Ferritins Can Regulate the Secretion of Apolipoprotein B

Sarah Hevi and Steven L. Chuck‡

From the Molecular Medicine Unit Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215

Apolipoprotein B (apoB) is the large (molecular mass greater than 500 kDa), hydrophobic protein that is secreted with cholesterol esters and triglycerides in very low density lipoprotein particles (1). Regulation of the secretion of apoB from hepatocytes occurs largely post-translationally (2). Newly synthesized apoB can be assembled with lipids into lipoprotein particles (1). Regulation of the secretion of apoB is largely post-translational. The metabolic pathways for iron storage and intercellular cholesterol and triglyceride transport could intersect.

In HepG2 cells, unassembled apoB can be targeted for endoplasmic reticulum-associated degradation (ERAD) via the ubiquitin-proteasome pathway (5–8). ApoB is marked with ubiquitin for degradation even before it is finished being translated (7, 9, 10) while the protein is still in the translocation channel (8, 11). Furthermore, factors that slow the translocation of apoB increase the number of ubiquitin-conjugated apoB molecules marked for degradation by proteasomes (8) suggesting that translocation is a point of regulation of secretion. Other factors also regulate secretion since some ubiquitin-conjugated apoB can be deubiquitylated and secreted (11). In HepG2 cells, other non-ERAD routes for disposal exist but are not sensitive to agents that inhibit proteasomes (12–15).

The intertwined processes of lipidation, folding, translocation, intracellular degradation, quality control, and regulation of the secretion of apoB appear to involve many different hepatic proteins. In search of novel proteins that play such roles, we previously conducted a proteomic screen to identify proteins that bind apoB in hepatocytes. We identified ferritin heavy (H) and light (L) chains among many proteins that could be cross-linked to apoB in rat liver microsomes (16). Fusion proteins containing either ferritin H or L also bind apoB directly in vitro (16).

Ferritin H and L store iron in the cytosol of cells. Despite some differences in their primary structure, ferritin H (molecular mass of 21 kDa) and L (molecular mass of 19 kDa) share a similar tertiary structure that permits the assembly of cages composed of 24 H and L subunits that can store over 4,000 iron atoms (17). In iron-storage tissues such as the liver and spleen, ferritin L subunits predominate. Both types of subunits are necessary for efficient storage of iron. Ferritin H contains a ferroxidase site, whereas ferritin L has residues that may nucleate ferricydrate formation.

In light of the physical interaction between these proteins, we investigated the role of ferritin H and L in the biogenesis of apoB. We show that ferritin H or L specifically inhibit the secretion of apoB.

EXPERIMENTAL PROCEDURES

Reagents—LipopectAMINE 2000, Geneticin, and protein-A agarose were purchased from Invitrogen. Polyclonal antibody against apoB was obtained from Chemicon International, antibody against ferritins was from Fitzgerald Industries International, antisemur against factor XIII was purchased from Calbiochem, and antibody against ferritins was purchased from Invitrogen. Polyclonal antibody against apoB was purchased from Mann. Protein G-agarose was purchased from VWR and antibody against ferritins was purchased from Fitzgerald Industries International.

Plasmid Construction—The plasmid pcFerritinLight was constructed by amplifying the coding region for ferritin L by PCR using primers BamKFrtn, 5'-CGGGATCCGCCACCATGACGACCGCGTC-3' and EcoHI and EcoRt, 5'-CGGAATTCATGAGCTCAGGACGACCGGTC-3', digesting the PCR product with BamHI and EcoRI, and ligating the purified insert into pcDNA3 at the same restriction sites. The plasmid pcFerritinHeavy was constructed in a similar manner but using the primers BamKFrtn, 5'-CGGGATCCGCCACCATGAGCAGCCGTC-
Ferritins Can Regulate the Secretion of Apolipoprotein B

CACCCTCG-3′, and R1PrflnH, 5′-CGGAATTCCTATGGGAAATTAGCC-CGGAGG-5′. The plasmids were sequenced for verification.

**Cell Culture and Preparation of Stable, Transfected Cell Lines—**HepG2 cells were obtained from the ATCC and maintained in an α modification of Eagle’s medium with 10% fetal bovine serum, 2 mM L-glutamine, and penicillin/streptomycin at 37 °C in 5% CO₂. HepG2 cells were transfected in 10-cm dishes using 10 μg of plasmid DNA and 50 μl of LipofectAMINE 2000 overnight. After 2 days, the cells were split and grown in α-modification of Eagle’s medium containing 800 μg/ml Geneticin. After selection of individual clones, the cells were maintained in the same medium.

**Metabolic Labeling, Immunoprecipitation, and Visualization—**Cells were incubated in methionine-free Dulbecco’s modification of Eagle’s medium for 1 h, labeled with 100 μCi/ml [35S]methionine, and chased in complete medium for 1 h unless otherwise noted. The medium was removed, centrifuged at 14,000 rpm in a microfuge at 4 °C for 10 min to remove cells and debris, and transferred to a new tube. The medium was made up to 1× Triton X-100 salt wash buffer (5) with 1 mM phenylmethylsulfonyl fluoride. The cells were lysed in 1× Triton X-100 salt wash buffer with 1 mM phenylmethylsulfonyl fluoride and centrifuged as the medium to remove cellular debris. The clarified cell lysates and medium were subjected to immunoprecipitation using polyclonal antibodies as specified. After rotating the samples with protein A- or G-agarose overnight in the cold room, the immunoprecipitates were washed twice with 1× Triton X-100 salt wash buffer and twice with Tris-NaCl (5). The washed immunoprecipitates were boiled in SDS-loading buffer with 0.5 μM dithiothreitol and resolved by SDS-PAGE. Gels were soaked in destain and then in 1 M sodium salicylate, dried, and exposed to film. The radioactivity of some samples was measured in an Amersham Biosciences PhosphorImager. Each experiment was repeated a minimum of three times.

**Precipitation of Secreted Proteins by Trichloroacetic Acid—**The serum-free medium (see Fig. 2E) was recovered after a 1-h chase, and cellular debris was removed in a microcentrifuge at maximum speed for 10 min. Proteins in the supernatant were precipitated by the addition of an equal volume of ice-cold 20% trichloroacetic acid and the sample was incubated on ice for 30 min. The precipitated proteins were recovered by centrifugation at 5 min at maximum speed in a microcentrifuge at 4 °C and washed twice with cold acetone. After drying, the precipitated proteins were dissolved in SDS-loading buffer, resolved by electrophoresis, and visualized.

**TLC—**Dishes of cells were incubated in medium containing 20 μCi/ml [3H]acetate for 2 h. Cellular lipids were extracted with chloroform/methanol (2:1), and equal aliquots were resolved by TLC (8). Lipid spots were visualized with iodine vapor, outlined with a pencil, scraped into scintillation vials, and counted. The c.p.m. were adjusted for the amount of cellular protein as measured by a protein assay kit from Bio-Rad.

**RESULTS**

**Increasing Ferritins by Iron Loading Leads to Decreased ApoB Secretion—**We first assessed the secretion of apoB in the presence of increased levels of ferritin H and L induced by iron treatment. Translation of ferritin proteins is regulated by iron-regulatory proteins that bind to a stem-loop structure in the 5′-untranslated region of ferritin mRNA. This binding prevents translation; in the presence of iron, however, iron-regulatory proteins are released, and synthesis of ferritin H and L chains proceeds (17, 19, 20). To induce synthesis of both ferritin H and L proteins, we treated HepG2 cells briefly with 10 μM FeSO₄/10 μM 8-hydroxyquinoline which has been shown to be non-toxic to cells (21). This treatment causes a rapid and marked increase in the synthesis of ferritin H and L chains as compared with untreated HepG2 cells (Fig. 1A). Concomitant with this increase in ferritin synthesis, we observed that the amount of apoB in the medium of cells treated with ferrous sulfate was reduced (Fig. 1B). To distinguish whether this reduction is due to diminished secretion or decreased synthesis of apoB, HepG2 cells incubated in the absence or presence of ferrous sulfate were pulse-labeled with radioactive methionine and immediately lysed. Equivalent amounts of completed and elongating apoB are found regardless of treatment with iron (Fig. 1C). Furthermore, we evaluated whether enhanced postsecretory removal of apoB is responsible for the reduced apoB in the medium of iron-treated cells. Untreated HepG2 cells and cells treated with ferrous sulfate were incubated with conditioned medium containing radiolabeled apoB. The amount of radiolabeled apoB recovered from the untreated and iron-treated HepG2 cells was similar (Fig. 1D). Therefore, treatment of HepG2 cells with ferrous sulfate leads to decreased secretion of apoB rather than decreased synthesis or increased removal of the apolipoprotein from the medium. Secretion is not affected globally since albumin, another endogenous protein of hepatocytes, is secreted normally (Fig. 1D). Thus, the treatment of hepatocytes with iron is associated with an increase in ferritin H and L synthesis and a selective decrease in the secretion of apoB. These data prompted us to test each ferritin chain further for its effect on apoB secretion in the absence of iron loading.

**Either Ferritin H or L Specifically Inhibits the Secretion of ApoB—**To eliminate any redox effect of iron and to distinguish the role of each type of ferritin chain, we investigated the secretion of apoB in stable, transfected HepG2 cell lines. We
Ferritins Can Regulate the Secretion of Apolipoprotein B

The secretion of apoB is inhibited from HepG2 cells expressing increased ferritin H or L. HepG2 (Ctrl), H3 (expressing increased ferritin H), and L1 (expressing increased ferritin L) cells were pulse-labeled with \(^{35}\text{S}\)methionine for 15 min and chased in complete medium for 1 h. A, ferritin H and L chains immunoprecipitated from the cell lysates of untransfected (Ctrl) and transfected HepG2 cell lines (H3 and L1). B, apoB secretion from untransfected, H3, L1, and stable, HepG2 cell lines transfected with the empty vector, pcDNA3 (pc2 and pc6). Aliquots of the cell lysates (C) and medium (M) were subjected to immunoprecipitation using antibody against apoB.

Transfected HepG2 cells with plasmids encoding either ferritin H or L that lacked the 5'-untranslated region so that the genes would be expressed constitutively rather than be subject to regulation by iron. Geneticin-resistant cell lines with increased expression of either ferritin H (H3) or L chains (L1) were isolated (Fig. 2A). Using metabolic labeling with radiolabeled methionine, we found that the secretion of apoB from H3 and L1 cells is markedly reduced (Fig. 2B). Three other cell lines expressing similar levels of ferritin were examined, and all inhibited apoB secretion by a proportion similar to H3 cells (data not shown). Likewise, three other cell lines with similarly elevated levels of ferritin L showed inhibition of apoB to the same proportion as L1 cells (data not shown). We also created stable HepG2 cell lines that were transfected with the empty vector. Eight clones of these Genenticin-resistant HepG2 cells were examined; all showed normal secretion of apoB (two are shown in Fig. 2B). Furthermore, other stable, transfected HepG2 cell lines expressing only Geneticin resistance have been reported to show normal secretion of apoB (22). Therefore, neither Geneticin treatment nor overexpression of aminoglycoside phosphotransferase affect apoB secretion. Finally, the secretion of other proteins is not affected by ferritins. In H3 and L1 cells, elevated levels of ferritins do not affect the secretion of albumin or factor XIII (Fig. 3, A and B). Furthermore, the secretion of apoA-I, the principal protein in high density lipoproteins, is normal in H3 and L1 cells (Fig. 3C). Moreover, the profile of radiolabeled proteins secreted by HepG2, H3, and L1 cells appears grossly similar (Fig. 3D). Thus, increased levels of either ferritin H or L specifically inhibit the secretion of apoB without blocking secretion in general.

The Block in ApoB Secretion Induced by Ferritin H or L Is Not from Diminished Lipid Synthesis or Micronasal Triglyceride Transfer Protein (MTP) Activity—ApoB is secreted only when assembled with lipids as a lipoprotein particle. An adequate supply of the protein and lipids is necessary for normal secretion of apoB-containing lipoprotein particles. To assess whether ferritins diminish cholesterol and triglycerides and thereby impair apoB secretion, we measured lipid synthesis in HepG2, H3, and L1 cells using labeled acetate (18). The amount of labeled phosphatidylcholine, triglycerides, cholesterol, and cholesterol esters were not diminished in H3 or L1 cells as compared with HepG2 cells, indicating that synthesis of these lipids is not decreased (Fig. 4). In fact, the amount of labeled triglycerides was elevated significantly in H3 and L1 cells. This elevation could result from an increase in the synthesis or decrease in the removal of cellular triglycerides. In either case, elevated levels of ferritins inhibit secretion of apoB by a mechanism other than diminished lipid synthesis.

The lipid transfer activity of MTP, a heterodimer with protein disulfide isomerase, is essential for the secretion of apoB (23–25). To rule out insertionnal inactivation or ferritin-mediated suppression of MTP activity, we assayed the levels of MTP activity in HepG2, H3, and L1 cells. The three cell lines had nearly identical levels of MTP lipid transfer activity as assessed by a fluorometric lipid transfer assay (data not shown). These data indicate that disruption of MTP activity is not the cause of the inhibition of apoB secretion seen in H3 or L1 cells. Thus, elevated levels of ferritin do not regulate apoB secretion through a decrease in the availability or MTP-mediated transfer of lipids. These data strongly suggest that ferritins act directly on the secretion of the apoB protein.

The Block in ApoB Secretion Can Be Overcome Partially by Increased Triglycerides—Increased triglyceride synthesis normally stimulates the secretion of apoB. The decreased release of apoB in the face of elevated triglycerides in H3 and L1 cells raises the issue of whether the ferritin-induced block to secretion is responsive to further stimulation of triglyceride synthesis or is fixed at a maximum rate of secretion. To investigate this question, sets of dishes of untransfected HepG2, H3, and L1 cells were analyzed by pulse-chase labeling in the absence or presence of oleic acid to boost triglyceride synthesis. As expected, the secretion of apoB from HepG2 cells is markedly increased by oleic acid (Fig. 5). Treatment of H3 and L1 cells also raised secretion of apoB from these cells, and secretion...
Ferritins Can Regulate the Secretion of Apolipoprotein B

**The block in apoB secretion is not due to diminished synthesis of lipids.** Lipids from HepG2, H3, and L1 cells were labeled with 20 μCi/ml [3H]acetate, extracted with chloroform/methanol, separated by thin-layer chromatography, and measured by scintillation counting of the scraped spots (18) (see “Experimental Procedures” for details). Solid black bars, HepG2 cells; gray bars, H3 cells; white bars, L1 cells; PC, phosphatidylcholine; TG, triglycerides; Chol, cholesterol; CE, cholesterol esters; PE, phosphatidylethanolamine. The graph shows the average of three experiments, and the error bars indicate the standard error. Analysis of variance showed a significant difference only for triglycerides as indicated in the graph. This difference is significant for H3 and HepG2 or L1 and HepG2 by the Tukey-Kramer Student’s t-tests.

The inhibition of apoB secretion is primary and leads to secondary degradation of the protein. As shown in A, HepG2 (Ctrl), H3, and L1 cells were pulse-labeled with [35S]methionine for 15 min and chased in complete medium for 1 h. Some dishes were treated with 0.8 mM oleic acid (+) beginning 2 h prior to labeling and throughout the experiment, whereas other dishes were left untreated (−). The cell lysates (C) and medium (M) were subjected to immunoprecipitation using antibody against apoB. Bottom panel, graph of the apoB secretion from the cells shown in the top panel. A Phosphor-Imager was used to measure the secretion of apoB.

**The Post-translational Block in ApoB Secretion Leads to Secondary Degradation of ApoB—Control of apoB secretion primarily occurs through post-translational mechanisms. Therefore, we hypothesized that ferritins post-transcriptionally regulate apoB secretion. If ferritins do not affect the translation of apoB, then the effect on secretion must be post-translational. Furthermore, we reasoned that an increased amount of apoB in H3 and L1 cells must be degraded intracellularly as either a primary or secondary event. If a block in apoB secretion is the principal event, then degradation would simply follow as a secondary event to dispose of excess intracellular chains of apoB. However, if increased degradation is the primary event, fewer apoB molecules would be available for secretion in H3 and L1 cells. In this secondary scenario, inhibiting degradation would lead to increased secretion of apoB.

We investigated whether ferritins affect the translation of apoB and whether increased amounts of apoB are degraded in H3 and L1 cells. We pulse-labeled cells for 15 min followed by a chase period of 10 or 60 min. The short 10-min chase period permits elongating chains to become full-length, and since apoB requires more than 10 min to exit hepatocytes, the nascent proteins are all found in the cell lysates (Fig. 6A). apoB is degraded in HepG2, H3, and L1 cells as evidenced by the difference in intracellular apoB between 10 and 60 min of chase even after accounting for the secreted proteins (Fig. 6A). However, the amount of apoB present in H3 and L1 cells at 10 min of chase is less than the amount of apoB found in control HepG2 cells. This difference could result from decreased synthesis or increased degradation of apoB in H3 and L1 cells. Therefore, we treated the three cell lines with ALLN, an inhibitor of proteasomes and other proteases that has been shown to decrease the degradation of apoB in HepG2 cells (26). When treated with ALLN, H3 and L1 cells contain similar amounts of apoB as control HepG2 cells after the short chase period (Fig.
Ferritins Can Regulate the Secretion of Apolipoprotein B

**DISCUSSION**

We demonstrate that ferritin H and L inhibit the secretion of apoB post-translationally. This effect of ferritins is not due to diminished lipid synthesis or alterations in MTP activity. As a consequence of this inhibition of secretion, increased apoB undergoes ERAD via the ubiquitin-proteasome pathway.

The block in secretion caused by either ferritin H or L appears specific for apoB. This conclusion is based on the normal secretion of a cross-section of proteins including albumin (an abundant protein), factor XIII (a less abundant protein that is secreted rapidly), and apoA-I (another apolipoprotein). Furthermore, the profiles of secreted proteins appear similar among HepG2, H3, and L1 cells. Although we cannot exclude the possibility that another protein is likewise affected, it appears that ferritins specifically inhibit the secretion of apoB.

The precise mechanism by which ferritins specifically block the secretion of apoB is an area of ongoing investigation in our laboratory.

Protein secretion is generally thought to be either constitutive or regulated via exocytosis from secretory vesicles. However, apoB secretion is regulated by post-translational mechanisms without the protein being stored in secretory vesicles. In addition to lipids and MTP activity, several stimuli including insulin, apolipoproteins, chaperone proteins, and cytokines such as tumor necrosis factor and interleukin-1β can alter the secretion of apoB (27, 28). Tumor necrosis factor and interleukin-1β decrease apoB secretion via epidermal growth factor receptor signaling (28), and these two cytokines also increase levels of ferritins (20). Our studies suggest that ferritins could be the intracellular effectors that decrease apoB secretion by these two cytokines.

The inhibition of apoB export by ferritins is a new example of regulation of protein secretion. Chaperone proteins anchored within the ER lumen, such as BiP, facilitate secretion of many proteins. One lumenal chaperone protein, MTP, appears to promote the secretion of apoB specifically (23–25). Counterbalancing these factors, the cytosolic chaperone proteins, hsp70 and hsp90, stimulate ERAD of apoB (29) and other proteins (30–32). In contrast, ferritins are normally cytosolic proteins that inhibit the secretion of apoB specifically. Furthermore, ferritins are not known to serve as protein chaperones.

This report is the first evidence of the intersection between the seemingly disparate pathways for iron storage and lipoprotein secretion. Although this connection is new, abnormalities in **intracellular** iron and cholesterol transport have been linked previously. Several patients with Niemann-Pick disease, a disorder marked by defective intracellular transport of cholesterol, have been described who lack hepatic ferritins (33–35). Although not directly related to our data, these reports indicate a link between pathways for handling iron and lipids.

Future investigations of ferritins and apoB in vivo should be conducted using appropriate models because not all conditions with high liver ferritin levels are equivalent. In hemochromatosis, for example, dysregulated intestinal iron absorption leads to tissue iron deposition and increased ferritin synthesis. The chronic iron deposition causes lipid peroxidation (36) and functional and morphologic damage to the liver (37, 38). These abnormalities would make it difficult to separate the toxic effects of iron from ferritins on apoB secretion either in affected homozygous C282Y humans or in a mouse model of hemochromatosis (39). Thus, hepatocytes with elevated ferritins due to a high level of intracellular iron are not the same as cells expressing increased ferritins in the absence of an iron stimulus.
Ferritins Can Regulate the Secretion of Apolipoprotein B

(Our data using iron-loaded HepG2 cells (Fig. 1) simply prompted a more careful investigation of each ferritin chain on apoB secretion in stable cell lines, but these findings do not imply that the two types of cell systems are equivalent.) In H3 and L1 cells, ferritins are increased without iron loading. Similarly, increases in ferritin levels that do not stem from changes in intracellular iron can occur physiologically from cytokines and other non-iron stimuli of ferritin synthesis (20). Therefore, a more relevant animal model to explore the relationship between ferritins and apoB is a mouse engineered to have altered ferritins and apoB. Consequently, ferritin could alter the biogenesis or trafficking of other proteins along with apoB. In general, rather than acting only within the confines of discrete metabolic pathways, key proteins of different pathways could interact directly in intracellular metabolic networks.

Acknowledgments—We thank Sharmistha Ghosh, Jeff Flier, and Seth Alper for reading the manuscript and helpful feedback, K. Aftab Rashid for constructing the plasmids pFerritinLight and pFerritinHeavy, Alan Stuart-Tilley for help with the statistical analyses, and Jean Vance for advice on TLC.

REFERENCES

1. Chan, L., Chang, B. H., Liao, W., Oka, K., and Lau, P. P. (2000) Recent Prog. Horm. Res. 55, 95–125
2. Pullinger, C. R., North, J. D., Teng, B. B., Rifici, V. A., Ronhild de Brito, A. E., and Scott, J. (1989) J. Lipid Res. 30, 1065–1077
3. Sato, R., Imanaka, T., Takatsuki, A., and Takano, T. (1990) J. Biol. Chem. 265, 11880–11884
4. Fisher, E. A., and Ginsberg, H. N. (2002) J. Biol. Chem. 277, 17377–17380
5. Yeung, S. C., Chen, S. H., and Chan, L. (1996) Biochemistry 35, 13843–13848
6. Fisher, E. A., Zhou, M., Mitchell, D. M., Wu, X., Omura, S., Wang, H., Goldberg, A. L., and Ginsberg, H. N. (1997) J. Biol. Chem. 272, 20427–20434
7. Bennet, F., and Grand-Perret, P. (1997) J. Biol. Chem. 272, 20435–20442
8. Chen, Y., Le Caherec, F., and Chuck, S. L. (1996) J. Biol. Chem. 271, 11887–11894
9. Liao, W., Yeung, S. C., and Chan, L. (1998) J. Biol. Chem. 273, 27225–27230
10. Pariyarath, R., Wang, H., Aitchison, J. D., Ginsberg, H. N., Welch, W. J., Johnson, A. R., and Fisher, E. A. (2001) J. Biol. Chem. 276, 541–559
11. Mitchell, D. M., Zhou, M., Parivyarath, R., Wang, H., Aitchison, J. D., Ginsberg, H. N., and Fisher, E. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14733–14738
12. Wu, X., Sakata, N., Lele, K. M., Zhou, M., Jiang, H., and Ginsberg, H. N. (1997) J. Biol. Chem. 272, 11575–11580
13. Fleming, J. F., Spitsen, G. M., Hui, T. Y., Olivier, L., Du, E. Z., Raabe, M., and Davis, R. A. (1999) J. Biol. Chem. 274, 9509–9514
14. Cavallio, D., Ruby, D., Mohammadi, A., Macri, J., and Adeli, K. (1999) J. Biol. Chem. 274, 23135–23143
15. Fisher, E. A., Pan, M., Chen, X., Wu, X., Wang, H., Jamil, H., Sparks, J. D., and Williams, K. J. (2001) J. Biol. Chem. 276, 27855–27863
16. Rashid, K. A., Hevi, S., Chen, Y., Le Caherec, F., and Chuck, S. L. (2002) J. Biol. Chem. 277, 22010–22017
17. Harrison, P. M., and Arslis, P. (1996) Biochim. Biophys. Acta 1275, 161–203
18. Rusínol, A. E., Lysak, P. S., Sigurdson, G. T., and Vance, J. E. (1996) J. Lipid Res. 37, 2296–2304
19. Rouault, T., and Klauser, R. (1997) Curr. Top. Cell. Regul. 35, 1–19
20. Torti, F. M., and Torti, S. V. (2002) Blood 99, 3505–3516
21. Van Lenten, B. J., Prieve, J., Navab, M., Hama, S., Luna, A. J., and Fogelman, A. M. (1995) J. Clin. Invest. 95, 2104–2110
22. Xia, H., and Redman, C. (2000) Biochem. Biophys. Res. Commun. 273, 377–384
23. Wetterau, J. R., Agerbeck, L. P., Bouna, M. E., Eisenberg, C., Munck, A., Hernier, M., Schmitz, J., Gay, G., Rader, D. J., and Gregg, R. E. (1992) Science 258, 999–1001
24. Leiper, J. M., Bayliss, D. J., Pease, R. J., Brett, D. J., Scott, J., and Shoulders, C. C. (1994) J. Biol. Chem. 269, 21951–21954
25. Jamil, H., Gordon, D. A., Eustice, D. C., Brooks, C. M., Dickson, J. K. J., Chen, Y., Ricci, B., Chu, C.-H., Harrity, T. W., Cisek, C. P. J., Biller, S. A., Gregg, R. E., and Wetterau, J. R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11991–11995
26. Sakata, N., Wu, X., Dixon, J. L., and Ginsberg, H. N. (1993) J. Biol. Chem. 268, 22962–22970
27. Sparks, J. D., Sparks, C. E., Bolognino, M., Roncione, A. M., Jackson, T. K., and Amatruda, J. M. (1988) J. Clin. Invest. 82, 37–43
28. Murthy, S., Mathur, S. N., and Field, P. J. (2000) J. Biol. Chem. 275, 9222–9229
29. Gusarova, V., Caplan, A. J., Brodsky, J. L., and Fisher, E. A. (2001) J. Biol. Chem. 276, 24891–24900
30. Zhang, Y., Nijhrok, G., Dzian, M. L., McCracken, A. A., Watkins, S. C., Michaelis, S., and Brodsky, J. L. (2001) Mol. Biol. Cell 12, 1303–1314
31. Brodsky, J. L., and McCracken, A. A. (1999) Semin. Cell Dev. Biol. 10, 567–573
32. Brodsky, J. L., Warner, J. L., Kruse, K. B., and McCracken, A. A. (1999) J. Biol. Chem. 274, 3453–3460
33. Christomanou, H., Kellermann, J., Linke, R. P., and Harzer, K. (1995) Biochem. Mol. Med. 53, 105–113
34. Christomanou, H., and Harzer, K. (1996) Biochem. Mol. Med. 58, 176–183
35. Christomanou, H., Vanier, M. T., Santambrogio, P., Arosio, P., Kleier, W. J., and Harzer, K. (2000) Mol. Genet. Metab. 70, 196–202
36. Britton, R. S. (1996) Semin. Liver Dis. 16, 3–12
37. Bothwell, T. H., and MacPhail, A. P. (1998) Semin. Hematol. 35, 55–71
38. Hussain, S. P., Raja, K., Amstad, P. A., Sawyer, M., Trudel, L. J., Wogan, G. N., Hofseith, L. J., Shields, P. G., Billar, T. R., Trautven, C., Hohler, T., Galle, P. R., Phillips, D. H., Markin, R., Marrogi, A. J., and Harris, C. C. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12770–12775
39. Zhou, X. Y., Tomatsu, S., Fleming, R. E., Parkkila, S., Waheed, A., Jiang, J., Fei, Y., Brunt, E. M., Ruddy, D. A., Prass, C. E., Schatzman, R. C., O'Neill, R., Britton, R. S., Bacon, B. R., and Sly, W. S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2492–2497
40. Ferreira, C., Santambrogio, P., Martin, M. E., Andreu, V., Feldmann, G., Henin, D., and Beaumont, C. (2001) Blood 98, 525–532
