Biosynthesis of Apolipoprotein B48-containing Lipoproteins

REGULATION BY NOVEL POST-TRANSCRIPTIOINAL MECHANISMS*

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Apolipoprotein (apo) B plays a central role in lipoprotein metabolism. It is the only apolipoprotein in low density lipoproteins (LDL), the lipoproteins that transport two-thirds of the plasma cholesterol in humans. Elevated concentrations of LDL cholesterol are one of the major risk factors for the development of atherosclerosis. The possibility of lowering plasma LDL concentrations by limiting the production of very low density lipoproteins (VLDL), the precursor of LDL, has fueled interest in the biosynthesis of VLDL. The production of apoB and the formation of VLDL are not regulated at the level of apoB gene transcription but rather by other post-transcriptional processes, some of which are unique to apoB and offer opportunities for therapeutic intervention. Two approaches have received the greatest attention. The first approach involves the introduction of a unique RNA editing enzyme into the liver that would reduce the formation of apoB100 and LDL. The second involves the assembly and intracellular sorting of apoB-containing lipoproteins and, specifically, the inhibition of lipid addition to apoB100. The prime target for such an inhibition is the microsomal transfer protein, a protein that is necessary for VLDL and chylomicron assembly and secretion. This paper will review the molecular mechanisms that have inspired the efforts to address these two possibilities.

Apolipoprotein B and mRNA Editing

Apolipoprotein B100, one of the largest proteins known (4,536 amino acids) (1, 2), is synthesized and used to assemble VLDL in the liver (3). After secretion into the circulation, the VLDL are converted into intermediate density lipoproteins (VLDL) in the liver (3). After secretion into the circulation, the apoB100 wrapped around the particle, some-what like a belt (4). The interaction between the protein and the lipids occurs via amphipathic a-helices and amphipathic 3-sheets (5). Amphipathic 3-sheets have been suggested to confer irreversible lipid binding to apoB. With the apparent exception of the first 80–100 kDa (the N-terminal cysteine-rich region of apoB), these amphipathic structures are clustered in different regions over the entire length of apoB (5).

The small intestine synthesizes a shorter form of apoB from the same gene (3). The shorter form, called apoB48, consists of the N-terminal 2,152 amino acids of apoB100 and is necessary for the synthesis, assembly, and secretion of the triglyceride-enriched chylomicrons (6). Much less is known about the conformation of apoB48 on chylomicrons, but the first region of amphipathic -strand may enable apoB48 to associate strongly with chylomicrons during the assembly of the lipoproteins.

Apolipoprotein B48 is synthesized by a novel post-transcriptional modification of the mRNA, designated mRNA editing (7–9). The editing process deaminates a specific cytidine (nucleotide (nt) 6666) to form a uridine (10, 11). This changes the codon at position 2153 from a genomically encoded CAA (glutamate) to an in-frame stop codon (UAA) (12). Apolipoprotein B mRNA editing occurs in the small intestines of all mammals and in the livers of rats, mice, dogs, and horses (13). Hepatic editing activity is regulated by growth hormone, thyroxine, cortisol, fasting, and diet (14–17).

Apolipoprotein B mRNA Editing Sequence Specificity

Nucleotide 6666 on apoB mRNA is normally deaminated with great precision. The nucleotide sequence elements on apoB mRNA necessary for this high specificity have been identified by site-directed mutagenesis (10, 18–22). Mutations at any of 10 specific nucleotides in an 11-nt region (Fig. 1) either abolished or greatly reduced in vitro apoB mRNA editing, indicating that mRNA editing is dependent upon this sequence element (18, 21, 22). Additionally, this recognition sequence, or “mooring sequence,” will promote editing at an upstream cytidine when it is inserted into a heterologous gene or introduced into a heterologous site in apoB mRNA (20, 22). A spacer sequence of four nucleotides between the cytidine and the mooring sequence is optimal for editing (22, 23). Surprisingly, mutations of most of the nucleotides 3–4 positions on either side of the edited base do not greatly reduce editing activity in vitro (19), but mutations in the sequence more upstream from the editing site reduce editing efficiency (22, 23) (Fig. 1, Efficiency). In addition to the sequence elements described above, the sequences further upstream and downstream of the the mooring and/or editing sites need to be AU-rich to achieve efficient editing (20).

Identification of the Catalytic Subunit of the ApoB mRNA Editing Complex

The cloning of one of the proteins that catalyze the apoB mRNA process was a major advance in understanding the mechanism of apoB mRNA editing. A functional cloning approach was used to identify and isolate a cDNA that encoded a 229-amino acid protein from a rat small intestine cDNA library (24). This protein, APOBEC-1 (apoB mRNA-editing enzyme catalytic polypeptide 1) (25), is the catalytic subunit of the apoB mRNA editing complex that deaminates nt 6666 in apoB mRNA (Fig. 1) (26). APOBEC-1 has been cloned from a number...
The auxiliary protein(s) apparently bind to the APOBEC-1 and RNA and provide specificity for the process. The mooring sequence is an 11-nucleotide sequence to which the auxiliary factor(s) apparently bind, and the efficiency sequence is a segment of the RNA upstream from the editing site that increases editing efficiency.

Even though APOBEC-1 is capable of binding RNA, the enzyme is not able to edit apoB RNA in vitro without the addition of complementary or auxiliary protein(s) (24, 30, 32). The number and identity of these protein(s) are unknown, but they apparently are part of a multicomponent editing complex and have a widespread distribution. They are found not only in apoB-synthesizing tissues but also in tissues and organs that synthesize little, if any, apoB and contain undetectable apoB mRNA-editing activity (24, 30–32). Their widespread distribution suggests that the auxiliary factor(s) may have a more extensive function, possibly as components of another type of RNA editing process.

The auxiliary protein(s) have recently been shown to bind to APOBEC-1 (35), but they also may be specific apoB-RNA-binding proteins. Cross-linking studies using UV radiation have identified proteins from liver and intestinal extracts that bind to apoB mRNA (10, 22, 36, 37), with molecular masses ranging from 40 to 66 kDa. Navaratnam et al. (10) found two proteins from rat enterocyte S100 supernatant editing extract with molecular masses of 43 and 60 kDa that UV cross-linked to rat apoB RNA. One of these proteins, p60 (60 kDa), cross-linked to a site centered at nt 6671–6674 of the apoB mRNA (10). As described previously, this site is within the 11 nt mooring sequence that mutational analysis has demonstrated to be essential for in vitro editing (21). Although the specificity of p60 binding has been questioned (22), apoB RNA with mutations in the mooring sequence compete poorly with wild-type apoB RNA for binding to this protein, suggesting that p60 binds to the mooring sequence. Thus, it appears that the auxiliary factor(s) interact with both APOBEC-1 and the apoB mRNA.

The question of whether APOBEC-1 normally functions as a component in the editing of mRNAs other than apoB mRNA has not been resolved. No other substrate for APOBEC-1 has been identified in normal animals. In humans and rabbits, the tissue distribution of APOBEC-1 mRNA reflects the sites in which apoB mRNA is edited (i.e. predominantly in the small intestine (27, 28, 30)), with low abundance in the colon (rabbit) and only trace amounts in other tissues (27, 28, 30). In rats, APOBEC-1 mRNA is located in the liver and small intestine and at lower levels in the colon, kidney, spleen, and lung, with trace amounts found in every tissue examined (14). In mice, the highest levels of mRNA expression are found in the small intestine, liver, and spleen, followed by the kidney, lung, muscle, and heart (29). Apolipoprotein B mRNA editing activity also has been identified in five osteosarcomatoid and epidermoid cell lines (38). Thus, evidence of editing or APOBEC-1 mRNA in cell lines and tissues that do not synthesize apoB suggests other possible functions for APOBEC-1.

**RNA Editing and Cancer: Overexpression of APOBEC-1 in the Liver of Transgenic Mice Causes Hepatic Tumors**

Recently, APOBEC-1 overexpressed in transgenic animals was shown to edit other mRNAs (39). Transgenic rabbits and mice expressing rabbit APOBEC-1 in their livers were generated to determine whether the hepatic expression of APOBEC-1 would lower LDL concentrations by reducing apoB100 synthesis by the liver. The apoB mRNAs from the livers of the transgenic animals were extensively edited, and their apoB100 and LDL concentrations were reduced compared with control animals. Unexpectedly, all of the transgenic mice and a transgenic rabbit displayed liver dysplasia, and many transgenic mice developed hepatocellular carcinomas. Thus, even though adenovirus-mediated transfer of APOBEC-1 to the livers of mice lowers LDL levels (40), the finding of tumorigenesis associated with the hepatic overexpression of APOBEC-1 severely compromises its potential use in gene therapy for lowering LDL concentrations to prevent atherosclerosis.

Other hepatic mRNAs with mooring sequence motifs similar to that of apoB mRNA were examined for cytidine deamination editing. The RNAs for a tyrosine kinase and a homologue of a translation initiation factor were found to be edited in the transgenic mouse livers but not in the livers of control mice (39). These results suggest that aberrant editing of hepatic mRNAs that encode proteins with important cellular functions could cause the liver dysplasia and subsequent tumors. The identification of these mRNAs should reveal novel proteins with important biological properties.

**Assembly of Apolipoprotein B48 into Lipoproteins**

The editing process determines the type of apoB molecule synthesized, which, in turn, dictates the type of lipoprotein assembled. As shown in Fig. 2, the assembly pathways for the lipid addition to apoB48 involve two main steps. Studies in hepatoma cells have demonstrated that these steps regulate the amount of nascent lipoproteins secreted by the cells. Lipids are added to apoB during the translation/translocation of the protein (41–43), and the amount of lipids added to apoB in the co-translational step depends upon the length of the apoB (41, 43). Lipoprotein assembly begins when the nascent chains reach a length of ~700–900 amino acids (41). The N-terminal 80-kDa section of apoB, which contains 12 cysteines, appears to have a low affinity for lipids (42). Twelve cysteines in this region form disulfide bonds (5, 44) that stabilize and fold the domain. The resulting compact globular structure (5) exists as a water-soluble, lipid-poor (or lipid-free) protein when expressed as a truncated protein in cells in culture (42). The N-terminal domain also may participate in the assembly process by interacting with proteins involved in lipidation of apoB, such as the microsomal transfer protein (MTP) (45). Incorrect lipidation results in increased degradation. One possibility is that a protease cleavage site becomes exposed under this condition, resulting in a loss of the N-terminal globular domain of the protein (46, 47) and sorting of the protein to post-translational degradation.

During the co-translational assembly process, a direct rela-
tionship apparently exists between the size of the apoB nascent polypeptide, the amount of lipid added, and the resulting size of the assembled lipoprotein (41–43). Lipoproteins assembled cotranslationally by apoB48 have the size (approximately 10 nm) and density of high density lipoprotein (HDL) particles (48–50) (Fig. 2). Yet, apolipoprotein B48 directs the assembly of intestinal chylomicrons that are particles much larger (75–1200 nm) than the VLDL assembled by apoB100 (30–80 nm) in the liver (51). In the rodent, hepatic apoB48 assembles VLDL particles as large as those assembled by apoB100 (51). Pulse-chase studies in Ma-RH7777 cells have provided an explanation for this paradox (49). Apolipoprotein B48 VLDL are formed in two well-defined steps (Fig. 2). Initially, small apoB48 "HDL-like" particles (i.e. lipoprotein particles having the size (10 nm) and density of HDL particles) are formed cotranslationally. These particles can be either secreted from the cell or retained in the cell and converted to large triglyceride-rich apoB48 VLDL/chylomicron particles, when triglyceride biosynthesis is stimulated. A similar model was suggested as the interpretation of the immunoelectron microscopic observations of intracellular apoB-containing lipoproteins (52). Thus, the assembly of apoB48 VLDL occurs in two steps, with the major amount of lipid added in the second step (49, 53) (Fig. 2). Chylomicron particle assembly in the intestine probably occurs by a similar mechanism.

**Lipid Addition during the Assembly Process**

Structural studies indicate that apoB interacts with phospholipids, which presumably are added during the translation/translocation process. It is also known that phospholipid synthesis is essential for secretion of apoB-containing lipoproteins (54). Both apoB100 and apoB48 are associated with the ER membrane (55–59), and this association occurs cotranslationally (57). Although it may be exposed on the cytoplasmic side (58, 59), apoB may also interact with the inner leaflet of the ER membrane (60, 61), and this interaction may provide the phospholipids needed to create the lipoprotein surface.

The addition of triglyceride to the lipoprotein particle is essential for the formation of lipoprotein particles and modulates the amount and type of lipoprotein particles secreted (62). Recently, a microsomal enzyme, MTP, has been isolated from the ER (63, 64) and found to be essential for the assembly and secretion of apoB-containing lipoproteins. In vitro, MTP has been shown to transfer lipids, mainly triglycerides but also phospholipids and cholesterol esters (65), between amphiphilic surfaces of liposomes (66). Mutations in the MTP gene cause the genetic disorder abetalipoproteinemia (67), which is characterized by an absence of apoB-containing lipoproteins in plasma (51) but normal biosynthesis of apoB (68, 69). Transfer of apoB and MTP into cells that do not normally assemble lipoproteins induces basal co-translational lipidation resulting in the assembly and secretion of dense apoB-containing lipoproteins (45, 70, 71) (Fig. 2). Thus, it appears that MTP and apoB can use the secretory pathway of any eukaryotic cell to assemble lipoproteins and that MTP is involved in the first basal lipidation step.

The first step of apoB48 VLDL assembly is clearly separated from the second step (addition of the lipid core) (Fig. 2) (49). The second step of apoB48 VLDL assembly does not seem to involve MTP.3 Immunoelectron microscopic studies (52) of rat hepatocytes suggest the presence of preformed neutral lipid droplets in the smooth ER that do not contain immunoreactive apoB. Nascent VLDL particles (with immunoreactive apoB) are present in the smooth termini of the rough ER. Immunoreactive apoB also is seen in the rough ER, suggesting that the second step involves the fusion of the dense apoB48 particle with a preformed lipid core.

The second step in the assembly process is inhibited by brefeldin A (72) at concentrations that still allow transport through the secretory pathway, as well as the formation and secretion of the apoB48-containing dense particles (i.e. the tentative VLDL precursors). Recent studies indicate that brefeldin A acts by inhibiting an unknown protein important to the function of the guanine nucleotide exchange protein (73). Thus, the selective inhibition of the second step by brefeldin A suggests that a GTP/GDP-dependent cycle is involved in this step (Fig. 2).

More than 60% of the triglyceride used for the assembly of apoB-containing lipoproteins is derived from a storage pool in the cytoplasm (74, 75). It is first hydrolyzed to diacylglycerol and then re-esterified to triglyceride before assembly into VLDL. The cytoplasmic pool of triglycerides is not used for lipoprotein assembly in HepG2 cells (76), and this deficiency may be a major reason why these cells do not perform the second step in the assembly of full-sized VLDL.

**Biosynthesis of Apolipoprotein B-containing Lipoproteins Is Regulated Post-transcriptionally and Post-translationally**

Apolipoprotein B is constitutively expressed (56, 77, 78), and apoB-gene transcription is not highly variable. As discussed above, apoB mRNA editing is a post-transcriptional process that determines the size of apoB synthesized and, consequently, the type of particles assembled and secreted (16). The other main post-translational control point is the targeting of apoB for either degradation or secretion. In hepatoma cells, oleic acid induces an increased secretion of apoB-containing lipoproteins without any detectable change in the amount of apoB mRNA. The increased secretion is due to an increase in the proportion of the protein secreted (56) versus that which is post-translationally degraded. One possible explanation is that the relation between apoB and the lipid core is important for the secretion of lipoproteins (62). If apoB is incorrectly lipidated it will not fold correctly and will therefore be recognized

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3 D. Gordon, personal communication.
by mechanisms that retain or degrade misfolded proteins in the cell.

**Conclusion**

In this review, molecular mechanisms of two major steps in VLDL biosynthesis are considered: the apoB mRNA-editing step and the inhibition of lipid addition to apoB. Of the two different approaches for intervention with VLDL biosynthesis, the latter seems to be the more feasible. The therapeutic potential of hepatic expression of APOBEC-1 appears to be compromised because the overexpression of this protein in animals causes liver dysplasia and hepatocellular carcinoma. The use of MTP inhibitors may be a unique tool for interference with the initial step(s) of lipoprotein formation and may prove to be an efficient way to decrease the number of apoB-containing lipoprotein particles formed in the cell. This approach is especially attractive for patients with absent or defective LDL receptors and for patients with VLDL overproduction syndromes.

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**REFERENCES**

1. Chen, S.-H., Yang, C.-Y., Chen, P.-F., Setzer, D., Tanimura, M., Li, W.-H., Gotto, A. M., Jr., and Chan, L. (1986) J. Biol. Chem. 261, 12988–12991

2. Knott, T. J., Pease, R. J., Powell, L. M., Wallis, S. C., Rall, S. C., Jr., Innerarity, T. L., Tice, L. B., Taylor, W. H. M., Yudkoff, M., Riddle, L. J., Johnson, D. Fuller, M., Lusis, A. J., McCarthy, B. J., Malley, R. W., Levy-Wilson, B., and Scott, J. (1986) Nature 323, 734–738

3. Glickman, R. M., Rogers, M., and Glickman, J. N. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 5296–5300

4. Chatterton, J. E., Phillips, M. L., Curtiss, L. K., Milne, R., Fruchart, J.-C., and Davidson, N. O. (1987) J. Lipid Res. 28, 222–226

5. Segrest, J. P., Jones, M. K., Mishra, V. K., Anantharamaiah, G. M., and Scott, J. (1989) J. Biol. Chem. 264, 1663–1668

6. Kane, J. P. (1983) J. Clin. Invest. 71, 409–418

7. Backus, J. W., and Smith, H. C. (1991) J. Biol. Chem. 266, 14839–14845

8. Backus, J. W., and Smith, H. C. (1991) Trends Biochem. Sci. 16, 226–229

9. Powell, L. M., Wallis, S. C., Pease, R. J., Edwards, Y. H., Knott, T. J., and Scott, J. (1987) Cell 50, 831–840

10. Navaratnam, N., Shah, R., Patel, D., Fay, V., and Scott, J. (1993) J. Biol. Chem. 268, 26149–26154

11. Chen, S.-H., Yang, C.-Y., Chen, P.-F., Setzer, D., Tanimura, M., Li, W.-H., Gotto, A. M., Jr., and Chan, L. (1988) J. Biol. Chem. 263, 1674–1685

12. Knott, T. J., Pease, R. J., Powell, L. M., Wallis, S. C., Rall, S. C., Jr., Innerarity, T. L., Tice, L. B., Taylor, W. H. M., Yudkoff, M., Riddle, L. J., Johnson, D. Fuller, M., Lusis, A. J., McCarthy, B. J., Malley, R. W., Levy-Wilson, B., and Scott, J. (1986) J. Biol. Chem. 261, 12988–12991

13. Backus, J. W., and Smith, H. C. (1991) J. Biol. Chem. 266, 16531–16534

14. Hodges, P., and Scott, J. (1992) Trends Biochem. Sci. 17, 77–81

15. Gretch, D. G., Sturley, S. L., Wetterau, J., Wang, L., Grunwald, K., and Attie, A. D. (1995) J. Biol. Chem. 270, 7382–7392

16. Sorci-Thomas, M., Wilson, M. D., Johnson, F. L., Williams, D. L., and Rudel, L. L. (1989) J. Biol. Chem. 264, 2681–2688

17. Pullinger, C. R., North, J., Teng, B.-B., Rifidi, V. A., Ronhild de Brito, A. E., and Scott, J. (1999) J. Lipid Res. 40, 1055–1067

18. Tsai, S.-C., Adamik, R., Moss, J., and Vaughan, M. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 3052–3055

19. Wright, D. S., and Idol, J. R. (1995) Nature 378, 552–554

20. Gordon, D. A., Jamil, H., Sharp, D., Mullaney, D., Yao, Z., Gregg, R. E., and Wetterau, J. R., Jr. (1993) J. Clin. Invest. 92, 1176–1184

21. Adeli, K. (1994) J. Biol. Chem. 269, 1661–1666

22. Prentki, M., and Reaven, G. M. (1994) Diabetologia 37, 167–173

23. Spring, D. J., Chen-Liu, W. L., Chatterton, J. E., Elson, J., and Schumaker, V. N. (1992) J. Lipid Res. 33, 233–240

24. Teng, B., Blumenthal, S., Forte, T., Navaratnam, N., Scott, J., Gotto, A. M., Jr., and Chan, L. (1994) J. Biol. Chem. 269, 29395–29404

25. Bostrom, K., Garcia, Z., Poksay, K. S., Johnson, D. F., Lusis, A. J., and Davidson, N. O. (1990) J. Biol. Chem. 265, 22452–22457

26. Teng, B., Blumenthal, S., Forte, T., Navaratnam, N., Scott, J., Gotto, A. M., Jr., and Chan, L. (1994) J. Biol. Chem. 269, 29395–29404

27. Backus, J. W., and Smith, H. C. (1991) J. Biol. Chem. 266, 14839–14845

28. Backus, J. W., and Smith, H. C. (1991) J. Biol. Chem. 266, 14839–14845

29. Backus, J. W., and Smith, H. C. (1991) Biochemistry. Acta 1217, 65–73

30. Shah, R., Riddle, L. J., Legro, R. J., Navaratnam, N., Glickman, J. N., and Scott, J. (1992) J. Biol. Chem. 267, 16301–16304

31. Driscoll, D., Lakhe-Reddy, S., Oleksa, L. M., and Martinez, D. (1993) Mol. Cell. Biol. 13, 7288–7294

32. Backus, J. W., and Smith, H. C. (1992) Nucleic Acids Res. 20, 6007–6014

33. Teng, B., Burant, C. F., and Davidson, N. O. (1993) Nucleic Acids Res. 21, 1816–1819

34. Davidson, N., Innerarity, T. L., Scott, J., Smith, M., Driscoll, D. M., Tice, L. B., and Chan, L. (1995) RNA 1, 73

35. Navaratnam, N., Morrison, J. R., Bhattacharya, S., Patel, D., Funahashi, T., Giannoni, F., Teng, B.-B., Davidson, N., and Scott, J. (1993) J. Biol. Chem. 268, 20709–20712

36. Lau, P. P., Zhu, H.-J., Baldini, A., Charnsangavej, C., and Chan, L. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 8522–8526

37. Hladiljaciou, C., Giannoni, F., Funahashi, T., Skarosi, S. F., and Davidson, N. O. (1994) J. Biol. Chem. 269, 13800–13806

38. Nakamura, M., Oka, K., Kushniki, J., Kobayashi, K., Yamamoto, T., Yamashita, S., and Chan, L. (1995) J. Biol. Chem. 270, 13042–13056

39. Yamakawa, M., Okazaki, K., Saito, K., Balseira, M., Gotoh, M., Zeng, G.-Q., and Innerarity, T. L. (1994) J. Biol. Chem. 269, 21725–21734

40. Navaratnam, N., Bhattacharya, S., Fujino, T., Patel, D., Armar, A. L., and Scott, J. (1995) Cell 81, 187–195

41. Driscoll, D. M., and Zhang, Q. (1994) Mol. Cell. Biol. 14, 2845–2852

42. MacGinnitie, A. J., Anant, S., and Davidson, N. O. (1995) J. Biol. Chem. 270, 14768–14775