XA        | 27.8 ± 3.1 | 18.7 ± 2.6* | 43.9 ± 5.2 |
| lro1Δ      | 7.0 ± 0.6* | 38.6 ± 5.6* | 51.2 ± 4.5* |
| 5XΔ        | 5.9 ± 2.9* | 29.7 ± 3.1* | 55.8 ± 6.4* |

### Table III

Effects of LRO1 expression on [3H]oleate incorporation into TG

Normal and lro1Δ strains were transformed with a vector control, low copy (LRO1/pRS413), or high copy (LRO1/pRS426-GP) expression plasmids. The strains were grown to log phase and pulse labeled with [3H]oleate as described under “Experimental Procedures.” The method of data expression is as described for Table I. Asterisks denote significant difference (p < 0.01) compared with normal strain transformed with vector control.

| Genotype   | Transformed with | TG %  | DG %  | PL %  |
|------------|------------------|-------|-------|-------|
| lro1Δ      | Vector           | 6.4 ± 1.3* | 24.7 ± 2.8* | 50.4 ± 5.8* |
| Normal     | Vector           | 26.1 ± 2.3 | 14.7 ± 3.8 | 41.8 ± 4.5 |
| Normal     | LRO1/pRS413      | 30.0 ± 0.6* | 16.2 ± 1.3 | 40.6 ± 3.4 |
| Normal     | LRO1/pRS413      | 28.5 ± 1.6 | 10.5 ± 1.4* | 44.0 ± 3.8 |
| lro1Δ      | LRO1/pRS426GP    | 36.4 ± 2.2* | 11.5 ± 0.9* | 37.7 ± 2.7* |
| Normal     | LRO1/pRS426GP    | 44.7 ± 3.7* | 9.4 ± 1.0* | 32.7 ± 4.0* |
tionary phase. We have shown that yeast mainly utilizes enzymes without sequence similarity to mammalian DGAT to catalyze the terminal step in TG synthesis. DGAT-independent TG synthesis also occurs in mammals in that a deletion mutant mouse for DGAT retains significant triglyceride synthesis activity (34). Two qualitatively different DGAT activities identified in rat liver (35, 36) and an oleoyl-CoA-independent DG transacylase in rat intestine (24) further support the presence of more than one mammalian enzyme, with more than one mechanism, that can catalyze the terminal step in TG synthesis.

Lro1 may provide a paradigm for such an enzyme. Our in vitro assays identified a novel mechanism of synthesizing TG from lecithin that was absent in lro1 strains. The sequence similarity to mammalian LCAT and the use of a phospholipid substrate suggest that Lro1 uses an LCAT-like mechanism involving a phospholipase and an acyltransferase activity. This reaction accounts for the majority of TG synthesis in yeast but plays no detectable role in sterol esterification. Because the amount of TG synthesis was not further decreased when the four DGAT-like genes were deleted along with LRO1, there clearly exists uncharacterized acyl-CoA-dependent and -independent mechanisms(s) for TG synthesis in yeast.

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