We have identified two yeast genes with similarity to a human cDNA encoding acyl-coenzyme A:cholesterol acyltransferase (ACAT). Deletion of both yeast genes results in a viable cell with undetectable esterifiable sterol (Yang, H., Bard, M., Bruner, D. A., Gleeson, A., Deckelbaum, R. J., Aljinovic, G., Pohl, T., Rothstein, R., and Sturley, S. L. (1996) Science 272, 1353–1356). Here, we expressed the human cDNA in the yeast double mutant, resulting in high level production of ACAT protein, but low in vivo esterification of ergosterol, the predominant yeast sterol. The activity of the enzyme was increased by incubation of these cells with 25-hydroxy, cholesterol, an established positive regulator of mammalian sterol esterification. In contrast, the yeast enzymes were unaffected by this reagent. In vitro microsomal assays indicated no sterol esterification in extracts from the double mutant. However, significant activity was detected from strains expressing human ACAT when cholesterol was equilibrated with the microsomal membranes. The human enzyme in yeast utilized cholesterol as the preferred sterol and was sensitive to competitive (S58035) and non-competitive (DuP 128) ACAT inhibitors. The yeast esterifying enzymes exhibited a diminished sterol substrate preference and were sensitive only to S58035. Human ACAT had a broad acyl-CoA substrate specificity, the other substrate for this reaction. By contrast, the yeast enzymes had a marked preference for specific acyl-CoAs, particularly unsaturated C18 forms. These results confirm the yeast genes as functional homologs of the human gene and demonstrate that the enzymes confer substrate specificity to the esterification reaction in both organisms.

Free sterols are essential components of all eukaryotic membranes and exert a major influence on membrane fluidity and permeability and the activity of membrane-bound proteins (2). The esterification of sterol, i.e. the conjugation of fatty acids with sterols, plays an important role in sterol homeostasis, since it converts excess free sterol into a cytoplasmic storage form. In human cells, this esterification reaction is mediated by the enzyme, acyl-coenzyme A:cholesterol acyltransferase (ACAT (3)). ACAT activity has been localized to the rough endoplasmic reticulum and is present in all tissues tested (4). ACAT has several physiological functions with possible pathological consequences. In addition to maintaining intracellular cholesterol homeostasis, the ACAT reaction has been suggested to be involved in absorption of cholesterol from the intestinal lumen (5). Furthermore, the cholesterol ester synthesis rate in the liver may affect the secretion of very low density lipoprotein and thus plasma cholesterol and triglyceride levels (6). In adrenal cells, cholesterol ester is a sterol source for acute steroid hormone production. Most profoundly, the up-regulation of ACAT in macrophages and smooth muscle cells, accompanied by the accumulation of cholesterol ester in the arterial wall (foam cells), is an early event in the formation of an atherosclerotic plaque (7, 8).

Based on these functions of ACAT, pharmacological inhibitors of the esterification reaction have been proposed and developed as potential hypocholesterolemic and anti-atherogenic agents (9, 10). Such inhibitors could reduce cholesterol uptake in the intestine, increase the conversion of free cholesterol to bile acids in the liver, reduce very low density lipoprotein secretion, and decrease the accumulation of cholesterol ester in cells in the vascular wall. Despite the strong inhibitory effect of these compounds in vitro and in animal models, they have not been efficacious in lowering serum cholesterol in human trials (11–13). Whether the absorbable ACAT inhibitors may prevent foam cell formation is currently unknown. Sandoz 58035, one of the first specific ACAT inhibitors, is a competitive fatty acid homologue (14) while DuP 128 is a potent, non-competitive inhibitor of the reaction (IC50 of 10 nm (15, 16)). Compounds such as these, which inhibit mammalian ACAT by different mechanisms, may be useful in studying esterification in other organisms, and thus afford a more thorough overall understanding of the ACAT reaction.

The sterol esterification reaction is conserved from yeast to humans (17). In each organism, the predominant sterol is structurally distinct. In mammalian cells, the major sterol source is cholesterol; in yeast, it is ergosterol. Microsomal preparations from rat liver exhibit marked specificity of the ACAT reaction for cholesterol. Notably, the reaction was very sensitive to changes in the sterol side chain; the addition of a 24-β-methyl group, as in ergosterol, resulted in esterification at about 5% the rate of cholesterol. In contrast, yeast microsomal
preparations esterified both ergosterol and cholesterol to similar extents (18). The predominant fatty acyl-CoA in each species has not been as well studied. However, an order of preference for the rat liver enzyme was established as oleoyl > palmitoyl > stearoyl > linoleoyl-CoA (19). Whether the ACAT homologs in different species prefer one particular acyl-CoA substrate over others is unknown.

A comprehensive understanding of the ACAT reaction and the further development of useful ACAT inhibitors has been hampered by the inability to biochemically purify the ACAT enzyme to homogeneity. A human macrophage ACAT cDNA has recently been identified (20). By homology searching with homologs in different species prefer one particular acyl-CoA substrate over others is unknown.

The ensuing fragment contains a full-length human ACAT open reading frame directly downstream of the yeast GAL1/10 promoter (Fig. 1A).

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting Analysis—Yeast cells were grown overnight in SC-ura dextrose (2%) liquid medium. Cells were harvested at mid-log phase and washed with sterile water twice. The cells were resuspended in 10 ml of SC-ura medium containing 2% galactose and 1% raffinose and grown overnight. Cells were lysed by vortexing with glass beads in 0.01M borate, 0.15M NaCl, and 0.1% Tween 20 (TBST) and probed with 0.28 μg/ml of the

DM10 αACAT antibody (27) in TBST, 1% non-fat milk for 1 h. Detection of the immune complexes was attained using horseradish peroxidase-conjugated secondary anti-rabbit IgG antibody and the ECL Western blotting detection reagent (Amersham).

In Vivo Assays of Sterol Esterification—Yeast strains containing pRS426-ACAT or vector control were grown in 5 ml of SC-ura (2% glucose) medium to a density of 10^7 cells/ml. The cells were washed twice with sterile water, and then resuspended in 10 ml of SC-ura media containing 2% galactose and 1% raffinose. For experiments to test the effect of 25-hydroxycholesterol on sterol esterification, 10 or 25 μg/ml 25-hydroxycholesterol in ethanol was added to the growth medium at this point. After 4 h, 1 μCi/ml [3H]oleate in tyloxapoll/ethanol (1:1) was added and the cells were pulsed for a further 4 h. Total lipids were prepared and analyzed as described (1). Incorporation of label into ergosterol ester was determined after scintillation counting and normalization to a [14C]cholesterol internal standard and the dry weight of the cells. The data is expressed as means of triplicate assays over at least two experiments with the corresponding standard deviations.

In Vitro (Microsomal) Sterol Esterification Assay—Yeast cells containing pRS426-ACAT or the vector control were grown in 5 ml of SC-ura media containing 2% glucose to a density of about 10^6 cells/ml. The cultures were diluted to 500 ml of SC-ura media containing 2% glucose and grown overnight. The cells were harvested, washed twice with sterile water, and then resuspended in 500 ml of ura-CM media containing 2% galactose and 1% raffinose and allowed to grow for 6 h. Before freezing as a cell pellet. Frozen yeast pellets were quick-thawed at 37 °C, washed in 2 × pellet volumes of homogenization buffer (HB: 0.1 M potassium phosphate, 0.5 mM EDTA, 1 mM glutathione, 20 μM leupeptin, 10 μg/ml benzamidine, and 2 mM phenylmethylsulfonyl fluoride) and spun at 2,000 × g to re pellet the cells. The supernatant was removed and another 2 volumes of HB added to resuspend the cells. The suspension, was shaken with intermittent cooling in the presence of 1.0 g of 0.5-mm diameter glass beads in a mini-beadbeater (Biospec Products) at 5,000 rpm for 3 × 1-min intervals. The resulting homogenate was spun at 1,000 × g for 5 min and 15,000 × g for 15 min. The supernatant was removed and spun at 105,000 × g for 1 h to pellet the microsomes. This pellet was resuspended in ACAT buffer (0.1 M potassium phosphate, 1 mM GSH, pH 7.4) and protein concentration determined (28). Microsomes were aliquoted and frozen at −70 °C. Rat liver microsomes were prepared as described previously (29).

Enzyme activity was determined by the rate of incorporation of [14C]oleoyl-CoA or [14C]cholesterol into sterol ester (29). The standard assay, in duplicate or triplicate, contained 200 μg of microsomal protein, 1 mg of bovine serum albumin, 20 nmol of oleoyl-CoA, and 20 μg of cholesterol in a final volume of 200 μl of 0.1 M potassium phosphate buffer, pH 7.4, containing 1 mM glutathione. In control assays, labeled oleoyl-CoA or [14C]cholesterol was added after the reaction had been stopped. In assays where inhibitors were tested, the inhibitors were added in 5 μl of MeSO and compared to a solvent control of 5 μl of MeSO. SS8035 and DnF 128 were used at concentrations of 50 and 0.5 μM, respectively. For experiments on substrate preferences utilizing [14C]oleoyl-CoA (30,000 dpm/nmol), cold sterols were included whereas those utilizing [14C]cholesterol (40,000 dpm/nmol) were unlabeled acyl-CoAs. Where added, sterols were suspended in reaction buffer with the aid of Triton WR-1339 at a ratio of 30:1 (Triton/sterol, w/v) (29). After preincubation for 15 min at 37 °C, the reaction was initiated by the addition of oleoyl-CoA. The assay was stopped after 2.5 min by the addition of 4 ml of chloroform/methanol (2:1). Phase separation was indicated by the addition of water (800 μl) and [3H]oleoyl oleate (30,000 dpm) and 15 μg of cholesterol oleate were added as the internal standard and carrier, respectively. The chloroform layer containing the lipids was dried under nitrogen and resuspended in 100 μl of chloroform for spotting on ITLC-SA thin layer plates (Gelman Sciences). Lipids were separated and quantitated as described above. The specific activity of ACAT was determined as picomoles of either cholesterol or oleoyl-CoA converted to sterol ester/min/mg of protein.

RESULTS

To initiate a molecular analysis of sterol esterification in the eukaryotic micro-organism Saccharomyces cerevisiae (yeast), we identified two yeast gene products that exhibit significant structural and functional homology to the putative catalytic component of cholesterol esterification in human macrophages. We have shown that deletion of these genes produces a yeast cell lacking sterol ester (see Ref. 1 and Fig. 2). To confirm that
these genes encode catalytic components of the esterification complex we isolated microsomes from wild type, single and double are\(^{-}\) mutant yeast strains. The *in vitro* measurements of sterol esterification essentially mirrored the assays performed *in vivo*. Deletion of both *ARE1* and *ARE2* produces a strain devoid of microsomal ACAT activity. *ARE1* encodes the minor isoform in terms of its contribution to esterification, whereas Are2p is the major esterification enzyme (~80% of wild-type), *in vitro* and *in vivo*. The activity of the yeast isoforms in wild-type cells was elevated 2–3-fold after equilibration of exogenous cholesterol into microsomes prior to the assay (not shown). This increase in activity is similar to that observed in rat liver microsomes (29).

To compare and contrast the esterification activities conferred by the yeast and human enzymes, we expressed a human ACAT cDNA originally isolated from macrophages. The cDNA was fused to the divergent inducible promoter from the yeast GAL1/GAL10 genes in pRS426-ACAT as described above (Fig. 1A). A conditional promoter (in this case induced by galactose) was used to circumvent possible toxicity of high level expression of human ACAT in yeast. In fact, no toxicity was observed. The plasmid was used to transform yeast and human ACAT in yeast is of coincident mobility.

| Substrate and Inhibitor Specificity of Sterol Esterification | Esterification\(^a\) | pmol/min/mg |
|-------------------------------------------------------------|----------------------|-------------|
| Wild-type                                                  | 981 ± 55             |
| *ARE1 are2\(^{-}\)*                                        | 224 ± 49             |
| *are1* ARE2                                                 | 637 ± 133            |
| *are1* are2\(^{-}\)                                        | 31 ± 31              |
| Rat liver microsomes                                       | 1350 ± 50            |

\(^a\) Esterification in the presence of exogenous cholesterol; ± S.D. from triplicates.

sterol is ergosterol which differs from cholesterol by the presence of unsaturation at the C-7 position and at C-22, with an extra methyl group at C-24. It has been shown that ergosterol is not a good substrate for rat liver microsomal ACAT (18). To test if the human ACAT protein expressed in yeast is functional and that the observed activity represents low affinity of the human enzyme for ergosterol, we utilized an *in vitro* assay system to measure ACAT activity (Table II). The addition of exogenous cholesterol to the microsomal assay greatly increased esterification by the hACAT expressing strain approximately 6-fold. This suggests that one reason for the low activity observed *in vivo* is the lack of appropriate substrate for the mammalian enzyme. The *in vitro* ACAT activity of microsomes from yeast expressing the hACAT was sensitive to both competitive (Sandoz 58035) and non-competitive (DuP 128) inhibitors. Interestingly, the endogenous yeast enzymes were only affected by the competitive inhibitor (SS8035, Table II), even though DuP 128 was present at 50 times its IC\(_{50}\) for mammalian ACAT. This suggests that the binding site for DuP 128 is not conserved between yeast and humans. To test if the individual yeast ARE enzymes differ in their sensitivity to SS8035, we prepared and assayed microsomes from single *are1\(^{-}\)* and *are2\(^{-}\)* mutants (strains SCY060 and SCY061). Using a range of concentrations of the inhibitor, we estimated the IC\(_{50}\) of Are1p and Are2p to be 31 and 34 μM, respectively, whereas the IC\(_{50}\) of a preparation from wild type cells was 20 μM. The IC\(_{50}\) of rat ACAT in the same experiment was estimated as 40 μM.

Hoping to demonstrate that activity of the human ACAT cDNA in yeast, we used these observations to assess the affinity of the human and yeast enzymes for different sterol and acyl-CoA substrates (Tables III and IV). Despite the fact that the dominant sterol in yeast is ergosterol, the yeast ARE enzymes also readily esterified cholesterol. In contrast, the human macrophage ACAT expressed in the double *are2* mutant yeast showed a substrate selectivity similar to the mammalian enzyme as opposed to yeast, with little activity against ergosterol (Table III). Both yeast and rat enzymes esterified 7-dehydrocholesterol, while no apparent activity utilizing this substrate was observed with hACAT expressed in yeast.

To assess the acyl-CoA substrate specificity, the assay con-
Substrate and Inhibitor Specificity of Sterol Esterification

**DISCUSSION**

Our previous demonstration of the *in vivo* requirement of the yeast ARE genes in sterol esterification provokes two hypotheses with regard to their role in this reaction. These proteins are homologous to a mammalian protein shown to be required for sterol esterification, *in vitro* (30). However, it was hypothetically possible that the ARE gene products mediate substrate transport to the rough endoplasmic reticulum, conventionally the site of esterification. A more parsimonious explanation would be that ARE1 and ARE2 encode isoforms of the catalytic components of this enzyme. We now find this latter possibility to be the case. The fact that microsomes from yeast strains deficient in ARE1, ARE2, or both genes display identical relative levels of *in vitro* activity to those observed *in vivo*, rules out a direct role for these proteins in sterol translocation. Furthermore, the substrate specificities of both the yeast and human enzymes expressed in yeast are most consistent with the ARE and hACAT proteins acting as catalytic components of the enzymatic complex as opposed to regulatory factors.

The observation of two enzymes for sterol esterification in yeast is intriguing. The requirement for multiple enzymes for the same reaction would be advantageous to a cell if one enzyme was either differentially regulated, alternatively localized, or specific for a substrate. We are currently developing reagents such as isoform-specific antibodies which will enable us to address these issues. Presently, however, we are incapable of determining if the observed difference in levels of sterol esterification observed in *are1Δ* or *are2Δ* mutants represents different rate constants for the reaction or different levels of protein expression. Clearly both enzymes are active, since a null phenotype occurs only when both genes are deleted.

To some extent the differences between the ARE isoforms and human macrophage ACAT are as interesting as their similarities. Despite the overall 49% identity at the translational level, the highest degree of conservation with human macrophage ACAT is in the COOH-terminal domain. Thus far, we have been unable to distinguish Are1p and Are2p except in terms of their overall relative contributions to sterol ester mass. The human enzyme, however, is quite distinct. Yeast enzyme is neither induced by 25-hydroxycholesterol nor sensitive to the non-com-
petitive ACAT inhibitor, DuP 128. However, the human enzyme demonstrates the predicted response to these reagents, namely increased activity and inhibition, respectively. Since the transcription of the hACAT cDNA is driven by a yeast promoter regulated only by carbon source, these responses, particularly the induction by 25-hydroxycholesterol, clearly act at a post-transcriptional point. This is in confirmation of previous studies (30). The 71% inhibition observed with S58035 in wild type yeast could be due to preferential inhibition of Are2p or to partial inhibition of both isoforms. An analysis of microsomal preparations from single or double mutants refutes this latter hypothesis; Are1p and Are2p were equally sensitive to the sterol substrate specificity of ACAT.

In addition to demonstrating the differences between the yeast and human reaction, our work also confirms the fidelity of the yeast expression system with which to study the human enzyme. It is clear that the expression of hACAT in yeast occurs at high levels in terms of mass and that the reaction proceeds in a similar manner to that performed in mammalian cells. Thus, it seems likely that the sterol substrate specificities and pharmacological inhibitions described here for recombinant hACAT, essentially reproduce those observed with the enzyme in its normal cellular context.

The acyl-CoA specificity profile is more complex. Under the assay conditions employed, the hACAT expressed in yeast utilized a much broader range of fatty acids for esterification than either the yeast or the rat liver enzyme (Table IV). This may suggest that the enzyme, although functional, is in a slightly different membrane environment, such that the active site is accessible by CoAs containing acyl chains of different chain lengths and desaturation. Species and tissue differences cannot be ruled out, since in this study, the properties of human macrophage ACAT are compared to rat liver ACAT. In previous studies, DuP 128 inhibition and sterol substrate specificity were similar between liver or macrophage preparations from human or rat. The yeast enzymes appear to be very specific for C18 unsaturated fatty acids; this is in agreement with previous work (31, 32) but different to that found by Taylor (33) who saw no significant changes in in vivo esterification with different fatty acid substrates. The effect of linoleic acid on the ARE proteins is difficult to assess, since normally this fatty acid is not present in yeast. The fatty acid specificity of rat liver ACAT in our study is similar to that observed previously with the exception of linoleic acid. Previous studies (19, 34), found an esterification rate for linoleoyl-CoA between 15 and 40%, the rate of oleate, while in our experiments they were equivalent. There are several possible explanations for the observed differences. The specific activity of ACAT in the earlier experiments was about 10-fold less than under present conditions and the corresponding assay times were much longer; 2.5 min versus 30–60 min previously. The specificity of steryl ester hydrolyase could play a role over the longer assay period. This longer assay time could also affect the amount of fatty acid being incorporated into phospholipid, where linoleate was much preferred to oleate (19). In those experiments, the ratio of linoleate/oleate in sterol ester was low (0.15), however, the ratio in phospholipid was high (3.4) suggesting a preference for linoleate incorporation into phospholipid under these assay conditions. Furthermore, in the previous experiments, radiolabeled cholesterol was added as a tracer in acetone, while in our experiments, the cholesterol was equilibrated with microsomes using the detergent Triton WR-1339. The non-ionic detergent may facilitate the interaction of linoleoyl-CoA with the microsomal ACAT.

The demonstration of distinct substrate specificities of the human reaction is an important observation. The relative occurrences of these substrates in different tissues and in different species will reflect both physiological and nutritional influences and is probably variable. The presence of multiple ACAT isoforms with substrate specificity or differential tissue-specific expression would confer greater flexibility to this critical homeostatic process, which could be considered in some organs to be a detoxification process. It is noteworthy that the human macrophage ACAT mRNA is most abundant in adventinal tissues but barely detectable in liver and intestine, and yet these latter tissues exhibit exceptionally high ACAT activity (36). Of interest, we have recently identified other ACAT related gene products in the data base of expressed sequence tags (1), which may be candidates for isoforms that mediate sterol esterification in specific tissues or cell types, possibly with alternate substrate specificities. The study of these isoforms in the yeast are mutant background is currently being performed in this laboratory.

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REFERENCES

1. Yang, H., Bard, M., Bruner, D. A., Gleeson, A., Deckelbaum, R. J., Aljovinovic, G., Pohl, T., Rothstein, R., and Sturley, S. L. (1996) Science 272, 1353–1356
2. Blom, K. E. (1983) CRC Crit. Rev. Biochem. 14, 47–92
3. Chang, T. Y., Chang, C. C. Y., and Cadigan, K. M. (1994) Trends Cardiovasc. Med. 4, 223–230
4. Sukling, K. E., and Stange, E. F. (1985) J. Lipid Res. 26, 647–670

* J. T. Billheimer, unpublished data.

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5. Field, F. J., Kam, N. T., and Mathur, S. N. (1990) Gastroenterology 99, 539–551
6. Huff, M. W., Telford, D. E., Barrett, P. H. R., Billheimer, J. T., and Gillies, P. J. (1994) Arterioscler. Thromb. 14, 1498–1508
7. Ross, R. (1993) Nature 362, 801–809
8. Brown, M. S., and Goldstein, J. L. (1983) Annu. Rev. Biochem. 52, 223–261
9. Billheimer, J. T., and Wilde, R. (1991) in Anti-atherosclerotic Agents Current Patents Ltd. (Tarr, I. J., ed) pp. B5–B19, Current Patents Ltd., London, United Kingdom
10. Sliskovic, D. R., and White, A. D. (1991) Trends Pharmacol. Sci. 12, 194–199
11. Hainer, J. W., Terry, J., Connell, B., Zyruk, H., Jenkins, R., Shand, D., Gillies, P., Livak, K., Hunt, T., and Crouse, J. (1994) Clin. Pharmacol. Ther. 56, 65–74
12. Nakaya, N., Nakamichi, N., Sekino, H., Namura, M., Ishii, M., Tomono, Y., and Yamato, C. (1994) Atherosclerosis 109, 253
13. Yukawa, S. (1986) Gendai Iryo 18, 2837–2841
14. Ross A. C., Go, K. J., Heider J. G., and Rothblatt G. H. (1984) J. Biol. Chem. 259, 815–819
15. Billheimer, J. T., Cromley, D., Higley, C., Wexler, R., Robinson, C., and Gillies, P. (1991) Ninth International Symposium on Atherosclerosis October, 1990, 184, A94, Elsevier Science B.V., Amsterdam, The Netherlands
16. Higley, C., Wilde, R., Maduskuie, T., Johnson, A., Pennve, P., Billheimer, J., Robinson, C., Gillies, P., and Wexler, R. (1994) J. Med. Chem. 37, 3511–3522
17. Billheimer, J. T., and Gillies, P. J. (1992) in Advances in Cholesterol Research (Esfahani, M., and Swaney, J. B., eds) Telford Press, Philadelphia, PA
18. Tavani, D., Nes, W., and Billheimer, J. (1982) J. Lipid Res. 23, 774–781
19. Goodman, D. S., Deykin, D., and Shiraatori, T. (1964) J. Biol. Chem. 239, 1335–1345
20. Chang, C. C. Y., Hub, H. Y., Cadigan, K. M., and Chang, T. Y. (1993) J. Biol. Chem. 268, 20747–20755
21. Yu, C., Kennedy, N. J., Chang, C. C. Y., and Rothblatt, J. A. (1996) J. Biol. Chem. 271, 24157–24163
22. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) J. Bacteriol. 153, 163–168
23. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, New York
24. Sherman, F., and Hicks, J. (1991) Methods Enzymol. 194, 21–37
25. Wang, H., Germain, S. J., Benfield, P. P., and Gillies, P. J. (1996) Arterioscler. Thromb. Vasc. Biol. 16, 809–814
26. Laemmli, U. K. (1970) Nature 227, 680–685
27. Chang, C. C. Y., Chen, J., Thomas, M. A., Cheng, D., Del Priore, V. A., Newtown, R. S., Pape, M. E., and Chang, T. Y. (1995) J. Biol. Chem. 270, 29532–29540
28. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
29. Billheimer, J. T., Tavani, D., and Nes, W. R. (1981) Anal. Biochem. 111, 331–335
30. Cheng, D., Chang, C. C. Y., Qu, X., and Chang, T. Y. (1994) J. Biol. Chem. 270, 685–695
31. Taketani, S., Nagai, J., and Katsuki, H. (1978) Biochim. Biophys. Acta 528, 416–423
32. Maydastha, P., and Parks, L. (1969) Biochim. Biophys. Acta 176, 858–862
33. Taylor, F. R., and Parks, L. W. (1981) J. Biol. Chem. 256, 13048–13054
34. Sgoutas, D. (1970) Biochemistry 9, 1826–1833
35. Carr, T. P., Parks, J. S., and Ruzel, L. L. (1992) Arterioscler. Thromb. 12, 1275–1283
36. Uelman, P. J., Oka, R., Sullivan, M., Chang, C. C. Y., Chang, T. Y., and Chan, L. (1996) J. Biol. Chem. 271, 26192–26201

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