Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 709–713, February 1987
Biochemistry

Expression of the murine apolipoprotein E gene is coupled to the differentiated state of F9 embryonal carcinoma cells

(lipid transport/gene regulation/development)

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Communicated by Michael S. Brown, October 16, 1986 (received for review September 2, 1986)

ABSTRACT **Apolipoprotein E (apoE) expression was studied in F9 embryonal carcinoma cells that differentiate in response to retinoic acid into cells resembling either parietal endoderm or visceral endoderm of the parietal or visceral yolk sac. F9 cells secreted newly synthesized apoE when incubated with radiolabeled amino acid. Upon differentiation to parietal endoderm-like cells, apoE synthesis and secretion were markedly down-regulated. In contrast, apoE secretion was up-regulated upon differentiation to visceral endoderm-like cells. These changes in apoE expression were due, at least in part, to regulation of apoE mRNA abundance since visceral endoderm-like cells contained 5- to 7-fold more apoE mRNA than parietal endoderm-like cells. The apoE phenotype reflected the differentiated state of the F9 cell since either the up-regulated or down-regulated pattern was stable to the removal of the retinoic acid inducer. To determine how well apoE regulation in F9 cells reflects regulation in visceral and parietal yolk sacs, apoE mRNA was measured in yolk sac tissues of the 12-day mouse embryo. Visceral yolk sac contained 109 ± 11 pg of apoE mRNA per μg of RNA while parietal yolk sac contained 14.3 ± 3.1 pg/μg of RNA. This 7- to 8-fold difference is similar to the difference in the apoE mRNA contents of visceral endoderm-like and parietal endoderm-like F9 cells. RNA gel blot analysis showed that apoE mRNA is the same size in F9 cells as in yolk sac tissues and fetal or adult liver. In addition, primer-extension analysis showed that transcription is initiated at or near the same site on the apoE gene in F9 cells and mouse tissues. These data suggest that both quantitative and qualitative features of apoE gene expression in development are retained in the F9 cell. The F9 cell should provide a useful system to study the developmental activation of endogenous apolipoprotein genes as well as exogenous apolipoprotein genes introduced by transfection.**

Apolipoprotein E (apoE) plays an important role in cholesterol metabolism as a recognition signal for receptor-mediated uptake of lipoprotein particles. The binding of apoE by hepatic receptors appears to be a key component of the reverse cholesterol transport system by which cholesterol is transported from peripheral tissues to the liver for metabolism and elimination (1–4). In contrast to most plasma apolipoproteins that are synthesized in the liver and small intestine, apoE also is synthesized by human adrenal and kidney tissue (5), human monocyte–macrophage (6), and mouse peritoneal macrophage (7). These findings led to proposals that apoE made by macrophage or peripheral tissues acts to target lipoproteins for delivery to the liver or serves in the redistribution or shuttling of cholesterol among peripheral tissues (5–7). Further studies have confirmed that apoE is made in virtually all peripheral tissues in nonhuman primates (8, 9) and rodents (10–12).

In addition to adult peripheral tissues, apoE and other apolipoproteins are synthesized in visceral yolk sac endoderm of the mid-gestation mouse embryo (13, 14) suggesting that this fetal tissue may be a source of lipoproteins during postimplantation embryonic development (13). Human secondary yolk sac (15) and late-gestation rat yolk sac (16) also synthesize apolipoproteins.

In the present study we have examined the expression of the apoE gene in mouse embryos and F9 embryonal carcinoma cells. This clonal cell line resembles an embryonic stem cell that is arrested at an early differentiation stage prior to the emergence of the extraembryonic endoderm of the preimplantation mouse embryo (17–20). Under appropriate culture conditions, F9 cells irreversibly differentiate into cells which phenotypically resemble either parietal endoderm (PE) (19) or visceral endoderm (VE) (20) cells of the parietal or visceral yolk sac. Our results show that F9 stem cells synthesize apoE, and its expression is coupled to the differentiated state of the cell. Measurements of apoE mRNA in mouse yolk sac tissues indicate that changes in apoE expression resulting from F9 cell differentiation *in vitro* accurately reflect the *in vivo* pattern of apoE expression in the parietal and visceral yolk sacs.

MATERIALS AND METHODS

Cell Culture. F9 cells were grown on gelatinized Falcon culture flasks in Dulbecco's modified Eagle's medium (DMEM) containing 15% (vol/vol) heat-inactivated fetal bovine serum as described (19). Cells were differentiated into PE-like cells by growth as monolayers in the presence of 0.1 μM retinoic acid and 1 mM N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate (Bt_2cAMP) for 3–4 days (19). Differentiation to VE-like cells was achieved by growing small aggregates of 20–40 cells for 7 days in suspension in bacteriological Petri dishes in the above medium containing 0.1 μM retinoic acid (20). Monolayers were harvested for RNA isolation by rinsing three times with cold, phosphate-buffered saline (pH 7.4), solubilizing the cells in guanidine isothiocyanate buffer (21), and sedimentation of the extract through CsCl (21). RNA was further subjected to two phenol/chloroform, 1:1 (vol/vol), extractions and repeated ethanol precipitation from sodium acetate (9). Aggregates of VE-like cells were washed twice with cold, buffered saline by centrifugation prior to RNA extraction.

Metabolic Labeling, Immunoprecipitation, and Electrophoresis. Monolayer cultures or cell aggregates were washed twice with culture medium lacking methionine and serum and incubated for 6 hr in DMEM lacking methionine and supplemented with [^{35}S]methionine (100 μCi/ml; 1200 Ci/mmol; 1 Ci = 37 GBq; Amersham) and 15% (vol/vol) dialyzed fetal

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Abbreviations: apoE, apolipoprotein E; PE, parietal endoderm; VE, visceral endoderm; Bt_2cAMP, N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate.

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bovine serum. After incubation, medium was adjusted to 0.2 mg of phenylmethylsulfonyl fluoride per ml, centrifuged to remove cells, and dialyzed against 0.02 M sodium phosphate, pH 7.4/0.15 M NaCl/phenylmethylsulfonyl fluoride at 0.1 mg/ml/1 mM methionine. Monolayers or cell aggregates were washed twice with cold, buffered saline, homogenized, and centrifuged to prepare a high-speed supernatant as described (22). Mouse liver was incubated with [^{35}S]methionine in short-term organ culture (23).

Cell extracts and culture medium were analyzed with a double-antibody procedure (22, 23) employing the rabbit anti-apoE antibody described (24) and goat anti-rabbit γ-globulin as the second antibody. Primary antibody was used in excess to ensure quantitative immunoprecipitation. Control immunoprecipitations used preimmune rabbit serum as primary antibody. Immunoprecipitates were analyzed by $NaDodSO_4$/10% polyacrylamide gel electrophoresis and fluorography (25, 26). Radiolabeled protein standards (Bethesda Research Laboratories) were routinely used for calibration.

RNA Gel Blot Analysis, Solution Hybridization, and Primer Extension. Formaldehyde-treated RNA samples were analyzed by electrophoresis in 1.2% agarose gels (27), transferred to nitrocellulose (28), and hybridized with the uniformly labeled apoE cDNA described below. A DNA excess solution hybridization assay for mouse apoE mRNA was constructed with a cDNA probe corresponding to nucleotides 87–186 of mouse apoE mRNA (29). The cDNA fragment in bacteriophage M13mp8 (M13E100) was used to synthesize a ^{32}P-labeled single-stranded probe (9). Template DNA or total RNA was hybridized to completion with excess probe, and S1-nuclease-resistant hybrids were acid precipitated, collected on glass fiber filters, and counted by scintillation spectrometry as described (9, 30). ApoE mRNA values were determined by reference to a standard curve constructed with template DNA (M13E100) as the hybridization standard (30). Primer extension analysis was carried out as described (31) using the solution hybridization probe for which the 3′ end corresponds to nucleotide 87 of apoE mRNA (29).

Miscellaneous. Protein was determined by the method of Lowry (32) with bovine serum albumin as standard. Tissue plasminogen activator activity was determined on samples of culture medium (33). α-Fetoprotein mRNA was monitored by dot-blot analysis (34) using a nick-translated (35) cDNA (36). The isolation of embryonic tissues was as described (37). Mature female CD-1 and male J/129 mice were obtained from Charles River Breeding Laboratories and The Jackson Laboratory, respectively.

RESULTS

To determine whether F9 stem cells synthesize and secrete apoE, cultures were incubated with [^{35}S]methionine followed by immunoprecipitation and gel electrophoresis of cell extracts and medium. Fig. 1, lane A, shows that anti-apoE binds to a newly synthesized protein of M_r 33,000 in cell extracts that comigrated with newly synthesized liver apoE. The apoE band was not detected in the preimmune serum control (lane B). Immunoprecipitation of the culture medium also showed the apoE band in the anti-apoE sample (lane C) but not in the preimmune serum control (lane D). Immunoprecipitation of an equal quantity of protein radioactivity from F9 cells that had differentiated to PE-like cells yielded much less newly synthesized apoE (lane E). In comparison to the apoE secreted by F9 stem cells (lane C), the PE-like cells also secreted much less apoE (lane G). Densitometric analysis of the fluorograms indicated a 15-times difference in the synthesis and secretion of radiolabeled apoE between F9 stem cells and PE-like cells (data not shown).

Analysis of the culture medium from cell aggregates that had differentiated to VE-like cells also showed a newly synthesized apoE band (lane J). In comparison to cells that had been cultured as aggregates in the absence of retinoic acid (lane I), the VE-like cells (lane J) secreted ≈7.5-fold more newly synthesized apoE. These results suggest that apoE synthesis in the F9 stem cells is up-regulated following differentiation to VE-like cells and down-regulated following differentiation to PE-like cells.

A DNA-excess solution hybridization assay was constructed to quantitate absolute amounts of apoE mRNA in F9 stem cells, PE-like cells, and VE-like cells. As shown in Fig. 2*A* the apoE mRNA content is similar in VE-like cells and adult

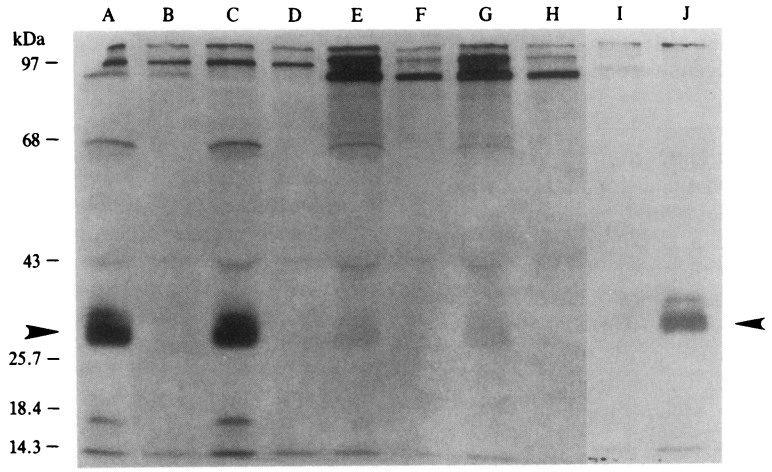

FIG. 1. ApoE synthesis and secretion by F9 cells. F9 cells were differentiated to PE-like or VE-like cells for 4 days or 7 days, respectively. Cells were incubated with [^{35}S]methionine for 6 hr, and cell extracts and culture media were analyzed for newly synthesized apoE by immunoprecipitation followed by $NaDodSO_4$/PAGE and fluorography. The fluorogram shows anti-apoE immunoprecipitates from 100,000 cpm of protein radioactivity from extracts of undifferentiated F9 cells (lane A) and PE-like cells (lane E) and immunoprecipitates from 500,000 cpm of protein radioactivity from culture medium of undifferentiated F9 cells (lane C) and PE-like cells (lane G). The lanes adjacent to each sample (lanes B, D, F, and H) are the respective control immunoprecipitations with preimmune serum. Lanes I and J show the anti-apoE immunoprecipitates from 80,000 cpm of protein radioactivity from culture medium of F9 cell aggregates (lane I) and VE-like cells (lane J). The samples in lanes I and J are not directly comparable to the samples from monolayer cultures (lanes A–H) because of differences in specific activities of radiolabeled methionine. Newly synthesized mouse liver apoE was immunoprecipitated and electrophoresed in an adjacent lane; its mobility is indicated by the arrowhead. The mobilities of molecular weight standards are indicated on the left of the fluorogram.

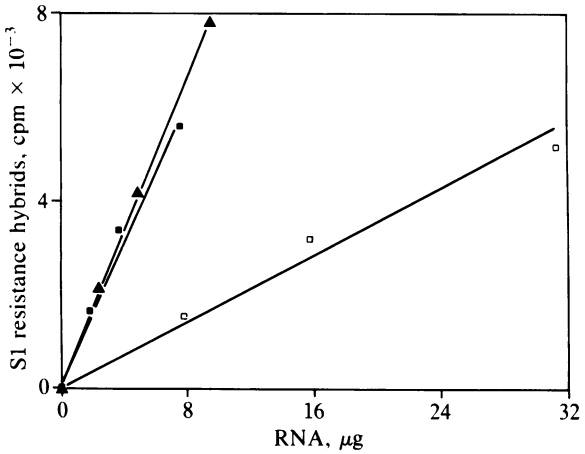

FIG. 2. ApoE mRNA in mouse liver, PE-like cells, and VE-like cells. Total RNA from the indicated cells was hybridized to completion with excess single-stranded apoE cDNA, samples were digested with S1 nuclease, S1-nuclease-resistant hybrids were collected by acid-precipitation, and radioactivity was determined by scintillation spectrometry. The figure shows the S1-nuclease-resistant hybrids as a function of input RNA in the assay. ▲, mouse liver mRNA. □, PE-like mRNA. ■, VE-like mRNA.

mouse liver. The VE-like cells contain ≈5-fold more apoE mRNA than PE-like cells. Table 1 summarizes data from three experiments that show that apoE mRNA increased 3- to 4-fold upon differentiation to VE-like cells and decreased by 50% upon differentiation to PE-like cells. Growth of F9 cells as aggregates in the absence of retinoic acid did not significantly alter the apoE mRNA content. Thus, the changes in apoE synthesis and secretion upon differentiation to PE-like and VE-like cells are due, at least in part, to the cellular abundance of apoE mRNA. The differentiation state of the F9 cells in these experiments was confirmed by monitoring tissue plasminogen activator activity (33) and α-fetoprotein mRNA (36), which are markers for the PE-like and VE-like phenotypes, respectively (17–20). Secreted tissue plasminogen activator was detected with PE-like cells but not with F9 stem cells, whereas α-fetoprotein mRNA was detected in VE-like cells but not in F9 stem cells or F9 aggregates grown in the absence of retinoic acid (data not shown).

A characteristic of the differentiated F9 phenotype is that, once established, it is stable to removal of the retinoic acid inducer (19, 20). Table 2 shows that apoE mRNA levels are similarly reduced upon incubation with retinoic acid and Bt_2cAMP for 1 day followed by 3 days without inducers as compared to continuous exposure to inducers. Tissue plasminogen activator also was produced after only 1 day of exposure to inducers but accumulated to higher levels upon continuous exposure (Table 2). Similar results were obtained upon differentiation to the VE-like phenotype. Exposure to retinoic acid for 2 days followed by 5 days without inducer elevated apoE mRNA as effectively as continuous exposure

Table 1. ApoE mRNA in F9 stem cells, PE-like cells, and VE-like cells

	ApoE mRNA, pg of mRNA per μg of RNA			
Experiment	Stem cells	PE-like cells	Stem cells in aggregates	VE-like cells
1	13.9 ± 1.2	6.8 ± 1.3	18.8 ± 1.6	46.4 ± 6.0
2	12.6 ± 0.2	6.4 ± 0.8	11.9 ± 1.2	31.6 ± 4.4
3	8.4 ± 0.4	4.5 ± 0.4	9.1 ± 1.9	34.2 ± 4.2

ApoE mRNA (mean ± SD) was measured in total RNA prepared from 2 to 4 dishes.

Table 2. Stability of the F9 apoE phenotype

	Days			ApoE mRNA, pg/μg of	TPA activity, ΔA_{405} per
Treatment	+	−	Phenotype	RNA	min
None	0	4	Stem	12.6 ± 0.2	0
RA/Bt_2cAMP	4	0	PE-like	5.9 ± 0.4	0.33
RA/Bt_2cAMP	1	3	PE-like	6.9 ± 0.3	0.07
RA	7	0	VE-like	33.2 ± 7	ND
RA	2	5	VE-like	43.5 ± 6	ND

Cells were grown for the indicated time in the presence (+) or absence (−) of retinoic acid (RA) and Bt_2cAMP. Culture medium was changed every 2 days in stem cells, PE-like cells, and VE-like cells and was changed daily in VE-like cells after 4 days. ApoE mRNA and tissue plasminogen activator (TPA) activity were measured. Data represent mean ± SD of measurements from two to four dishes. ND, not determined.

to inducer for 7 days. These results indicate that the up- or down-regulated level of apoE mRNA is associated with the respective differentiated phenotype and not the continuous presence of inducer.

Exposure of F9 cells to retinoic acid alone induces several markers characteristic of the PE-like phenotype, but full expression of such markers requires both retinoic acid and Bt_2cAMP (19). The phenotype produced with retinoic acid alone has been described as a primitive endoderm that may proceed to either PE-like or VE-like cells depending upon the presence of Bt_2cAMP and the culture conditions (17–20). These characteristics were tested with regard to the down-regulation of apoE expression in a thymidine kinase-deficient clonal line of F9 cells (38). These cells show the same up- and down-regulation of apoE expression upon differentiation but have a higher level of apoE mRNA in the undifferentiated stem cell as compared to F9 stem cells. As shown in Table 3 incubation with Bt_2cAMP produced a small decrease in apoE mRNA as well as a small but detectable accumulation of tissue plasminogen activator. Retinoic acid yielded a 50% reduction in apoE mRNA and a substantially greater accumulation of tissue plasminogen activator. Exposure to both retinoic acid and Bt_2cAMP was required to give maximal down-regulation of apoE mRNA and maximal accumulation of tissue plasminogen activator. The degree to which apoE mRNA is down-regulated appears to parallel the degree to which tissue plasminogen activator is up-regulated.

To determine how well apoE regulation in F9 cells reflects regulation in the visceral and parietal yolk sacs of the embryo, apoE mRNA was measured in the yolk sac tissues of the 12-day mouse embryo. As shown in Table 4, the apoE mRNA content of the visceral yolk sac was 7- to 8-fold greater than that of the parietal yolk sac. This difference is similar to that seen in the apoE mRNA contents of VE-like and PE-like F9 cells (Table 1) although the absolute values of apoE mRNA are lower in the F9 cells. Separation of the visceral yolk sac into its component tissues showed that apoE mRNA was present mostly in the VE and not the extraem-

Table 3. Hormone requirements for the apoE F9 PE-like phenotype

	Days			ApoE mRNA, pg/μg of	TPA activity, ΔA_{405} per
Treatment	+	−	Phenotype	RNA	min
None	0	4	Stem	26.1 ± 2.8	0
Bt_2cAMP	4	0	Stem	20.6 ± 0.9	0.01
RA	4	0	Primitive endoderm-like	14.0 ± 0.9	0.06
RA/Bt_2cAMP	4	0	PE-like	8.3 ± 1.0	0.19

Experimental details are as described in Table 2.

Table 4. ApoE mRNA in fetal liver and yolk sac tissues

Tissue	ApoE mRNA, pg/μg of total RNA			
	Day 12	Day 14	Day 16	Day 18
FL	23.2 ± 0.4	82.8 ± 1.7	168 ± 4	332 ± 2
VYS	109 ± 11	159 ± 12	54.5 ± 0.8	47.4 ± 7
PYS	14.3 ± 3.1			
VE	171 ± 12			
VM	16.4 ± 0.7			

Embryonic tissues were isolated as described (37). Each value is the mean ± SD of four measurements from 25–50 pooled embryos. FL, fetal liver; VYS, visceral yolk sac; PYS, parietal yolk sac; VM, extraembryonic mesoderm.

bryonic mesoderm (Table 4). The apoE mRNA content of the visceral yolk sac remained high in the 12-day and 14-day embryos but declined markedly in the late-gestation 16-day and 18-day embryos (Table 4). ApoE mRNA was present in the 12-day fetal liver at only 20% of its concentration in the visceral yolk sac. In the succeeding 6 days the apoE mRNA content of the fetal liver increased ≈15-fold (Table 4).

RNA gel blot analysis showed that apoE mRNA from F9 stem cells, VE-like cells, PE-like cells, visceral yolk sac, fetal liver, and adult liver comigrated as a single band with a mobility corresponding to ≈1100 nucleotides (data not shown). Primer extension analysis was used to compare the sites of transcription initiation on the apoE gene in F9 cells with adult and embryonic tissues. As shown in Fig. 3, lane E, electrophoresis of primer-extended products produced with adult liver apoE mRNA showed a major 282-nucleotide cDNA corresponding to a position 67 nucleotides on the 5′ side of the translation start codon (29). cDNA of the same size was produced with RNA from F9 stem cells (lane B), VE-like cells (lane D), and PE-like cells (lane C). Identical results were obtained with apoE mRNA from fetal liver and visceral yolk sac (data not shown). These results indicate that transcription is initiated at or very near the same site on the apoE gene in undifferentiated and differentiated F9 cells as well as in fetal yolk sac and liver.

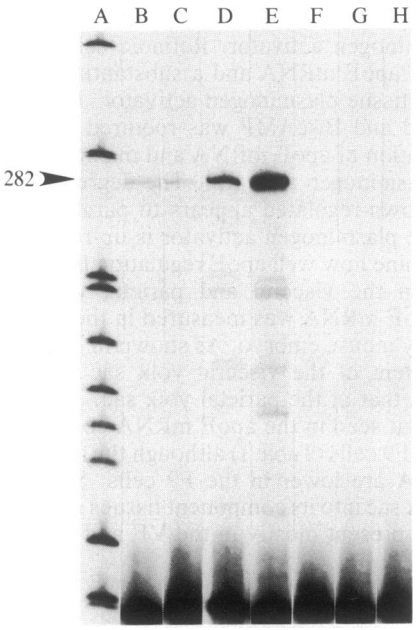

FIG. 3. Primer extension analysis of apoE mRNA from F9 cells and mouse tissues. Primer-extended cDNA samples were prepared and analyzed by 8 M urea/6% polyacrylamide gel electrophoresis. The figure shows the autoradiogram of the gel. RNA samples were from undifferentiated F9 cells (lane B), PE-like cells (lane C), VE-like cells (lane D), and adult mouse liver (lane E). Control reactions lacking RNA (lane F) or lacking reverse transcriptase (lane G) are shown. Lane H shows probe–primer alone, and lane A shows molecular weight standards prepared by end-labeling an *Msp* I digest of pBR322. The arrowhead indicates the mobility of the primer-extended cDNA corresponding to ≈282 nucleotides.

DISCUSSION

The results of this study show that expression of the apoE gene is decreased upon differentiation of F9 cells to the PE-like phenotype and increased upon differentiation to the VE-like phenotype. The changes in apoE expression accompanying differentiation appear to reflect the differentiated state of the cell, since either the up-regulated or down-regulated pattern of apoE expression is stable to removal of the retinoic acid inducer (Table 2). Similarly, retinoic acid treatment in the absence of Bt_2cAMP results in either the up-regulated or down-regulated apoE phenotype depending upon whether the cells are cultured in monolayer or as aggregatres in suspension (Table 3). Thus, the pattern of apoE expression in differentiated F9 cells shows a coupling to the differentiated state as occurs with a variety of markers characteristic of either the VE or PE of the mouse embryo (17–20).

These studies also showed that changes in apoE expression following differentiation of F9 cells are due, at least in part, to regulation of the apoE mRNA content. Interestingly, upon differentiation to PE-like cells, the relative synthesis of apoE (Fig. 1) decreased much more than expected from the 50% reduction in apoE mRNA (Table 1), suggesting that some of the decrease in apoE synthesis may reflect translational regulation. Further studies will be required to determine whether this is the case, however, since changes in the relative synthesis of apoE (Fig. 1) do not take into account changes in the absolute rate of protein synthesis that may result from differentiation to either PE-like or VE-like cells.

ApoE mRNA was 7- to 8-fold more abundant in visceral as compared to parietal yolk sac and 6- to 7-fold more abundant in F9 VE-like cells as compared to PE-like cells. Thus, differentiation of F9 cells in culture results in similar changes in apoE mRNA content as occurs in the formation of visceral and parietal yolk sac tissues in the mouse embryo. In addition, apoE mRNA is the same size in F9 cells as in yolk sac tissues and fetal or adult liver, and transcription appears to be initiated from the same site on the gene in F9 cells and mouse tissues (Fig. 3). These data suggest that both quantitative and qualitative features of *in vivo* apoE gene expression are retained in the F9 cell.

ApoE mRNA in the 12-day embryo was present primarily in the visceral yolk sac as compared to the parietal yolk sac and in the endoderm as compared to the mesoderm layer of the visceral yolk sac (Table 4). This distribution is similar to that reported by Meehan *et al.* (14) for apolipoprotein AI mRNA in the 13.5-day mouse embryo. Visceral yolk sac endoderm from 10.5-day embryos secrete newly synthesized apolipoprotein AI, as well as proteins corresponding in molecular weight to apolipoproteins B, E, and AIV that are coprecipitated with apolipoprotein AI antiserum (13). These findings led Shi and Heath (13) to propose that VE is a source of apolipoproteins during postimplantation development. The visceral yolk sac completely invests the embryo, and prior to establishment of the placenta is believed to have a major role in nutrition of the embryo (39). Apolipoprotein and/or lipoprotein production by visceral yolk sac may be required for the transport of maternal lipid across the visceral yolk sac and distribution to fetal cells.

At later times in development, the apoE mRNA content of visceral yolk sac reached a maximum at 14 days and subsequently declined while liver apoE mRNA rapidly accumulated (Table 4). A similar accumulation of apolipoprotein AI

mRNA in fetal mouse liver has been seen between 13 and 18 days of development (14). These data are consistent with the idea that the visceral yolk sac deteriorates during late gestation (40) while fetal liver increases its synthesis of proteins previously made by yolk sac (41). The presence of significant amounts of apoE mRNA in the fetal liver on the 12th day of gestation indicates that the apoE gene is active much earlier than the 14th day at which time the liver is believed to be functionally active (41, 42). Further studies are required to determine whether apoE mRNA is translated and whether lipoproteins are actually produced by the liver at this early time.

The finding that F9 stem cells synthesize apoE may indicate that apolipoproteins and/or lipoproteins are made by the embryo even prior to or at the time of implantation. In this regard it is of interest that one potential function of apoE made in peripheral tissues is as a targeting protein for local redistribution or shuttling of cholesterol among peripheral tissues (5). In one case of tissue remodeling, local apoE synthesis and mRNA accumulation are induced in rat optic nerve undergoing Wallerian degeneration (24). In this situation, enhanced apoE synthesis accompanies the massive mobilization of cholesterol resulting from myelin breakdown in the nerve. Similarly, apoE may function in the local transport or reutilization of cholesterol or other lipids released during the tissue destruction or remodeling associated with implantation of the embryo in the uterine wall.

Differentiation of F9 cells to the VE-like phenotype should provide a useful system with which to study the developmental activation of the apoE gene as well as other apolipoprotein genes. In preliminary studies we have found that apolipoprotein AI mRNA also is induced in VE-like F9 cells as compared to F9 stem cells. Further studies will be required to determine whether the entire complement of apolipoprotein genes is coordinately regulated in these cells. The F9 cell should prove useful for the analysis of DNA sequences and proteins involved in the regulation of the endogenous apolipoprotein genes as well as apolipoprotein genes introduced into these cells by transfection.

We thank Philomena Ostapchuk for advice and assistance in culturing F9 cells. cDNA clones for mouse apoE and α-fetoprotein were kindly provided by Aldons J. Lusis and Shirley M. Tilghman, respectively. This research was supported by grants from the National Institutes of Health (HL-32868, AM 18171, and HD-17875) and the American Cancer Society (CD-48).

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