Characterization of Two Human Genes Encoding Acyl Coenzyme A: Cholesterol Acyltransferase-related Enzymes*

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The enzyme acyl coenzyme A:cholesterol acyltransferase 1 (ACAT1) mediates sterol esterification, a crucial component of intracellular lipid homeostasis. Two enzymes catalyze this activity in Saccharomyces cerevisiae (yeast), and several lines of evidence suggest that this enzyme performs the last acylation in triacylglycerol biosynthesis.

The intracellular formation of sterol esters from fatty acid and sterol is mediated by acyl-CoA:cholesterol acyltransferase (ACAT). The pathological accumulation of cholesterol esters in atherosclerotic lesions has lead to intense pursuit of ACAT inhibitors as pharmacological agents. Microsomal ACAT preparations from various tissues display differential sensitivities to some of these agents (1) including histidine modifiers (2). This suggests that more than one protein mediates the esterification reaction, such as occurs in yeast (reviewed in Ref. 3). Saccharomyces cerevisiae (budding yeast) has two ACAT-related enzymes, Are1 and Are2, which are derived from separate genes and have been shown to independently esterify sterols (4, 5). In terms of contribution to the sterol ester mass of the cell, Are1 is the minor isoform relative to Are2. These genes were identified based on sequence conservation to a human gene, ACAT1, which encodes an ACAT enzyme with homologs in many mammalian species (6, 7). The human ACAT1 gene encodes a 550-amino acid polypeptide and is expressed in most tissues, predominantly placenta, lung, kidney, and pancreas (6). ACAT1 has been predicted to have two transmembrane domains (6) and has been immunolocalized to the endoplasmic reticulum (8, 9). When murine ACAT1 was disrupted in induced mutant mice, homozygotes for the deletion were found to essentially lack ACAT activity in embryonic fibroblasts and have negligible amounts of cholesterol ester in the adrenal cortex and peritoneal macrophages (10). However, cholesterol ester accumulation was normal in hepatocytes while dietary cholesterol absorption, an indirect marker for intestinal cholesterol esterification, was indistinguishable from control litters. This is consistent with the concept of a multigene family for this activity.

ACAT isoforms may be required to perform the variety of physiological roles mediated by cholesterol esterification. In increases in cellular free cholesterol above certain levels are cytotoxic and are ameliorated by cholesterol ester formation (11). In hepatocytes, the bulk of cholesterol secreted in very low density lipoprotein is esterified intracellularly and determines the sterol ester mass of the cell (12–14). Cholesterol esterification in the enteroctye may be necessary for cholesterol absorption from the lumen and secretion in chylomicrons into the lymph (15). The formation of cholesterol ester stores could also provide a readily available substrate for steroid hormone synthesis in steroidogenic tissues (16, 17). It is likely that different ACAT isoforms mediate each of these processes, and the data presented here support that hypothesis.

We reasoned that additional human ACAT proteins would have sequence similarity to regions conserved between human ACAT1 and yeast Are1 and Are2. (4). Accordingly, an ACAT consensus sequence was used to screen the database of expressed sequence tags (dbEST). Several cDNA entries were identified which were transcribed from two independent human genes. This study is a description of the isolation of full-length cDNA clones for two ACAT-related gene products (ARGP1 and ARGP2), examination of their pattern of tissue...
expression, and assays of enzymatic activity. We show that ARGP2 can catalyze the formation of sterol ester from cholesterol and oleoyl-CoA, leading us to rename this gene, ACAT2. By contrast, ARGP1 did not detectably esterify cholesterol and we propose that it performs acyl-CoA-dependent acylation of other molecules, such as diacylglycerol.

**EXPERIMENTAL PROCEDURES**

**General**—Molecular biology techniques were performed by conventional protocols (18, 19) and DNA modifying reagents were purchased from Life Technologies, Inc., New England Biolabs, or Promega as indicated. The 5′-end labeled probe specific for ARGP1 (synthesized using a 420-bp replica of the plate) were probed by hybridization with a digoxigenin density of 5000 colonies per plate. Membrane (Hybond-N, Amersham) (18) was prehybridized with 0.1 mg/ml salmon sperm DNA, and 2% (w/v) blocking reagent (Boehringer Mannheim) at 65 °C for 14–18 h. The membranes were washed with 0.1× SSC, 0.5% SDS at 50 °C. After stripping the membrane was probed with ARGP2 (dbEST clone 10272 insert and the ARGP2 5′ RACE product) using the conditions for the 5′ RACE—A 30-mer ACAT consensus peptide sequence (FAEMLRFLQDDFRKYKDVWNSSTSYYTVN) was used as the query in a tblastn (which compares a protein sequence against a database) search of the database of expressed sequence tags at NCBI (dbEST). Three clones, H24971, R07932, and R99213, derived from a common gene (named ACAT related gene product 1, ARGP1), were identified (p < 10−5). The entire human ACAT1 protein was then used in an identical search. In addition to clones of ACAT1 and ARGP1, two entries, R10272 and W76421, with significant similarity were identified (p < 10−5). They were derived from a gene we named ARGP2. Rescreening the dbEST with these clones identified two more ARGP2 entries. *Escherichia coli* clones with the largest inserts corresponding to these sequences were obtained from the L.M.A.G.E. consortium and sequencing with T3, T7, or gene specific primers.

5′ Rapid Amplification of cDNA Ends (RACE) of ARGP1—Oligo(dT) primed, double stranded cDNA was reverse transcribed from human, ileal, poly(A)+ mRNA, kindly provided by Dr. Paul Dawson, and ligated to adapters using a commercially available kit (CLONTECH, Palo Alto, CA). Touchdown PCR (28) was performed for 35 cycles with a forward primer complementary to the adapter (5′-CAACCGTCCTTGAGTGGACACC-3′) and a reverse primer (End4A, 5′-CCACCTGGAAGCCTGGAAGAAC-3′) complementary to the ARGP1 dbEST clone Z43867. The PCR mixture included 200 nM each oligo, 200 μM dNTPs, 400 nM of each primer, and 2 units of Taq polymerase, and sequenced.

5′ RACE of ARGP2—A human, fetal (20 weeks post-conception) liver/spleen, oligo(dT)-primed, cDNA library in the vector pT7T3 (Stratagene) was kindly provided by Dr. Bento Soares. PCR was performed with the cDNA, a forward primer (M13 reverse, 5′-CAACCTGGAAGCCTGGAAGAAC-3′), a reverse primer (End4B, 5′-CCACCTGGAAGCCTGGAAGAAC-3′), and a 5′-untranslated region and 1 bp of 3′-untranslated region, in pRS426GP described previously (27). Yeast strain, SC059 (MATα, ade1-1, can1-1, trpl-1, ura3-1, his3-11, 15, leu2-3, 112, met14A14, his3-A16, ale1-A16, leu2-A16) (32) with deletions in the ARL1 and ARE2 yeast homologs of human ACAT1 (4), which are known to be critical for the expression of the yeast ACAT1, was transformed with 5′-end labeled probe specific for ARGP1 (synthesized using a 420-bp NotI, PstI digestion product of the 5′ RACE product) or ARGP2 (synthesized using the 5′ RACE product) in 5× SSC, 0.5% SDS, 0.1% Na-lauroylsarcosine, 0.1 mg/ml salmon sperm DNA, and 2% (w/v) blocking reagent (Boehringer Mannheim) at 65 °C for 14–18 h was washed in 0.2× SSC, 0.1% SDS at 60 °C for 20 min, incubated with an anti-digoxigenin antibody (1:10,000), washed in Tris-buffered saline, incubated with the peroxidase substrate CSPD (Boehringer Mannheim), washed in Tris-buffered saline, incubated with the peroxidase substrate CSPD (Boehringer Mannheim), and detected by enhanced chemiluminescence (ECL). For ARGP1, 4 single positive clones were isolated after screening ~20,000 clones. For ARGP2, 4 single positive clones were isolated after screening ~30,000 clones. The longest clones for each were sequenced multiple times on both strands using vector and gene-specific oligonucleotides.

**Tissue Culture**—Cultured human Caco2, HeLa, HepG2, and THP1 cell lines were obtained by Dr. R. J. Deckelbaum and originally obtained from the ATCC. HepG2, HeLa, and Caco2 cells were maintained as cell monolayers in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) + 10% fetal bovine serum (HyClone) in 5% CO2. THP1 monocyte cells were maintained in suspension in RPMI (Life Technologies, Inc.) + 10% fetal bovine serum in 5% CO2. Differentiation of THP1 cells was stimulated with 150 ng/ml tetracystrate phorbol ester and 140 μg/ml β-mercaptoethanol. Whole cell RNA was isolated from confluent monolayer cultures or pelleted THP1 cells using TRIzol (Life Technologies, Inc.). The Caco2 cells had been confluent for approximately 21 days.

**Human Adult and Fetal Multi-tissue Northern Blot Analysis**—Commercially obtained multi-tissue Northern blot (CLONTECH) contained 2 μg of poly(A)+ RNA from human adult or fetal (18–24 weeks post-conception) tissues originally resolved on a 1.2% agarose, formaldehyde gel. The adult tissue membrane was hybridized with a random-hexamer primed, 32PdCTP-labeled probe, generated using the insert of the ARGP1 dbEST clone R99213, in ExpressHyb buffer (CLONTECH) for 1 h at 68 °C. The membrane was washed in 0.1× SSC, 0.5% SDS at 50 °C. After stripping the membrane was probed with ARGP2 (dbEST clone 10272 insert and the ARGP2 5′ RACE product) using the conditions for the 5′ RACE.

**Reverse Transcription PCR**—Human cdNA obtained from a Quick Screen cDNA Panel of Human tissues (CLONTECH) was reverse transcribed with Life Technologies, Inc.) human ideal poly(A)+ mRNA was used as template in a PCR reaction with primers specific for ARGP1 (106, GCCATCTGACTGTGAGTGGTG; 110, AGGCTGAGCATGGCTGCTGG), ARGP2 (202, GAGCTGCCGCCCACTTTCAATACTGACCC; 206, CAGCTGCTCTGTCTTTCGA), or β-actin (Act1, GAGCTGCCGTACGCGCCTGCT; Act2, CACATCTGCTGAGATGGTCAC). The PCR mixture included 1.5 mM MgCl2, 200 μM dNTPs, 400 ng/ml of each primer, and 2 units of Taq (Life Technologies, Inc.). Following 35 cycles (94 °C, 45 s; 60 °C, 45 s; 72 °C, 2 min), the products were resolved on a 1% agarose gel and visualized by ethidium bromide staining. DNA sequences from the purified products were sequenced using reverse transcribease (SuperScriptII, Life Technologies, Inc.) extraction. The Caco2 cells had been confluent for approximately 21 days.

**RESULTS**

**Isolation of Full-length cDNA Clones for Two ACAT Related Human Genes**—A comparison of the human ACAT1 protein and the two yeast ACAT orthologs (Are1, Are2) identified a highly conserved (70% identical) region of 30 amino acids (ACAT1 amino acids 391–420) near the carboxy terminus. This peptide was used to screen the data base of expressed sequence tags (dbEST). The search identified several human cdNAS, the longest being 890 bp (GenBank accession number H45993), derived from a common gene we call the ARGP1.
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A 1976-bp ARGP1 cDNA clone was identified by colony hybridization screening of a HepG2 cDNA library. Translation of this clone predicts the 522-amino acid polypeptide shown. The residues in bold are conserved with human ACAT1. The underlined portions are predicted transmembrane domains, two potential N-linked glycosylation sites are boxed, and a putative tyrosine phosphorylation motif is in brackets. The sequence has been deposited at GenBank, accession number AF059203.

date, 26 clones for human ARGP1 are present in the dbEST from fetal liver/spleen, brain, breast, cerebellum, kidney, lung tumor, ovary tumor, placenta, testis, and tumor libraries and are derived from a common gene we call ARGP2. As of this writing, 26 clones for human ARGP1 are present in the dbEST (GenBank accession number AA410072).

Northern blot analysis of human tissues (Figs. 3 and 4) showed that the initial dbEST clones for ARGP1 and ARGP2 were truncated, relative to the observed transcripts, by approximately 1000 and 1400 bp, respectively. To isolate full-length cDNAs, 5' and 3' RACE products were then used as probes to screen a size-fractionated cDNA library. The longest dbEST clones, were identified in fetal liver/spleen and fetal heart tissue libraries and are derived from a common gene we call ARGP2.

The dbEST was then searched using the entire ACAT1 protein sequence. Four human cDNAs, distinct from ARGP1 cDNA clones, 5' and 3' RACE products were then used as probes to screen a size-fractionated cDNA library. The longest dbEST clones, were identified in fetal liver/spleen and fetal heart tissue libraries and are derived from a common gene we call ARGP2.

The longest ARGP2 open reading frame, flanked by a 244 nucleotide 5'-untranslated region and a 265-nucleotide 3'-untranslated region, encodes a 522-amino acid protein (Fig. 2) with a calculated molecular mass of 52,216 daltons. The predicted initiator methionine lies within a consensus for initiation of translation (29) and downstream of an in-frame termination codon. Comparison to ACAT1 revealed 22% amino acid sequence identity (29% similarity) over the entire molecule. The conservation of these molecules is greatest toward the COOH terminus, such that ACAT1 and ARGP1 are 26% identical over the last 250 residues. This pattern of sequence similarity is strikingly similar to that observed from comparison of ACAT1 with the yeast Are1 and Are2 proteins. ARGP1 is predicted to be a membrane bound protein with nine putative transmembrane domains and one N-linked glycosylation site. Uniquely, ARGP1 contains a diacylglycerol/phorbol ester binding signature sequence (H.[FWY].[KR].F..P) at amino acids 382–392 which was originally identified by comparison of protein kinase C isoforms and diacylglycerol kinases (Fig. 7) (36, 37). This motif is also conserved in the murine homolog of ARGP1 residing at the dbEST (GenBank accession number AA764382).
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To further examine the expression of ACAT2 in adults, a RT-PCR was performed using cDNA generated from a variety of tissues (Fig. 5). As shown, ARGP2 is expressed in human adult heart, kidney, liver, lung, pancreas, and ileum. The identity of the PCR product was verified by Southern blotting and hybridization with an ARGP2-specific cDNA probe (data not shown). An RT-PCR analysis of ARGP1 on these same samples gave a similar pattern of expression to that determined by the Northern blot in Fig. 3.

ARGP1 and ARGP2 expression in human tissue culture cell lines was also examined by RT-PCR (Fig. 6). ARGP1 was expressed in cell culture models for human endothelial (HeLa), hepatocyte (HepG2), monocyte (undifferentiated THP1), macrophage (differentiated THP1), and intestinal epithelial (Caco2) cells. Expression of ARGP2 was limited to HepG2 and Caco2 cells. This reinforces the concept that ARGP1 is widely expressed while the expression of ARGP2 is more restricted. ACAT1 was expressed in all of these cell lines confirming previous observations (7, 31) (data not shown).

Assay of ACAT Activity in ACAT Negative Yeast Transformed with ARGP1 and ARGP2—The ability of ARGP1 and ARGP2 to esterify sterols was assayed in a sterol esterification deficient yeast strain (SCY059) in which the endogenous ARE genes were deleted (27). Microsomes from these yeast, transformed with an expression vector harboring no insert or cDNA inserts for ARGP1, ARGP2, or human ACAT1 were assayed in vitro for the incorporation of [14C]oleate into sterol ester. Since we previously demonstrated that cholesterol is the preferred substrate for mammalian ACAT enzymes (27, 32), assays were performed with exogenous cholesterol supplied in Triton WR-1339. As shown in Table I, ARGP2 forms cholesterol ester at a
rate of 49 pmol/min/mg of microsomal protein. This is 24-fold over background and about 15% of the activity detected in microsomes from ACAT1 transformants. We therefore renamed ARGP2 as ACAT2. ARGP1 did not display significant ACAT activity. None of the enzymes showed the ability to use ergosterol, the major sterol in yeast microsomes, as a substrate (data not shown). While the ACAT1 and ACAT2 mediated activities were equally sensitive (75% inhibition) to the ACAT inhibitor Dup128 (0.5 μM; not shown), they showed significantly different sensitivity to the histidine/tyrosine modifying agent diethylpyrocarbonate (DEPC, Table I). This reagent was previously demonstrated to distinguish liver and adrenal ACAT activities, the latter being significantly more sensitive. Since adrenal ACAT would primarily represent ACAT1, our data are consistent with ACAT2 representing the DEPC-resistant isoform identified by Kinnunen et al. (2).

DISCUSSION

We have isolated two independent human cDNAs, ARGP1 and ACAT2, which encode proteins with significant sequence similarities to human ACAT1. The level of nucleotide sequence conservation between ACAT1 and ACAT2 (55%) suggests their common evolution possibly arising from a gene duplication event, as clearly occurred in the case of the yeast ARE gene family. However, ARGP1 is more distantly related, bearing 39 and 43% nucleotide identity with ACAT1 and ACAT2, respectively, and may have evolved independently. The uniform similarity between the human genes and the two yeast ARE genes precludes any assignment of lineage across species.

The similarity among the three human ACAT-like proteins is most distinct over their COOH-terminal regions just as is the case when comparing the yeast Are proteins to ACAT1. The predicted ARGP1 protein displays 28% identity with ACAT1 over this portion of the molecule and includes a FY.DWN motif present in all cloned ACATs and shown to be important for enzymatic activity (Fig. 7A)2. However, ARGP1 is the most divergent member of this gene family. For example, a HSF motif (residues 268–270) is invariant in ACAT1 and yeast Are enzymes and was critical to ACAT1 activity in CHO cells. Replacement of Ser by Leu produced an inactive and unstable molecule (33). This motif is not conserved in ARGP1, although several serines are present in the region (e.g. Ser357, Fig. 7B).

ARGP1 is also unique in its predicted possession of a diacylglycerol/phorbol ester-binding site (Fig. 7A), leading us to speculate that this enzyme might esterify diacylglycerol to produce triglyceride. Sequence similarity between diacylglycerol acyltransferase and ACAT enzymes might be expected since both have a common substrate, acyl-CoA, but differ in the alcohol (cholesterol or diacylglycerol) used as a second substrate.

Of the two new gene products described here, ACAT2 displays significantly greater sequence similarity to ACAT1, with an overall identity of 47% and 63% invariance over the COOH-terminal half of the molecules. The FY.DWN motif common to this family of proteins is maintained in ACAT2 to the extent that the flanking residues render the tyrosine a candidate for phosphorylation as observed in ACAT1 and in yeast (Fig. 7A). Tyrosine phosphorylation may be a regulator of ACAT activity, although serine and threonine phosphorylation is unlikely to be involved (94, 35). The HSF motif found in ACAT1, Are1 and Are2 is conservatively replaced in ACAT2 by YSF (residues 244–246; Fig. 7B). Interestingly, histidine modifying agents selectively inactivate adrenal microsomal ACAT activity but display a significantly higher Ki (1500 versus 250 μM) against liver microsomes (2). It is intriguing to speculate that sequence variation in the (H/Y)SF motif may explain this observation. In accordance with this, we showed that ACAT1 was significantly more sensitive to DEPC than ACAT2. In common with ACAT1, Are1 and Are2, the ACAT2 sequence predicts a leucine heptad motif which may play a role in multiprotein complex formation. Radiolabel inactivation studies in rat liver microsomes have shown that the ACAT enzymatic complex is about 200 kDa (36, 37), much larger than the predicted monomer for ACAT 1 (65 kDa) or ACAT2 (60 kDa). There is also evidence that ACAT1 interacts with itself in a yeast two-hybrid system (38) and ACAT2 may be similar in this regard. ARGP1 and ACAT2 are also similar to ACAT1 in terms of hydrophobicity. While previous studies suggested that ACAT1 contains two transmembrane domains (6), the PredictProtein algorithm (39) indicates

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A The “DWWN” region of the ACAT gene family

| Accession | Sequence |
|-----------|----------|
| ARE1 482  | ELTrPaRDYF YgddWncVSf eeFariWNVP VHKRiIHRYY hsmgml.hl skS |
| ARE2 514  | ELTrPGdRodYF YgddWncVSv adFariWNIP VHKRiIHRYY hsmgml.kl nKS |
| ACAT1 394  | EmlRFGdRmF ydmWnsSST snyyRTNNV VHDWLyTTy KdfLWfFkr fKS |
| ACAT2 369  | EmlRFGdRmF ydmWnsSST snyyRTNNV VHDWLyTTy dgsLrllgar arg |
| ARGP1 351  | ElmgFgDRmF ydmWnsSST tyFqwnNLLP VHRWolhRFT kgmlLrgrss .Kr |

Consensus: EL-RFGR-F Y-DWNS-S- --F-R-WNP YHKWL-RHVV --L------- -KS

B The “HSF” region of the ACAT gene family

| Accession | Sequence |
|-----------|----------|
| ARE1 306  | FvMKHSHSfAf yNgLyLaDkg |
| ARE2 335  | 1MKHSHSfAf yNgLyLaDke |
| ACAT1 265  | FvMKHAHsFr eNHvpLmAS |
| ACAT2 240  | FLMKSySFrl eavpLT.l.rA |
| ARGP1 206  | FL.KLsyrD vNawc..rA |

Consensus: FLMKSHSFA --N--LWL-I-A

Fig. 7. Consensus sequences in the ACAT multigene family. Two regions of structural and functional conservation are shown. The amino acid position of each initial residue is shown. **Uppercase residues** indicate those of the consensus calculated with a plurality of 2. **A.** The DWWN motif is invariant in all members identified to date of this gene family, the tyrosine and tryptophans being critical to activity. In all but ARGP1, the Tyr constitutes a candidate target for phosphorylation (indicated in bold and by π). In ARGP1, the underlined sequence HKWCIRHFYP represents a candidate for diacylglycerol binding as found in protein kinase C and diacylglycerol kinases (motif, H.[FWY][IKR].F..P). The asterisks identify those residues critical to definition of this motif that distinguish ARGP1 from the other members of the family. B, the HSF region. The central serine residue (indicated σ) was found to be critical to the activity and stability of Chinese hamster ovary ACAT1.

Eight such domains in ACAT1, similar to the number predicted for ARGP1 (nine) and ACAT2 (seven). Membrane spanning domains are expected characteristics of ACAT and diacylglycerol acyltransferases since both activities are associated with microsomal membranes (40–42).

In addition to sequence similarity with ACAT1, we expect alternate ACAT enzymes to be expressed in the tissues which retain ACAT activity in the induced mutant ACAT1 mouse, namely the liver and intestine. ARGP1 met this criteria, however, it is also highly expressed in human adult adrenal cortex which was depleted of cholesterol esters in the induced mutant mouse. Monocytes from acac−/− mice were also devoid of cholesterol ester and yet ARGP1 mRNA was detected in the human THP1 monocye cell line. This evidence is contrary to ARGP1 being an ACAT, barring species-specific differences in expression. By the sensitive technique of RT-PCR, ACAT2 expression was observed in human adult liver and intestine and in cell culture models of the hepatocyte and intestinal enterocyte but was undetectable in THP1 monocytes and macrophages. This profile of expression is consistent with a role for ACAT2 in the livers and intestine of mammals, particularly ACAT1 knockout mice.

In confirmation of ACAT2 being a candidate for a second ACAT, heterologous expression of ACAT2 in an ACAT-negative yeast strain conferred significant microsomal cholesterol esterification with oleoyl-CoA at a level comparable to the 20–50 pmol/min/mg of protein observed in human liver microsomes supplied with exogenous cholesterol (43). The ACAT2-mediated esterification activity was significantly (85%) less than that mediated by ACAT1 in yeast. This may be due to differences in protein expression (although both mRNAs were produced at high levels as detected by RT-PCR, data not shown), protein stability, or a genuine difference between the two enzymes.

Liver ACAT, predicted to comprise both ACAT1 and ACAT2, utilizes a limited range of sterol substrates but a wide variety (16:0, 18:0, 18:1, 18:2, and 20:4) of fatty acyl-CoAs (27, 44). Determining substrate-specific differences between ACAT1 and ACAT2 may thus explain their redundancy. The redundancy may also be related to substrate affinity such as seen between the hexokinase types I-III and hexokinase type IV (glucokinase) (45). In such a scenario, one ACAT would have a lower affinity for cholesterol and only catalyze esterification at high cholesterol concentrations.

In addition to potential differences in activity, the two enzymes may have different physiological roles. For storage, cholesterol esters concentrate as cytoplasmic neutral lipid droplets, whereas for lipoprotein synthesis, cholesterol esters are incorporated into lipoprotein particles in the endoplasmic reticulum lumen. Redundant ACAT enzymes might allow one to be specific for cytoplasmic release of the cholesterol ester product and another to mediate endoplasmic reticulum lumenal release. Since lipoprotein synthesis occurs primarily in the liver and intestine, we speculate that ACAT2 may release cholesterol ester into the endoplasmic reticulum lumen, leaving ACAT1 to esterify and store sterols in the cytoplasm. The large amount of cholesterol ester, likely as cytoplasmic droplets, in the livers of high fat, high cholesterol fed acac−/− mice, is contrary to this hypothesis. Alternatively, ACAT2’s role may be important in the fetus since it was easily detected by Northern blot in human fetal liver.

The abundance of ARGP1 entries in the dbEST from a wide variety of cDNA libraries is reflective of the ubiquitous nature of ARGP1 expression in human adult tissues and tissue culture cell lines. This suggests that ARGP1 serves a function important to many cell types. Expression of two independent clones of ARGP1 under the regulation of two yeast promoters, GAL1/10 and GAPDH (not shown), failed to detectably esterify cholesterol or ergosterol. ARGP1-specific mRNA was identified by RT-PCR in each case. We take this as further evidence that unlike ACAT1 and ACAT2, ARGP1 is not involved in cholesterol esterification, at least when expressed in yeast. Based on the conservation of amino acids in ARGP1 that are important for ACAT1 to be active, ARGP1 likely catalyzes a reaction similar to ACAT. Other esterification reactions which use fatty acyl-CoAs as substrates include retinol esterification, methyl ester formation, triterpene esterification, monoaoylglycerol transferase, and diacylglycerol transferase. In the latter case our observations of a diacylglycerol-binding site in ARGP1 biases us to the possibility of ARGP1 being diacylglycerol acyltransferase, which to date has not been isolated at the molec-
ular level. We are presently investigating whether ARGP1 can mediate these reactions.

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