Regulation of Sterol Regulatory Element-binding Proteins in Hamster Intestine by Changes in Cholesterol Flux

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A control chow diet or diets containing 1% cholesterol (cholesterol-enriched) or 4% cholestyramine and 0.15% lovastatin (cholesterol-depletion) were fed to hamsters for 2 weeks. Sterol regulatory element-binding protein (SREBP)-1a, SREBP-1c, SREBP-2, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, 3-hydroxy-3-methylglutaryl-coenzyme A synthase, and LDL receptor mRNA levels and SREBP-1 and -2 protein expression were estimated in villus cell populations from duodenum, jejunum, and ileum. SREBP-1a was a minor transcript in hamster intestine, and its gene expression was not altered by changes in dietary cholesterol flux. In contrast, SREBP-1c gene expression was increased by dietary cholesterol and decreased by cholesterol depletion. mRNA levels for SREBP-2 and the other sterol-responsive genes were increased in intestines of animals on the cholesterol depletion diet but minimally suppressed if at all, by the diet enriched in cholesterol. In general, the amount of the precursor form of SREBP-1 was higher in cells of the upper villus and lower in cells of the lower villus. SREBP-2 precursor was higher in cells of the lower villus and lower in cells of the upper villus. Protein expression of precursor correlated with the location of gene expression for SREBPs. The amount of precursor mass of SREBP-2 was not altered by cholesterol feeding but was increased by cholesterol depletion. The mature form of SREBP-2 was in very low abundance and difficult to detect in intestines of animals fed control chow or cholesterol. It was readily detectable and increased in intestines of animals on the cholesterol-depletion diet. The diets did not significantly alter the amount of precursor or mature forms of SREBP-1. Cholesterol feeding had no effect on cholesterol or fatty acid synthesis, whereas synthesis of these lipids was increased in intestines of hamsters on the cholesterol-depleted diet. These results suggest that SREBP-1a has little or no role in regulating intestinal cholesterol synthesis. It is postulated that under basal conditions, SREBP-1c regulates intestinal fatty acid synthesis and SREBP-2 regulates cholesterol synthesis. Following marked changes in cholesterol flux across the intestine, SREBP-2 assumes the role of SREBP-1 and regulates both cholesterol and fatty acid synthesis in intestine.

The cholesterol content of all cells is tightly controlled. When cholesterol is in excess, cells decrease both lipoprotein cho-

terol uptake and cholesterol synthesis to prevent accumulation of this potentially toxic sterol. In contrast, when cells have insufficient cholesterol to meet their sterol requirements, lipoprotein cholesterol uptake is increased as are rates of cholesterol biosynthesis. This end product feedback mechanism of regulating the amount of cellular cholesterol has been extensively studied and characterized (for a review, see Ref. 1). Understanding of how a cell senses and regulates the amount of cholesterol has been advanced by the characterization of two transcription factors called sterol regulatory element-binding proteins (SREBPs).1 SREBP-1 and -2 (for a review, see Ref. 2). SREBPs are bound to membranes of the endoplasmic reticulum and nuclear envelope. Under conditions of cholesterol deficiency, a two-step proteolytic process releases from the membrane “precursor” protein a “mature” form of the protein (3). This active form of the protein enters the nucleus and binds to a 10-base pair sterol regulatory element that enhances the transcription of target genes that encode enzymes regulating cholesterol and fatty acid synthesis (4–8). SREBP-1 and -2 are derived from two separate genes. Two isoforms of SREBP-1 exist, 1a and 1c (9). SREBP-1a appears to be the more potent transcription factor and regulates genes of both fatty acid and cholesterol pathways (10). SREBP-1c, in contrast, tends to be more active in regulating genes of the fatty acid biosynthetic pathway (10). SREBP-2 preferentially enhances several genes of the cholesterol biosynthetic pathway (11–13).

We have recently characterized gene expression for SREBP-1 and -2 in hamster small intestine and have implicated SREBP-2 as responsible for regulating cholesterol synthesis and SREBP-1c as responsible for regulating fatty acid synthesis in intestine (14). We have also demonstrated that changes in cholesterol flux across the intestinal cell regulate gene expression of SREBP-1c and several sterol-responsive genes, including SREBP-2. In the present study, we have extended our investigations into the regulation of intestinal SREBPs by studying the regulation of SREBP gene and protein expression in hamster small intestine by changes in cholesterol flux. The results demonstrate that a diet enriched in cholesterol increases gene expression of SREBP-1c, whereas a cholesterol-depletion diet markedly decreases its expression. In contrast, a cholesterol-depletion diet results in a substantial increase in the expression of SREBP-2 and other sterol-responsive genes, HMG-CoA reductase, HMG-CoA synthase, and the LDL receptor, while dietary cholesterol had little, if any effect on the expression of these genes. In the intestines of hamsters on control or cholesterol diets, the mature form of SREBP-2 is in very low abundance. In intestines of animals on the cholesterol-depletion diet, the precursor and mature forms of SREBP-2 are increased.

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1 The abbreviations used are: SREBP, sterol regulatory element-binding protein; LDL, low density lipoproteins; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A.
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MATERIALS AND METHODS

Trinitated water and [32P]UTP were purchased from PerkinElmer Life Sciences. Protease inhibitors, TRI REAGENT, INFINITY cholesterol reagent, and cholesterol were purchased from Sigma. MAXiscript T7 and RPA III kits were supplied by Ambion (Austin, TX). Medium M199 was from Life Technologies, Inc. Lovastatin was obtained from Merck. Cholesterol esterase was obtained from Bristol-Myers Squibb Co.

Animals—Male Golden Syrian hamsters weighing 90–120 g were purchased from Harlan Sprague-Dawley, Inc. They were maintained for a week on NIH-31 modified mouse/rat diet number 7013 (Harlan/Teklad, Madison, WI) before starting the test diets. The animals were fed the test diets ad libitum, and the light cycle was 0600–1800 h. The control chow diet was prepared by dissolving 1% cholesterol in the olive oil and thoroughly mixing with the control chow diet. The control chow diet was supplemented with 4% (w/w) cholesterol and 0.15% (w/w) lovastatin (dissolved in olive oil). The start of the dietary regimen was staggered in the hamsters so that all animals were fed the respective diets for exactly 14 days.

Isolation of Enterocytes—Hamsters were killed between 0800 and 0900 h by inhalation of CO2. The small intestine was removed in its entirety and placed in a beaker containing cold 0.9% saline. Isolated intestinal cells were prepared by a procedure described by Cartwright and Higgins that was modified for hamster intestine (14, 15). The tunic of the intestine was flushed with solution A (117 mM NaCl, 5.4 mM KCl, 0.96 mM NaH2PO4, 26 mM NaHCO3, 5.5 mM HEPES, 5.5 mM glucose) to remove fecal material and debris. The intestine was then filled with solution B (117 mM NaCl, 1.5 mM KCl, 0.96 mM NaH2PO4, 26 mM NaHCO3, 5.5 mM HEPES, 5.5 mM glucose, 27 mM sodium citrate) and placed in a beaker containing 0.9% saline at 37 °C. The beaker was gently agitated for 15 min. Solution B was removed, and the intestine was divided into three equal segments representing duodenum, jejunum, and ileum. Each segment was filled with solution C (115 mM NaCl, 5.4 mM KCl, 0.96 mM NaH2PO4, 26 mM NaHCO3, 1.5 mM EDTA, 0.5 mM Na2EDTA, 5 mM HEPES, 5.5 mM glucose) and placed in a beaker containing 0.9% saline at 37 °C. The beakers were gently agitated in a shaking water bath to isolate cells along the villus. Solution C was emptied into plastic conical centrifuge tubes every 5 min and replaced. The tubes containing the cells were kept on ice until 12 fractions were collected. Two sequential fractions were then pooled from the first eight fractions, and the last four fractions were combined to obtain a total of five fractions (16). Cells were collected by centrifugation at 1000 × g for 10 min at 4 °C.

Cholesterol and Fatty Acid Synthesis—Cholesterol and fatty acid synthesis were estimated by incorporation of tritiated water into these lipids 4 h after intraperitoneal injection of 100 mCi of tritiated water (1 Ci/mg; PerkinElmer Life Sciences) (17). Intestinal cell fractions were collected from duodenum, jejunum, and ileum as described above. The cells were homogenized in 2 ml of 0.5 M NaOH and kept in boiling water for 1 h to saponify the lipids. After adding an equal volume of acidic water, the lipids were extracted twice at pH 3.0 with 4 ml of hexane. To remove residual labeled water, the hexane extract was washed once with 1 ml of acidic water. The hexanes were evaporated under a stream of nitrogen, and the lipids were dissolved in 0.1 ml of chloroform. Fatty acids and cholesterol were separated by thin layer chromatography on silica gel plates using hexanes/diethyl ether/acetic acid (70:30:1, V/v/V) as solvent. The bands were visualized by exposure to iodine, and the radioactivity in fatty acids and cholesterol fractions was determined by scraping the bands in the 80–150-kDa range were observed in the absence of pri-
Gene Expression—Following 2 weeks on the respective diets, the intestines were divided into three equal segments representing duodenum, jejunum, and ileum. Isolated intestinal cells were prepared from duodenum, jejunum, and ileum of hamsters fed for 2 weeks a control chow diet or a cholesterol-rich (cholesterol) or cholesterol-depletion (cholesterol plusLovastatin) diet as described under “Materials and Methods.” RNA was isolated from each of the five fractions, and mRNA levels were estimated by RNase protection assay. 18 S rRNA was used as an internal control to confirm that equal amounts of RNA were used for the assays. Fraction 1 represents mRNA from cells of the uppermost villus, and fraction 5 represents mRNA from cells of the crypts. a, a representative RNase protection assay from a single hamster on each diet; b, mRNA levels for the genes in the different cell populations of the villus from duodenum, jejunum, and ileum. The data represent the means ± S.E. from three animals fed the respective diets. c, cumulative mRNA levels in duodenum, jejunum, and ileum. Open bar, control chow; diagonal bar, cholesterol-rich; cross-hatch bar, cholesterol-depletion. *, cholesterol-rich values are significantly different from control chow values at p < 0.05. **, cholesterol-depletion values are significantly different from control chow values at p < 0.05. ***, cholesterol-depletion values are significantly different from cholesterol-rich values at p < 0.05.

Compared with the expression of SREBP-1a in intestines of animals on the control chow diet, neither the cholesterol diet nor the cholesterol-depletion diet altered SREBP-1a gene expression (Fig. 1, a and b). In contrast, expression of SREBP-2 and the other sterol-responsive genes was lowest in cells of the upper villus and increased as cells of the lower villus were reached. Compared with duodenum, expression of these genes was modestly higher in jejunum and ileum.

In animals fed the cholesterol-depletion diet, SREBP-1c was highest in cells of the villus tip and decreased as cells of the lower villus were reached (Fig. 1, a and b). In contrast, expression of SREBP-2 and the other sterol-responsive genes was lowest in cells of the upper villus and increased as cells of the lower villus were reached. Compared with duodenum, expression of these genes was modestly higher in jejunum and ileum.

In animals fed the cholesterol-depletion diet, SREBP-2,
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Fig. 2. Effect of diets on hepatic SREBP-1 and -2 mass. Total membranes were prepared from livers of hamsters fed control chow, cholesterol-rich or cholesterol-depletion diets. The membranes were analyzed for SREBP-1 and SREBP-2 mass by immunoblot as described under “Materials and Methods.” a, an immunoblot from a single animal on each of the diets. 6–100 μg of protein was applied per lane. P, precursor form; M, mature forms. b, an immunoblot of total membranes prepared from livers of three individual animals fed the respective diets. P, precursor; M, mature. 50 μg of protein was applied per lane.

HMG-CoA synthase, HMG-CoA reductase, and LDL receptor gene expressions were dramatically increased in all cell populations of the villus and in all three segments of the small intestine (Fig. 1, b and c). In contrast, cholesterol feeding had minimal if any effect on mRNA levels of these genes.

Protein Expression—In preliminary experiments, we found the mature form of both intestinal SREBP’s to be in very low abundance and detection difficult. To help in identifying the bands representing precursor and mature peptides of SREBP’s and to support our results in intestine, protein expression of SREBP-1 and -2 from livers and intestines of hamsters on the respective diets was estimated. Fig. 2a shows a representative immunoblot of SREBP-1 and SREBP-2 from a single animal in each of the dietary groups. Because of the low abundance of the mature form of both SREBP’s and to accurately measure changes in the amount of the precursor form, the blots were exposed to film for different times. The exposure time for estimating the amount of the precursor form was 3–4 times less than the exposure time used to detect and estimate the amount of the mature form. Fig. 2b shows the amount of the precursor form of SREBP-1 and -2 in the different cell populations of the villus in the three segments. Fig. 2c shows the cumulative average of the precursor mass in duodenum, jejunum, and ileum. The results depicted in the representative blot demonstrate that in membranes prepared from intestines of hamsters on all three diets, the precursor forms of both SREBP-1 and -2 were readily detectable. In general, in animals fed control chow and the cholesterol-enriched diets, the amount of the precursor form of SREBP-2 tended to be lower in membranes prepared from cells of the upper villus and higher in cells of the lower to middle villus. The reverse tended to be true for the precursor form of SREBP-1, particularly in the duodenum (Fig. 3, a and b). It should be appreciated here that the antibody for SREBP-1 detects the mass of both SREBP-1a and SREBP-1c. From the cumulative data shown in Figs. 3, b and c, it is apparent that the amount of precursor form of SREBP-2 in intestinal cells of animals fed the cholesterol-enriched diet was similar to the amount present in intestines of animals on the control chow diet. There was, however, significantly more SREBP-2 precursor in duodenum and jejunum of hamsters fed the cholesterol-depleted diet than in hamsters fed either the control chow or cholesterol diet. In ileum, significant differences in SREBP-2 precursor forms were only observed between hamsters ingesting the cholesterol-depleted diet and animals on control chow. The mature form of SREBP-2 was difficult to detect in intestines of animals fed the control chow or the cholesterol diet. In all five animals, a distinct band above background noise was never fully appreciated (Fig. 3a). However, in intestines of animals on the cholesterol-depleted diet, particularly in the proximal intestine, the mature form of SREBP-2 was readily detected.
detectable and was consistently increased. In contrast, although there was a tendency for SREBP-1 precursor to be present in higher amounts in jejunum of hamsters fed the cholesterol-enriched diet, because of variability among animals, this did not reach statistical significance (Fig. 3, b and c).

Like the mature form of SREBP-2, the mature form of SREBP-1 was also in low abundance and difficult to detect. Although in Fig. 3a, it appears that the mature form of SREBP1 was increased in intestines of animals fed the cholesterol diet, the mature protein did not consistently appear as a distinct band. Thus, we were unable to make any conclusions regarding the regulation of mature SREBP-1 by dietary cholesterol.

**Cholesterol Mass**—To determine whether the diets altered the cholesterol content of cells along the intestine, cholesterol mass was estimated in the five villus cell populations of duodenum, jejunum, and ileum (Fig. 4). In all three segments of intestines of hamsters fed the control chow diet, there was a trend indicating more cholesterol mass in cells of the upper rather than the lower villus. Cholesterol feeding did not alter this trend but caused significant accumulation of cholesterol in all cell populations of the villus. More cholesterol accumulated in cells of the jejunum and ileum than duodenum. The cholesterol-depleted diet decreased the amount of cholesterol in intestines of these animals, except perhaps, in lower villus cells of the ileum. The cholesteryl ester content mirrored the results of total cholesterol, accounting for ~3%, 0.9%, and 9% of total cholesterol in intestinal cell fractions of animals on chow, cholesterol-depletion, and cholesterol-enriched diets, respectively.

**Cholesterol and Fatty Acid Synthesis**—Rates of cholesterol
and fatty acid synthesis in intestinal cell fractions were estimated by tritiated water incorporation into cholesterol and fatty acids following the dietary periods (Fig. 5). In animals on all three diets, rates of cholesterol synthesis were lowest in cells of the villus tips and increased as cells of the lower villus were reached. Rates of cholesterol synthesis were similar in intestines of animals fed the chow or cholesterol-enriched diets. In contrast, rates of cholesterol synthesis were markedly increased in intestines of hamsters on the cholesterol-depletion diet.

Rates of fatty acid synthesis were also similar in intestines of animals on control chow or cholesterol diets. In contrast, in all cells along the villus and in all three segments of small intestine, rates of fatty acid synthesis were increased in animals on the cholesterol-depletion diet.

**DISCUSSION**

The results of this study clearly show that SREBP-1a is a minor transcript in intestinal cells. Moreover, gene expression of SREBP-1a in intestine was not altered by rather drastic changes in cholesterol flux. These findings, taken together with earlier results in isolated intestinal segments, which showed that gene expression of SREBP-1a was not regulated by cholesterol flux, suggest that SREBP-1a does not regulate cholesterol synthesis in intestine (14). In a previous study, gene expression of SREBP-1a in livers of hamsters ingesting a cholesterol-enriched diet was also not altered (conditions that would have had profound effects on hepatic cholesterol synthesis) (12). Because the liver and intestine account for most cholesterol synthesis within the body, the results from both studies suggest that SREBP-1a has only a small role in regulating total body cholesterol synthesis. This is not to imply that SREBP-1a does not regulate genes of the cholesterol biosynthetic pathway. This is clearly not the case. In livers of transgenic mice that overexpress a truncated form of SREBP-1a, massive accumulation of cholesterol occurs and rates of cholesterol synthesis and expressions of sterol-responsive genes are markedly increased (20). Furthermore, transfection of cultured cells with SREBP-1a increases rates of transcription of sterol-responsive genes (10). Despite these findings that support the view that SREBP-1a is a rather potent transcription factor for genes that regulate cholesterol synthesis, SREBP-1a does not appear to be an important regulator of cholesterol synthesis in hamster intestine or liver.

In contrast to SREBP-1a, changes in cholesterol flux do regulate gene expression of intestinal SREBP-1c and SREBP-2. The regulation of these genes by cholesterol flux, however, is not a simple matter. In cell culture experiments, changes in cholesterol flux cause coordinate regulation of expression of both SREBP-1 and -2 (21–23). It is quite obvious from the results presented here in intact animals that these transcripts are not coordinately regulated by cholesterol flux; in fact, quite the opposite is true. SREBP-1c is thought to be a weaker transcription factor than SREBP-1a and more specific for enhancing the transcription of genes in the fatty acid synthetic pathway (10). Transgenic animals overexpressing SREBP-1c in liver display a moderate increase in fatty acid synthesis without altering cholesterol synthesis (10). In our previous study, we postulated that SREBP-1c was responsible for regulating fatty acid synthesis but not cholesterol synthesis in intestine (14). The present results also support the notion that SREBP-1c plays little or no role in regulating cholesterol synthesis in intestine. In response to changes in cholesterol flux, changes in gene expression of SREBP-1c were reciprocal to the changes observed in expressions of the three sterol-responsive genes and to cholesterol synthesis. Whereas the previously observed correlation between SREBP-1c gene expression and fatty acid synthesis holds true in intestines of chow-fed animals (14), the present results might argue against a regulatory role for SREBP-1c in fatty acid synthesis in intestine following changes in cholesterol flux. Despite a profound decrease in SREBP-1c gene expression in intestines of hamsters ingesting the cholesterol-depletion diet, fatty acid synthesis in the intestines of these animals was enhanced severalfold. Moreover, cholesterol feeding caused a dramatic increase in gene expres-
sion of intestinal SREBP-1c, yet fatty acid synthesis in intestines of animals on the cholesterol-enriched diet was similar to animals on the chow diet. Thus, it would appear that, at least at a transcriptional level, SREBP-1c is responsible for maintaining basal levels of fatty acid synthesis in intestine. In response to changes in cholesterol flux, however, mechanisms other than those involving transcription of SREBP-1c need to be entertained.

A mechanism has now been provided that explains the regulation of SREBP-1c gene expression by changes in cholesterol flux. In a recent study performed in mice, Repa et al. (24) have shown that the SREBP-1c gene is a target of the nuclear receptor RXR/LXR transcription factor. By feeding mice either a high cholesterol diet or RXR/LXR agonists, both hepatic and intestinal mRNA levels and protein expression of SREBP-1c were increased. An RXR/LXR DNA-binding site in the promoter of SREBP-1c was identified and found to be required for this regulation. Despite an increase in SREBP-1c gene expression by cholesterol feeding, hepatic levels of the lipogenic target genes, fatty acid synthase and acetyl-CoA carboxylase, were not increased. Only stearyl-CoA desaturase gene expression was increased in livers of mice ingesting cholesterol. Perhaps this lack of effect of cholesterol feeding on these lipogenic target genes explains why in the present study, fatty acid synthesis was not increased in intestines of animals having increased gene expression of SREBP-1c.

The present results would suggest that with changes in cholesterol flux, particularly cholesterol depletion, SREBP-2 assumes the role for regulating cholesterol and fatty acid synthesis in intestine. In intestines of hamsters fed the cholesterol-depletion diet, rates of cholesterol and fatty acid synthesis were increased, as were mRNA levels for SREBP-2 and the sterol-responsive genes. Moreover, the amount of precursor and mature forms of SREBP-2 was also increased. In transgenic animals overproducing the mature form of SREBP-2, hepatic fatty acid synthesis and triglyceride content were increased 4-fold (13). Thus, although SREBP-2 appears to be more specific for increasing the expression of genes of the cholesterol biosynthetic pathway, SREBP-2 can enhance fatty acid synthesis as well. Thus, under conditions of cholesterol depletion, we would postulate that SREBP-2 supplants the function of SREBP-1 in intestine and augments cholesterol and fatty acid synthesis.

In intestines of hamsters ingesting a cholesterol-enriched diet, a diet that increased plasma cholesterol levels 2-fold, mRNA levels of the sterol-responsive genes and SREBP-2 were minimally decreased if at all. Similarly, rates of cholesterol and fatty acid synthesis were not altered. This was somewhat unexpected. With a large influx of cholesterol, a more dramatic suppression of genes that regulate cholesterol metabolism and rates of cholesterol synthesis was expected. Perhaps this did not occur because cholesterol taken up from the lumen does not enter a critical "regulatory pool." The absorbed sterol escapes putative "cholesterol sensors" in endoplasmic reticulum or plasma membrane that would, in turn, down-regulate the SREBP pathway. A similar possible mechanism was postulated in experiments using CaCo-2 cells (25). Cholesterol taken up from micelles was shown to displace cholesterol of the plasma membrane (or endoplasmic reticulum) is reached. Another possible explanation for the modest changes observed in sterol-responsive gene expression by dietary cholesterol comes from results in chow-fed animals. In intestinal cells of these animals, there were abundant amounts of the precursor form of SREBP-2. The mature form, however, was not detectable. This implies that there is an adequate supply of cholesterol in these cells that prevents cleavage of the precursor protein. Thus, the expression of sterol-responsive genes, including SREBP-2 itself, and rates of cholesterol synthesis would be chronically suppressed. To demonstrate further suppression by dietary cholesterol might not be possible. This is not the first study that has failed to observe a significant decrease in intestinal cholesterol synthesis by dietary cholesterol in intact animals (for a review, see Ref. 26).

It has been postulated that in an intestinal absorptive cell there exists a regulatory pool of cholesterol whose size determines rates of cholesterol synthesis. Newly synthesized cholesterol, plasma membrane cholesterol, absorbed cholesterol, and cholesterol derived from lipoproteins contribute, albeit in different proportions, to this pool (27). The present data support this notion. In intestines of hamsters fed a chow diet, there was a clear trend for the rates of cholesterol synthesis and the expressions of sterol-responsive genes, SREBP-2, HMG-CoA reductase, HMG-CoA synthase, and the LDL receptor, to be lower in cells of the upper villus (most differentiated) and higher in cells of the distal villus (least differentiated). This suggests that compared with differentiated cells, the regulatory pool of cholesterol is smaller in undifferentiated cells, which in turn, enhances cholesterol synthesis by increasing the expression of genes in the cholesterol synthetic pathway via activation of the SREBP pathway. Indeed, assuming that cholesterol mass reflect differences in this regulatory cholesterol pool, cells of the upper villus did contain more cholesterol than cells of the lower villus. Moreover, feeding cholesterol markedly increased the amount of cellular cholesterol, particularly in cells of jejunum and ileum, without altering this gradient in cells along the villus. Thus, with cholesterol feeding, rates of cholesterol synthesis and expressions of sterol-responsive genes maintained a gradient along the villus axis that was similar to that observed in control chow-fed animals. In contrast, by feeding a cholesterol-depletion diet, this regulatory cholesterol pool would decrease, stimulating the SREBP pathway and enhancing transcription of genes that regulate cholesterol synthesis. Marked stimulation of the SREBP pathway obscured the gradient of cholesterol synthesis and gene expression that was appreciated along the villus axis in intestines of animals on chow and cholesterol diets. It seems clear in intestine, at least, that activation of the SREBP pathway by decreasing the regulatory cholesterol pool is a much stronger stimulus than suppressing the SREBP pathway by expanding the regulatory cholesterol pool by dietary cholesterol.

In the classic SREBP pathway described in cultured cells, cholesterol and fatty acid synthesis are enhanced by the cleavage of a membrane-bound precursor form of SREBP (regulated intramembrane proteolysis), releasing a nuclear or active form (28). This "mature" protein then enters the nucleus, binds to a sterol regulatory element in promoters of genes in these two pathways, and enhances their transcription (4–8). The results of this study, however, suggest another possible mechanism for the regulation of cholesterol and fatty acid synthesis by SREBPs in intestine. The correlation that was observed between gene expression and the amount of precursor mass of SREBP-1 and -2 in individual cell populations of the intestine, although not exact, was fairly striking. Because our antibody does not distinguish between SREBP-1a and -1c and will detect both, this correlation is best illustrated for SREBP-2. Both mRNA levels and precursor mass of SREBP-2 were lower in cells of the upper villus and higher in cells of the lower to middle villus. Moreover, in intestines of animals ingesting the
chol erol-depletion diet, there was a marked increase in both mRNA abundance and precursor mass of SREBP-2. It was only in intestines of this group of animals that the mature form of SREBP-2 could be detected. If regulated intramembrane proteolysis were the only mechanism for formation of the mature form following cholesterol depletion, it would have been expected that the amount of precursor mass would decrease as the amount of mature form increased. This did not occur. In fact, the amount of precursor mass markedly increased with cholesterol depletion. These findings are at least suggestive that in intestine, the mass of SREBP-2, both precursor and mature, is regulated at the level of transcription. Other investigators have also observed a dissociation between the amount of mature form of SREBP-1 and -2 and gene expression for enzymes involved in cholesterol and fatty acid synthesis, suggesting that the amount of the mature form can be regulated by changes in synthesis of its precursor (29, 30).

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