The Hepatitis B Virus X Protein Inhibits Secretion of Apolipoprotein B by Enhancing the Expression of N-Acetylglucosaminyltransferase III*  

Sung-Koo Kang‡, Tae-Wook Chung‡, Ji-Young Lee‡, Young-Choon Lee§, Richard E. Morton*, and Cheorl-Ho Kim‡

From the ‡National Research Laboratory for Glycobiology, and Department of Biochemistry and Molecular Biology, College of Oriental Medicine, Dongguk University, Kyungju, Kyungbuk 780-714, Korea, and §Faculty of Biotechnology, Dong-A University, Saha-gu, Busan 604-714, Korea, and ¶Department of Cell Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195

The X protein of hepatitis B virus (HBx) plays a major role on hepatocellular carcinoma (HCC). Apolipoprotein B (apoB) in the liver is an important glycoprotein for transportation of very low density lipoproteins and low density lipoproteins. Although lipid accumulation in the liver is known as one of the factors for the HCC, the relationship between HBx and apoB during the HCC development is poorly understood. To better understand the biological significance of HBx in HCC, liver Chang cells that specifically express HBx were established and characterized. In this study we demonstrate that overexpression of HBx significantly up-regulates the expression of UDP-N-acetylglucosamine:β-mannoside-1,4-N-acetylglucosaminyltransferase-III (GnT-III), an enzyme that functions as a bisecting-β-N-acetylglucosamine (GlcNAc) transferase in apoB, and increases GnT-III promoter activity in a chloramphenicol acetyltransferase assay. GnT-III expression levels of HBx-transfected cells appeared to be higher than that of hepatocarcinoma cells as well as GnT-III-transfected cells, indicating that HBx may have a strong GnT-III promoter-enhancing activity. Intracellular levels of apoBs, which contained the increased bisecting GlcNAc, were accumulated in HBx-transfected liver cells. These cells as well as GnT-III-transfected liver cells revealed the inhibition of apoB secretion and the increased accumulation of intracellular triglyceride and cholesterol compared with vector-transfected cells. Moreover, overexpression of GnT-III and HBx in liver cells was shown to down-regulate the transcriptional level of microsomal triglyceride transfer protein, which regulates the assembly and secretion of apoB. Therefore, our study strongly suggested that the HBx increase in intracellular accumulation of aberrantly glycosylated apoB resulted in inhibition of secretion of apoB as well as intracellular lipid accumulation by elevating the expression of GnT-III.

In Western countries 75–90% of hepatocellular carcinomas (HCCs)1 are associated with chronic liver diseases (1). Hepatitis B virus is a major causative agent of acute and chronic hepatitis in humans (2) and is closely associated with the incidence of human liver cancer. Among the four proteins that originate from the hepatitis B virus genome, HBx protein is a 17-kDa multifunctional regulatory protein and has been detected with high frequency in liver cells from patients with chronic hepatitis, cirrhosis, and liver cancer (3). In our previous study HBx has an inhibitory effect on the p53-mediated transcription of the 3′-inositol phosphatase and tensin homologue deleted on chromosome 10, which is associated with tumor suppression (4). Therefore, HBx is thought to be associated with the development of HCC. However, the precise function of HBx in the tumorigenic transformation of liver cells remains unclear.

The liver is the major organ for both the production of plasma lipoproteins and their uptake from plasma and catabolism (5). The production of apolipoprotein B (apoB, a 500-kDa protein)-containing lipoproteins by the liver is required for the assembly and secretion of very low density lipoproteins and low density lipoproteins (6–10). The assembly of apoB with lipid to form a secretion-competent particle is a complex process (11, 12). It is widely accepted that hepatic lipid availability is obligatory for apoB-containing lipoprotein assembly within the liver. This finding has been supported by studies demonstrating the necessity of triglyceride (12, 13) and phospholipid (14). The microsomal triglyceride transfer protein (MTP) also plays a key role in apoB secretion by catalyzing the transfer of lipids to the nascent apoB molecule as it is co-translationally translocated across the endoplasmic reticulum membrane (15, 16).

GnT-III catalyzes the attachment of a GlcNAc residue to mannose in the β(1–4) configuration in the region of N-glycans and forms a bisecting GlcNAc (17), as shown in Scheme 1. Recent investigations revealed that the bisecting GlcNAc residue, a product of GnT-III activity, correlated with a number of biological events including the suppression of metastasis of mouse melanoma cells (18) and has been reported to be significantly elevated in the serum of human subjects with hepatomas, liver cirrhosis, as well as in HCC (19–25). Therefore, GnT-III also could be a factor for the development of HCC.

1 The abbreviations used are: HCC, hepatocellular carcinoma; HBx, X protein of hepatitis B virus; apoB, apolipoprotein B; GnT-III, UDP-N-acetylglucosaminyltransferase-III; MTP, microsomal triglyceride transfer protein; RT, reverse transcriptase; CAT, chloramphenicol acetyltransferase; t-RA, all-trans-retinoic acid; CREB, cAMP-response element-binding protein; E-PHA, erythrosquillactinating phytomenadione.

* This work was supported by National Research Laboratory Program, Ministry of Science and Technology, South Korea Grant M102030000024-02J0600-01300 (to C.-H.K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: NRLG and Dept. of Biochemistry and Molecular Biology, Dongguk University, Sukjang-Dong 707, Kyungju City, Kyungbu 780-714, Korea. Tel.: 82-54-770-2663; Fax: 82-54-770-2281; E-mail: chkimhio@dongguk.ac.kr.

2 This paper is available on line at http://www.jbc.org

28106 This paper is available on line at http://www.jbc.org
ApoB is a glycoprotein and contains high mannose N-glycans or biantennary-type oligosaccharides in the case of human low density lipoproteins (26, 27). Some investigators show that the glycosylation of proteins and lipids are associated with development, differentiation, and carcinogenesis (28–30). Recently, aberrant glycosylation as the direct result of the formation of bisecting-GlcNAc by GnT-III has been shown to disrupt the function of apoB, leading to the generation of fatty liver (31, 32). On the basis of the findings reported herein, we provide evidence of the molecular mechanism underlying the inhibition of apoB secretion and intracellular accumulation of triglyceride and cholesterol in vitro model by HBx/GnT-III expression.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Chang cells (ATCC number CCL-13), a human liver cell line, were maintained using Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum at 37 °C in a humidified 5% CO2 incubator. The cells were used for stable transfection with HBx and GnT-III cDNA using LipofectAMINE reagent by cell culture medium containing 600 μg/ml G418 sulfate. Total RNA was prepared using the TRIzol reagent (Invitrogen, USA) filtration, and finally, preparative gel electrophoresis to obtain purified monoclonal antibody as described previously (35).

Western Blot and Lectin Blot Analysis—Liver Chang cells were lysed in radioimmunoprecipitation assay buffer containing 150 mM NaCl, 20 mM Tris (pH 7.5), 1% Triton X-100, 2 mM EDTA, 10% (v/v) glycerol, 0.1% SDS, 0.5% deoxycholate, 1 mM Na3VO4, 20 μg/ml phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 100 kallikrein inhibitor units of aprotonin per ml. Cells lysates were clarified by centrifugation at 14,000 × g for 10 min at 4 °C. Protein (25 μg) was separated on SDS-PAGE gels and then transferred to nitrocellulose membrane. After blocking non-specific binding sites, the membranes were incubated with specific antibodies, anti-GnT-III, anti-glyceraldehyde-3-phosphate dehydrogenase (Chemicon), anti-HBV (Koma Biotechnology, Korea), and anti-apoB (Calbiochem). After washing the membranes with phosphate-buffered saline three times, they were further incubated with horseradish peroxidase-conjugated antibody. For detection of bisecting-GlcNAc residues, the membrane was incubated with 2 μg/ml biotinylated ethyleneglycol-biotinylated glycoprotein from E.coli (Roche Applied Science). Lectins were detected by using horseradish peroxidase-conjugated lectin (Seikagakukougyo, Japan). Immunoblotting was revealed by the enhanced chemiluminescence detection kit (Amersham Biosciences).

Immunoprecipitation—The cell lysate (0.5 mg/ml) was preclreated with 50 μl of protein A-Sepharose beads at 4 °C for 1 h and clarified by centrifugation at 14,000 rpm for 10 min. The preclreated lysate was incubated with an anti-apoB antibody for 1 h, then 50 μl of protein A-Sepharose beads were added, and the mixture was incubated for 1 h. After extensive washing with radioimmunoprecipitation assay buffer, the immunoprecipitated apoB were eluted from beads with 50 μg/ml SDS sample buffer and subjected to 6% SDS-PAGE under reducing conditions, Western blot and lectin blot were performed with anti-apoB and E-PHA, respectively, as described above.

Construction of Plasmids and Transfections of Chang Cells with GnT-III Promoter-Chloramphenicol Acetyltransferase (CAT) Gene Fusion Vector—pSV0-CAT, which expresses chloramphenicol acetyltransferase (CAT), was from the laboratory of Molecular Glycobiology, Korea Research Institute of Bioscience and Technology (KIRIB), Daejon, Korea. pGNT-CAT plasmid was constructed by ligating DNA fragment ranging from –1 to –1058 bp of GnT-III upstream region of promoter in pSV0-CAT. Cells were co-transfected with 10 μg of pGNT-CAT plasmid and different concentration of cDNA-HBx gene by LipofectAMINE-based transfection method. Transfected cells were cultured at 37 °C in 3% CO2 for 24 h followed by 5% CO2 for 24 h and used for the CAT assay.

Statistics—Results are expressed as the mean ± S.D. and averages of three to five experiments. Means were compared by t tests to determine statistical significance. A p value of <0.05 was considered significant.

RESULTS

HBx Enhances the Expression of GnT-III by the Promoter Activity of the GnT-III Gene—Chang cells were transfected with the HBx cDNA using pcDNA expression vector. The ex-
pression of both HBx mRNA and protein was verified before the investigation of HBx-induced effects. The expression of HBx mRNA was confirmed by Northern blot analysis, and the RNA controls of the corresponding blots are shown in Fig. 1A. Furthermore, the expression of HBx protein was confirmed by Western blot analysis using monoclonal anti-HBx antibodies (Fig. 1B).

Although we were searching for some factors that induced GnT-III, we found that HBx protein enhanced the mRNA and protein expression of GnT-III in the HBx-transfected cells, Chang-HBx. To elucidate whether the endogenous GnT-III gene is activated in the Chang-HBx, the GnT-III mRNA and protein levels were measured by Northern blot (Fig. 2A), RT-PCR (Fig. 2B), and Western blot analysis (Fig. 2C). As shown by these data, the expression of GnT-III gene and protein was significantly elevated in Chang-HBx cells compared with Chang and Chang-pcDNA3 cells.

Here, we postulate whether HBx may enhance the promoter activity of the GnT-III gene in the endogenous system. In a previous study we isolated and characterized the 5′-flanking region of GnT-III gene from human placental genome library (37). A schematic diagram of the GnT-III promoter from −1058 to −1 is shown in Fig. 3A. Putative promoter elements are based on sequence comparison to known motifs: GRE, half-palindromic glucocorticoid-response element (TGTCCT), recognition sites for CREB (CGTGACGA), AP-2 (GGCCTGGGGA), and SP1 (GGGGCC). The EMBL data library accession number is L48489. To examine the effect of HBx on the promoter activity of human GnT-III gene, the plasmid pGNT-CAT containing GnT-III promoter in front of the CAT reporter gene was co-transfected in human normal liver Chang cells with an increasing amount of HBx expression vector (pcDNA-HBx) (see “Experimental Procedures”). Indeed, as shown in Fig. 3B, the CAT activity of the GnT-III promoter was gradually elevated up to 5-fold by increasing the HBx expression vector, although detailed characterization of the promoter activity is limited due to its comprehensive properties of the GnT-III promoter region (38, 39). Therefore, these results showed that the HBx protein transactivated GnT-III gene transcriptionally and translationally.

**Comparison of the Levels of HBx and GnT-III mRNA and Protein in Normal Cells, Hepatocarcinoma Cells, and Our Transfected Cells**—To compare the expression levels of HBx and GnT-III among the several cell lines, GnT-III cDNA was transfected into liver Chang cells, and RT-PCR and Western blot analysis were carried out using normal liver Chang cells, the hepatocarcinoma cell line (HepG2), and our transfected cells as described under “Experimental Procedures.” As shown in Fig. 4A, we could not detect the HBx level in HepG2 cells by RT-PCR (Fig. 4A) and Western blot (Fig. 4B) because HepG2 is well known as hepatocarcinoma cell line, which is not integrated with hepatitis B virus genome. The level of GnT-III expression in HepG2 cells was similar to that of GnT-III-transfected cells but appeared to be lower than that in HBx-transfected cells. Moreover, in densitometry analysis (Fig. 4C) based on protein levels, GnT-III levels in HBx-transfected cells was increased up to 2-fold over that in GnT-III-transfected cells.

**Accumulation of ApoB with Increased Bisecting-GlcNAc in HBx-transfected Cells**—To investigate the increase of accumulation of intracellular apoB in HBx-transfected liver cells, immunoprecipitation analysis was performed using cell lysates with anti-apoB antibody as described under “Experimental Procedures.” As shown in Fig. 5A, compared with pcDNA-transfected cells as control, HBx-transfected cells contained higher levels of intact apoB bands, 150- and 50-kDa apoB species. Next, to determine whether these apoB species are aberrantly glycosylated, lectin blot analysis were performed using E-PHA lectins, which are known to react preferentially with bisecting-GlcNAc (40). In Fig. 5B, immunoprecipitated apoB species showed increased bisecting-GlcNAc, which is a consistent result with Western blot analysis in HBx-transfected cells. However, although an ~80-kDa protein reacted with E-PHA, this band appeared to be nonspecific because it could not detect in Western blot analysis (Fig. 5A). Furthermore, the reactivity of immunoprecipitated apoB to E-PHA was blocked in the presence of an authentic inhibitor, GalNAc (41). Therefore, these results indicated that HBx-transfected cells significantly increased the intracellular accumulation of apoB species, which contained increased bisecting GlcNAc.

**Enhanced Expression of GnT-III by HBx Decreases the Secretion of ApoB by Regulation of GnT-III**
was higher than that in GnT-III-transfected cell as shown in Fig. 4, we hypothesized that the aberrant glycosylation of apoB by HBx-induced GnT-III expression may be involved in apoB secretion; the apoB protein level was measured by Western blot from culture media in the control vector, GnT-III, and HBx-transfected cells because apoB plays an important role for delivery of triglyceride from liver to peripheral tissue. Cells were treated with t-RA for 2 days in serum-free media because secretion of apoB induced by t-RA was increased in dose and time-dependent manner (data not shown). The same result was observed in treatment with oleic acid, and there was no apoptotic fragmentation in the cell (data not shown). The secretion of apoB was significantly
HBx Inhibits Secretion of ApoB by Regulation of GnT-III

**Fig. 6.** GnT-III expression levels, inhibition of apoB secretion from culture media, and changes in cellular triglyceride and cholesterol contents in GnT-III- and HBx-transfected liver cells. A, after being permanently transfected with HBx and GnT-III cDNAs, Chang cells were incubated with 1 μm t-RA in serum-free media for 2 days. Proteins (25 μg) from media were subjected to 6% SDS-PAGE. Secretion of apoB from the media was analyzed by Western blot and bands were quantitated by densitometry. B, changes in cellular triglyceride (TG) and cholesterol mass were measured in Chang cells using enzymatic reagents. Values are the means for three to five experiments.

As a result of the Western blot experiment of inhibition of apoB secretion in HBx- and GnT-III-transfected cells, as shown in Fig. 6A, we postulated that cellular triglyceride and cholesterol mass may be increased in the HBx- and GnT-III-transfected cells. To test this, we measured the accumulation of triglyceride and cholesterol in the cytosolic fraction in Chang cells transfected with HBx and GnT-III. As expected, in Fig. 6C cellular triglyceride levels in GnT-III- and HBx-transfected cells were higher than vector-transfected cells up to 25% (p < 0.05) and 85% (p < 0.01), respectively. Cellular cholesterol levels in GnT-III- and HBx-transfected cells were slightly increased by 21% (p < 0.05) and 35% (p < 0.01), respectively. These results clearly indicated that increased accumulation of intracellular apoBs caused an accumulation of triglyceride and cholesterol by enhanced expression of HBx-induced GnT-III.

**DISCUSSION**

Here, we provide evidence of the relationship between HBx and GnT-III responsible for inhibition of apoB secretion. However, the HepG2 cell line is already a HCC, and increase of the expression of GnT-III gene was reported in human hepatocarcinoma tissues, fetal liver tissues, and hepatoma cell lines (42). In this study we have chosen a liver Chang cell line that is originally derived from normal liver tissue but is subsequently established via HeLa cell contamination. Miyoshi et al. (43) observe that during hepatocarcinogenesis, GnT-III messenger RNA levels were increased in LEC rats, and Ishibashi et al. (21) also reported that GnT-III activity in human serum, and liver and hepatoma tissues were increased in liver cirrhosis and hepatoma patients. We observed that HBx increases GnT-III expression by transcription as well as translation levels. In the previous study we found that the promoter region of GnT-III has seven AP-2 sites by sequence homology search (37). Elevation of GnT-III gene expression by HBx may be modulated by AP-2 activation since HBx has been shown to play a critical role for apoB assembly and secretion, to determine whether MTP expression may be affected in the GnT-III- and HBx-transfected liver cells, MTP mRNA levels were measured by RT-PCR and Northern blot analysis (Fig. 7, A and B). The expression of MTP was significantly decreased in GnT-III- and HBx-transfected cells compared with pcDNA-transfected cells after treatment of t-RA for 48 h in serum-free media. When the cells were treated with t-RA, however, there was no difference in apoB gene expression by RT-PCR (data not shown). This result suggested that the relationship between HBx and GnT-III may regulate MTP expression for apoB assembly and secretion.

Expression Levels of MTP mRNA—Because MTP has been shown to play a critical role for apoB assembly and secretion, to determine whether MTP expression may be affected in the GnT-III- and HBx-transfected liver cells, MTP mRNA levels were measured by RT-PCR and Northern blot analysis (Fig. 7, A and B). The expression of MTP was significantly decreased in GnT-III- and HBx-transfected cells compared with pcDNA-transfected cells after treatment of t-RA for 48 h in serum-free media. When the cells were treated with t-RA, however, there was no difference in apoB gene expression by RT-PCR (data not shown). This result suggested that the relationship between HBx and GnT-III may regulate MTP expression for apoB assembly and secretion.
GnT-III expression levels in HBx-transfected cells is higher than that in hepatocarcinoma HepG2 cells as well as GnT-III-transfected cells, indicating that HBx induces GnT-III expression with strong GnT-III promoter activity. Therefore, this finding supports several studies which show that GnT-III expression may be involved in the development of HCC (23, 25). Unfortunately, this study did not determine the precise promoter region involved in the activation for GnT-III expression by HBx because of the limitation of the comprehensive properties of the GnT-III promoter region (38, 39), but this is the first report that HBx transactivates GnT-III expression.

The hepatotoxic effect of apoB-containing lipoproteins is regulated largely at posttranscriptional levels, with nascent apoB molecules secreted or degraded intracellularly (11). Based on our findings that intracellular accumulation of aberrantly glycosylated apoB species, which exhibit strong reactivities to E-PHA, is increased and low molecular weight immunoreactive apoB species (especially 150 and 50 kDa) are detected in HBx-transfected cells, the present study supports our previous results (31) and those of Ihara et al. (32), who report that overexpression of GnT-III in transgenic hepatocytes induced aberrant glycosylation of apoB and disrupted apoB secretion. They also showed that the 130- and 50-kDa apoB species were immunoprecipitated and detected with lectin blot analysis. In addition, apoB mRNA levels in liver Chang cells are not affected by HBx transfection by RT-PCR (data not shown), consistent with the concept that, under most conditions of altered apoB secretion from HepG2 cells, apoB mRNA levels remain unchanged (13, 51, 52).

Some investigators suggest that hepatic triglyceride accumulation has a greater influence on apoB secretion (53) and cholesteryl ester is the major lipid species stimulating very low density lipoprotein secretion (54). However, in our study, even though there is intracellular accumulation of triglyceride and cholesterol in GnT-III- as well as HBx-transfected liver Chang cells, inhibition of apoB secretion occurs in these cells. Therefore, intracellular accumulation of apoB caused by the ability of HBx to induce GnT-III expression is the major determinant for the inhibition of apoB secretion and intracellular lipid accumulation.

With regard to the association of apoB with tumorigenesis, lipid accumulation in the liver resulted in the development of dysplasia and carcinoma of the liver in mice expressing aberrantly truncated apoB (55). Our study also revealed that an increase of the accumulation of triglyceride and cholesterol in liver cells is detected in GnT-III- as well as HBx-transfected cells, which is consistent with previous studies by Lee et al. (31) and Ihara et al. (32), who showed that lipid accumulation in the liver in GnT-III transgenic mice leads to the generation of liver abnormalities. Thus, intracellular accumulation of triglyceride and cholesterol in liver cells caused by HBx-induced GnT-III expression may give us a new insight for HBx-mediated HCC development.

In addition, MTP, an intraluminal protein in the endoplasmic reticulum plays an essential role in regulating the assembly and secretion of apoB-containing lipoproteins (44, 50, 56). It is interesting to note that HBx- and GnT-III- transfected cells showed down-expression of MTP mRNA but not in vector-transfected cell. Because cells were treated with t-RA in our experiment, MTP expression levels by RT-PCR and secretion of apoB by Western blot were increased in dose- and time-dependent manners (data not shown). However, these data questioned whether down-regulation of MTP transcriptional levels by overexpression of GnT-III and HBx may result from the accumulation of triglyceride and cholesterol in liver cells primarily or secondarily. The contribution of down-regulation of MTP expression by GnT-III and HBx remains to be clarified.
41. Serafini-Cessi, F., Franceschi, C., and Sperti, S. (1979) Biochem. J. 183, 381–388
42. Song, E. Y., Lee, Y. C., Park, Y. G., Chung, T. W., and Kim, C. H. (2001) Cancer Invest. 19, 797–805
43. Miyoshi, E., Nishikawa, A., Ibara, Y., Gu, J., Sugiyama, T., Hayashi, N., Fusamoto, H., Kamada, T., and Taniguchi, N. (1993) Cancer Res. 53, 3899–3902
44. Gordon, D. A., Jamil, H., Gregg, R. E., Olofsson, S.-O., and Boren, J. (1996) J. Biol. Chem. 271, 33047–33053
45. Imagawa, M., Chiu, R., and Karin, M. (1987) Cell 51, 251–260
46. Hymans, S. E., Comb, M., Pearlberg, J., and Goodman, H. M. (1989) Mol. Cell. Biol. 9, 321–324
47. Kannan, P., Buettner, R., Chiao, P. J., Yim, S. O., Sarkiss, M., and Tainsky, M. A. (1994) Genes Dev. 8, 1258–1269
48. Caselmann, W. H., and Keshy, R. (1998) Hepatitis B Virus: Molecular Mechanisms in Disease and Novel Strategies for Therapy, pp 161–181, Imperial College Press, London
49. Havv, I., Shamay, M., Datzh, G., and Shaul, Y. (1998) Mol. Cell. Biol. 18, 1562–1569
50. Jamil, H., Gordon, D. A., Eustice, D. C., Broods, C. M., Dickson, J. J. K., Chen, Y., Ricci, B., Chu, C-H., Harrity, T. W., Ciosek, J. C. P., Biller, S. A., Gregg, R. E., and Wetterau, J. R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11991–11995
51. Wilcox, L. J., Barrett, P. H. R., Newton, R. S., and Huff, M. W. (1999) Arterioscler. Thromb. Vasc. Biol. 19, 939–949
52. Wilcox, L. J., Barrett, P. H. R., and Huff, M. W. (1999) J. Lipid Res. 40, 1078–1089
53. Benoist, F., and Grand-Perret, T. (1996) Arterioscler. Thromb. Vasc. Biol. 16, 1229–1235
54. Avaramoglu, R. K., Cianflone, K., and Sniderman, A. D. (1995) J. Lipid Res. 36, 2513–2528
55. Yamanaka, S., Balestra, M. E., Ferrell, L. D., Fan, J., Arnold, K. S., Taylor, S., Taylor, J. M., and Innerarity, T. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8483–8487
56. Hassain, M. M., Shi, J., and Dreizen, P. (2003) J. Lipid Res. 44, 22–32