Microsomal Triglyceride Transfer Protein Promotes the Secretion of Xenopus laevis Vitellogenin A1*

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Vitellogenins (Vtg) are ancient lipid transport and storage proteins and members of the large lipid transfer protein (LLTP) gene family, which includes insect apolipophorin II/I, apolipoprotein B (apoB), and the microsomal triglyceride transfer protein (MTP). Lipidation of Vtg occurs at its site of synthesis in vertebrate liver, insect fat body, and nematode intestine; however, the mechanism of Vtg lipid acquisition is unknown. To explore whether Vtg biogenesis requires the apoB cofactor and LLTP family member, MTP, Vtg was expressed in COS cells with and without coexpression of the 97-kDa subunit of human MTP. Expression of Vtg alone gave rise to a ~220-kDa apoprotein, which was predominantly confined to an intracelular location. Coexpression of Vtg with human MTP enhanced Vtg secretion by 5-fold, without dramatically affecting its intracellular stability. A comparison of wild type and a triglyceride transfer-defective form of MTP revealed that both were capable of promoting Vtg secretion, whereas only wild type MTP could promote the secretion of apoB41 (amino-terminal 41% of apoB). These studies demonstrate that the biogenesis of Vtg is MTP-dependent and that MTP is the likely ancestral member of the LLTP gene family.

The vitellogenins (Vtgs)1 are egg yolk storage precursor proteins that transport minerals, amino acids, lipids, and other nutrients from extravarian tissue to the developing oocyte in nematodes, arthropods, and oviparous vertebrates. As Vtg exists in species whose last common ancestor diverged over 550 million years ago, they may have played a pivotal role in the appearance of both apoB and MTP. As such, the sequestration of lipid by Vtg was presumed to be MTP-independent and probably the result of autonomous lipid recruitment (8, 9). It was recently shown, however, that MTP exists both in insects and nematodes, neither of which express a known apoB ortholog (10, 11). Although Drosophila MTP was shown to be capable of lipidating human apoB41 in a cotransfection assay, the endogenous substrate(s) of insect MTP have not been identified (10). The current studies reveal that the biogenesis of what had previously appeared to be the oldest member of the LLTP family gene, Vtg, is in fact MTP-dependent. Hence, MTP is likely the ancestral member of the LLTP gene family and may function in the biogenesis of all of its evolutionary descendants.

EXPERIMENTAL PROCEDURES

Construction of Plasmids—A clone containing full-length Xenopus laevis Vtg-A1 cDNA in the pCDV-1 vector (Amerham Biosciences) was produced as described (12). For expression, the entire cDNA insert was ligated into the expression vector pCMV5 (13). MTP-N780Y was constructed by PCR-based site-directed mutagenesis as described by Ohashi et al. (14).

Cell Culture and Metabolic Radiolabeling—COS-1 cells were grown in 100-mm dishes in Dulbecco’s modified Eagle’s medium with 4.5 mg/liter glucose, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% FBS (Mediatech). Cells were transfected at 50–60% confluence with a total of 9 μg of DNA/dish by the FuGENE 6 method (Roche Applied Science), using a 2:1 (vol:mass) ratio of FuGENE 6:DNA. For coexpression, cells were transfected with 6 μg of Vtg or apoB41 plasmid (15) and 3 μg of truncated human placental alkaline phosphatase (AP), proteins (LLTP). Members of this family include Vtg, insect apolipophorin II/I, apolipoprotein B (apoB), and the microsomal triglyceride transfer protein (MTP) (3). Although these proteins are homologous, they perform rather distinct functions, suggesting that they are paralogs rather than orthologs.

Lamprey lipovitellin, the processed form of Vtg, is the only known LLTP family member whose crystal structure has been solved (4). Lamprey lipovitellin contains an amino-terminal β barrel, an extended α helical region, and a C-terminal domain, which sequesters ~20–40 molecules of mainly phospholipid within a funnel-shaped lipid binding cavity (4, 5). The primary structure of Vtg is partially conserved within the amino-termini ~750–1000 amino acids of apoB, apolipophorin-II/I, and MTP, suggesting that the amino termini of all LLTP family members assume a Vtg-like tertiary structure (2, 6, 7). On the other hand, the amounts and types of lipids carried by various LLTP family members varies considerably, suggesting that the lipid binding domains of these proteins may have evolved distinct structural and functional properties consistent with their disparate roles in lipid transport.

Until recently, it was assumed that Vtg is the primordial member of the LLTP gene family, evolutionarily preceding the appearance of both apoB and MTP. As such, the sequestration of lipid by Vtg was presumed to be MTP-independent and probably the result of autonomous lipid recruitment (8, 9). It was recently shown, however, that MTP exists both in insects and nematodes, neither of which express a known apoB ortholog (10, 11). Although Drosophila MTP was shown to be capable of lipidating human apoB41 in a cotransfection assay, the endogenous substrate(s) of insect MTP have not been identified (10). The current studies reveal that the biogenesis of what had previously appeared to be the oldest member of the LLTP family gene, Vtg, is in fact MTP-dependent. Hence, MTP is likely the ancestral member of the LLTP gene family and may function in the biogenesis of all of its evolutionary descendants.

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1 The abbreviations used are: Vtg, vitellogenin; AP, truncated human placental alkaline phosphatase; apo, apolipoprotein; LLTP, large lipid transfer protein; MTP, microsomal triglyceride transfer protein.
MTP-dependent Vitellogenin Biogenesis

Xenopus Vtg was transfected in duplicate with either AP or the human MTP 97-kDa subunit, or MTP-N780Y. Cells were incubated with transfection mixture for 24 h and then radiolabeled with 100 μCi/ml [35S]Met/Cys (EasyTag Express Protein Labeling Mix, PerkinElmer Life Sciences) in Met/Cys-deficient Dulbecco’s modified Eagle’s medium (ICN Biomedicals) for the indicated times. Following labeling, media were recovered, and the cells washed with phosphate-buffered saline. Cells were lysed on the plates with 1 ml of lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, and pepstatin A). Media samples were clarified by centrifugation at 10,000 × g for 5 min and adjusted to lysis buffer conditions. Media and cell lysates were subjected to immunoprecipitation with antibody to Vtg following SDS-PAGE and fluorography. Arrow indicates position of Vtg polypeptide; line shows position of 175-kDa gel marker.

![Expression of Vtg-A1](image)

| AP | Vtg | MTP |
|----|-----|-----|
| ++ | +   | -   |
| +  | ++  | +   |
| -  | +   | ++  |
| +  | +   | +   |

RESULTS

Xenopus Vtg was transfected in duplicate with either AP or the human MTP 97-kDa subunit (17, 18). Cells were radiolabeled with [35S]Met/Cys for 4 h, and cell lysates and media samples were subjected to immunoprecipitation with anti-Xenopus Vtg antibodies. Cotransfection with AP and Vtg gave rise to a protein of ≈220 kDa, not present when cells were transfected with AP alone. (Fig. 1, compare lane 1 with lanes 3 and 5). The gel mobility was consistent with a calculated molecular mass of 200 kDa and some covalent modification, including N-linked glycosylation and phosphorylation within the Vtg phosphovitelin domain (19). Surprisingly, only trace amounts of Vtg were detected in media, unless cotransfection was performed with MTP (compare lanes 4 and 6 with lanes 8 and 10). These data suggest that MTP might be required to promote the efficient secretion of Vtg.

DISCUSSION

The stimulation of Vtg secretion by MTP observed in the current study is reminiscent of the effects of MTP on apoB. In the absence of MTP, apoB fails to acquire lipid during transport, causing misfolding and intracellular retention and degradation (25, 26). In contrast, coexpression of apoB with MTP promotes nascent lipoprotein formation, which stabilizes apoB and promotes anterograde transport and secretion (23, 27). Under the conditions employed here, a dramatic increase in Vtg secretion was observed upon coexpression with MTP (Figs. 1 and 2). However, this increased secretion was not accompanied by a detectable degree of Vtg lipidation as judged by density gradient centrifugation. This is to be expected given that the small amount of lipid (20–40 molecules/monomer) associated with lipovitellin forms a very high density lipoprotein, which is difficult to resolve from lipid-poor proteins based on density alone (5, 28). Hence additional methods beyond those commonly used to study buoyant lipoprotein assembly will be required to monitor Vtg

wild-type human MTP 97-kDa subunit, or MTP-N780Y. Cells were incubated with transfection mixture for 24 h and then radiolabeled with 100 μCi/ml [35S]Met/Cys (EasyTag Express Protein Labeling Mix, PerkinElmer Life Sciences) in Met/Cys-deficient Dulbecco’s modified Eagle’s medium (ICN Biomedicals) for the indicated times. Following labeling, media were recovered, and the cells washed with phosphate-buffered saline. Cells were lysed on the plates with 1 ml of lysis buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, and pepstatin A). Media samples were clarified by centrifugation at 10,000 × g for 5 min and adjusted to lysis buffer conditions. Media and cell lysates were subjected to immunoprecipitation with antibody to Vtg following SDS-PAGE and fluorography. Arrow indicates position of Vtg polypeptide; line shows position of 175-kDa gel marker.

![Expression of Vtg-A1](image)

| AP | Vtg | MTP |
|----|-----|-----|
| ++ | +   | -   |
| +  | ++  | +   |
| -  | +   | ++  |
| +  | +   | +   |

FIG. 1. Expression of Vtg-A1. COS cells were transfected with AP, Vtg, and MTP, as indicated. Transfected cells were radiolabeled with [35S]Met/Cys for 5 h. Cell lysate (C) and media (M) samples were subjected to immunoprecipitation with anti-Xenopus Vtg polyclonal antibodies followed by SDS-PAGE and fluorography. Arrow indicates position of Vtg polypeptide; line shows position of 175-kDa gel marker.

The ability of MTP-N780Y to promote apoB41 secretion is severely reduced and approaches the background observed with no MTP coexpression (compare lanes 2 and 6). However, unlike apoB41, the secretion of Vtg was nearly identical using both the wild type and the N780Y forms of MTP (Fig. 3, compare lanes 10 and 12). These data suggest that the requirements for Vtg and apoB biogenesis contributed by MTP are overlapping but not identical.

To explore whether MTP-mediated stimulation of Vtg secretion is accompanied by its acquisition of lipid, equilibrium density gradient centrifugation at d = 1.25 g/ml KBr was performed as described (24). Vtg recovered from AP- or MTP-transfected cells was recovered in the d > 1.25 g/ml, lipid-poor bottom gradient fraction (Fig. 4, lanes 1–8). We also observed that Vtg was incapable of floatation at d = 1.29 g/ml using CsCl gradients (data not shown). These results suggest that even in the presence of MTP, Vtg does not acquire sufficient lipid to form a buoyant lipoprotein. As a positive control, apoB20.1 was analyzed. This form of apoB was shown previously to form a small emulsion particle with a density of −1.24 g/ml when coexpressed with MTP (24). Under the same conditions used for the Vtg analysis, MTP coexpression converted ~20% of the total secreted apoB20.1 into a form that floated at d < 1.25 g/ml (Fig. 4, compare lanes 9 and 11 with lanes 13 and 15). Together these data suggest that the amount of lipid added to Vtg by MTP is insufficient to create a buoyant lipoprotein or that the effects of MTP on Vtg secretion competence are mediated by events other than bulk lipidation.
lipidation in cell-based systems. Despite the inability to detect lipid associated with Vtg, MTP exerted a profound stimulatory effect on Vtg secretion, which is comparable to that seen for apoB41. This raises the possibility that MTP functions by loading lipid into the Vtg lipid-binding cavity, perhaps during or shortly after translation. On the other hand, it is intriguing that a triglyceride transfer-defective abetalipoproteinemia allele of MTP (N780Y) is also effective in promoting Vtg secretion, suggesting that a non-bulk lipid transfer-related activity of MTP may be important for Vtg assembly. A chaperone-like activity of MTP has long been proposed to play a role in the folding dynamics of apoB, and perhaps by analogy, Vtg (29). It has also been noted that the MTP-dependent addition of chaperone-like lipids to apoB may facilitate subsequent folding and bulk lipid acquisition (30). In this regard, it of interest that the crystal structure of lipovitellin revealed a single molecule of lipid fully surrounded by protein atoms at a site far removed from the main lipid-binding cavity (5). An intriguing possibility is that MTP, utilizing an activity distinct from its ability to engage in bulk lipid transfer, deposits a single phospholipid molecule in this site, which then facilitates the formation of the native tertiary structure of Vtg. By analogy, the CD1 major histocompatibility complex lipid antigen-presenting proteins acquire a single phospholipid molecule in the ER, which may facilitate their assembly and transport to endosomes (31). Surprisingly, recent studies in MTP knock-out mice revealed that antigenic glycolipid transfer to CD1d in hepatocytes and intestinal epithelial cells, is MTP-dependent (32).

The finding that Vtg is a substrate for MTP has important implications for understanding the evolutionary origins and mechanisms of apoB-containing lipoprotein formation. Until recently, it was presumed that so-called “primitive liproproteins”, such as Vtg and insect lipophorins, were formed by autonomous lipid recruitment (8, 9). The studies here, how-

**FIG. 2.** Secretion of Vtg-A1 is stimulated by MTP. A, COS cells were transfected with Vtg and AP (lanes 1–12) or Vtg and MTP (lanes 13–24). Cells were metabolically radiolabeled for 1 h with [35S]Met/Cys and then chased with medium containing an excess of cold Met and Cys for either 0 or 4 h, as indicated. Cell lysates and media samples were subjected to immunoprecipitation with anti-Vtg antibodies, followed by SDS-PAGE and fluorography. Arrows (right) indicate position of Vtg and co-immunoprecipitated MTP polypeptides. Lines (left) show positions of 83- and 175-kDa gel markers. Gels from A and an additional independent experiment (data not shown) were subjected to phosphorimaging analysis to determine the quantitative impact of MTP on Vtg secretion efficiency (B) and presecretory degradation (C). Data in B and C are mean ± S.D. (n = 5 or 6).

**FIG. 3.** Vtg-A1 is secreted from COS cells cotransfected with a triglyceride transfer-defective form of COS cells cotransfected with a triglyceride transfer-defective form of MTP. COS cells in 100-mm dishes were cotransfected with apoB41 (lanes 1–6) or Vtg (lanes 7–12) and AP, wild type (wt) MTP, or the triglyceride transfer-defective MTP-N780Y, as indicated. Cells were radiolabeled for 12 h with [35S]Met/Cys followed by immunoprecipitation of cell lysate (C) and media (M) samples with anti-Vtg (lanes 1–6) or anti-apoB (lanes 7–12) antibodies. Immunoprecipitates were analyzed by SDS-PAGE and fluorography.

**FIG. 4.** Differential effects of MTP on lipidation status of Vtg-A1 and apoB20.1. Duplicate sets of COS cells were cotransfected with Vtg (lanes 1–8) or apoB20.1 (lanes 9–16) and either AP (+) or MTP (+), as indicated. Cells were metabolically radiolabeled with [35S]Met/Cys for 4 h, and medium samples were recovered, concentrated, and adjusted to 1.25 g/ml KBr. After centrifugation for 16 h, the d > 1.25 g/ml top 1-ml (T) and the d > 1.25 g/ml bottom 2-ml (B) gradient fractions were recovered by tube slicing. Vtg and apoB20.1 were recovered by immunoprecipitation and analyzed by SDS-PAGE and fluorography. Each apoB20.1 determination was performed with medium from one 100-mm dish. Each Vtg analysis was performed using media pooled from two 150-mm dishes. Note that in lane 16, only one-half of the available sample was loaded.
ever, clearly demonstrate that even Vtg-containing lipoprotein formation cannot proceed without the participation of MTP. As Vtg and MTP are highly conserved in most oviparous animals, the observed capacity of human MTP to promote the secretion of X. laevis Vtg-A1, probably extends to invertebrates as well. Indeed, genetic studies in Caenorhabditis elegans have drawn an indirect functional connection between MTP and Vtg. Mutations or silencing of dsc-4, the progenitor member of the LLTP gene family and likely ortholog of MTP, partially suppressed a pleiotropic phenotype caused by disruption of the ubiquinone biosynthetic gene, CLK-1. siRNA-mediated silencing of some Vtg genes created a similar phenotype, suggesting the possibility that suppression of clk-1 by dsc-4 is related to disruption of Vtg-containing lipoprotein assembly (11).

In conclusion, the present studies demonstrate that MTP is the progenitor member of the LLTP gene family and likely acts on all LLTP family descendants, including Vtg, apoB, and perhaps apolipophorin-II/I. Its differential interactions with multiple substrates has numerous implications for understanding the evolution of MTP and its many acquired roles in intracellular lipid mobilization, lipoprotein assembly and secretion, antigenic lipid presentation, and perhaps other as yet unknown functions.

REFERENCES
1. Byrne, B. M., Gruber, M., and AB, G. (1989) Prog. Biophys. Molec. Biol. 53, 33–69
2. Mann, C. J., Anderson, T. A., Read, J., Chester, S. A., Harrison, G. B., Köch, S., Ritchie, P. J., Bradbury, P., Hussain, F. S., Amey, J., Vanloo, B., Roseneu, M., Infante, R., Hanceck, J. M., Levitt, D. G., Banaszak, L. J., Scott, J., and Shoulders, C. C. (1999) J. Mol. Biol. 285, 391–408
3. Babin, P. J., Bogerid, J., Koiman, F. P., Van Marrewijk, W. J. A., and Van der Horst, D. J. (1999) J. Mol. Evol. 49, 150–160
4. Anderson, T. A., Levitt, D. G., and Banaszak, L. J. (1998) Structure (Lond.) 6, 895–909
5. Thompson, J. R., and Banaszak, L. J. (2002) Biochemistry 41, 9398–9409
6. Segrest, J. P., Jones, M. K., and Dashiti, N. (1999) J. Lipid Res. 40, 1401–1416
7. Smolenars, M. M., Kasperaitis, M. A., Richardson, P. E., Rodenburg, K. W., and Van der Horst, D. J. (2005) J. Lipid Res. 46, 412–421
8. Canavoso, L. E., Jouni, Z. E., Karnas, K. J., Pennington, J. R., and Wells, M. A. (2001) Anna. Rev. Nutr. 21, 23–46
9. Hui, T. Y., Olivier, L. M., Kang, S., and Davis, R. A. (2002) J. Lipid Res. 43, 785–793
10. Sellers, J. A., Hou, L., Athar, H., Hussain, M. M., and Shelnas, G. S. (2003) J. Biol. Chem. 278, 20367–20373
11. Shibata, Y., Brancicky, R., Landaverde, I. O., and Hekimi, S. (2003) Science 299, 1779–1782
12. Batistuzzo de Medeiros, S. B. (1993) Etudes Structurales, Fonctionnelles et Evolutives sur le Gene A1 de la Vitelogenine chez le Crapaud a Griffes Xenopus laevis. Ph.D thesis, Institute de Biologie Animale, Université de Lausanne-Facultés des Sciences, Lausanne-Suisse (Switzerland)
13. Andersson, S., Davis, D. L., Dahlback, H., Jornvall, H., and Russell, D. W. (1989) J. Biol. Chem. 264, 8222–8229
14. Ohashi, K., Ishibashi, S., Osga, T., Tazawa, R., Harada, K., Yahagi, N., Shinomi, F., Irioka, Y., Tamura, Y., Nagai, R., Ilklingworth, D. R., Gotoda, T., and Yamada, N. (2000) J. Lipid Res. 41, 1199–1204
15. Sellers, J. A., and Shelnas, G. S. (2001) J. Lipid Res. 42, 1897–1904
16. Shelnas, G. S., Morris-Rogers, K. C., and Ingram, M. F. (1994) J. Biol. Chem. 269, 9311–9318
17. Gordon, D. A., and Jamil, H. (2000) Biochim. Biophys. Acta Mol. Cell. Biol. Lipids 1486, 72–83
18. Hussain, M. M., Shi, J., and Dreizen, P. (2003) J. Lipid Res. 44, 22–32
19. Nardelli, D., van het Schip, F. D., Gerber-Huber, S., Haefliger, J. A., Gruber, M., AB, G., and Wahl, W. (1987) J. Biol. Chem. 262, 15377–15385
20. Wu, X. J., Zhou, M. Y., Huang, L. S., Wetterau, J., and Ginsberg, H. N. (1996) J. Biol. Chem. 271, 10277–10281
21. Patel, S. B., and Grundy, S. M. (1996) J. Biol. Chem. 271, 18686–18694
22. Read, J., Anderson, T. A., Ritchie, P. J., Vanloo, B., Amey, J., Levitt, D., Rosseneu, M., Scott, J., and Shoulders, C. C. (2000) J. Biol. Chem. 275, 36372–36377
23. Leiper, J. M., Bayliss, J. D., Pease, R. J., Brett, D. J., Scott, J., and Shoulders, C. C. (1994) J. Biol. Chem. 269, 21951–21954
24. Shelnas, G. S., Hou, L., Ledford, A. S., Parks, J. S., and Weinberg, R. B. (2003) J. Biol. Chem. 278, 44702–44707
25. Shelnas, G. S., and Sellers, J. A. (2001) Curr. Opin. Lipidol. 12, 151–157
26. Fisher, E. A., Pan, M., Chen, X. L., Wu, X. Y., Wang, H. X., Jamil, H., Sparks, J. D., and Williams, K. J. (2001) J. Biol. Chem. 276, 27855–27863
27. Gordon, D. A., Jamil, H., Sharp, D., Mullaney, D., Yao, Z., Gregg, R. E., and Wetterau, J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7628–7632
28. Chapman, M. J. (1980) J. Lipid Res. 21, 789–793
29. Gordon, D. A. (1997) Curr. Opin. Lipidol. 8, 131–137
30. Wetterau, J. R., Lin, M. C. M., and Jamil, H. (1997) Biochim. Biophys. Acta Lipids Lipid Metab. 1345, 136–150
31. Park, J. J., Kang, S. J., De Silva, A. D., Stanic, A. K., Casorati, G., Hachey, D. L., Creswell, P., and Joyce, S. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 1922–1926
32. Brozovic, S., Nagaishi, T., Yoshida, M., Betz, S., Salas, A., Chen, D., Kaser, A., Glickman, J., Kuo, T., Little, A., Morrison, J., Corazza, N., Kim, J. Y., Colgan, S. P., Young, S. G., Exley, M., and Blumberg, R. S. (2004) Nat. Med. 10, 535–539