Developmental Regulation of Apolipoprotein B mRNA Editing Is an Autonomous Function of Small Intestine Involving Homeobox Gene Cdx1*

Received for publication, February 15, 2002, and in revised form, November 20, 2002
Published, JBC Papers in Press, December 18, 2002, DOI 10.1074/jbc.M201601200

Amy P. Patterson‡‡, Zhigang Chen‡, Deborah C. Rubin‡, Virginie Moucadel¶, Juan Lucio Iovanna‡, H. Bryan Brewer, Jr.**, and Thomas L. Eggerman‡‡‡§§

From the §NHLBI, National Institutes of Health, Bethesda, Maryland 20892, ‡Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110, ¶Laboratoire de Recherche de Physiologie et Pathologie Digestives, INSERM E.116, 13009 Marseille, France, **Molecular Disease Branch, NHLBI, National Institutes of Health, Bethesda, Maryland 20892, ‡‡Division of Diabetes, Endocrinology and Metabolic Diseases, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, and §§Department of Laboratory Medicine, W. G. Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland 20892

Apolipoprotein B mRNA editing is developmentally regulated in the human and rodent small intestine, changing from <1% at day 14 to ~90% by day 20 in the rat fetus. This regulation is coincident with the developmental formation of the crypt-to-villus axis functional unit, a continuous and rapidly renewing system involving cell generation, migration, and differentiation. Utilizing small intestine isografts implanted into the subcutaneous tissue of adult recipients, apolipoprotein B mRNA editing was developmentally up-regulated, parallel to that seen with an intact control. In contrast, apoB mRNA expression remains nearly constant in the small intestine beyond the observation of increased editing level in the rat small intestine increases from <1% at day 14 to ~90% by day 20. Post-natally, 90–95% of apoB mRNA editing is observed in the rat small intestine, which is the major lipoprotein in very low density lipoproteins (VLDL) and functions as a ligand for the low density lipoprotein receptor. Both apoB-48 and apoB-100 are encoded by a single gene located on human chromosome 2. The two forms are generated as a result of apoB mRNA editing, a post-transcriptional specific conversion of a cytidine to a uracil at nucleotide position 6666, resulting in the replacement of a CAA(Q) codon with an in-frame translational UAA (stop) codon (2).

ApoB mRNA editing is dependent on a group of trans protein factors that recognize the cis elements around the mRNA editing site (2) and consequently is very site-specific. However, the editing level can be regulated by a number of factors including thyroid hormone (3), growth hormone (4, 5), fasting and refeeding (6), developmental stage (7–9), estrogen (10), ethanol (11, 12), and insulin (13). Most of regulatory studies are performed with rat liver or rat liver cell cultures. Expression level modulation or protein phosphorylation are proposed as the mechanisms regulating apoB mRNA editing (14). However, little is known about apoB mRNA editing regulation in the small intestine beyond the observation of increased editing during development, especially the rapid up-regulation 2–3 days before birth in rat (7–9). As our laboratory and others have shown (7, 8), during rat fetal development, apoB mRNA editing level in the rat small intestine increases from <1% at 14 days post-conception to 90% by day 20. Post-natally, 90–95% of apoB transcripts are edited. In contrast, editing in the rat liver remains at low levels (~8–10%) during fetal and neonatal development until the third post-natal week when editing increases dramatically and attains adult levels (40–60%) by 35 days of age (7).

The small intestine undergoes significant change during fetal development. The human fetal small intestine is covered with a multilayered stratified epithelium before 7–8 weeks of age. By 9–10 weeks, villi lined by simple columnar epithelium have begun to form and become vascularized in the duodenum and proximal jejunum (15, 16). Functional adrenergic receptors...
Developmental Regulation of Apolipoprotein B mRNA Editing

and innervation by Auerbach’s plexus appear at 9 weeks. Primitive crypts appear at 10–12 weeks, followed by lymphopoiesis at 15 weeks (17). Amniotic fluid, continually swallowed by the human fetus as early as 16 weeks of gestation, contains a significant quantity of lipid (15, 17). In addition, biliary lipid secretion occurs as early as 22 weeks (17). During this time, the human fetal intestine increasingly edits apoB transcripts from <10% at 11 weeks to an adult-like 85% level by 20 weeks of gestation (7, 9). The developmental regulation of apoB mRNA editing is coincident with the onset of intestinal ontogeny in human, which is characterized with the formation and maturation of the crypt-to-villus axis (7, 9). Similarly, the rodent intestinal endoderm undergoes cytodifferentiation to form an epithelial monolayer overlaying nascent villi. The axis of differentiation from the crypt to villus becomes first evident between 17 and 18 days of gestation when apoB mRNA editing increases rapidly to adult levels (7, 9, 18).

The crypt-to-villus axis is the functional unit of the intestinal epithelium, a continuous and rapidly renewing system involving cell generation, migration, and differentiation, from the stem cell population located at the bottom of the crypt to the excretion of the terminally differentiated cells at the tip of the villus (19). The crypt-to-villus renewing system is sustained in older life after initially being formed in the fetus. Villi are primarily lined by functional absorptive and goblet cells whereas the crypts contain stem cells, poorly differentiated and proliferative cells, a subset of differentiated secretory cells, and the Paneth cells (20, 21). Gene expression in intestinal cells is tightly regulated to control cell proliferation, migration, and differentiation along the crypt-to-villus axis and are still not completely understood (20, 22, 23). Several lines of evidence suggest that homeobox genes related to the caudal gene are involved in the regulation of intestinal differentiation (24). Cdxl and Cdx2 are the predominant homeobox transcription factors of the small intestine and are specifically expressed in the small intestine and colon (25, 26). Cdxl is a negatively regulated target of p53 in intestinal cells and is involved in the regulatory networks of apoptosis, proliferation, and differentiation (27–29). Both Cdxl and Cdx2 affect cellular proliferation and differentiation of IEC-6 cells, an undifferentiated rat intestinal epithelial cell line and regulate the intestinal epithelial cell phenotype (30–32).

This study focused on how the developmental regulation of apoB mRNA editing relates to the formation of the crypt-to-villus axis in small intestinal epithelium and the potential effect of Cdxl and Cdx2 upon editing. Our data demonstrated that like the self-renewing crypt-to-villus axis, small intestinal apoB mRNA editing is an autonomous process that involves the action of Cdxl protein.

MATERIALS AND METHODS

Intestinal Isograft Implantation—Fetal Balb/c mice were obtained on day 15 to 16 of gestation. Small intestines were excised and implanted in the subcutaneous tissue of adult Balb/c mice as described previously (33). The isografts were retrieved from the subcutaneous tissue of recipient mice at 6, 14, 21, and 31 days following transplantation. Small intestines harvested from normal Balb/c mice served as controls. All tissues were stored in liquid nitrogen before RNA extraction. Total RNA was isolated from the tissues using the guanidinium thiocyanate/isoamyl alcohol method (7). For light microscopy of paraffin-embedded tissue, small intestine prior to implantation and isograft ex situ developed for 4 weeks were fixed in Bouin’s fixative and embedded in paraffin. Sections were cut at 6 μm, mounted on glass slides, and stained with hematoxylin and eosin.

Immunohistochemical Analysis—Tissues prepared from human small intestine were fixed in Bouin’s solution for immunohistochemical analysis, sectioned at 5 μm thick, and deparaffinized as described previously (33). Endogenous peroxidase activity was quenched with 30 min incubation in 3% H2O2/97% methanol. Slides were washed three times in Tris-buffered saline, pretreated with 3% goat serum to block nonspecific antibody binding, and incubated 2 h at room temperature with monoclonal anti-human apolipoprotein B antibodies (clones ABBl and AB5 for apoB-100 only, and clones AB-BL and AB-B2 for both apoB-48 and apoB-100; Canadian Biobicial Ltd., Scarborough, Canada). Slides were then washed with Tris-buffered saline and subsequently incubated with biotinylated rabbit anti-IgG as secondary antibody for 30 min at room temperature. Slides were washed with Tris-buffered saline and developed in 3,3’-diaminobenzidine tetrahydrochloride (Vector Laboratories). The tissue was counterstained with hematoxylin, mounted with Permount, and viewed by fluorescent microscopy.

Preparation of Fully Differentiated Villus-like Cells—Viable epithelial cells from small intestine villi were isolated as described previously (33). Briefly, the small intestinal villi were isolated by trypsinization, longitudinally and washed in phosphate-buffered saline. The small intestine fragment was transferred to a beaker containing 25 ml of ice-cold Matrisperse cell release solution (Becton Dickinson Labware) and incubated at 4 °C overnight without agitation. Then, the beaker was gently shaken for 10 min to separate the epithelium. The remaining fragment was removed, and the cells released from small intestinal fragment were collected by centrifugation at 1500 rpm, 4 °C for 5 min. The total RNA was isolated from the cell preparation using the Trizol reagent (Invitrogen) according to the manufacturer’s instructions.

Construction of Cdxl and Cdx2 Expression Vectors and Establishment of Stable Cell Lines—The sequences encoding the reading frame of human Cdx1 and Cdx2 including a Kozak consensus sequence, start codon, and termination codon was synthesized by reverse transcription-polymerase chain reaction using human small intestine RNA as template and subcloned into EcoRl/BamHI sites of pcDNA3.1+ (Invitrogen) and pIREShyg2 (Clontech) to yield the Cdxl and Cdx2 expression constructs with a cytomegalovirus promoter. The nucleotide sequence of Cdx1 and Cdx2 in the expression plasmids was confirmed by sequencing from both directions.

IEC-6 cells were obtained from the American Type Culture Collection, Manassas, VA and maintained under an atmosphere of 5% CO2 in Dulbecco’s modified Eagle’s medium containing 5% fetal bovine serum, 10 μg/ml insulin, 4 mM glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin. The Cdx1 and Cdx2 plasmids were transfected into IEC-6 cells using LipofectAMINE reagent as described by the supplier (Invitrogen). The stably transfected cells were selected by resistance to 0.6 mg/ml G418 in the culture medium. Surviving colonies were pooled, and total RNA was isolated with Trizol reagent (Invitrogen) for the analyses of apoB mRNA editing and gene expression. For Cdxl and Cdx2 co-expression, Cdxl-pIREShyg2 was first transfected into IEC-6 cells. The stably transfected Cdxl/IEC-6 cells selected by 0.6 mg/ml G418 were then transfected by Cdx2-pIREShyg2 and selected by 40 μg/ml hygromycin. The dual expression of Cdxl and Cdx2 in the final surviving cell colonies was confirmed by RT-PCR with primers against the expressing genes.

ApoB mRNA Editing and RNA Expression Level Assay—The apoB mRNA editing assay was performed as described previously (14). Briefly, total RNA samples (2–5 μg) were pretreated with 10 units of DNase (Promega) at 37 °C for 1 h to remove potential genomic DNA contaminants and reverse-transcribed by Moloney murine leukemia virus reverse transcriptase using a random primer at 37 °C for 1 h. The resulting cDNA was used for apoB PCR amplification to determine apoB mRNA editing level or quantitation of RNA levels by PCR method.

To determine apoB mRNA editing, apoB covering the editing site region was PCR amplified as follows: 1) mouse apoB, 5 cycles of 50 °C at 94 °C, 1 min at 58 °C, 2 min at 72 °C and 30 cycles of 50 °C at 94 °C, 1 min at 54 °C, 2 min at 72 °C; and 2) rat apoB, 35–40 cycles of 50 °C at 94 °C, 1 min at 56 °C, 2 min at 72 °C. The apoB PCR products were purified by GeneClean kit (Bio101) and annealed to 32P end-labeled extension primer at 42 °C for 1–2 h after denaturation at 94 °C for 3 min. The primer annealed apoB was extended by Sequenase in the presence of dDGTP at 37 °C for 10 min, and the ratio between apoB-48 and apoB-100 was determined by PhosphorImager evaluating the extension product produced by 8% urea-polyacrylamide gel.

For the determination of apoB mRNA editing in IEC-6 cells, PCR primer extension instead of direct Sequenase extension described above was utilized because of the low abundance of apoB mRNA. 3 of 10 μl of purified apoB PCR products were mixed with 32P end-labeled extension primer and thermal stable Sequenase (Applied Biosystems), and the primer extension was performed by PCR according to the manufacturer’s instruction. The primer extension products were separated, and apoB mRNA editing was determined by PhosphorImager as described above.

To quantitate the mRNA expression level by PCR, aliquots of cDNA were amplified with the following primers in the presence of 32P end-labeled primers at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. Each sample was run in triplicate, and the average was calculated for quantitation.
RESULTS

The Developmental Regulation of Small Intestine Apolipoprotein B mRNA Editing Was Recapitulated in Organ Isografts—The progressive increase of small intestine apoB mRNA editing up to an adult level coincides with the fetal intestinal epithelium cytodifferentiation. The luminal contents and enterohepatic circulation in small intestine potentially could play a role in editing regulation. For example, epidermal growth factor is present in a significant quantity in amniotic fluid, saliva, and bile (35). Insulin concentrations increases steadily from an undetectable level before 12 weeks of gestation up to 30 milli-units/ml between 12 and 35 weeks of gestation in humans. Epidermal growth factor and glucocorticoids can regulate production of apoB-48, apoB-100, and lipid particles including chylomicrons, very low density lipoproteins, and high density lipoprotein in jejunal explants (35). To assess whether the increased apoB mRNA editing observed during small intestinal development is because of endoderm cytodifferentiation or regulation from intraluminal contents including bile, local hormones, and neural intervention, segments of small intestine were taken from fetal Balb/c mice at age 15–16 days, prior to epithelial cytodifferentiation, and transplanted to the subcutaneous tissue of adult Balb/c recipients. The isografts were allowed to develop ex situ and were subsequently harvested for analysis at 6, 14, 21, and 31 days following transplantation. As shown in Fig. 1A, the histology of the grafts at the time of transplantation (16 days) consisted of a simple stratified endoderm surrounded by loosely packed mesenchyme. Four weeks later following transplantation, the isograft showed an ex situ development of grossly normal vasculature, crypts, and villi, recapitulating the normal development of small intestine (33). The editing analyses showed that apoB mRNA editing in isografts increased sharply from 6% at 16 days, the initial time point for transplantation, to ~93% at 6 days following transplantation, and the editing level remained thereafter (Fig. 1B). This pattern is identical to that seen with the normal control. These data suggest that the developmental regulation of apoB mRNA editing is an autonomous function of small intestine, independent of luminal contents, enterohepatic circulation, innervation, and anatomic location.

On the other hand, the expression level of apoB mRNA in isografts was different from that of the normal control. As shown in Fig. 1C, apoB mRNA expression in the normal control...
Developmental Regulation of Apolipoprotein B mRNA Editing

Fig. 2. Expression and distribution of apolipoprotein B along the crypt-villus axis. Cryosections of human adult small intestine were stained with antibodies that recognized apoB-48 and B-100 total proteins (A) or apoB-100 only (B). C and D are enlargements of A and B regions, which contain crypts.

was low at day 16 of gestation but increased from −2 to −84% of peak value at birth. The expression peaked at post-natal 7 days and then declined gradually as observed previously (36). In contrast, the apoB mRNA level in small intestinal isografts gradually rose during this period with the highest value about 26% of the peak normal value in normal control. These data indicate that luminal contents, enterohepatic circulation, innervation, and/or anatomic location may play an important role in regulating apoB mRNA expression level. APOBEC-1 and ACF are the core enzyme components in apoB mRNA editing complex. Expression of both gene products was readily detected in isografts as shown in Fig. 1D. This indicates that there is sufficient APOBEC-1 in the presence of ACF to support the editing in isografts although they had different relative gene expression levels when compared with an intact control.

ApoB Protein Production and ApoB mRNA Editing Is Fully Attained in Well Differentiated Enterocytes of the Villus Surface—The developmental regulation of apoB mRNA editing in the small intestine is coincident with the endoderm cytodifferentiation that forms the crypt-to-villus axis. The crypt-to-villus axis is a self-renewing system with cell generation and migration from the bottom crypt-like cells to the top villus with well differentiated cells. We examined the histological distribution of apoB-48 and apoB-100 in human small intestine to see where the edited apoB protein is located. As shown in Fig. 2, A and B, apoB proteins were predominantly distributed in the well differentiated enterocytes along the villus surface when using an antibody recognizing both apoB-48 and apoB-100. When using an antibody recognizing apoB-100 only, the apoB staining was mainly found in the lamina propria and crypts. Taking the staining around the crypt as a reference, the intensity of apoB protein staining in villus surface cells was higher when stained for both apoB-48 and apoB-100 but was lower when stained for apoB-100 only (Fig. 2, C and D), indicating the presence of apoB-48 in the enterocyte along villus surface. These data suggest that apoB mRNA editing mainly occurs in well differentiated cells along the villus surface.

IEC-6 is a commercially available crypt-like undifferentiated rat intestinal epithelial cell line (37). Fully differentiated villus-like cells can be prepared from rat small intestine by a method described previously (34). Therefore, both fully differentiated villus-like cells from rat small intestine and undifferentiated crypt-like IEC-6 cells were obtained to compare their apoB mRNA editing levels. As shown in Fig. 2B, the RNA expression of sucrase isomaltase, a specific marker for intestinal differentiation, was only detected in the enterocytes prepared from the proximal small intestine but not in IEC-6 cells. The apoB mRNA expression level was barely detectable even with a high PCR cycle number in IEC-6 cells whereas the level in villus enterocytes was very high (Fig. 2B). The apoB mRNA editing analyses demonstrated that the villus-like cells from the small intestine had a editing level (~91.6%) comparable with the whole tissue (~93%) (Fig. 3A). In contrast, IEC-6 cells only had a background level of apoB mRNA editing (~0.3%), which was detected by a more sensitive primer extension based on PCR cycles (see “Materials and Methods”) because of a low abundance of apoB mRNA expression. These data are consist-
The regulation of apoB mRNA editing has been investigated extensively in the last decade with most of the regulatory studies being carried out on rat liver or hepatocytes. However, very little has been established about the small intestine, the other major source of apolipoprotein B. ApoB mRNA editing in the small intestine dramatically increases 2–3 days before birth in rodents (7–9). Coincident with this editing change is a significant increase of apoB mRNA editing in normal small intestinal development and the up-regulation of editing in Cdx1 overexpressing IEC-6 cells suggest that Cdx1 may play an important role in regulating apoB mRNA expression. The concurrent increase of Cdx1 expression and apoB mRNA editing in normal small intestinal development and the up-regulation of editing in Cdx1 overexpressing IEC-6 cells suggest that Cdx1 may play an important role in the developmental regulation of apoB mRNA editing.

**DISCUSSION**

The regulation of apoB mRNA editing has been investigated extensively in the last decade with most of the regulatory studies being carried out on rat liver or hepatocytes. However, very little has been established about the small intestine, the other major source of apolipoprotein B. ApoB mRNA editing in the small intestine dramatically increases 2–3 days before birth in rodents (7–9). Coincident with this editing change is

---

**Fig. 4.** The comparative ontogeny between apoB mRNA editing and the expression of Cdx1 and Cdx2. Total RNA was isolated from small intestine tissues of different ages and analyzed for apoB mRNA editing (A) and expression of APOBEC-1 (B), ACF (C), Cdx1 (D), and Cdx2 (E) by RT-PCR normalized to β-actin. Values are means ± S.D. and represent two to four determinations for each group.

**Fig. 5.** The effect of Cdx1 overexpression on apoB mRNA editing in IEC-6 cells. A, total RNA was isolated from stable Cdx1 transfectants and analyzed for apoB mRNA editing by PCR-primer extension in vector control (lanes 1 and 2) and Cdx1 overexpressing cells (lanes 3–5). B, the expression levels of Cdx1, Cdx2, APOBEC-1, ACF, and β-2-microglobulin were determined by semi-quantitative RT-PCR.

Cdx1 + Cdx2 (3.5% ± 1.6% (S.E.), n = 6). Compared with vector control (1.2% ± 0.1% (S.E.), n = 8), there was a significantly statistical difference for Cdx1 overexpression (p < 0.01 by analysis of variance using a StatView program) but not for Cdx2 or the co-expression of Cdx1 and Cdx2.

To investigate the potential mechanism by which Cdx1 increases apoB mRNA editing, expression of the editing components, APOBEC-1 and ACF, were evaluated. In these experiments, the vector control had editing ~0.8% whereas Cdx1 overexpression increased the editing to 22–27% (Fig. 5A). Cdx1 overexpression also significantly increased the expression of activating factor ACF, a component of the apoB mRNA editing complex. In contrast, the catalytic component APOBEC-1 remained relatively unchanged (Fig. 5B). The expression of apoB mRNA was not significantly modulated by either Cdx1 or Cdx2 overexpression (data not shown), consistent with the intestine isograft data, which suggested that other factors such as luminal contents or enterohepatic circulation may play an important role in regulating apoB mRNA expression. The concurrent increase of Cdx1 expression and apoB mRNA editing in normal small intestinal development and the up-regulation of editing in Cdx1 overexpressing IEC-6 cells suggest that Cdx1 may play an important role in the developmental regulation of apoB mRNA editing.
the onset of intestinal ontogenesis in both the human and rat (9).

The major event of fetal intestinal ontogenesis is the formation of the crypt-to-villus axis, the functional unit of the small intestine (33). The development of crypt-to-villus axis in fetus can be recapitulated in the intestinal isograft (35). Therefore, the isograft method was utilized to examine whether the normal luminal environment, innervation, anatomic location, or intestinal endoderm cytodifferentiation is required for the development of apoB mRNA editing. We found that apoB mRNA editing developed similarly in the isograft as it did in the intact control. These data suggest that the development of apoB mRNA editing is an autonomous function of small intestine like the generation of intestinal epithelium from endoderm. In contrast, the pattern of apoB mRNA expression was altered in the isografts. In the intact control, apoB mRNA expression increased dramatically and peaked 7 days after birth, which is similar to results described previously (36). The dramatic increase of apoB mRNA expression was not observed in the small intestinal isografts. Enterocytes that develop *ex situ* expressed significantly lower amounts of apoB mRNA during the small intestinal isografts. Enterocytes that develop which is similar to results described previously (36). The expression of other intestinal gene products in the isograft model has been reported having appropriate expression for fatty acid binding protein (L-FABP) and apolipoprotein (apo) AIV or increased expression for epimorphin/syntaxin 2 (33, 38). In this study, the mRNA expression levels of the housekeeping gene β-2-microglobulin are comparable between isografts and intact control in this setting (see Fig. 1D), indicating a specificity of the apoB effect. The sum of these data suggests that intestinal luminal contents, neonatal developmental factors, enterohepatic circulation, and/or local factors reflecting the anatomic location such as innervation control the developmental up and down-regulation of apoB mRNA levels during *in situ* development.

The crypt-to-villus axis in the small intestine is a continuous and rapidly renewing system involving cell proliferation, migration, and differentiation from the stem cell population located at the bottom of the crypt to the extrusion of the terminally differentiated cells at the tip of the villus (20). The crypt-to-villus axis formed during fetal development is sustained throughout life, continually replicating the normal ontogeny. With immunohistological analyses of adult human small intestine, we found that apoB-48 was predominantly located in surface villus cells, which are differentiated mature cells that migrated from the crypts (20). The well differentiated villus cells prepared from rat adult small intestine had an apoB mRNA editing level comparable with small intestine tissue whereas the crypt-like cells, IEC-6, had no detectable editing (see Fig. 3). These two lines of evidence suggest that the intestinal stem-like cells in crypts have little apoB mRNA editing. Subsequently the cells increase editing as they proliferate, differentiate, and migrate from the bottom of crypts during development.

The rat intestinal cell line IEC-6 retains the characteristics of normal rat crypt jejunal cells (37). We found that apoB mRNA expression was barely detectable and required a high cycle PCR for detection, and no apoB mRNA editing was detected. This is consistent with the report that IEC-6 cells are unable to synthesize apolipoproteins and lipoproteins (39). On the other hand, human crypt intestinal epithelial cells are able to synthesize apolipoproteins and lipoproteins with a predominance of apoB-100 (40). This indicates that human crypt intestinal epithelial cells, in contrast to IEC-6 cells, can edit apoB mRNA transcripts at a low level. Taken together, the very limited apoB mRNA editing observed in crypt cell lines and the adult tissue level of editing in well differentiated villus cells support the concept that as differentiation occurs when the crypt cells develop into villus cells, there is a corresponding increase of apoB mRNA editing. This proposal is also supported by Caco-2 studies where maturation increases apoB mRNA editing (41).

A fully developed intestinal epithelium results from a complex series of cellular transitions. Cytodifferentiation of endoderm to form the crypt-to-villus axis is a particularly critical time in mouse intestinal development (42) and is when the rapid up-regulation of apoB mRNA editing occurs. Homeobox genes are essential in controlling normal embryonic development (43). Cdx1 and Cdx2 are the predominant homeobox transcription factors specifically expressed in the small intestine and colon and have been proposed as important regulatory factors during endoderm cytodifferentiation (24, 44). The expression pattern of Cdx1 and Cdx2 is largely preserved even in the fetal small intestine isograft model (45). Expression of Cdx1 and Cdx2 induces crypt-like IEC-6 cells to develop into differentiated mature villus-like cells (30, 31). In this study, we found that Cdx1 mRNA expression increased 18-fold when apoB mRNA editing increased from ~2% to an adult level (~90%) whereas Cdx2 expression did not change significantly (see Fig. 4). Overexpression of Cdx1 in crypt-like IEC-6 cells increased apoB mRNA editing from 0.8% to as high as 22–27% (see Fig. 5). These findings suggest that Cdx1 may play an important role in the development regulation of apoB mRNA editing. Overexpression of Cdx2, as well as Cdx1 plus Cdx2, also increased the editing in IEC-6 cells but not to statistically significant levels. The absence of a combination effect may reflect the reported antagonism between Cdx1 and Cdx2 (46).

There was considerable variability in the degree of editing induction in the Cdx1 and Cdx2 overexpression studies in IEC-6 cells. One possible explanation for this variability could be differences in transfection efficiencies with subsequent variable expression levels. Alternatively, this variation could be because of the complex nature of Cdx1 and Cdx2 as transcription factors including potential antagonism between factors regulated by Cdx1 and Cdx2. It has been reported that persistent Cdx1 expression promotes IEC-6 cell proliferation and differentiation whereas conditional inductive Cdx1 expression inhibits proliferation but has no effect on differentiation (31, 32). An effect of Cdx2 upon differentiation has been reported for conditional inductive Cdx2 expression in IEC-6 cells and persistent expression in Caco-2 cells (30, 47). In addition, Cdx2 can be inactivated by phosphorylation at serine 60 (48, 49). Thus, the effect of Cdx1 or Cdx2 can be modified by multiple factors that could contribute to the variation seen in apoB mRNA editing observed in overexpressing IEC-6 cells.

The apoB mRNA expression level was not changed by overexpressing either Cdx1 or Cdx2 (data not shown). This contrasts to the ontogeny in normal small intestine where both apoB mRNA expression and editing were increased significantly. The lower level of apoB mRNA expression in isografts and the absence of Cdx1 and Cdx2 overexpression affecting apoB mRNA levels in IEC-6 cells suggest that factors other than Cdx1 or Cdx2 are involved in the developmental regulation of apoB mRNA expression.

ApoB mRNA editing is dependent on a group of proteins that recognize the apoB mRNA substrate to form an editing enzyme complex. APOBEC-1 and ACP are the core enzyme components of the complex. APOBEC-1 is the catalytic enzyme component and forms an *in vitro* functional editing enzyme, together with
ACF. A number of other proteins have also been found recently (50) including editing inhibitory factors, CUGBP2, and GRY-RBP. However, the exact roles for each protein component in regulating editing remain to be elucidated. The potential roles of APOBEC-1 and ACF in the developmental regulation of apoB mRNA editing in small intestine was investigated with rat/human small intestine tissue and Caco-2 cells by comparing their mRNA abundance and editing levels (8, 51). APOBEC-1 increased progressively whereas ACF mRNA abundance was relatively consistent, indicating that APOBEC-1 expression potentially determined the level of apoB mRNA editing. In this study, ACF appeared to have a role in determining apoB mRNA editing. We found that during mouse small intestine ontogeny editing increased from -2 to -90% whereas both ACF and APOBEC-1 expression nearly doubled (see Fig. 4). In addition, when using Cdx1 overexpressing rat IEC-6 cells, APOBEC-1 remained relatively unchanged, and ACF expression significantly increased when apoB mRNA editing also increased significantly (see Fig. 5).

In summary, our findings suggest that 1) the developmental regulation of apoB mRNA editing in fetus is an autonomous function of the small intestine; 2) apoB mRNA editing level increases as the intestinal epithelial cells mature and ascend from the crypt, simulating the developmental regulation of editing observed during small intestinal cytodifferentiation; 3) the increased levels of homeobox gene Cdx1 expression coincides with the increased apoB mRNA editing observed during normal small intestine development; and 4) Cdx1 overexpression increases apoB mRNA editing and ACF expression in crypt-like IEC-6 cells. These results suggest that Cdx1 may play an important role in the developmental regulation of apoB mRNA editing.

REFERENCES
1. Young, S. G. (1990) Circulation 82, 1574–1594
2. Anant, S., and Davidson, N. O. (2001) Curr. Opin. Lipidol. 12, 159–165
3. Inui, Y., Giannoni, F., Funahashi, T., and Davidson, N. O. (1994) J. Lipid Res. 35, 1477–1489
4. Sjoberg, A., Oscarsson, J., Bostrom, K., Innerarity, T. L., Eden, S., and Olofsson, S. O. (1992) J. Biol. Chem. 267, 1522–1527
5. Patterson, A. P., Tennyson, G. E., Hoeg, J. M., Sviridov, D. D., and Brewer, H. B., Jr. (1992) Arterioscler. Thromb. 12, 468–473
6. Giannoni, F., Chou, S. C., Skaros, S. F., Verp, M. S., Field, F. J., Coleman, R. A., and Davidson, N. O. (1995) J. Lipid Res. 36, 1664–1675
7. modifier, N., and Smith, H. C. (1998) Biochem. Biophys. Res. Commun. 252, 334–339
8. Thorngate, P. E., Raghow, R., Wilcox, H. G., Werner, C. S., Heimberg, M., and Elam, M. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5392–5396
9. Chen, Z., Eggerman, T. L., and Patterson, A. P. (2001) Biochem. J. 357, 661–672
10. Grand, R. J., Watkins, J. B., and Torti, F. M. (1976) Gastroenterology 70, 790–810
11. Lau, P. P., Cahill, D. J., Zhu, H. J., and Chan, L. (1995) J. Lipid Res. 36, 2069–2076
12. Van Mater, D., Sowden, M. P., Cianci, J., Sparks, J. D., Sparks, C. E., Ballatore, N., and Smith, H. C. (1998) Biochem. Biophys. Res. Commun. 252, 334–339
13. Thorngate, P. E., Raghow, R., Wilcox, H. G., Werner, C. S., Heimberg, M., and Elam, M. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5392–5396
14. Chen, Z., Eggerman, T. L., and Patterson, A. P. (2001) Biochem. J. 357, 661–672
15. Grand, R. J., Watkins, J. B., and Torti, F. M. (1976) Gastroenterology 70, 790–810
16. Mourey, P. C., and Trier, J. S. (1978) Anat. Rec. 191, 269–285
17. Davidson, N. O., Drewek, M. J., Gordon, J. L., and Elovson, J. (1988) J. Clin. Invest. 82, 300–308
18. Mathan, M., Mouey, P. C., and Trier, J. S. (1976) Am. J. Anat. 146, 73–92
19. Babahtsaksy, M. W., and Podolosky, D. K. (1999) in Textbook of Gastroenterology (Yamada, T., ed) pp. 547–584, J. B. Lippincott, Philadelphia
20. Pageot, L. P., Perreault, N., Baoua, N., Franchescu, C., Magny, P., and Beaulieu, J. F. (2000) Microbes Res. 49, 394–406
21. Cloutherty, J. P., and Subramanian, V. (2001) Mech. Dev. 101, 3–9
22. Beaulieu, J. F. (1999) Front Biosci. 4, D310–D321
23. Shaw, S. C., and Walters, J. R. (1997) Gut 40, 5–8
24. Silberg, D. G., Swain, G. P., Suh, E. R., and Traber, P. G. (2000) Gastroenterology 119, 961–971