Essential Amino Acids Regulate Fatty Acid Synthase Expression through an Uncharged Transfer RNA-dependent Mechanism*

(Received for publication, August 3, 1995, and in revised form, September 21, 1995)

Steven M. Dudek† and Clay F. Semenkovich§

From the Departments of Medicine and Cell Biology & Physiology, Washington University School of Medicine, St. Louis, Missouri 63110

To better understand the regulation of gene expression by amino acids, we studied the effects of these macronutrients on fatty acid synthase (FAS), an enzyme crucial for energy storage. When HepG2 cells were fed serum-free media selectively deficient in each amino acid, the omission of any single classic essential amino acid as well as Arg or His (essential in some rapidly growing cells) resulted in FAS mRNA levels that were about half of those in complete medium. Control message levels were unaffected and omission of nonessential amino acids did not alter FAS expression. FAS mRNA levels peaked 12–16 h after feeding complete and Ser (nonessential)-deficient media but did not increase in cells fed Lys (essential)-deficient medium. With Lys, FAS mRNA increased over the physiologic concentration range of 15–150 μM, and low concentrations of lysine decreased FAS but not apoB protein mass. Transcription inhibitors mimicked treatment with Lys-deficient media, and nuclear run-off assays showed that Lys-deficient media abolished FAS but not apoB transcription. After treatment with Lys-deficient media, the intracellular Lys pool was rapidly depleted in association with an increase of uncharged (deacylated) tRNA<sup>lys</sup> from <1 to 64% of available tRNA<sup>lys</sup>. Even in the presence of the essential amino acid His, increasing the levels of uncharged tRNA<sup>his</sup> with histidinol, a competitive inhibitor of the histidinyl-tRNA synthetase, blocked FAS expression. Tyrosinol treatment did not alter FAS mRNA levels. These results suggest that essential amino acids regulate FAS expression by altering uncharged tRNA levels, a novel mechanism for nutrient control of gene expression in mammalian cells.

Fatty acid synthase (FAS)<sup>1</sup> is a large multifunctional protein that synthesizes the fatty acid palmitate from acetyl-CoA, malonyl-CoA, and NADPH (1). This process is essential for the conversion of dietary calories into a storage form suitable for use during periods of fasting. Although several enzymes are critical for the synthesis of fatty acids, it appears that FAS is rate-limiting in the long term control of lipogenesis (2).

Feeding increases FAS expression (3). The intake of high carbohydrate diets following periods of fasting increases FAS mRNA levels and enzyme concentration (4). Hormones, many of which are affected by feeding and fasting, play a role in this regulation. In diabetic rats, insulin increases hepatic FAS transcription, mRNA levels, and enzyme levels 5–15-fold (5). Dexamethasone enhances FAS mRNA induction by insulin in primary rat hepatocyte cultures (6). Triiodothyronine stimulates FAS gene transcription in cultured chick embryo hepatocytes (7) and mouse 3T3-L1 adipocytes (8). Progesterone stabilizes FAS mRNA levels (9).

Hormonal regulation of FAS expression has appropriately been the focus of many previous studies. However, individual dietary components, especially macronutrients, can also affect FAS expression. The carbohydrate glucose stabilizes FAS mRNA levels in HepG2 cells cultured in serum-free media (10, 11). Polyunsaturated fatty acids are potent inhibitors of FAS gene transcription in rats (12). But the role of amino acids in FAS regulation is ill-defined. Maximal induction of transcription, mRNA levels, and enzyme activity in fasted rats requires feeding of both carbohydrate and protein (5). In cultured rat hepatocytes, a combination of essential and nonessential amino acids is necessary for maximal induction of FAS protein levels (13), but nothing else is known about the effects of individual amino acids on FAS gene expression.

An active role for amino acids in gene regulation is not without precedence. Starvation for a single essential amino acid induces differentiation of the human promyelocytic leukemia line HL-60 into mature granulocytes (14). Asparagine synthetase mRNA levels are increased by essential amino acid starvation in baby hamster kidney and HeLa cells (15). In yeast, deficiencies of various amino acids will induce the respective amino acid synthetic pathway by activating the transcription factor GCN4 (16). Therefore, we used human HepG2 cells, known to functionally resemble hepatocytes (17), to test the hypothesis that amino acids regulate FAS expression. In this paper, we present data supporting this hypothesis and show that the effect of amino acids is likely mediated by changes in the intracellular concentrations of uncharged tRNA.

MATERIALS AND METHODS

Tissue Culture Reagents and Culture Conditions—HepG2 cells were maintained in minimum essential medium prepared by the Washington University Tissue Culture Support Center containing 10% fetal bovine serum purchased from Intergen. Experiments were performed using a modified RPMI 1640 media prepared in our laboratory with Life Technologies, Inc. Select-Amine kits. Amino acid concentrations in the modified RPMI 1640 (see Table I) were specifically selected to correspond to human serum levels (18, 19). Bovine albumin, actinomycin D, cyclosporin, dichloro-bifluoromethylbenzimidazole, histidinol, and tyrosinol were...
purchased from Sigma. For most experiments, cells were split 1:25 from 90% confluent T-75 flasks to 100-mm tissue culture dishes on day 1. On day 3 or 4, cells were fed modified RPMI 1640 with 10% fetal bovine serum. After 2 or 3 days when the cells were 50–75% confluent, the media were removed, the dishes were washed with phosphate-buffered saline (PBS), and the cells were fed modified RPMI 1640 with 1% fetal bovine serum. When an amino acid is omitted (10), the omission is left unoxidized, allowing the determination of the total charging capacity of tRNA. The percentage of charged tRNA is determined by dividing the counts from in vitro charging reactions (using radiolabeled amino acids) using oxidized samples by the counts from reactions using unoxidized samples.

For each experiment, cells were fed complete, serine-deficient, or lysine-deficient media (200-mm diameter dishes/condition). 2 h later, total RNA was isolated from these cells under mildly acidic conditions and divided into two aliquots for each condition. One aliquot was oxidized with 2 mM sodium periodate at 25 °C for 15 min in the dark, and the other was left unoxidized. All samples were ethanol precipitated, washed with 70% ethanol, dissolved in 0.1 M Tris (pH 8.8), incubated for 3 h at 37 °C to strip tRNA of bound amino acids, and then again ethanol precipitated.

The actual charging assay was performed as follows after preliminary experiments showed these conditions yielded results within the linear response range of the assay. The assay mixture for each sample contained 500 μg of total RNA, 30 μg of partially purified aminoacyl-tRNA synthetases (2.5 mATP, 0.25 mM CTP, 50 mM Tris (pH 7.5), 150 mM NaCl, 0.1 mM EDTA, 2 mM MgCl₂, 2.5 μCi of [³⁵S]-lysine or [³⁵S]-serine, 100 μM unlabeled lysine (or serine) in a total volume of 500 μl. The samples were incubated at 37 °C for 30 min, precipitated with 10% trichloroacetic acid solution containing 1 mg/ml unlabeled lysine (or serine), applied to 2.4-mm filters (Whatman 540), washed twice with 5% trichloroacetic acid and once with 95% ethanol, and then dried. Radioactivity was quantitated with a Beckman LS 2800 scintillation counter.

The degree of in vivo charged tRNA was calculated by dividing the charging capacity of periodate-oxidized RNA by the charging capacity of unoxidized RNA. The data are expressed in Table III as the percentage of unchanged tRNA (determined by subtracting the percentage of charged tRNA from 100).

**RESULTS**

When HepG2 cells were incubated for 12 h in serum-free experimental media resembling the human amino acid composition of human serum (Table I), the omission of a single essential amino acid, lysine, resulted in lower FAS message levels compared with complete medium as shown by the RNase protection gel depicted in Fig. 1. Omission of histidine, essential in infancy in humans (24), had the same effect, but the omission of serine, a nonessential amino acid, did not affect FAS mRNA levels.

These analyses were extended to all 20 amino acids (Fig. 2). HepG2 cells were cultured for 12 h in complete medium and in 20 separate media, each selectively deficient in a single amino acid. Fig. 2 shows the relative levels of FAS (Fig. 2A) and apolipoprotein B (apoB) (Fig. 2B) mRNA expression after incubation in selectively deficient media with data expressed relative to complete medium containing all 20 amino acids. Each one-letter amino acid abbreviation on the horizontal axis of
Essential Amino Acids and FAS Expression

Fig. 2 denotes the amino acid on the xerine. All are nonessential; they can be synthesized from pre-
teine, glutamic acid, glutamine, glycine, proline, serine, or ty-

raptidely growing cell lines (26). The effect appeared to be rela-
tively specific for FAS, because message levels for apoB (B) and γ-actin (data not shown) were unchanged by amino acid deficiency.

Omission of a single essential amino acid blocked the time-
dependent induction of FAS expression after feeding (Fig. 3). 2–4 hours after feeding complete or serine (nonessential)-defi-
cient media, FAS mRNA levels (Fig. 3A) began to increase and peaked 12–16 h post-feeding. No FAS induction occurred when cells were fed lysine (essential)-deficient medium (Fig. 3A, open circles). No differences in apoB expression were observed after feeding the experimental media (Fig. 3B).

Two lines of evidence suggested that decreased cell viability did not explain the effect of lysine-deficiency on FAS expression. First, when cells were cultured for 12 h in lysine-deficient, serine-deficient, or complete media followed by addition of MTT (cleaved by mitochondrial dehydrogenases of living cells), MTT cleavage products (quantitated as described under “Materials and Methods”) in each of the three media were virtually identical (data not shown). Second, the inhibition of FAS mRNA induction by essential amino acid deficiency was reversible (Fig. 4). Cells incubated in lysine-deficient medium from 0–12 h showed no increase in FAS mRNA, but replacing lysine-deficient medium with complete medium at 12 h resulted in the expected increase in FAS message at 24 h (Fig. 4). Cells incubated in lysine-deficient medium from 0–12 h showed no increase in FAS mRNA, but replacing lysine-deficient medium with complete medium at 12 h resulted in the expected increase in FAS message at 24 h (Fig. 4).

Fig. 2 Deprivation of any single essential amino acid is associated with decreased FAS mRNA levels. HepG2 cells were treated as described in the legend to Fig. 1. Separate dishes received 21 different media, complete medium as well as 20 media each deficient in a single amino acid. The one-letter code on the horizontal axis denotes the amino acid deficient from the medium in which the cells were cultured. FAS (A) and apoB (B) mRNA levels were assayed by RNase protection as in Fig. 1 except protected fragments were collected on filters and counted as described under “Materials and Methods.” Results represent mRNA levels after 12 h in each selectively-deficient medium and are expressed relative to message detected in complete medium (containing all amino acids). Data shown represent the mean of two independent experiments.

Fig. 2 denotes the amino acid absent from the medium. FAS mRNA levels were unchanged relative to complete medium after 12 h of deficiency in any one of the 10 amino acids on the left half of the panel: alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, or tyrosine. All are nonessential; they can be synthesized from precursors. However, deficiency in any one of the 10 amino acids on the right half of the panel resulted in a ~50% decrease in FAS message level. This group includes the 8 classic essential amino acids: isoleucine, leucine, lysine, methionine, phenylala-
nine, threonine, tryptophan, and valine (25). Deficiency of arginine or histidine also resulted in lower FAS mRNA. These two amino acids have been shown to be functionally essential in rapidly growing cell lines (26). The effect appeared to be rela-

Table I

Amino acid compositions

The first column lists ranges for human fasting plasma levels for the 20 standard amino acids (18, 19). The second column lists the amino acid composition for the complete medium used in these experiments. The last column lists standard RPMI 1640 medium for comparison.

| Human serum | Modified RPMI 1640 | Standard RPMI 1640 |
|-------------|-------------------|--------------------|
| Alanine     | 300–500           | 300                |
| Arginine    | 50–115            | 100                |
| Asparagine  | 30–50             | 80                 |
| Aspartate   | 10–30             | 10                 |
| Cysteine    | 30–75             | 100                |
| Glutamate   | 50–70             | 70                 |
| Glutamine   | 550–600           | 550                |
| Glycine     | 200–300           | 133                |
| Histidine   | 50–115            | 60                 |
| Isoleucine  | 50–80             | 60                 |
| Leucine     | 100–160           | 110                |
| Lysine      | 100–200           | 150                |
| Methionine  | 20–30             | 20                 |
| Phenylalanine | 50–90           | 50                 |
| Proline     | 150–300           | 150                |
| Serine      | 110–130           | 130                |
| Threonine   | 110–150           | 120                |
| Tryptophan  | 60–75             | 30                 |
| Tyrosine    | 50–90             | 50                 |
| Valine      | 180–250           | 171                |
FAS expression increased linearly over this physiologic range, reaching a peak at approximately 150 μM lysine. ApoB mRNA levels were unaffected by lysine concentration.

To determine if the regulation of FAS mRNA by essential amino acids is reflected at the protein level, FAS mass was assayed by Western blotting (Fig. 6). Obviously, cells deprived of an essential amino acid will be unable to synthesize proteins containing that amino acid. So instead of lysine-deficient medium, cells were cultured in medium containing 1.5 μM lysine, a low concentration empirically found to be sufficient for protein synthesis. Fig. 6 shows Western blots of FAS and apoB protein after incubation in serine-deficient and 1.5 μM lysine medium for 12 h, longer than the half-life of the FAS protein in HepG2 cells (10). ApoB is a suitable control protein for this experiment because it is extremely short-lived (27). FAS protein mass was strikingly lower in the 1.5 μM lysine medium whereas apoB protein was not, confirming that the cells had sufficient lysine for protein synthesis. In fact, apoB protein levels were higher in the low lysine medium, consistent with previous studies showing that incubation of HepG2 cells in low concentrations of amino acids increases apoB synthesis (28). For both FAS and apoB protein, mass was the same in complete and serine-deficient media (not shown). These results suggest that changes in FAS message levels induced by changes in essential amino acid concentrations can be reflected at the level of the FAS protein.

To address the possibility of transcriptional regulation of FAS expression by essential amino acids, cells were cultured for 4 h in complete, serine-deficient, or lysine-deficient medium in the presence of the transcriptional inhibitors actinomycin D, cordycepin, and dichlorobifluorosylbenzimidazole. Although these compounds inhibit transcription by different mechanisms, each mimicked the effect of essential amino acid deficiency on FAS message levels (Fig. 7). In the same cells, there were no differences in apoB mRNA levels between cells cultured in complete, serine-deficient, or lysine-deficient medium in the absence of transcriptional inhibitors, and there were no differences in apoB mRNA levels between cells cultured in the different media in the presence of transcriptional inhibitors (data not shown). These results suggest that essential amino acid regulation of FAS is transcriptional.
Nuclear run-off assays provided more direct evidence of transcriptional regulation. As shown in Table II, there was no detectable initiation of FAS transcription after 4 h of incubation in lysine-deficient medium. ApoB transcription also decreased under these conditions but to a lesser extent than FAS. Unlike FAS, the decrease in apoB transcription was not associated with changes in apoB message levels (see Figs. 2 and 3).

To explore how deficiency of a single essential amino acid might affect FAS transcription, the role of uncharged or deacetylated transfer RNA (tRNA not bound to its cognate amino acid) was studied. Uncharged tRNA is known to regulate transcription in bacteria (29). One way to alter uncharged tRNA levels is to change intracellular amino acid concentrations (30). If amino acids alter uncharged tRNA levels to regulate FAS expression, the effect must be rapid. Message levels increase within 2–4 h of feeding (Fig. 3), and substantial changes in amino acid pool sizes should precede this increase. As shown in Fig. 8, feeding lysine-deficient medium depleted intracellular lysine pools within 1 h (Fig. 3A). In contrast, there was no decrease in intracellular serine pools (Fig. 3B) after incubation in serine-deficient medium, an expected result because cells readily synthesize this amino acid.

To determine if the decrease in intracellular lysine affects uncharged tRNA levels in HepG2 cells, the amount of uncharged tRNA was assayed by a periodate oxidation method described under "Materials and Methods." 2 h after feeding lysine-deficient medium, 64% of available tRNA\textsuperscript{Lys} was uncharged as shown in Table III. At the same time point, the uncharged fraction accounted for less than 1% of available tRNA\textsuperscript{Lys} in cells fed complete or serine-deficient medium. As expected, the percentage of uncharged tRNA\textsuperscript{Ser} did not increase in serine-deficient medium. Uncharged tRNA\textsuperscript{Ser} levels in complete medium were substantially higher than those of uncharged tRNA\textsuperscript{Lys} but similar to uncharged tRNA\textsuperscript{Ser} levels reported in Escherichia coli (31).

If uncharged tRNAs for essential amino acids regulate FAS expression, then inhibiting the charging reaction for an essential amino acid, even in the presence of normal concentrations of that nutrient, should mimic the effect of essential amino acid deficiency. Amino acid alcohols are competitive inhibitors of the specific aminoacyl-tRNA synthetases that facilitate the binding of amino acids to their cognate tRNAs (23, 32). Compounds such as histidinol (the alcohol of the essential amino acid histidine) and tyrosinol (the alcohol of the nonessential amino acid tyrosine) increase uncharged tRNA\textsuperscript{H\textsubscript{is}} and tRNA\textsuperscript{Tyr}, respectively, even at normal amino acid concentrations. As shown in Fig. 9A, culturing HepG2 cells for 12 h in complete medium with 2 \text{mM} histidinol had the same effect on FAS expression as histidine-deficient medium; FAS mRNA levels were unaffected by incubation in complete medium with 2 \text{mM} tyrosinol and tyrosine-deficient medium. The effects of histidinol on FAS expression were dose-dependent (data not shown) and these amino acid alcohols did not affect HepG2 viability within the time frame of these experiments. These data support the hypothesis that essential amino acids regulate FAS expression by altering levels of uncharged tRNA.

**Table II**

Essential amino acid deficiency abolishes FAS transcription initiation

HepG2 cells were washed with PBS and fed either serine-deficient or lysine-deficient medium. 4 h later, nuclei were isolated, and the nuclear run-on assay was performed as described under "Materials and Methods." Data from the experiment shown represent the signals generated by nuclei of nearly confluent cells from 60–100-mm tissue culture dishes for each condition (serine-deficient and lysine-deficient). Transcription assayed in this way is inhibited by 2 \text{ng/ml} \text{a-amanitin}. Bands appearing after exposure of the slot blot to film for 7 days in the presence of an intensifying screen were quantitated using Image-Pro Plus densitometry software. Results obtained after subtraction of nonspecific hybridization to pBluescript are expressed relative to FAS transcription in serine-deficient medium, represented as 100%. The same results were obtained in two independent experiments.

|                          | FAS transcription in Ser-deficient medium |
|--------------------------|------------------------------------------|
|                          | Serine-deficient | Lysine-deficient |
| FAS                      | 100             | 0                |
| Apo B                    | 359             | 91               |

**Fig. 6.** Essential amino acid effects are reflected at the protein level in HepG2 cells. Cells were incubated in serine-deficient (Minus Serine) or low lysine (1.5 \text{ mM} Lysine) media for 12 h followed by preparation of postnuclear cell extracts. 20 \text{ g} of protein was loaded in each lane, subjected to SDS-PAGE, and then Western blotted using anti-FAS and anti-apoB antibodies. The arrowheads denote the positions of the FAS (M, \sim 260,000) and apoB (M, \sim 550,000) proteins.

**Fig. 7.** Treatment with different transcriptional inhibitors mimics the effect of essential amino acid deficiency on FAS mRNA levels. HepG2 cells were washed and fed either complete, serine-deficient, or lysine-deficient media in the presence of carrier (Controls), 4 \text{ \mu M} actinomycin D, 80 \text{ \mu M} cordycepin, or 65 \text{ \mu M} dichlororibofuranosylbenzimidazole (DRB). 4 h later, RNA was isolated from triplicate dishes for each condition, and FAS mRNA was determined by RNase protection. FAS mRNA levels are expressed relative to message in cells fed lysine-deficient control medium. Data are expressed as means \pm S.E. The same results were obtained in two independent experiments.
In this paper, we present evidence that essential amino acids in HepG2 cells regulate the expression of a critical enzyme in lipogenesis, fatty acid synthase. Deficiency of a single essential amino acid in serum-free medium reversibly blocks the induction of FAS normally associated with feeding, and essential amino acid effects are concentration-dependent. Essential amino acids appear to regulate FAS expression via a transcriptional mechanism mediated through uncharged tRNA.

There is precedence for the involvement of amino acids in gene regulation. Global deprivation of amino acids predictably produces a myriad of effects. Removal of all amino acids from the culture medium up-regulates several types of amino acid transporters in the bovine renal epithelial cell line NBL-1 (33). In primary rat hepatocyte cultures, such amino acid deprivation decreases gene transcription of IGF-I while simultaneously increasing mRNA abundance for one of its binding proteins (34, 35).

Deficiencies of single amino acids also evoke responses. In bacteria, starvation for an individual amino acid typically increases the level of its cognate aminoacyl-tRNA synthetase (29, 36). Asparagine deprivation increases expression of asparaginyl-tRNA synthetase in baby hamster kidney and HeLa cells (15), mammalian cell lines. Amino acid synthetic pathways are induced in yeast by deficiency of the respective amino acid (16). Deficiency of a single essential amino acid induces differentiation of the human promyelocytic leukemia line HL-60 into mature granulocytes (14). This effect is mimicked by treatment with the aminoacyl-tRNA synthetase inhibitor histidinol (also used in the current study, see Fig. 9), suggesting an important role for tRNA during HL-60 differentiation.

The tRNA molecule carries out the adapter function necessary for translation of the genetic code from nucleic acid to protein. Aminoacyl-tRNA synthetases (37–39) catalyze the aminocylation of tRNAs with the correct amino acids. tRNA is not simply a passive conduit in this reaction. Each tRNA contains sequence, independent of the anticodon trinucleotide, and structure information targeting it to the appropriate aminoacyl-tRNA synthetase (40). The tRNA itself can also signal...
amino acid availability through its covalent modification by amino acids (41). Uncharged tRNA (tRNA not bound to its cognate amino acid) increases when the intracellular pool of that amino acid falls (30). This species can regulate gene expression in some systems. In addition to the induction of HL-60 differentiation discussed above (14), uncharged tRNA is involved in transcriptional regulation of aminoacyl-tRNA synthetase genes in Bacillus subtilis (29). In this system, uncharged tRNA likely has a conformation that stabilizes an antiterminator structure allowing transcription of the tyrosyl-tRNA synthetase gene. Thus when Tyr levels are low in this bacteria, uncharged tRNA signals the increased expression of the enzyme that attaches Tyr to its tRNA resulting in more efficient utilization of the remaining low levels of Tyr.

Our data suggest that uncharged tRNA is also involved in the regulation of FAS expression. An amino acid itself or a related factor could be responsible, but the aminoacyl-tRNA synthetase inhibition studies (Fig 9) argue otherwise. Even in the presence of histidine, increasing uncharged tRNA levels by inhibiting the histidinyl-tRNA synthetase mimicked the effect of essential amino acid deficiency. Our data further suggest that there are fundamental differences between the tRNAs for essential and nonessential amino acids because increased uncharged tRNAs for nonessential amino acids have no effect on FAS expression. About half of tRNASer (nonessential) molecules were always uncharged (similar to results reported in bacteria) (31) without affecting FAS expression; a similar level of uncharged tRNAlys (essential) was associated with inhibition of FAS (Table III). Increasing uncharged tRNA Tyr (nonessential) levels with tyrosinol (Fig. 9) did not affect FAS message levels.

How might uncharged tRNAs regulate FAS expression? Similar to the antitermination mechanism discussed above, uncharged tRNA may regulate FAS by interacting with the FAS transcriptional apparatus. However, unlike prokaryotes, components of transcription and translation are separated by the nuclear membrane in eukaryotes, and it is unknown if cytoplasmic uncharged tRNAs have access to the nucleus.

A second potential mechanism involves tRNA genes as transcriptional repressor elements. Scattered throughout eukaryotic genomes are moderately to highly repeated RNA polymerase II promoters (42), sequences that include tRNA genes. These may influence RNA polymerase II promoters (43, 44) to affect mRNA levels. At least 1300 tRNA genes and pseudo-genes are scattered throughout the human genome (45), but their locations are largely unknown. If a tRNA gene or pseudogene exists near the human FAS gene, activation of this locus in response to decreased levels of essential amino acids could influence transcription of the nearby FAS gene. Consistent with this mechanism, tRNA genes have been recently shown to act as repressor elements for RNA polymerase II transcription in yeast (46).

A third and perhaps the most likely mechanism underlying uncharged tRNA regulation of FAS expression would involve a transcription factor essential for FAS expression. This transcription factor would be under predominantly translational control; elevated levels of uncharged tRNAs for essential amino acids would inhibit translation of this factor, thereby blocking FAS expression. A similar mechanism is responsible for the activation of the transcription factor GCN4 in yeast after amino acid starvation (16). Uncharged tRNA binds and activates a kinase, GCN2, which phosphorylates eukaryotic initiation factor 2, which stimulates translation of GCN4 (47–49). We speculate that uncharged tRNAs for essential amino acids interact with translational components in the cytoplasm to block translation of a critical FAS transcription factor and prevent FAS induction with feeding.

In summary, depriving HepG2 cells of any essential amino acid prevents the induction of FAS expression normally associated with feeding. The likely role of tRNA in this process represents a novel form of gene regulation in mammalian cells. Obvious next steps include the overexpression of authentic uncharged transfer RNAs for essential amino acids in hepatocytes, and an appropriate search for tRNA response elements in the FAS gene.

Acknowledgments—We thank Trey Coleman for technical assistance and Megan Dudek for support.
Essential Amino Acids Regulate Fatty Acid Synthase Expression through an Uncharged Transfer RNA-dependent Mechanism
Steven M. Dudek and Clay F. Semenkovich

J. Biol. Chem. 1995, 270:29323-29329. doi: 10.1074/jbc.270.49.29323

Access the most updated version of this article at http://www.jbc.org/content/270/49/29323

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 48 references, 20 of which can be accessed free at http://www.jbc.org/content/270/49/29323.full.html#ref-list-1