Microsomal triglyceride transfer protein expression during mouse development

John M. Shelton,[1],[†] Mi-Hye Lee,[1],[*] James A. Richardson,[§] and Shailendra B. Patel[2],[*]

Division of Endocrinology, Diabetes, and Medical Genetics,[*] Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425-2222, and Department of Internal Medicine, Division of Cardiology,[†] and Department of Pathology,[§] University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235

Abstract   Feto-maternal transfer of lipophilic nutrients is an important factor in the normal development of the fetus and may be mediated by lipoproteins as carriers of these nutrients. Two proteins that may be important in this process are apolipoprotein B (apoB, the major structural protein of secreted lipoproteins) and microsomal triglyceride transfer protein (MTP) whose normal activity is required for the secretion of apoB-containing lipoproteins. Although no abnormalities of conception and embryonic lethality are known in humans who inherit genetic deficiencies of either of these proteins, homozygous mice bearing knockouts of either apoB or MTP show early embryonic lethality. To characterize the ontogeny of MTP expression during embryonic mouse development, we have used in situ hybridization to characterize the pattern of expression. By using microwave heating of tissue sections to optimize hybridization, we show that there is robust MTP expression in the yolk sac tissues followed by expression in the primordial liver cell nests as early as day 9 post-coitum (E9.5). Intestinal expression is detected around E12.5 and attains full adult expression patterns by E14.5. No expression in any other tissues was observed, including developing heart, kidney, placenta, and maternal decidua.▪ Thus the pattern of MTP expression is compatible with a role in the transfer of lipophilic nutrients from the yolk sac, prior to hepatic development and to the liver, once the circulatory system has been established.—Shelton, J. M., M-H. Lee, J. A. Richardson, and S. B. Patel. **Microsomal triglyceride transfer protein expression during mouse development.** *J. Lipid Res.* **2000.** 41: 532–537.

Supplementary key words   embryonic development • feto-maternal unit • microwave RNA retrieval • riboprobes • in situ hybridization

The secretion of lipoproteins by the liver and the intestine is dependent upon two proteins: apolipoprotein B (apoB) and microsomal triglyceride transfer protein (MTP) (1, 2). Genetic deficiency of MTP in humans results in the autosomal recessive condition of abetalipoproteinemia (3). A similar condition, homozygous hypobetalipoproteinemia, results from defects involving the major structural protein of liver or intestinally secreted lipoproteins,

apoB (4). MTP is an ER protein whose function is to transfer neutral lipids, particularly triglyceride and cholesteryl ester, into the nascent apoB and thus form a lipoprotein for secretion. Such lipoproteins are important for transport of both triglycerides, which allow for delivery of energy to the periphery, but also carry vital fat-soluble vitamins and essential fatty acids important for normal cell function. Thus, in the human condition of abetalipoproteinemia, it is the impaired absorption and peripheral delivery of vitamins A and E that are responsible for much of the pathophysiology of this inherited condition (1, 5). Therapeutic replenishment of such vitamins can ameliorate, if not entirely prevent, almost all of the complications when therapy has been initiated early in the course of this disease. Interestingly, although no formal studies have been reported, there does not appear to be an increased incidence of early fetal loss or apparent infertility in couples that are obligate carriers for this disease (1). A similar clinical pattern is also present in homozygous hypobetalipoproteinemia, where the defect involves the structural gene for apoB, but also results in very impaired lipoprotein secretion (4, 6). Affected individuals also exhibit fat-soluble vitamin deficiencies. Early studies of human embryos 5–11 weeks post-fertilization showed the expression of apoB RNA in the yolk sac, fetal liver, and intestine, but not other embryonic tissues, detected by in situ hybridization as well as by metabolic labeling studies (7–9). In contrast, a number of studies have now been reported that show that complete disruption of apoB or MTP in mice result in embryonic lethality (10–15). One explanation for this difference may be the dependence of the developing mouse embryo on the yolk sac for early nutrient delivery and thus the need for lipoprotein-mediated synthesis and secretion of the fat-soluble nutrients from the yolk sac to

---

Abbreviations: MTP, microsomal triglyceride transfer protein; apo, apolipoprotein; ER, endoplasmic reticulum; RPA, ribonuclease protection assay; RT-PCR, reverse transcription-polymerase chain reaction.

[1] These authors contributed equally to this work.

[2] To whom correspondence should be addressed.

the embryo as proposed by Farese et al. (16). In the developing human embryo, it would appear that the yolk sac may be a vestigial remnant.

In the mouse, disruption of the apoB gene leads to resorption of the embryos by 11.5 days post-coitum (E11.5) and histological analyses at earlier stages showed an increased incidence of hydrocephalus and exencephalus (10, 11). In the MTP knockout animals, a similar phenotype was also identified and the increase in exencephalus was noted by E10.5 (14). Interestingly, using a sensitive RN′ase protection assay, Raabe et al. (14) were able to show the presence of MTP message in not only liver and intestine, but also in kidney and heart of normal embryonic mice.

We report here application of a modification to conventional methodology that has allowed us to improve upon detection of MTP mRNA in situ in the developing mouse embryo, and augment previous biochemical developmental data (16, 17). Utilizing microwave heating RNA retrieval to improve in situ hybridization in paraffin-embedded tissues (18–22), we demonstrate that expression of MTP is initially present in the yolk sac membranes, followed by the primordial liver, with intestine as the last tissue to show robust expression. No detectable expression was noted in the developing cardiac and renal tissues, nor in the developing placenta and maternal decidua.

MATERIALS AND METHODS

Preparation of mouse tissues and embryos

Tissues were harvested from ICR mice (Harlan, Indianapolis, IN) according to NIH and institutional guidelines for animal use. Timed pregnant female animals were anesthetized and then killed by cervical dislocation. Embryos were dissected from the uterus, rinsed in cold DEPC-saline, and immediately fixed in chilled 4% paraformaldehyde/DEPC-PBS, pH 7.4. Early embryos (E9.5) were dissected intact within the maternal decidua, while later embryos were dissected free of extraembryonic membranes prior to fixation. Adult tissues were dissected from anesthetized non-pregnant female mice after transcardial perfusion with cold heparinized DEPC-saline followed by chilled 4% paraformaldehyde/DEPC-PBS, pH 7.4. Samples were fixed for 16 h with rocking at 4°C before transfer to DEPC-saline. Tissues were then dehydrated, paraffin embedded, sectioned at 4 μm onto microscope slides treated with Vectabond (Vector Laboratories, Burlingame, CA). Slides were stored desiccated at 4°C.

Vector cloning and riboprobe synthesis

Two probes (probe A, 3′ and B, 5′) were selected from mouse MTP cDNA and were amplified by PCR. The oligonucleotide sequences used for amplification are as follows;

Probe A (~277 bp)
mouse MTP1 (forward) 5′ GAG TTC ATC TCC ACA GTG CAG
mouse MTP2 (reverse) 5′ TGG TGG AAG GGA ACC CAC
Probe B (~540 bp)
mouse MTP3 (forward) 5′ GGA AGC TTA TGTG GCA GAG GGA GC
mouse MTP4 (reverse) 5′ CCC AGA GAT ATC TAC CTC

For probe A, the PCR products amplified from mouse full-length MTP cDNA (kind gift from Dr. Hariharan, Bristol-Myers-Squibb, Princeton, NJ) were subcloned into pBluescript II KS (Stratagene, La Jolla, CA) at the EcoRV site, using TA cloning (23, 24). Two positive clones, in sense and anti-sense orientation with respect to the T7 promoter were identified, verified by sequencing, linearized with Hind III, and used to synthesize sense and antisense probes by in vitro transcription (see below).

For probe B, amplified products were digested with Hind III and EcoRV and cloned into Hind II and EcoRV digested pBluescript II KS vector. Template DNA for antisense and sense were linearized with Hind III and EcoRV restriction enzymes, respectively, and riboprobes were generated by in vitro transcription with the Ambion Maxiscript kit (Ambion, Austin, TX) according to the kit instructions. Briefly, in 20 μl reactions, 200 ng of template DNA was transcribed with T7 (antisense) or T3 (sense) polymerase in the presence of 280 μCi of [α-^{35}S]-UTP (800 Ci/mmol) (Amersham, Piscataway, NJ) for 30 min at 37°C. After DN′ase digestion and termination of all enzymatic activity, the transcription products were suspended in final volumes of 75 μl (DEPC-H_2O) and purified over G-50 spin columns (5′–3′, Boulder, CO). Pre- and post-column samples were assessed for quality by scintillation counting and denaturing polyacrylamide gel electrophoresis, and the remainder of the transcription products were stored at −80°C.

In situ hybridization

For pre-hybridization, slides were heated to 58°C for 30 min. Utilizing RN′ase-free glass staining dishes and metal racks (Shandon, Pittsburgh, PA), slides were deparaffinized in xylene and hydrated through a series of graded ethanol/DEPC-saline rinses (95%, 85%, 60%, 30%) to DEPC-saline. To accomplish microwave RNA retrieval, the slides were transferred to upright plastic racks and immersed in plastic containers (Miles Tissue-Tek, Elkhart, IN) filled with DEPC-1X Antigen Retrieval Citra pH 6.0 (Biogenex, San Ramon, CA). Empty slots were filled with blank slides, and the plastic slide dish was covered loosely. The slides were heated in a 750 watt microwave at 90% power for 5 min. Any evaporated solution was replaced with DEPC-H_2O, and the container was heated at 60% power for an additional 5 min. The slides were cooled for 20 min, then returned to their metal racks and washed twice in DEPC-PBS, pH 7.4, for 5 min each. Subsequently, the slides were fixed for 20 min in 4% paraformaldehyde/DEPC-PBS, pH 7.4, and washed twice in DEPC-PBS, pH 7.4, for 5 min each. To further unmask RNA, the slides were permeabilized for 7.5 min with 20 μg/ml pronase-E in 50 mm Tris-HCl, pH 8.0/5 mm EDTA, pH 8.0/DEPC-H_2O. Excess pronase-E was removed by a 5-min DEPC-PBS, pH 7.4, wash before re-fixing in 4% paraformaldehyde/ DEPC-PBS, pH 7.4, for 5 min. Slides were washed in DEPC-PBS, pH 7.4, for 3 min. The slides were then acetylated in 0.25% acetic anhydride/0.1 m triethanolamine–HCl, pH 7.5, twice for 5-min. Next, the slides were equilibrated in 1 × SSC, pH 7.0, for 5 min followed by incubation in 50 mm n-ethylmaleimide/1 × SSC, pH 7.0, for 20 min. Five-min washes in DEPC-PBS, pH 7.4, and DEPC-saline followed and then the slides were dehydrated through graded ethanol/DEPC-saline rinses (30%, 60%, 85%, 95%) to absolute ethanol, and dried under vacuum for 2 h.

Riboprobes and hybridization mixture containing 50% formamide, 0.3 m NaCl, 20 mm Tris-HCl, pH 8.0, 5 mm EDTA, pH 8.0, 10 mm NaPO$_4$, pH 8.0, 10% dextran sulfate, 1 × Denhardt's, and 0.5 mg/ml tRNA were thawed from −80°C storage. Probes were diluted in aliquots of hybridization mixture sufficient to achieve 7.5×10^3 cpm/μl and the mixture heated to 95°C for 5 min. Diluted probes were then cooled to 37°C and 1 m DTT was added to achieve a final concentration of 10 mm DTT. Riboprobe was applied directly over the section, and slides were placed in a Nalgene utility box lined with 5 × SSC/50% formamide-saturated gel blot paper. Each slide was coverslipped with parafilm. The box was sealed and slides were hybridized for 14 h at 55°C.
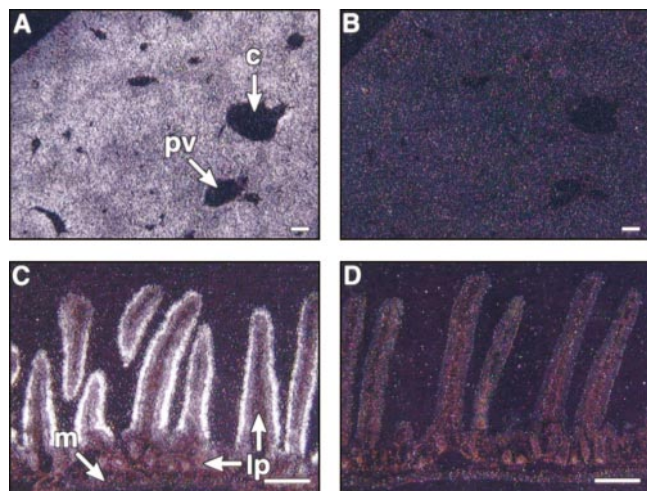
Fig. 1. In situ hybridization detection of MTP expression in adult tissues. Figure 1 shows the expression pattern of MTP in adult mouse liver and small intestine visualized by dark-field microscopy, using antisense (panels A and C) and sense (panels B and D) probes; (c) central vein, (lp) lamina propria, (m) muscularis; (pv) portal vein; bars of measure equal 150 μm.

After hybridization, parafilm coverslips were removed and the slides were placed in upright plastic racks and immersed in a 5 × SSC/10 mm DTT wash at 55°C for 40 min. Subsequently, the slides were washed for 30 min at 65°C in HS (2 × SSC/50% formamide/100 mm DTT), followed by three 10-min washes in NTE (0.5 m NaCl/10 mm Tris-HCl, pH 8.0/5 mm EDTA, pH 8.0) at 37°C. Slides were transferred to a fourth NTE wash containing RN′ase-A (2 μg/ml) and incubated 30 min at 37°C. Excess RN′ase-A was removed in a fifth NTE wash for 15 min at 37°C, before the slides were returned to HS for another 30 min at 65°C. After this second HS, the slides were washed for 15 min at 37°C each in 2 × SSC and 0.1 × SSC. Finally, the slides were dehydrated in graded ethanol rinses (30%, 60%, 85%, 95%) to absolute ethanol, and dried under vacuum.

Darkroom

Dried slides were dipped in dilute Ilford K.5 nuclear emulsion (Polysciences, Warrington, PA) pre-warmed to 42°C. Slides were hung vertically and slowly dried at room temperature at 75% humidity for 3 h. The slides were placed into 25-count microscope slide storage boxes with desiccant. The boxes were sealed with tape, wrapped with foil, and placed at 4°C. After 28 days autoradiographic exposure, the slides were developed in D19 (Eastman Kodak, Rochester, NY) at 14°C and the latent image was fixed with Kodak Fixer. The slides were thoroughly rinsed, counterstained with hematoxylin (Richard-Allen, Kalamazoo, MI), dehydrated, and coverslipped with permanent mounting media.

Microscopy and photomicrography

Visualization of MTP expression was achieved with a Leitz Laborlux-S microscope stand equipped with Plan-EF optics, a standard bright-field condenser, and a Mears low-magnification dark-field condenser. Photomicrographic record was made with an Optronics VI-470 CCD camera and a Power Macintosh G3 equipped with a Scion CG-7 frame grabber and Scion Image 1.62 software.

RESULTS AND DISCUSSION

Initial attempts using probe A as template on adult liver sections were consistently unsuccessful and resulted in both a high background and an anti-sense signal indistin-
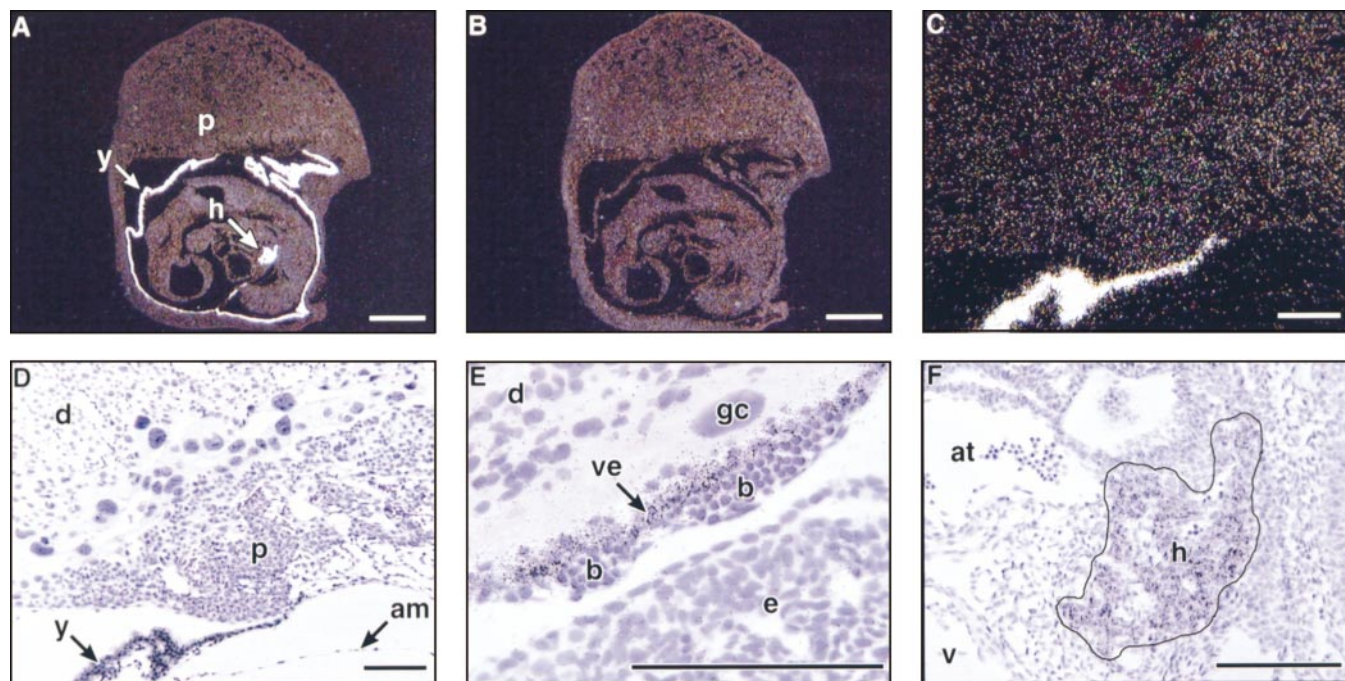
Fig. 2. In situ hybridization detection of MTP expression E9.5. Figure 2 shows the expression pattern of MTP in E9.5 embryonic mouse tissues, using antisense (all panels except B) and sense probes (panel B). Panels A–C were acquired using dark-field microscopy while panels D–F are bright-field. Panels C–F are high magnifications of panel A; (am) amnion, (at) atrium, (b) blood island, (d) decidua, (e) embryo, (gc) giant cell trophoblast, (h) hepatic primordium in septum transversum, (p) placenta, (v) ventricle, (ve) visceral endoderm, (y) yolk sac; bars of measure equal 800 μm (panels A and B); 150 μm (panels C–F).

guishable from the sense probe (data not shown). Although an improvement in the signal was obtained after microwave heating of the sections prior to hybridization, switching to the riboprobes generated from probe B located at the 5′ end of the cDNA gave a much more robust and reproducible signal from adult tissues (**Fig. 1 A–D**), and hence probe B was used in all ensuing experiments. Sense probe B served as the negative control and no specific hybridization was detected. A strong signal was obtained with adult liver (Fig. 1A) compared with the control sense probe (Fig. 1B). While all hepatocytes expressed MTP, there was a subtle heterogeneity of expression across the lobule. Hepatocytes associated with the central vein had a higher level of expression than those in the portal region. Northern analyses of mouse liver showed the abundance of MTP to be comparable to that of hepatic lipase (data not shown).

MTP expression was also detected in the small intestine as expected (Fig. 1C). Note that for the intestine, a very

clear demarcation of the MTP signal was evident, the expression being in the enterocytes lining the villi but not in lamina propria or the muscularis layers. This finding confirms the cell-specific expression pattern for MTP in the small intestine.

At the earliest time point examined, E9.5, the yolk sac showed well demarcated robust MTP expression (**Fig. 2A**); the sense control was negative (Fig. 2B). No significant expression was detected in the amnion, nor the maternal decidua and developing placenta, and only the yolk sac showed expression (Fig. 2C and D). Expression in the yolk sac was limited to the visceral endoderm, and no expression was detected in the adjacent extraembryonic mesoderm containing the developing blood islands (Fig. 2E).

Evaluation at this early stage of development (E9.5) also revealed MTP expression in the hepatic primordium. Although the liver is not recognizable as a separate organ at this time, nests of primordial cells destined to form the liver could be distinguished within the septum transversum (Fig 2F).
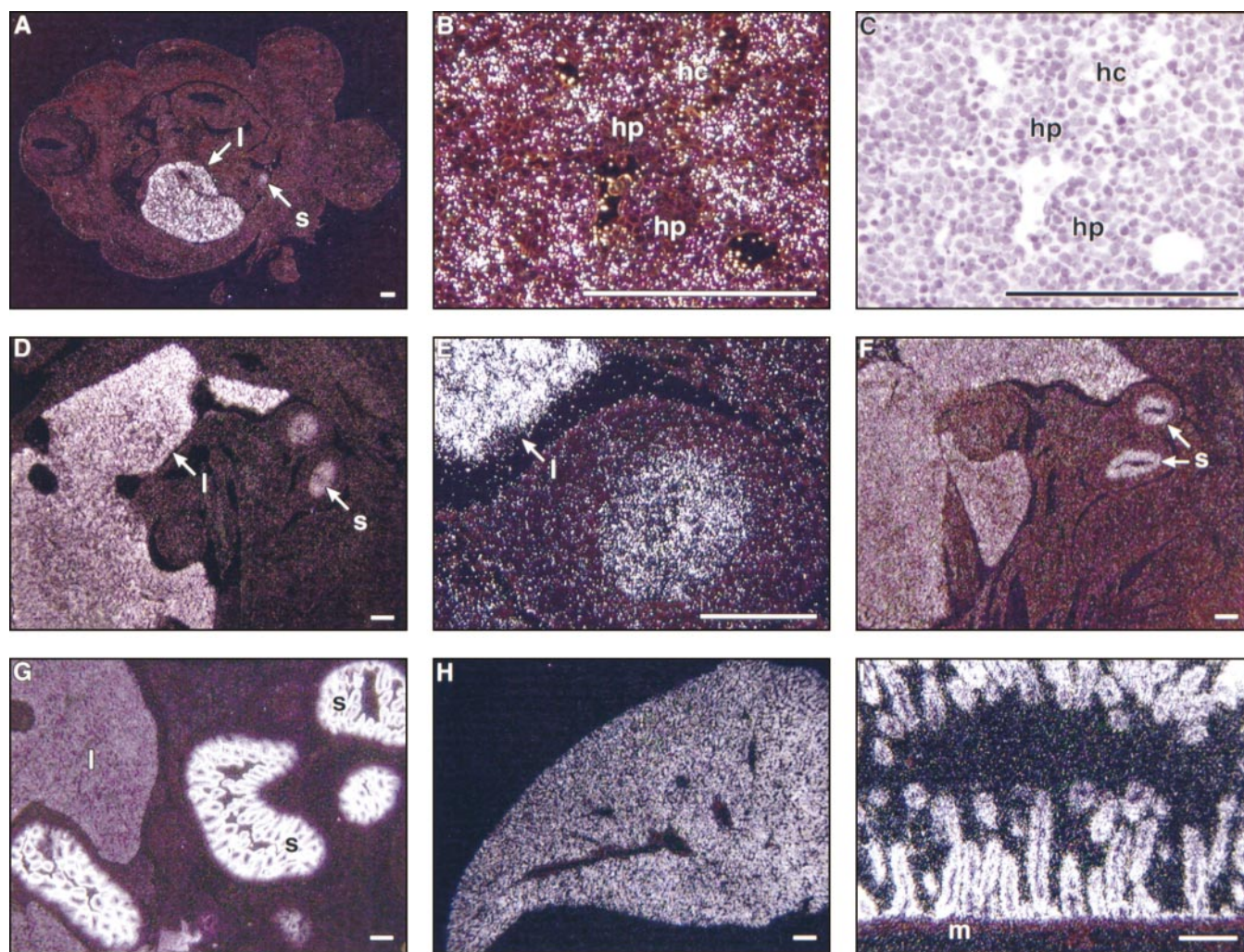

Fig. 3. In situ hybridization detection of MTP expression in later developing tissues. Figure 3 shows the expression pattern of MTP in E12.5 (panels A–C), E13.5 (panels D and E), E14.5 (panel F), E16.5 (panel G) embryonic, and day 1.5 (panels H and I) post-natal mouse tissues, using antisense probe. All images were acquired using dark-field microscopy except for panel C (bright-field); (hc) hepatic cords, (hp) hematopoetic nests, (l) liver, (m) muscularis, (s) small intestine; bars of measure equal 150 μm.

Figure 3 is a composite of the in situ expression patterns during later development using embryonic tissues from E12.5 to E16.5 (panels A–G) and post-natal tissues at 1.5 days (panels H and I). At E12.5, the liver is well developed and consists of both hepatocytes as well as nests of hematopoietic cells (Fig. 3A). Where clear distinction between these two types of cells could be made, no signal over the hematopoietic cells was detected (Fig. 3B and C). A positive signal in the intestinal epithelium was also evident at this stage of development (Fig. 3A).

Although in situ hybridization is not applicable to precise quantitation, it does provide qualitative data regarding relative gene expression. At E12.5, MTP expression was much more evident in the liver than in the developing intestine (Fig. 3A); this pattern remained at E13.5 (Fig. 3D and E). By E14.5, the expression in the liver and intestine were similar (Fig. 3F). But at E16.5, MTP expression in the liver was dramatically overshadowed by up-regulation of gene expression in the intestine (Fig. 3G). This relative expression pattern was maintained at post-natal day 1.5 (Fig. 3H and I) and into adulthood (Fig. 1A and C). This pattern of greater intestinal expression relative to liver is compatible with previous studies using Northern analyses.
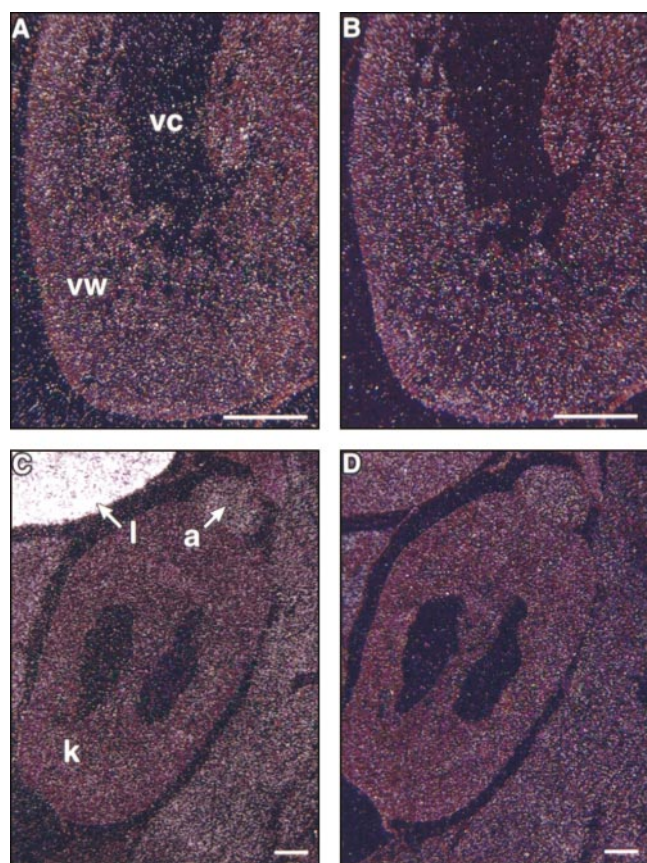

Fig. 4. In situ hybridization detection of MTP expression in late embryonic cardiac and renal tissues. Figure 4 shows the lack of detectable MTP expression in E16.5 embryonic mouse heart (panels A and B) and kidney (panels C and D) visualized by dark-field microscopy, using antisense (panels A and C) and sense (panels B and D) probes; (a) adrenal gland, (k) kidney, (l) liver, (vc) ventricular chamber, (vw) ventricular wall; bars of measure equal 200 μm.

Although MTP expression in adult murine cardiac tissue has been clearly demonstrated by both detection of mRNA, as well as of the protein by immunoprecipitation (12, 25), in our studies we did not detect hybridization signals over late (E16.5) embryonic cardiac or renal tissues that were significantly different from the background obtained with the sense probe (**Fig. 4**). While a small number of silver grains was evident over adrenal gland, the signal could not be appreciated above sense background (Fig. 4C and D).

Our earlier attempts to detect MTP mRNA using conventional in situ hybridization techniques were unsuccessful and complicated by increased background when duration of autoradiographic exposure was increased. We therefore modified the conventional technique in two ways: *1*) microwave heating of the tissue sections prior to probe hybridization and *2*) the addition of n-ethylmaleimide. Microwave heating has been previously reported for augmenting immuno-histochemical staining of proteins (18–22). However, this technique may also be used for improving nucleic acid detection. Although the exact mechanism of action has not been fully characterized, one postulated mechanism is that microwave heating may result in exposure of both protein epitopes and nucleic acids that are previously masked by proteins cross-linked by aldehyde fixation. This 'microwave retrieval' then allows for access to RNA or protein by riboprobes or antibodies respectively.

Much of the background seen with longer autoradiographic exposure is due to disulfide bonds between sulfur moieties of the riboprobes and tissues (26). Thus to increase the exposure time of autoradiography, the addition of n-ethylmaleimide allowed us to block non-specific sulfur-binding sites in the tissues during the hybridization.

Even though microwave RNA retrieval significantly increases in situ hybridization sensitivity and the addition of n-ethylmaleimide increases the ratio of signal-to-noise, the relative sensitivity of in situ hybridization remains below that of ribonuclease protection assay (RPA) or solution phase RT-PCR. Using in situ hybridization we were unsuccessful in localizing expression in the embryonic kidney and heart even though investigators using RT-PCR and RPA have found MTP expression in these tissues (17).

TABLE 1. Summary of MTP expression during mouse development

Age	Liver	Gut	Yolk Sac	Kidney	Heart
E9.5	+	−	+	−	−
E10.5	+	−	n/d	−	−
E11.5	+	−	n/d	−	−
E12.5	+	+	n/d	−	−
E13.5	+	+	n/d	−	−
E14.5	+	+	n/d	−	−
E16.5	+	+	n/d	−	−
P1.5	+	+	n/d	n/d	n/d
Adult	+	+	n/d	n/d	n/d

Table 1 shows the temporo-spatial detection of MTP expression by in situ hybridization. MTP was initially detected in the yolk sac and primordial liver at E9.5. Detection of expression in the developing intestinal tract followed at E12.5. No expression was detectable in developing cardiac or renal tissues; (n/d) indicates tissues/timepoints not evaluated.

In conclusion, using an improved in situ technique, incorporating microwave heating RNA retrieval and n-ethlymaleimide to reduce background, we report here the ontogeny of MTP expression in the developing mouse summarized in **Table 1**. Expression is initially seen in the yolk sac, followed by the primordial liver and subsequently in the developing intestinal tract. Despite modifications of standard in situ hybridization protocols to improve sensitivity and to reduce background, no detectable expression was seen in late embryonic cardiac or renal tissues, nor in the early developing placenta and maternal decidua.██

REFERENCES

1. Kane, J. P., and R. J. Havel. 1989. Disorders of the biogenesis and secretion of lipoproteins containing the B lipoproteins. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill Inc., NY. 1139–1164.
2. Wetterau, J. R., M. C. Lin, and H. Jamil. 1997. Microsomal triglyceride transfer protein. *Biochim. Biophys. Acta.* **1345:** 136–150.
3. Wetterau, J. R., L. P. Aggerbeck, M. E. Bouma, C. Eisenberg, A. Munck, M. Hermier, J. Schmitz, G. Gay, D. J. Rader, and R. E. Gregg. 1992. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. *Science.* **258:** 999–1001.
4. Linton, M. F., R. Farese, Jr., and S. G. Young. 1993. Familial hypobetalipoproteinemia. [Review]. *J. Lipid Res.* **34:** 521–541.
5. Kayden, H. J., and R. Silber. 1965. The role of vitamin E deficiency in the abnormal autohemolysis of acanthocytosis. *Trans. Assoc. Am. Phys.* **78:** 334–342.
6. Schonfeld, G. 1995. The hypobetalipoproteinemias. [Review]. *Annu. Rev. Nutr.* **15:** 23–34.
7. Shi, W. K., B. Hopkins, S. Thompson, J. K. Heath, B. M. Luke, and C. F. Graham. 1985. Synthesis of apolipoproteins, alphafoetoprotein, albumin, and transferrin by the human foetal yolk sack and other foetal organs. *J. Embryol. Exp. Morph.* **85:** 191–206.
8. Hopkins, B., C. R. Sharpe, F. E. Baralle, and C. F. Graham. 1986. Organ distribution of apolipoprotein gene transcripts in 6–12 week postfertilization human embryos. *J. Embryol. Exp. Morph.* **97:** 177–187.
9. Hopkins, B., A. L. Brice, P. N. Schofield, F. E. Baralle, and C. F. Graham. 1987. Identity of cells containing apolipoprotein B messenger RNA, in 6– to 12–week postfertilization human embryos. *Development.* **100:** 83–93.
10. Homanics, G. E., T. J. Smith, S. H. Zhang, D. Lee, S. G. Young, and N. Maeda. 1993. Targeted modification of the apolipoprotein B gene results in hypobetalipoproteinemia and developmental abnormalities in mice. *Proc. Natl. Acad. Sci. USA.* **90:** 2389–2393.
11. Farese, R., Jr., S. L. Ruland, L. M. Flynn, R. P. Stokowski, and S. G. Young. 1995. Knockout of the mouse apolipoprotein B gene results in embryonic lethality in homozygotes and protection against diet-induced hypercholesterolemia in heterozygotes. *Proc. Natl. Acad. Sci. USA.* **92:** 1774–1778.
12. Nielsen, L. B., M. Veniant, J. Boren, M. Raabe, J. S. Wong, C. Tam, L. Flynn, T. Vanni-Reyes, M. D. Gunn, I. J. Goldberg, R. L. Hamilton, and S. G. Young. 1998. Genes for apolipoprotein B and microsomal triglyceride transfer protein are expressed in the heart: evidence that the heart has the capacity to synthesize and secrete lipoproteins. *Circulation.* **98:** 13–16.
13. Raabe, M., E. Kim, M. Veniant, L. B. Nielsen, and S. G. Young. 1998. Using genetically engineered mice to understand apolipoprotein-B deficiency syndromes in humans. *Proc. Assoc. Am. Physiol.* **110:** 521–530.
14. Raabe, M., L. M. Flynn, C. H. Zlot, J. S. Wong, M. M. Veniant, R. L. Hamilton, and S. G. Young. 1998. Knockout of the abetalipoproteinemia gene in mice: reduced lipoprotein secretion in heterozygotes and embryonic lethality in homozygotes. *Proc. Natl. Acad. Sci. USA.* **95:** 8686–8691.
15. Chang, B. H., W. Liao, L. Li, M. Nakamuta, D. Mack, and L. Chan. 1999. Liver-specific inactivation of the abetalipoproteinemia gene completely abrogates very low density lipoprotein/low density lipoprotein production in a viable conditional knockout mouse. *J. Biol. Chem.* **274:** 6051–6055.
16. Farese, R., Jr., S. Cases, S. L. Ruland, H. J. Kayden, S. J. Wong, S. G. Young, and R. L. Hamilton. 1996. A novel function for apolipoprotein B: lipoprotein synthesis in the yolk sac is critical for maternal-fetal lipid transport in mice. *J. Lipid Res.* **37:** 347–360.
17. Terasawa, Y., S. J. Cases, J. S. Wong, H. Jamil, S. Jothi, M. G. Traber, L. Packer, D. A. Gordon, R. L. Hamilton, and R. V. Farese, Jr. 1999. Apolipoprotein B-related gene expression and ultrastructural characteristics of lipoprotein secretion in mouse yolk sac during embryonic development. *J. Lipid Res.* **40:** 1967–1977.
18. Sperry, A., L. Jin, and R. V. Lloyd. 1996. Microwave treatment enhances detection of RNA and DNA by in situ hybridization [see comments]. *Diag. Molec. Path.* **5:** 291–296.
19. McMahon, J., and S. McQuaid. 1996. The use of microwave irradiation as a pretreatment to in situ hybridization for the detection of measles virus and chicken anaemia virus in formalin-fixed paraffin-embedded tissue. *Histochem. J.* **28:** 157–164.
20. Lan, H. Y., W. Mu, Y. Y. Ng, D. J. Nikolic-Paterson, and R. C. Atkins. 1996. A simple, reliable, and sensitive method for nonradioactive in situ hybridization: use of microwave heating to improve hybridization efficiency and preserve tissue morphology. *J. Histochem. Cytochem.* **44:** 281–287.
21. Oliver, K. R., R. P. Heavens, and D. J. Sirinathsinghji. 1997. Quantitative comparison of pretreatment regimens used to sensitize in situ hybridization using oligonucleotide probes on paraffin-embedded brain tissue. *J. Histochem. Cytochem.* **45:** 1707–1713.
22. Wilkens, L., R. von Wasielewski, M. Werner, M. Nolte, and A. Georgii. 1996. Microwave pretreatment improves RNA-ISH in various formalin-fixed tissues using a uniform protocol. *Pathol. Res. Pract.* **192:** 588–594.
23. Mead, D. A., N. K. Pey, C. Herrnstadt, R. A. Marcil, and L. M. Smith. 1991. A universal method for the direct cloning of PCR amplified nucleic acid. *Bio/Technology.* **9:** 657–663.
24. Marchuk, D., M. Drumm, A. Saulino, and F. S. Collins. 1991. Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. *Nucleic Acids Res.* **19:** 1154.
25. Boren, J., M. M. Veniant, and S. G. Young. 1998. Apo B100-containing lipoproteins are secreted by the heart. *J. Clin. Invest.* **101:** 1197–1202.
26. Zoeller, R. T., D. L. Fletcher, O. Butnariu, C. A. Lowry, and F. L. Moore. 1997. N-ethylmaleimide (NEM) can significantly improve in situ hybridization results using 35S-labeled oligodeoxynucleotide or complementary RNA probes. *J. Histochem. Cytochem.* **45:** 1035–1041.