Annu. Rev. Nutr. 1997. 17:405–33

REGULATION OF THE EXPRESSION OF LIPOGENIC ENZYME GENES BY CARBOHYDRATE

Howard C. Towle and Elizabeth N. Kaytor
Department of Biochemistry and the Institute of Human Genetics, University of Minnesota, Minneapolis, Minnesota 55455

Hsiu-Ming Shih
School and Graduate Institute of Medical Technology, College of Medicine, National Taiwan University, Taipei 10016, Taiwan

KEY WORDS: lipogenesis, glucose, insulin, transcription, transfection

ABSTRACT

Diets high in simple carbohydrates and low in fats lead in the mammalian liver to induction of a set of enzymes involved in lipogenesis. This induction occurs, in part, through transcriptional mechanisms that lead to elevated levels of the mRNA for these enzymes. For most of the lipogenic enzymes, an increase in glucose metabolism is required to trigger the transcriptional response. The intracellular mediator of this signaling pathway is unknown, although evidence suggests either glucose-6-phosphate or xylulose-5-phosphate. Studies to map the regulatory sequences of lipogenic enzyme genes involved in the transcriptional response have been performed for the L-type pyruvate kinase, S_{14}, and acetyl-coenzyme A carboxylase genes. These studies have identified the DNA sequences necessary to link the signal generated by carbohydrate metabolism to specific nuclear transcription factors.

CONTENTS

INTRODUCTION 406
LIPOGENIC ENZYMES 407
Members of the Lipogenic Enzyme Family 407
Site of Action—Transcriptional Versus Posttranscriptional 409
The Pyruvate Kinase Model 410
The S_{14} Model 410
The Acetyl-Coenzyme A Carboxylase Model 411

0199-9885/97/0715-000405$08.00

INCREASED CARBOHYDRATE METABOLISM AS A PRIMARY SIGNAL FOR INDUCTION 412
Metabolic versus Hormonal Signals for Lipogenic Gene Activation 412
Primary Hepatocytes as a Model for Studying the Role of Carbohydrate Metabolism . . . 413
Role of Insulin in the Process of Induction 414
Possible Intracellular Mediators of the Carbohydrate Response 415
REGULATORY SEQUENCES AND *TRANS*-ACTING FACTORS FOR METABOLIC SIGNALING OF TRANSCRIPTIONAL INDUCTION 417
The Carbohydrate Response Element—L-PK and S_{14} Genes 417
Role of USF in the Carbohydrate Response 419
Acetyl-Coenzyme A Carboxylase and the Role of Sp1 421
INTERACTIONS OF CARBOHYDRATE METABOLISM WITH OTHER REGULATORY PATHWAYS 422
Polyunsaturated Fatty Acids 422
Glucagon and cAMP 423
Thyroid Hormones 424
TRANSCRIPTIONAL REGULATION BY METABOLIC SIGNALING IN THE PANCREATIC β-CELL 425
CONCLUSIONS 427

INTRODUCTION

When a mammal consumes a meal rich in simple carbohydrates and low in fats, the excess carbohydrate that reaches the liver is converted to triglycerides. The high-carbohydrate meal invokes in the liver a well-characterized response that allows lipogenesis to occur optimally. This response is exerted at two levels. The first level involves rapid activation of the key rate-limiting enzymes. Both hormonal and metabolic signals modulate enzyme activities via allosteric mechanisms and posttranslational modifications. These changes occur within minutes of altered glucose levels in the liver. The second level involves changes in the cellular concentrations of critical enzymes and proteins through alterations in gene expression. These changes occur over longer time frames, hours rather than minutes, and presumably allow the organism to utilize limited sources of simple carbohydrate in the natural environment by converting them to triglycerides, the major energy storage form in mammals.

Hormonal signals play an important part in the disposition of glucose, regulating, at the levels of both catalytic efficiency and gene expression. In particular, the pancreatic hormones involved in glucose homeostasis, insulin and glucagon, can initiate events that lead directly to the activation or repression of gene expression. Nutrients and metabolic products are also critical to regulation of gene expression in mammals. Particularly important in changes in gene expression is glucose metabolism, though the pathways responsible for these changes are only beginning to be unraveled. In this review, we examine regulation of expression of lipogenic genes in the hepatocyte in response to altered glucose metabolism. Several recent reviews have examined the enzyme

responses to glucose (6, 34, 36, 38, 66), so those areas are summarized briefly. Emphasis is directed at recent studies on the nature of this novel signaling pathway in the liver.

LIPOGENIC ENZYMES

Members of the Lipogenic Enzyme Family

Lipogenic enzymes are responsible for converting glucose to triglycerides. In rats and mice, many of these enzymes are induced by a high-carbohydrate diet. Most experiments have compared fasted animals with those that were fasted and then refed high-carbohydrate, fat-free diets. These regimens give minimal and maximal rates of lipogenesis, respectively. While most studies examined the process in the liver, a few studies explored adipose tissue, the other major site of lipogenesis in mammals. In general, the enzymes induced in the liver by a high-carbohydrate diet are the same ones induced in adipose tissue (31). For each of the enzymes discussed below, measurement of mRNA level has indicated that induction is due to an increased rate of enzyme synthesis rather than to a change in enzyme degradation.

Enzymes central to the process of lipogenesis are those that catalyze fatty acid biosynthesis: acetyl-coenzyme A carboxylase (ACC); fatty acid synthase; and ATP-citrate lyase, which plays a role in the transfer of acetyl-coenzyme A (acetyl-CoA) from the mitochondrion to the cytosol, where fatty acid synthesis occurs (Figure 1). All three of these enzymes are induced by diets favoring lipogenesis, and the extent of the responses of these enzymes and their mRNAs is quite dramatic. Increases of 25- to over 100-fold have been reported between the fasted and fasted/refed states (19, 96, 97). Fatty acid synthesis is dependent on NADPH for the required reducing potential. The major sources of NADPH production are the reactions catalyzed by glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and malic enzyme. Again, mRNAs encoding each of these enzymes are induced by a high-carbohydrate diet (18, 83, 102). Increased lipogenesis from glucose is also dependent on effective glycolysis to provide acetyl-CoA, and several key glycolytic enzymes are induced by a high-carbohydrate diet. These include the liver-specific forms of glucokinase (40), 6-phosphofructo-1-kinase (28), aldolase B (135), and pyruvate kinase (37, 135), as well as the high K_m glucose transporter, GLUT2 (124). In general, the induction of mRNAs for these glycolytic enzymes is more modest than that observed for the enzymes of fatty acid biosynthesis, ranging from 4- to 10-fold.

Several enzymes involved in triglyceride formation and maturation are also induced by a high-carbohydrate diet, including glycerol-3-phosphate acyltransferase (112), acyl-CoA synthetase (long chain) (120), and stearoyl-CoA desaturase (type I) (122). Apolipoprotein B, a key component of very low

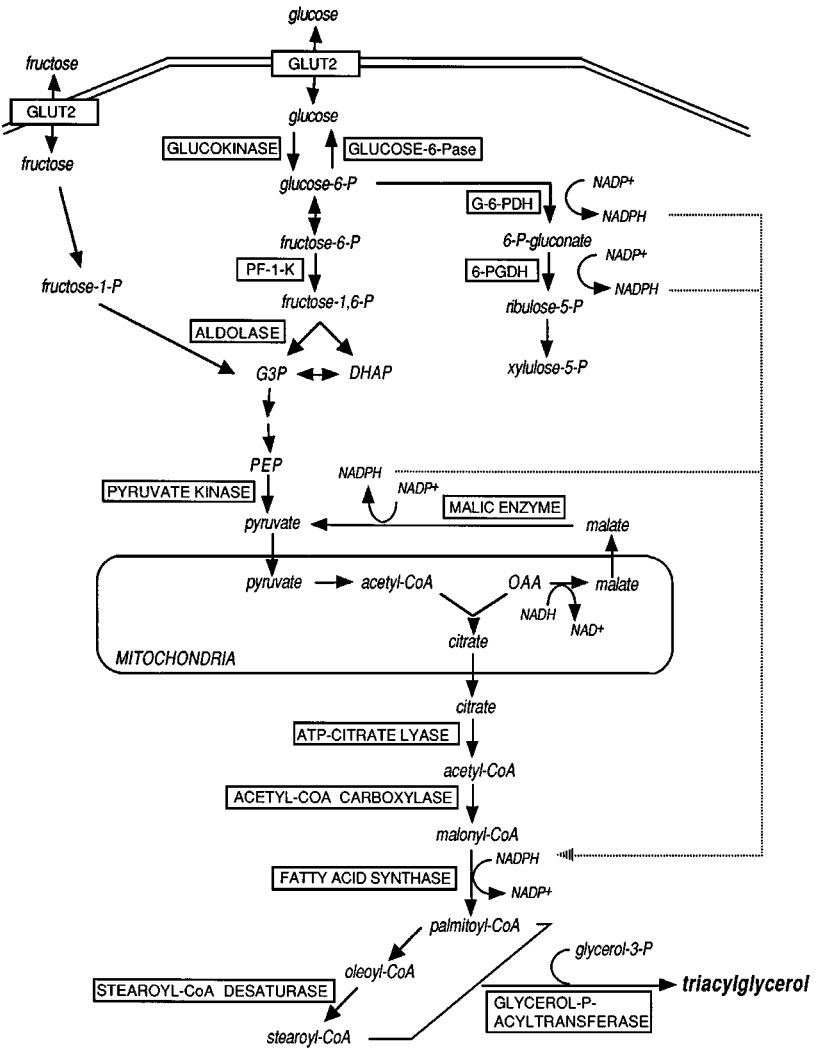

Figure 1 A simplified diagram of lipogenesis in the liver. The principal pathway for the flow of carbons from glucose to triacylglycerols and for the generation of NADPH is outlined. Enzymes in boxes are those that are induced by the feeding of a high-carbohydrate diet to rats or mice (see text for references). Abbreviations: GLUCOSE-6-Pase, glucose-6-phosphatase; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase; PF-1-K, 6-phosphofructo-1-kinase; G3P, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; PEP, phosphoenolpyruvate; OAA, oxalacetate.

density lipoproteins, is not regulated by a high-carbohydrate diet (for review see 114). However, the ratio of $apoB_{100}$ to the edited $apoB_{48}$ falls in rats fed a high-carbohydrate diet (1). This change is mediated by an increase in the mRNA encoding the catalytic subunit of the apolipoprotein B editing complex, which is responsible for converting $apoB_{100}$ to B_{48} (26).

Several other genes are induced in rats fed a high-carbohydrate diet, although the role of their products in lipogenesis is not as clearly defined. These include the rat gene product known as S_{14} (described below). The bifunctional 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, which is responsible for formation and degradation of the key allosteric effector, fructose-2,6-bisphosphate, is also induced by high carbohydrate (10). Unexpectedly, glucose-6-phosphatase was also found to be induced by a high-carbohydrate diet (80). This presents a paradox, as this enzyme is involved in gluconeogenesis, rather than lipogenesis. Generally, the enzymes of gluconeogenesis are regulated inversely to those of glycolysis. For instance, mRNA encoding phosphoenolpyruvate carboxykinase is decreased by glucose in cultured hepatoma cells (48). Thus, the physiological basis of the rise in glucose-6-phosphatase in high-glucose conditions is difficult to rationalize.

Site of Action—Transcriptional Versus Posttranscriptional

The intracellular site at which mRNA level is regulated by a high-carbohydrate diet has been explored by using the nuclear run-on assay to estimate rates of gene transcription. Comparison of changes in transcription rates with changes in mRNA levels has been used to estimate the fraction of a response contributed by transcriptional mechanisms. However, quantitative comparisons between these two values are problematic. Not only are the kinetics of change significantly different, it is also not practical to reach a true steady state in experiments involving feeding regimens. Thus, only in cases where there is a high degree of concordance or discordance between these measurements can reliable conclusions be reached.

In the majority of cases examined, transcriptional regulation is the major site for regulation of mRNA level caused by a high-carbohydrate diet. Examples include fatty acid synthase (98), ATP citrate lyase (57), glycerol-3-phosphate acyltransferase (112), 6-phosphofructo-1-kinase (105), and stearoyl-CoA desaturase I (93). However, posttranscriptional mechanisms are also involved in controlling lipogenic gene expression. Carbohydrate causes an increase in malic enzyme mRNA but not a significant elevation in transcription (18). Glucose-6-phosphate dehydrogenase shows only a small, transient rise (102) or no rise at all (117) in transcription despite dramatic increases in mRNA levels. In other cases, both transcriptional and posttranscriptional mechanisms are involved (38). Fructose fed to diabetic rats caused increased stability of L-type pyruvate

kinase (L-PK) mRNA, in addition to a transcriptional response (92). In HepG2 hepatoma cells, glucose increases fatty acid synthase mRNA by increasing the stability of the mRNA (107). When both transcriptional and posttranscriptional mechanisms are operative, the transcriptional mechanisms generally are responsible for the earlier changes in response to feeding, whereas posttranscriptional mechanisms are operative at later times and maintain elevated mRNA levels in continued conditions of carbohydrate excess. Unfortunately, given the complexities of studying posttranscriptional regulation, little is known regarding the molecular mechanisms involved.

The Pyruvate Kinase Model

Pyruvate kinase catalyzes the final step in glycolysis, the conversion of phosphoenolpyruvate to pyruvate. This reaction is essentially irreversible, so that unique enzymes are required to catalyze the reverse process in gluconeogenesis. Both the enzymatic activity and the synthesis of pyruvate kinase are regulated in the cell. Several isozymic forms of pyruvate kinase are expressed in mammals, allowing for specific control in different tissues. The predominant form in the liver is the L-type. Regulation of L-PK enzyme activity occurs through the actions of allosteric effectors: ATP and alanine are inhibitors, whereas fructose-1,6-bisphosphate is a positive effector. The activity of L-PK is also inhibited via phosphorylation in response to several hormones, such as glucagon, that favor gluconeogenesis (for review see 100).

The accumulation of L-PK mRNA in the liver is depressed by fasting and is stimulated by a high-carbohydrate diet (91, 135). Transcription of the L-PK gene increased dramatically when fasted rats were refed a carbohydrate-rich diet (130). These changes occurred within 2 h of treatment and reached a transient maximum by 12 h, slightly preceding increases in L-PK mRNA. A transcriptional site of control for L-PK gene expression was confirmed by using transfection assays. Segments of the L-PK gene from -4300 to $+12$ were fused to a reporter gene such that its expression was dependent on activity of the L-PK promoter. When such chimeric constructs were introduced into primary hepatocytes, expression of the reporter gene was higher in cells cultured in high glucose than in cells maintained in low-glucose conditions (2, 123). Since the L-PK gene segments used in these experiments lacked structural RNA sequences, transcription had to be regulated by the altered glucose conditions. This assay served as the basis for mapping the L-PK gene sequences that are involved in mediating the transcriptional induction.

The S_{14} Model

S_{14} was first identified by two-dimensional gel electrophoresis of translational products from rat liver mRNA based on its rapid response to thyroid hormone. A high-carbohydrate diet also caused a dramatic increase in hepatic S_{14} mRNA

levels (67). The regulation of S_{14} was of interest because of its rapid response following administration of sucrose to fasted rats. Changes in mRNA levels were detectable within 30 min and reached 25-fold within 4 h (78), which suggests that the induction was a primary response to carbohydrate and did not require the synthesis of an intermediate protein.

S_{14} mRNA encodes a polypeptide of 17,000 M_r that has no sequence similarity to other proteins currently in the databases (84). Although the physiological role of S_{14} is unknown, evidence suggests that it is involved in lipid metabolism. In addition to liver, S_{14} mRNA is found predominantly in tissues actively engaged in fatty acid synthesis: white and brown adipose tissue, and the lactating mammary gland (24, 46). The mRNA for S_{14} appears developmentally in the liver at the time of weaning, when the pup switches from a diet high in fat to one high in carbohydrate and begins to synthesize much of the fat it requires from dietary carbohydrate (99). Premature weaning causes an earlier appearance of the S_{14} mRNA (99), as do other lipogenic stimuli, such as thyroid hormone or insulin (42, 45). Conversely, circumstances favoring a catabolic state, such as fasting, diabetes, or glucagon, result in a rapid disappearance of hepatic S_{14} mRNA (59). Following carbohydrate feeding, hepatic localization of the S_{14} polypeptide is similar to that for the lipogenic enzymes (61). Thus, S_{14} behaves in a fashion analogous to that for lipogenic enzymes.

Introducing an antisense S_{14} oligonucleotide into hepatocytes blocks the induction of S_{14} protein by thyroid hormone and glucose (58). Interestingly, this antisense oligonucleotide also inhibits the increase in lipogenesis normally observed following this treatment. The induction of fatty acid synthase, ATP-citrate lyase, and malic enzyme are also abolished, implicating S_{14} protein in the transduction of hormonal and nutritional signals for increased lipid synthesis in hepatocytes. The rapid changes in S_{14} mRNA and the short half-life of the mRNA (74) are consistent with a regulatory role for this protein. The S_{14} protein is found in the nucleus, suggesting that it could be involved in some aspect of nuclear mRNA production (60). Further work, however, is required to identify its role in this process.

As observed for the L-PK gene, the induction of S_{14} mRNA following carbohydrate ingestion is largely mediated by changes in its transcriptional rate (43). This finding was substantiated by transfection of primary hepatocytes with constructs containing the 5′-flanking region of the S_{14} gene (-4300 to $+18$) fused to a reporter gene. S_{14} promoter activity is greatly stimulated upon addition of high glucose to the media (41). The rapid transcriptional induction of this gene has made it a useful model for exploring regulation by carbohydrate.

The Acetyl-Coenzyme A Carboxylase Model

The first committed step in fatty acid biosynthesis, the carboxylation of acetyl-CoA to malonyl-CoA, is catalyzed by ACC. This enzyme exerts a major

influence on the rate of fatty acid synthesis and thus is subject to many levels of control, including control at the level of transcription (for reviews see 35, 55). The ACC gene is transcribed from two distinct promoters, designated PI and PII, that are separated by 10 kb along the chromosome (71). Transcription from PII occurs in all tissues, presumably to maintain basal levels of this essential enzyme (56). In addition, transcription from PII is induced under lipogenic conditions in the mammary gland. The PI promoter is expressed in epididymal fat tissue and is induced by diets favoring lipogenesis. In the liver, both PI and PII are induced following dietary manipulation. Interestingly, the time course of induction of these two promoters differs substantially. PII is activated with little or no lag and within 18 h has reached maximal activity. The response of PI shows a lag of about 6 h and only reaches maximal activity after 24 h. These observations suggest that distinct molecular mechanisms are involved in transcriptional activation of the two ACC promoters in the liver. (For a more detailed description of the regulation of expression of the ACC gene, see article by Kim in this volume.)

INCREASED CARBOHYDRATE METABOLISM AS A PRIMARY SIGNAL FOR INDUCTION

Metabolic versus Hormonal Signals for Lipogenic Gene Activation

When animals are fasted or are refed a high-carbohydrate diet, multiple factors impinge on the metabolic program of the liver. Among these factors, the counterregulatory hormones insulin and glucagon play a major role. Following a carbohydrate-rich meal, plasma insulin levels rise to counteract postprandial hyperglycemia, while plasma glucagon levels fall. This results in the liver in increased anabolic processes that promote storage of glucose as glycogen and fatty acids. Conversely, hypoglycemia caused by fasting leads to increases in glucagon and to decreases in insulin and promotes glucose formation and secretion by the hepatocyte. Insulin and glucagon can activate and repress the expression of specific genes in a variety of cells (for reviews see 36, 94). Hence, the transition from low to high rates of expression of lipogenic enzyme genes between fasted and carbohydrate-fed states could be mediated by a direct response to these hormonal signals acting through their plasma membrane receptors.

In addition to the changes in insulin and glucagon that occur during refeeding of the fasted animal, an increased flux of glucose and simple carbohydrates into the hepatocyte provides substrates for glycogen and fatty acid synthesis. These biosynthetic pathways are initiated by the actions of two key proteins: the GLUT2 glucose transporter and glucokinase. Both proteins display a high

K_m for glucose, effectively limiting the initiation of glycolysis to the range of glucose concentrations reaching the liver following a high-carbohydrate meal. Thus, changes in substrate utilization provide a second potential pathway for controlling lipogenic gene expression. Differentiating the relative roles of insulin and carbohydrate metabolism in regulating gene activity is not straightforward. For example, administering insulin to diabetic animals results in both a rise in plasma insulin and a concomitant increase in insulin-stimulated carbohydrate metabolism in the liver. Thus insulin, though often implicated as such, cannot be pinpointed as the proximal site of action in gene activation. Conversely, if fasted animals are given a high-carbohydrate meal, both glucose metabolism in the liver and plasma insulin levels rise. Again, this model does not distinguish the relative contributions of these factors.

It is now evident that metabolic, as well as hormonal, signals are involved in regulation of gene expression. Early studies suggesting that substrate utilization contributes to the regulation of lipogenic gene expression arose from experiments in which diabetic animals were fed fructose. This strategy provided a means of separating the effects of insulin and metabolites, as fructose can be metabolized in the liver in an insulin-independent fashion (127). Fructose enters glycolysis at the level of triose phosphates, bypassing glucokinase and 6-phosphofructo-1-kinase, the two major insulin-sensitive steps in the pathway. Feeding fructose to diabetic animals restored rates of fatty acid synthesis (see, for example, 133) and increased production of mRNA for L-PK (37), malic enzyme (50), ACC (49), and fatty acid synthase (51) to that of the fed nondiabetic state. Thus, increased gene expression was suggested to result from elevated metabolism of carbohydrates in the liver. Hepatic glucokinase shows a distinct response from the above lipogenic enzymes: It is unresponsive when diabetic rats are fed fructose (121). However, insulin injection fully restored glucokinase activity and mRNA to normal levels in the liver (116). Thus, glucokinase represents a gene product that is directly affected by insulin.

Primary Hepatocytes as a Model for Studying the Role of Carbohydrate Metabolism

While feeding fructose to diabetic rats implicated carbohydrate metabolism in the activation of lipogenic gene expression, such studies are complicated by the metabolic alterations caused by the diabetic state. A more effective means of differentiating between the effects of insulin and carbohydrate metabolism is to study the problem in cultured cells. Increased glucose concentrations in the media lead to elevated enzyme activities, rates of enzyme synthesis, and mRNA levels for several lipogenic enzymes in cultured primary hepatocytes (13, 52, 76, 115). This effect of glucose is not observed with nonmetabolizable analogs, such as 2-deoxyglucose or 3-O-methylglucose, or in the presence

of mannoheptulose, an inhibitor of glucokinase. Thus, glucose metabolism is critical for induction of lipogenic enzymes in the hepatocyte. The effect of glucose is significantly enhanced in the presence of insulin, but it occurs in hepatocytes maintained at a constant insulin concentration. Thus, it was suggested that insulin allows the effective metabolism of glucose. Consistent with this interpretation, fructose was found in several studies to induce enzyme activity in an insulin-independent fashion (25, 115).

Again, the response of the glucokinase gene in cultured hepatocytes is distinct from that of the other lipogenic enzymes. Addition of insulin to the medium elicits a time-dependent increase in glucokinase mRNA with or without glucose (39). This response occurs at a physiological concentration of insulin and occurs at a transcriptional level. Increased glucose does not affect glucokinase gene expression. Thus, glucokinase, the first step in glucose metabolism, stands apart from the majority of lipogenic enzymes with respect to its control.

Role of Insulin in the Process of Induction

The direct regulation of glucokinase by insulin suggested that it represents the insulin-sensitive step in lipogenic gene expression in primary hepatocytes. Vaulont & Kahn established that this is the case for the L-PK gene (129). Three independent lines of evidence support this conclusion. First, glucokinase activity in the hepatocyte is inhibited by a regulatory protein with which it forms a reversible complex (128). This inhibition is reversed by binding of fructose-1-phosphate to the regulatory protein. Doiron et al (17) found that low concentrations (0.2 mM) of fructose added to cultured hepatocytes can lead to an induction of L-PK transcription by glucose independent of insulin. This concentration of fructose was sufficient to activate glucokinase but not to provide substrate for lipogenesis. A second line of evidence arose from experiments in which hepatocytes were transfected with a vector that constitutively expresses glucokinase (17). This led to high levels of glucokinase without the requirement for insulin and overcame inhibition by the regulatory protein, which would be present in lower amounts than the overexpressed glucokinase. Indeed, these cells displayed increased L-PK promoter activity that was independent of insulin but that required glucose. Finally, a hepatocyte-like cell line (mhAT3F), derived by targeted carcinogenesis using the simian virus 40 T-antigen, did not require insulin to support the activation of L-PK expression by glucose (63). Glucokinase expression in this cell line was extinguished, and its ability to metabolize glucose was instead dependent on an insulin-independent hexokinase. The loss of insulin-dependent glucokinase expression correlated with insulin-independent activation by glucose.

The situation in mhAT3F cells may be similar to that in the adipocyte (for review see 21). Adipocytes do not express glucokinase; they rely on hexokinase

II for their glucose phosphorylating potential. Since hexokinase II expression is not insulin dependent, addition of glucose can lead to an induction of mRNA for ACC or fatty acid synthase in the absence of insulin in cultured adipose tissue (22). Because of the higher affinity of hexokinase II for glucose, this response occurs at lower glucose concentrations than that observed in the hepatocyte. What then serves to control glucose-mediated lipogenesis in adipose tissue? While glucose can stimulate ACC and fatty acid synthase in the absence of insulin, the addition of insulin strongly potentiates this effect. This is likely due to the effects of insulin on the GLUT4 transporter of the adipocyte, which is recruited from intracellular sites to the plasma membrane in the presence of insulin. Thus, glucose-sensing in the adipocyte is indirectly controlled by the β-cell, which senses plasma glucose levels to control insulin secretion.

Possible Intracellular Mediators of the Carbohydrate Response

If glucose metabolism is capable of generating an intracellular signal that influences transcriptional activity of the lipogenic enzyme genes, it is likely that a specific metabolic step is responsible. Efforts to identify the metabolic coupler that links glucose metabolism and gene transcription have not yet revealed the nature of this compound. However, several candidates have been suggested and are worthy of consideration.

The earliest work on this question came from Mariash & Oppenheimer (77), who studied the synthesis of malic enzyme in hepatocytes. In addition to glucose, a variety of compounds—including fructose, lactate, glycerol, and dihydroxyacetone—increased malic enzyme production. These compounds were all capable of being metabolized through the glycolytic pathway. However, compounds that entered metabolic pathways downstream from pyruvate, such as acetate or citrate, did not give a response. This led to the hypothesis that the critical step in signal generation occurred at a mitochondrial step at or downstream from pyruvate oxidation. This experiment is complicated by the gluconeogenic potential of the hepatocyte. All compounds that stimulated were also capable of being converted to glucose, whereas those that could not stimulate were not gluconeogenic. However, a mitochondrial origin for the mediator was supported by the observation that dichloroacetic acid, a stimulator of pyruvate dehydrogenase, also induced malic enzyme synthesis (77). This response did not require insulin and occurred at levels of glucose not normally able to stimulate malic enzyme synthesis. Thus, pyruvate oxidation was considered essential for generation of the metabolic signal.

A second possible site for generation of the metabolic coupler was proposed by Foufelle et al (22). These workers observed that 2-deoxyglucose stimulated expression of fatty acid synthase and ACC genes in cultured adipose tissue. 2-Deoxyglucose can be converted to 2-deoxyglucose-6-phosphate but

is not further metabolized by the glycolytic pathway. Thus, the generation of glucose-6-phosphate was proposed as the critical step in the process. Consistent with this notion, 3-O-methylglucose, which cannot be phosphorylated at the 6 position, was incapable of stimulating induction in the adipocytes. In addition, glucose-6-phosphate levels in adipocytes treated with varying concentrations of glucose correlated with levels of fatty acid synthase mRNA accumulation. However, it is difficult to reconcile this hypothesis with the effects of 2-deoxyglucose in hepatocytes. Numerous studies have reported no effect of this glucose analog on production of the lipogenic enzymes. It could be argued that the presence of glucose-6-phosphatase in the hepatocyte might prevent the accumulation of 2-deoxyglucose-6-phosphate necessary for stimulation. In an effort to overcome this, Rencurel et al (104) used high (30 mM) concentrations of 2-deoxyglucose but found no stimulation of GLUT2 mRNA. Direct measurement of 2-deoxyglucose-6-phosphate in these cells showed a substantial accumulation of this compound. Thus, glucose-6-phosphate is not a likely candidate for the metabolic coupler in hepatocytes, but it may be important in the adipocyte. (A more detailed discussion of this issue can be found in the article by Girard et al in this volume.)

Doiron et al (16) recently suggested that the signal for generation of the metabolic coupler arises not from glycolysis but from the alternative pathway for glucose oxidation, the pentose phosphate pathway. This suggestion was based on the observation that xylitol at a concentration of 0.5 mM stimulated L-PK promoter activity to the same extent as 20 mM glucose in mhAT3F cells. Since xylitol is converted to xylulose-5-phosphate and enters the pentose phosphate pathway, xylulose-5-phosphate was proposed as the key intracellular signaling molecule. Intriguingly, Nishimura et al (89, 90) recently identified xylulose-5-phosphate as a mediator for early effects of glucose on the activity of fructose-6-phosphate kinase/fructose-2,6-bisphosphatase. This metabolite accumulated in the liver within 2 min of glucose perfusion, slightly preceding increases in fructose-2,6-bisphosphate activity. Xylulose-5 phosphate activated a form of protein phosphatase 2A that dephosphorylated the bifunctional enzyme to activate its kinase activity and led to generation of fructose-2,6-bisphosphate. It is conceivable that such a phosphatase might also act on a transcription factor that interacts with the lipogenic enzyme genes to activate their expression. Consistent with this possibility, inhibitors of protein phosphatase 2A, including okadaic acid, block induction of lipogenic enzymes (119).

Two notes about the pentose phosphate shunt as a potential site of metabolic coupling are worth mentioning. First, a major function of this pathway in the hepatocyte is to generate NADPH, which provides the reducing potential for fatty acid synthesis. If metabolic signaling involves flux through the pentose phosphate pathway, the cellular demands for NADPH to drive fatty acid

synthesis could be coupled with generation of the intracellular intermediate involved in increasing production of the enzymes of fatty acid synthesis. Second, both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, the first two steps in the pentose phosphate pathway, are among the lipogenic enzyme genes induced by carbohydrate. It might seem illogical that these enzymes induced by glucose metabolism would be necessary for generation of the signal for their own induction. However, examples of such "feed-forward" regulatory circuits are known in biological systems. Such a system would provide a means of increasing sensitivity to changes in cellular metabolites by accelerating the process of signal generation with initial small changes in the formation of the metabolic coupler.

REGULATORY SEQUENCES AND *TRANS*-ACTING FACTORS FOR METABOLIC SIGNALING OF TRANSCRIPTIONAL INDUCTION

The Carbohydrate Response Element—L-PK and S_{14} Genes

Identification of the transcription factor or factors responsible for mediating the effects of carbohydrate metabolism would greatly aid efforts to unravel this intracellular signaling pathway. One potential means of achieving this is to identify the sequences of lipogenic enzyme genes that are required for regulation. These response elements would be the binding sites for transcription factors that are either directly modulated in response to carbohydrate metabolism or a part of a complex including such a protein. Regulatory sequences have been mapped to date for the L-PK, S_{14}, and ACC genes.

For the L-PK and S_{14} genes, mapping of sequences responsible for carbohydrate regulation has been accomplished by using transfection analysis in primary hepatocytes in culture (Figure 2). For the L-PK gene, the critical region is between positions -172 and -124 of the promoter region (2, 70, 123). For the S_{14} gene, the regulatory region is between bases -1467 and -1422 upstream of the transcriptional initiation site (109–111). This portion of the S_{14} gene is within a liver-specific DNase I hypersensitive region (43). Comparison of the L-PK and S_{14} regulatory regions revealed a sequence with identity in 9 out of 10 bp, suggesting that a common factor might be involved in mediating the responses of these two genes to carbohydrate (110).

Hepatic nuclear factors binding to the -172 to -124 region of the L-PK promoter were examined by DNase I footprinting and electrophoretic mobility shift analyses; two binding sites were identified (132). One site, from -168 to -145, interacts in vitro with the upstream stimulating factor (USF) (15, 70, 132). The other site from -144 to -126 bound to a distinct nuclear factor, hepatic nuclear

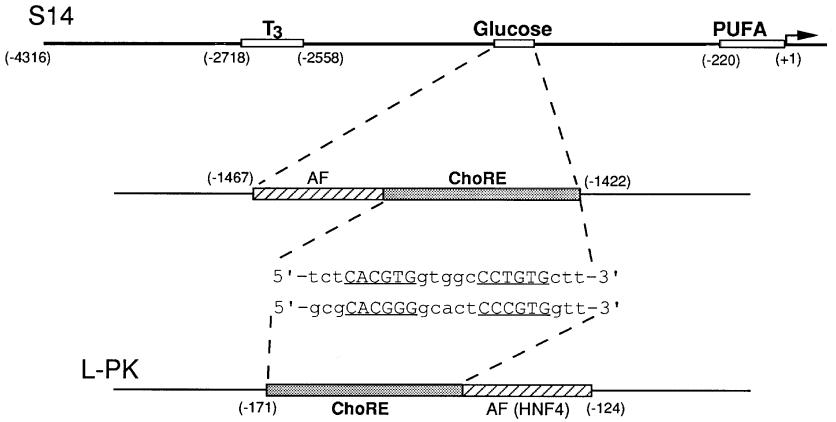

Figure 2 Organization of transcriptional regulatory sequences in the S_{14} and L-PK genes. The top line indicates the relative position of regulatory sequences in the S_{14} gene involved in transcriptional induction by thyroid hormone (T_3) and glucose metabolism or transcriptional repression by polyunsaturated fatty acids (PUFA). The bottom two lines indicate the relative position of carbohydrate response element (ChoREs) and accessory factor binding sites (AF) involved in the carbohydrate regulation of S_{14} and L-PK genes. Sequence comparison of the ChoREs is shown with CACGTG motifs in capital letters and underlined. Figure modified from Reference 125.

factor 4 (HNF4) (15, 70, 132). When oligonucleotides corresponding to these two binding sites were joined together, they were capable of restoring a glucose response to the basal L-PK promoter (defined as sequences from −96 to +12); however, each oligonucleotide alone was insufficient for control. Thus, two factors are essential for mediating glucose responsiveness in the L-PK promoter. A similar situation was found for the S_{14} gene (109). The region from −1467 to −1422 contains two factor binding sites. The site from −1448 to −1422 shares sequence similarity with the L-PK gene sequences and binds USF in vitro (111). The nature of the factor binding to the second site of the S_{14} regulatory region is unknown, but it is distinct from HNF4.

To evaluate the individual roles of the two binding sites present in the L-PK and S_{14} genes, multiple copies of each binding site were linked in tandem to a basal promoter and were tested for their abilities to confer a glucose response to a linked reporter gene. This strategy often allows the activity of a single transcription factor binding site that is part of a more complex regulatory region to be observed. Linking multiple copies of the PK USF binding site upstream of the basal L-PK promoter increased activity in response to glucose (2, 70). Similarly, the activity of the S_{14} USF binding site was greatly enhanced when linked in multiple copies to a basal promoter (109). Thus, the USF binding

site of either the L-PK or S_{14} genes binds to the transcription factor receiving the signal from enhanced carbohydrate metabolism and was designated the carbohydrate response element (ChoRE) (110). This sequence in the L-PK gene has also been termed a glucose/insulin response element (G/IRE) (2). A more appropriate name may be metabolic response element, as suggested by German & Wang (30) for sequences of the insulin gene involved in its response to glucose metabolism (see below).

If the ChoRE binds to the factor that receives the direct signal from the metabolic pathway, what is the role of the additional nuclear factor binding site found within each regulatory region? When linked in multiple copies to the basal L-PK promoter, these sites do not confer a response to glucose. These sites, however, synergize with the ChoRE to give a functional response. Such functional synergism is, in fact, a common feature of a number of hormonal and nutritional regulatory sequences. For example, the ability of the sterol response element binding protein to stimulate transcription of the low-density lipoprotein receptor gene depends on the presence of nearby Sp1 binding sites in the promoter (106). We refer to these sites in the L-PK and S_{14} genes as accessory sites. These sites could be involved in controlling other signals that influence expression of the L-PK and S_{14} genes. For example, in the case of the L-PK gene, the accessory site may be involved in mediating the repressive actions of polyunsaturated fatty acids on promoter activity (68).

Role of USF in the Carbohydrate Response

Examination of the ChoRE sequences of the L-PK and S_{14} genes reveals some striking similarities (Figure 2). Both genes contain two copies of a motif related to the consensus binding site: (5′)CACGTG. This E box motif binds to transcription factors of the basic/helix-loop-helix/leucine zipper (b/HLH/LZ) family. This family includes USF1 and USF2, c-myc and its related family members, TFEB, TFE3, AP-4, and SREBP1 and 2. In these proteins, the basic region is responsible for contacting the DNA double helix, while HLH and LZ domains promote dimerization, which is essential for DNA binding (47).

In both the L-PK and S_{14} ChoREs, the two CACGTG motifs are separated by 5 bp. In the L-PK ChoRE, the two motifs are found as palindromic sequences, $CACGGG(N)_5CCCGTG$. In the S_{14} gene, one motif contains a six-out-of-six match with the consensus binding site, while the other contains only a four-out-of-six match. In either case, mutation of specific bases within the CACGTG motifs interferes with the ability to respond to glucose, so that both sites are critical for regulation (2, 70, 109). This suggests that two b/HLH/LZ factors must bind to confer a glucose response. When the two motifs are spaced by 4 instead of 5 bp, the response to glucose is completely lost (109). It is reasonable to conjecture that the binding of two b/HLH/LZ factors might be

restricted when the two sites are closer than 5 bp. When the two CACGTG motifs are separated by more than 5 bp, the response to glucose is seriously blunted. This suggests that the two factors must interact in a highly specific manner to create a complex responsive to glucose. Reversing the direction of the palindromic motifs of the L-PK ChoRE to give either $GGGCAC(N)_5CACGGG$ or $CACGGG(N)_5CACGGG$ results in a loss of responsiveness (EN Kaytor, HC Towle, unpublished data). Thus, both the spacing and the orientation of CACGTG motifs are critical to control.

As mentioned above, one candidate for the b/HLH/LZ factor involved in mediating carbohydrate regulation is USF. USF is present in nuclear extracts from liver and binds to the ChoREs of either the L-PK or the S_{14} genes (15, 70, 111, 132). USF also binds to a site in the fatty acid synthase promoter that is critical for a response to insulin and/or glucose in mouse 3T3-L1 adipocytes (134). USF was first identified as a transcriptional factor based on its ability to activate the adenovirus major late promoter in HeLa cells. However, USF has been implicated in transcription of a number of cellular genes, including several genes expressed in the hepatocyte that do not respond to glucose (see, for example, 101). The ubiquitous expression of USF also argues against its function in this tissue-specific response pathway (113). However, the observation that two CACGTG motifs must be present at an appropriate distance and orientation for a glucose response raises the possibility that USF could be selectively involved in this regulation. For example, the specific positioning of two USF dimers could function to bind to a third component that is responsible for receiving the signal of glucose activation.

To find out if USF is involved in the carbohydrate response, Lefrancois-Martinez et al (64) tested whether a dominant negative form of this factor could inhibit the response to glucose. This form lacked the basic region of USF but retained the HLH and LZ regions. It thus was capable of heterodimerizing with endogenous USF but incapable of DNA binding. When a plasmid expressing this dominant negative form of USF was introduced into mhAT3F hepatoma cells, an inhibition of the glucose response of the L-PK promoter was observed. These authors concluded that USF proteins are components of the glucose response complex binding to the L-PK ChoRE. We performed similar experiments in primary hepatocytes using three different dominant negative forms of USF and got discrepant results (52a). While these forms were capable of inhibiting activity of a cotransfected plasmid expressing USF, they did not inhibit the glucose response of either the S_{14} or the L-PK promoters. It is difficult at present to reconcile these contradictory results. It is possible the difference in cells used could account for the discrepancy. For example, the hepatocytes might contain much higher levels of USF than the mhAT3F cell line and thus be more resistant to the effects of the dominant negative forms. Given these conflicting

results, we sought an additional means of addressing the role of USF in the glucose response. Point mutations were introduced into the ChoRE to determine whether functional activity of this element and USF binding were affected comparably. Several mutations were identified that strongly reduced USF binding without interfering with the glucose response. These results suggest that USF is not part of the carbohydrate responsive complex and that another b/HLH/LZ factor present in liver mediates this action. Further work is necessary to resolve the nature of the carbohydrate-responsive factor acting upon the L-PK and S_{14} genes.

Acetyl-Coenzyme A Carboxylase and the Role of Sp1

The possibility that multiple transcription factors are involved in the carbohydrate regulation of gene expression has been suggested by studies of the ACC gene. Expression of the ACC gene from its PII promoter is induced by glucose in the mouse 30A5 adipocyte cell line (11). By using deletional analysis, the region from −340 to −182 of the ACC PII promoter was shown to be necessary and sufficient for supporting a glucose response. This region contains two GC boxes, the consensus binding site for the Sp1 transcription factor. Specific mutation of either GC box disrupted the response of the ACC PII promoter to glucose. These two sites bound Sp1 in vitro, and the intensity of binding was greater in extracts from 30A5 cells incubated with 27.5 mM glucose than from cells incubated with 10 mM lactate. However, the amount of Sp1 protein present in 30A5 cells was not altered by treatment with glucose, suggesting that the increased binding activity of Sp1 was due to a posttranslational modification of the protein (12). This modification was suggested to be a dephosphorylation event, since okadaic acid inhibited the increase in binding activity observed in glucose-treated cells. Treatment of nuclear extract with antibodies against the catalytic subunit of protein phosphatase I partially suppressed the activation of Sp1 binding. Thus, glucose-induced dephosphorylation by the type 1 phosphatase was suggested to be critical for regulation.

These studies of the ACC gene raise several interesting questions. One question is how the specificity of regulation is achieved. Sp1 is expressed ubiquitously and is involved in the transcription of a great number of promoters. Thus, some unique feature of the ACC promoter must allow Sp1 to function in a glucose-responsive manner for this gene. Other sequences of the ACC regulatory region may act synergistically with Sp1 to provide promoter specificity. In fact, another mutation of the PII promoter between the two Sp1 binding sites also led to a loss of glucose inducibility (11). A second question of interest deals with the specificity of the glucose response between different genes of the lipogenic enzyme family. The results presented to date suggest that factors activating L-PK and S_{14} genes are distinct from those acting on ACC. This raises

two questions: Are similar signaling pathways involved in the adipocyte and hepatocyte to activate gene expression in response to glucose, and how many transcription factors might be activated by glucose metabolism?

INTERACTIONS OF CARBOHYDRATE METABOLISM WITH OTHER REGULATORY PATHWAYS

Polyunsaturated Fatty Acids

In addition to carbohydrate metabolism, the genes encoding lipogenic enzymes are regulated by a number of other nutritional and hormonal factors. Some of these work in conjunction with carbohydrates to induce lipogenic enzyme expression, whereas others repress the induction. The hepatocyte must effectively integrate these various signals to provide the optimal level of lipogenic enzyme production to meet the needs of the organism.

One highly effective inhibitor of the expression of lipogenic enzymes is dietary fat (for reviews see 7, 8). Since triglycerides are the product of the lipogenic pathway, this represents a situation analogous to end-product repression. Dietary fats have been known for many years to inhibit hepatic lipogenesis (see, for example, 9). Part of this inhibition is due to direct effects on the activities of lipogenic enzymes. For example, fatty acyl-CoA is an allosteric inhibitor of ACC. In addition, the inhibitory action of fats on hepatic lipogenesis includes the repression of lipogenic enzyme production. Lipogenic enzymes repressed by dietary fats include fatty acid synthase, ACC, glucose-6-phosphate dehydrogenase, stearoyl-CoA desaturase (types 1 and 2), L-PK, and S_{14}. This repression is specific for polyunsaturated fatty acids (PUFA); saturated and monounsaturated fatty acids have no inhibitory action. The effects of PUFA are dominant over carbohydrate activation. Furthermore, regulation occurs at the level of transcriptional repression, at least for those genes tested (3, 62, 93). The effects of dietary PUFA can be replicated in hepatocytes in culture (44). However, the intracellular pathway involved has not been elucidated.

A reasonable hypothesis is that inhibition of transcription by PUFA occurs by direct binding to a transcriptional factor that functions as a dominant repressor. To search for such a repressor, studies mapping the DNA regulatory sites necessary for repression have been initiated. For the S_{14} gene, sequences responsible for mediating the effect of PUFA were localized to the proximal promoter region between -220 and -80 (44). This region is distinct from either the carbohydrate or the thyroid hormone responsive elements of the gene (Figure 2). For the L-PK gene, the regulatory site was mapped to the HNF-4 accessory factor binding site (68). Thus, two adjacent sites of the L-PK gene are involved in mediating the actions of carbohydrate metabolism and PUFA.

A logical candidate for a *trans*-acting factor involved in mediating the actions of PUFA is the peroxisome proliferator-activated receptor (PPAR). The PPARα class, in particular, is activated by long-chain fatty acids (27, 53). PPARα turns on the expression of genes involved in oxidation of fatty acids, including peroxisomal acyl-CoA oxidase (54). Since other members of the PPAR family act as both activators and repressors, the question of whether PPARα is involved in repression of lipogenic gene expression was raised. Evidence accumulated to date indicates that this is not the case (8). While PPARα can be activated by saturated and monounsaturated fatty acids, as well as by PUFA, the suppression of lipogenic gene activation is specific for PUFA. Furthermore, the sequences of the S_{14} gene necessary for suppression by PUFA do not contain PPAR binding sites (103). Thus, the nature of the putative repressor activated by PUFA remains to be determined.

Glucagon and cAMP

Glucagon, acting through its intracellular mediator cAMP, is a second major regulator of lipogenic enzyme production in the liver. The production of virtually every member of the lipogenic enzyme family is repressed by cAMP (59, 77, 131). The repression is in all cases dominant over the inductive effects of carbohydrate. Thus, in a state of hypoglycemia, the liver is prevented from storing glucose as triglycerides.

Glucagon has dramatic effects on both the transcription and the stability of mRNAs for lipogenic enzymes. For example, transcription of the L-PK gene is reduced by over 90% within 15 min of glucagon administration (130). However, the mRNAs encoding many enzymes have long half-lives, so that turning off transcription alone would not be sufficient to reduce enzyme production in a timely manner. Thus, glucagon also decreases the stability of mRNAs for several lipogenic enzymes. For L-PK mRNA, the half-life was estimated to fall from 24 h in the presence of glucose and insulin to 1 h in the presence of glucagon (13).

Many genes induced by cAMP have regulatory sites known as cAMP-regulated enhancers (CREs), which bind to members of the ATF family of transcription factors. One of the best characterized of these is CREB, or CRE binding protein. The ability of CREB to activate transcription is dependent on the specific phosphorylation of serine 133 by cAMP-dependent protein kinase A (32). CREB can act as a repressor on certain promoter elements as well (65). Related family members also function as transcriptional repressors when binding to a CRE (23). Thus, one possible mechanism for repression of lipogenic enzyme expression is through the action of negative CREs. To test this possibility, regulatory sequences responsible for transcriptional repression by cAMP have been mapped in the L-PK and S_{14} genes. In both cases, the repressive effects

of cAMP localized to the ChoRE elements (2; EN Kaytor, HC Towle, unpublished data). Neither of these ChoREs contains sequences similar to known CREs. Two possibilities could explain the coincidence of regulatory sequences mediating effects of carbohydrate and glucagon. The first is that the effects of cAMP are indirect through its action to inhibit glucose metabolism and hence reduce formation of the intracellular mediator. The second possibility is that cAMP-dependent protein kinase A could phosphorylate a component of the carbohydrate-responsive complex distinct from known CRE binding proteins to cause inactivation.

Thyroid Hormones

As opposed to the inhibitory effects of PUFA and glucagon, thyroid hormones increase hepatic lipogenesis. The activation of lipogenic enzyme gene expression represents the major mechanism mediating this effect (for review see 126). Thyroid hormones act through a nuclear receptor of the steroid receptor family. Response elements responsible for inducing gene transcription by thyroid hormones have been mapped in the malic enzyme and S_{14} genes (14, 69). For the S_{14} gene, three distinct receptor binding sites are found between positions -2718 and -2558 of the 5′-flanking region. These sites function synergistically to moderate the strong induction of the S_{14} gene by thyroid hormone (69).

An interesting aspect of the regulation of lipogenic genes by thyroid hormone is its interaction with carbohydrate induction. For many lipogenic enzymes, these two effectors function in a highly synergistic fashion (75). This is illustrated by the S_{14} gene. Injection of 3,5,3′-triiodothyronine (T_3) into hypothyroid rats leads to a rapid increase in S_{14} mRNA levels that reaches 15- to 20-fold within 4 h. However, when hypothyroid animals that have been fasted overnight are similarly treated with T_3, little change in S_{14} mRNA level is detected within this time frame (78). Conversely, feeding a high-carbohydrate diet to hypothyroid animals gives a strongly diminished response of S_{14} mRNA compared with normal animals. What is the basis of the synergistic activation? ChoRE and thyroid hormone response elements of the S_{14} gene are separable and capable of functioning independently. However, the thyroid hormone receptor and carbohydrate-responsive factor may interact with a common transcriptional coactivator to stimulate gene transcription in a synergistic fashion. Alternatively, Sudo & Mariash (118) have observed a glucose-responsive change in S_{14} promoter activity that did not map to the ChoRE described earlier. This glucose response was only detected when an expression plasmid for thyroid hormone receptor was cotransfected into hepatocytes with the reporter plasmid and in the presence of T_3. This regulatory sequence could play a role in the synergistic activation of S_{14} gene expression.

TRANSCRIPTIONAL REGULATION BY METABOLIC SIGNALING IN THE PANCREATIC β-CELL

The pancreatic β-cell is responsible for secretion of insulin under conditions of hyperglycemia. That the stimulatory effect of glucose on insulin secretion is linked to its metabolism in β-cells is now dogma (for reviews see 81, 87). The metabolic pathways that couple glucose metabolism and insulin secretion in the β-cell are complex; however, the initial stages of this process are well understood and bear striking similarity to processes in the hepatocyte. Both cell types utilize GLUT2—the high K_m, high V_{max} transporter—to achieve rapid equilibration of glucose across the plasma membrane. Both also express the high K_m glucokinase to support glucose phosphorylation. This enzyme allows the rate of glucose metabolism to increase in proportion to plasma glucose levels. In the β-cell, the kinetic properties of glucokinase are responsible for glucose dependency curves of insulin secretion and, hence, this enzyme is considered by many to be the glucose sensor (81).

Not only do β-cells secrete insulin in response to hyperglycemia, they also increase production of insulin. This process is likely to be important for maintaining stores of insulin during periods of prolonged stimulation by glucose. Increased production of insulin is mediated at several sites, including gene transcription (88). In addition to the insulin gene, several other genes are glucose responsive in the β-cell. These include GLUT2 (5), pyruvate dehydrogenase E1α subunit (72), pyruvate carboxylase (72), and ACC (4).

Efforts to identify the transcription factors that mediate the glucose response in the β-cell have led to the unexpected finding that multiple factors contribute to the control. To date, three distinct factors have been implicated. (*a*) Melloul et al (82) found that a segment from the rat insulin I gene from -227 to -193 could support a response to glucose. Binding of nuclear factors to this element increased four- to sixfold in extracts of islets incubated at 20 mM glucose compared with extracts from islets at 2 mM glucose. MacFarlane et al (73) examined a homologous region of the human insulin gene and found that extracts from freshly isolated islets contained a binding activity that disappeared within 6 h if islets were incubated in 3 mM glucose but was maintained in 20 mM glucose. Interestingly, the loss of binding activity in low glucose was prevented when phosphatase inhibitors were included in the incubation, suggesting that binding is regulated by phosphorylation. Recently, the factor that reacts with this element was determined to be a β-cell–specific transcriptional factor of the homeodomain family, designated IPF1 (95). (*b*) German & Wang (30) demonstrated that a mutation of the E2 element, which lies just upstream to the IPF1 binding site, disrupted the glucose activation of the rat insulin I promoter. This

E2 region contains an E box with the sequence CATCTG. This element binds to members of the b/HLH family and, in particular, to a heterodimer between the ubiquitously expressed E12/E47 factor (86) and a β-cell–specific partner, designated β2 (85). Binding of this complex was also increased in extracts of islets incubated in high glucose (30). (*c*) Sharma & Stein (108) identified a region from -126 to -91 of the human and rat insulin II promoters important to glucose regulation. A factor designated RIPE3b1 binds within this region. The nature of this β-cell–specific protein is not yet known. The binding of RIPE3b1 was enhanced approximately eightfold in extracts prepared from an insulinoma cell line treated with 20 mM vs that in cells treated with 0.2 mM glucose.

Each of these three glucose response elements can act independently, but synergy between these sites is important to achieve the overall glucose response. Why does the β-cell use such a complex system to achieve glucose signaling of insulin gene transcription? The answer probably lies in the highly complex nature of the physiological stimuli to which the β-cell must respond to optimize production of this critical hormone. It is interesting to note apparent similarities between the glucose regulatory sequences of the insulin gene in β-cells and the L-PK and S_{14} genes in hepatocytes. In both cases, elements containing E box motifs play a role in regulation. In both cases, the E box–containing regulatory sites function synergistically with an adjacent site. Naturally, this leads to the question of whether common regulatory factors are involved. It would appear this is not the case. Factors binding to glucose response elements of the insulin gene are specific for the β-cell. Furthermore, the E boxes of the insulin gene are not duplicated in the unique arrangement found in the L-PK and S_{14} response elements. Given this, it is somewhat surprising that the L-PK promoter is capable of responding to glucose in the INS-1 β-cell line (79). This response of the L-PK promoter is dependent on the same DNA elements as in primary hepatocytes. This raises the question of what factors are involved in glucose activation of the L-PK promoter in the β-cell line. One possibility is that a β-cell factor that recognizes the insulin gene (most likely the E box binding factor) is able to recognize the response element of the L-PK gene despite the apparent dissimilarities. Alternatively, a distinct β-cell factor not utilized for controlling the insulin gene, and perhaps shared with the hepatocyte, may be capable of mediating the L-PK response to glucose.

As discussed for glucose induction in hepatocytes, the transcriptional response of the β-cell requires that glucose be metabolized to generate the intracellular signal. Mannoheptulose and glucosamine, which block the glucokinase reaction, inhibit glucose-mediated induction of transcription of insulin, ACC, and GLUT2 in β-cells (4, 20, 33). Likewise, nonmetabolizable glucose analogs, such as 3-O-methyl glucose, do not support a response. Beyond this

point, differences exist in the literature as to the ability of other secretagogues to induce β-cell transcription. Brun et al (4) found that a wide variety of other metabolizable nutrients were incapable of inducing ACC mRNA in the INS-1 cell line. However, they found that 2-deoxyglucose was capable of inducing ACC mRNA, suggesting that glucose does not have to be metabolized beyond glucose-6-phosphate in the glycolytic pathway to induce expression of this gene product. By contrast, Goodison et al (33) found that the insulin secretagogue 4-methyl-2-oxopentanoate (the deamination product of leucine) was stimulatory for insulin promoter activity in the HIT cell line. Ferrer et al (20) reported that glyceraldehyde was effective at stimulating the accumulation of GLUT2 mRNA but that 2-deoxyglucose was ineffective. German (29) also did not observe a stimulatory effect of 2-deoxyglucose on insulin promoter activity in fetal rat islets in culture. At present, it is difficult to reconcile the differences between these reports. It is possible that more than one step in the glycolytic pathway could lead to generation of signals and that different genes might utilize different pathways of activation. As for the hepatocyte, it is likely that examination of the essential transcription factors that mediate the response to glucose is necessary to resolve this issue.

CONCLUSIONS

The role of nutrients and metabolites in controlling gene expression in mammalian systems is an area of developing interest. In this review, we summarized studies aimed at characterizing the actions of carbohydrate metabolism on expression of genes for the lipogenic enzymes. These studies demonstrated a key role for metabolism of glucose in the signaling pathway and identified some potential candidates for the intracellular metabolites that may couple metabolism and transcription. Together with hormonal signals and other metabolic signals such as PUFA, the carbohydrate regulation of gene expression allows the hepatocyte to balance the utilization and storage of metabolic fuels by controlling production of key enzymes involved in these processes.

Over the past several years, efforts to identify the regulatory sequences responsible for mediating transcriptional regulation in response to altered carbohydrate metabolism have begun. Several distinct sites have been detected in genes regulated by glucose metabolism in hepatocytes and β-cells. These studies suggest that multiple factors are capable of being affected by glucose. Thus, the signaling pathway initiated by glucose metabolism can apparently influence several targets in the nucleus. Continuing efforts to identify the nature of these factors are critical for further defining the pathways involved in this intracellular signaling pathway.

ACKNOWLEDGMENTS

This work was supported by grant DK26919 from the National Institutes of Health. We are grateful to Dr. L. Karl Olson for critical reading.

Literature Cited

1. Baum CL, Teng B-B, Davidson NO. 1990. Apolipoprotein B messenger RNA editing in the rat liver. Modulation by fasting and refeeding a high carbohydrate diet. *J. Biol. Chem.* 265:19263–70
2. Bergot M-O, Diaz-Guerra M-JM, Puzenat N, Raymondjean M, Kahn A. 1992. *Cis*-regulation of the L-type pyruvate kinase gene promoter by glucose, insulin and cyclic AMP. *Nucleic Acids Res.* 20:1871–78
3. Blake WL, Clarke SD. 1990. Suppression of hepatic fatty acid synthase and S14 gene transcription by dietary polyunsaturated fat. *J. Nutr.* 120:1727–29
4. Brun T, Roche E, Kim K-H, Prentki M. 1993. Glucose regulates acetyl-CoA gene expression in a pancreatic beta-cell line (INS). *J. Biol. Chem.* 268:18905–11
5. Chen L, Alam T, Johnson JH, Hughes S, Newgard CB, Unger RH. 1990. Regulation of β-cell glucose transporter gene expression. *Proc. Natl. Acad. Sci. USA* 87:4088–92
6. Clarke SD, Abraham S. 1992. Gene expression: nutrient control of pre- and post-transcriptional events. *FASEB J.* 6:3146–52
7. Clarke SD, Jump DB. 1994. Dietary polyunsaturated fatty acid regulation of gene transcription. *Annu. Rev. Nutr.* 14:83–98
8. Clarke SD, Jump DB. 1996. Polyunsaturated fatty acid regulation of hepatic gene transcription. *J. Nutr.* 126:1105–9S
9. Clarke SD, Romsos DR, Leveille GA. 1976. Specific inhibition of hepatic fatty acid synthesis exerted by dietary linoleate and linolenate in essential fattty acid adequate rats. *Lipids* 11:485–90
10. Colosia AD, Marker AJ, Lange AJ, El-Maghrabi MR, Granner DK, et al. 1988. Induction of rat liver 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase mRNA by refeeding and insulin. *J. Biol. Chem.* 263:18669–77
11. Daniel S, Kim K-H. 1996. Sp1 mediates glucose activation of the acetyl-CoA carboxylase promoter. *J. Biol. Chem.* 271: 1385–92
12. Daniel S, Zhang S, DePaoli-Roach AA, Kim K-H. 1996. Dephosphorylation of Sp1 by protein phosphatase 1 is involved in the glucose-mediated activation of the acetyl-CoA carboxylase gene. *J. Biol. Chem.* 271:14692–97
13. Decaux J-F, Antoine B, Kahn A. 1989. Regulation of the expression of the L-type pyruvate kinase gene in adult rat hepatocytes in primary culture. *J. Biol. Chem.* 264:11584–90
14. Desvergne B, Petty K, Nikodem V. 1991. Functional characterization and receptor binding studies of the malic enzyme thyroid hormone response element. *J. Biol. Chem.* 266:1008–13
15. Diaz Guerra M-JM, Bergot M-O, Martinez A, Cuif M-H, Kahn A, Raymondjean M. 1993. Functional characterization of the L-type pyruvate kinase gene glucose response complex. *Mol. Cell. Biol.* 13:7725–33
16. Doiron B, Cuif M-H, Chen R, Kahn A. 1996. Transcriptional glucose signaling through the glucose response element is mediated by the pentose phosphate pathway. *J. Biol. Chem.* 271:5321–24
17. Doiron B, Cuif M-H, Kahn A, Diaz-Guerra M-J. 1994. Respective roles of glucose, fructose, and insulin in the regulation of the liver-specific pyruvate kinase gene promoter. *J. Biol. Chem.* 269:10213–16
18. Dozin B, Rall JE, Nikodem VM. 1986. Tissue-specific control of rat malic enzyme activity and messenger RNA levels by a high carbohydrate diet. *Proc. Natl. Acad. Sci. USA* 83:4705–9
19. Elshourbagy NA, Near JC, Kmetz PJ, Sathe GM, Southan C, et al. 1990. Rat ATP citrate lyase: molecular cloning and sequence analysis of a full-length cDNA and mRNA abundance as a function of diet, organ, and age. *J. Biol. Chem.* 265:1430–35

20. Ferrer J, Gomis R, Alverez JF, Casamitjana R, Vilardell E. 1993. Signals derived from glucose metabolism are required for glucose regulation of pancreatic islet GLUT2 mRNA and protein. *Diabetes* 42:1273–80
21. Foufelle F, Girard J, Ferre P. 1996. Regulation of lipogenic enzyme expression by glucose in liver and adipose tissue: Is glucose-6-phosphate the signalling molecule? *Biochem. Soc. Trans.* 24:372–78
22. Foufelle F, Gouhot B, Pegorier J-P, Perdereau D, Girard J, Ferre P. 1992. Glucose stimulation of lipogenic enzyme gene expression in cultured white adipose tissue. *J. Biol. Chem.* 267:20543–46
23. Foulkes NS, Borrelli E, Sassone-Corsi P. 1991. CREM gene: Use of alternative DNA-binding domains generates multiple antagonists of cAMP-induced transcription. *Cell* 64:739–49
24. Freake HC, Oppenheimer JH. 1987. Stimulation of the S14 mRNA and lipogenesis in brown fat by hypothyroidism, cold exposure, and cafeteria feeding: evidence supporting a general role for S14 in lipogenesis and lipogenesis in the maintenance of thermogenesis. *Proc. Natl. Acad. Sci. USA* 84:3070–74
25. Fukuda H, Katsurada A, Iritani N. 1992. Nutritional and hormonal regulation of mRNA levels of lipogenic enzymes in primary cultures of rat hepatocytes. *J. Biochem.* 111:25–30
26. Funahashi T, Giannoni G, DePaoli AM, Sharosi SF, Davidson NO. 1995. Tissue-specific, developmental and nutritional regulation of the gene encoding the catalytic subunit of the rat apolipoprotein B mRNA editing enzyme: functional role in the modulation of apoB mRNA editing. *J. Lipid Res.* 36:414–28
27. Gearing KL, Gottlicher M, Widmark E, Banner CD, Tollet P, et al. 1994. Fatty acid activation of the peroxisome proliferator activated receptor, a member of the nuclear receptor gene superfamily. *J. Nutr.* 124:1284–88S
28. Gehnrich SC, Gekakis N, Sul HS. 1988. Liver (B-type) phosphofructokinase mRNA: cloning, structure, and expression. *J. Biol. Chem.* 263:11755–59
29. German MS. 1993. Glucose sensing in pancreatic islet beta cells: the key role of glucokinase and the glycolytic intermediates. *Proc. Natl. Acad. Sci. USA* 90:1781–85
30. German MS, Wang J. 1994. The insulin gene contains multiple transcriptional elements that respond to glucose. *Mol. Cell. Biol.* 14:4067–75
31. Girard J, Perdereau D, Foufelle F, Prip-Buus C, Ferre P. 1994. Regulation of lipogenic enzyme gene expression by nutrients and hormones. *FASEB J.* 8:36–42
32. Gonzalez GA, Montminy MR. 1989. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* 59:675–80
33. Goodison S, Kenna S, Ashcroft SJH. 1992. Control of insulin gene expression by glucose. *Biochem. J.* 285:563–68
34. Goodridge A. 1987. Dietary regulation of gene expression: enzymes involved in carbohydrate and lipid metabolism. *Annu. Rev. Nutr.* 7:157–85
35. Hardie DG. 1989. Regulation of fatty acid synthesis via phosphorylation of acetyl-CoA carboxylase. *Prog. Lipid Res.* 28:117–46
36. Hillgartner FB, Salati LM, Goodridge AG. 1995. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. *Phys. Rev.* 75:47–76
37. Inoue H, Noguchi T, Tanaka T. 1984. Rapid regulation of L-type pyruvate kinase mRNA by fructose in diabetic rat liver. *J. Biochem.* 96:1457–62
38. Iritani N. 1992. Nutritional and hormonal regulation of lipogenic-enzyme gene expression in rat liver. *Eur. J. Biochem.* 205:433–42
39. Iynedjian PB, Jotterand D, Nouspikel T, Asfari M, Pilot PR. 1989. Transcriptional induction of glucokinase gene by insulin in cultured liver cells and its repression by the glucagon-cAMP system. *J. Biol. Chem.* 264:21824–29
40. Iynedjian PB, Ucla C, Mach B. 1987. Molecular cloning of glucokinase cDNA: developmental and dietary regulation of glucokinase mRNA in rat liver. *J. Biol. Chem.* 262:6032–38
41. Jacoby DB, Zilz ND, Towle HC. 1989. Sequences within the 5′-flanking region of the S_{14} gene confer responsiveness to glucose in primary hepatocytes. *J. Biol. Chem.* 264:17623–26
42. Jump DB, Bell A, Lepar G, Hu D. 1990. Insulin rapidly induces rat liver S14 gene transcription. *Mol. Endocrinol.* 4:1655–60
43. Jump DB, Bell A, Santiago V. 1990. Thyroid hormone and dietary carbohydrate interact to regulate rat liver S_{14} gene transcription and chromatin structure. *J. Biol. Chem.* 265:3474–78
44. Jump DB, Clarke SD, MacDougald O, Thelen A. 1993. Polyunsaturated fatty

acids inhibit S14 gene transcription in rat liver and cultured hepatocytes. *Proc. Natl. Acad. Sci. USA* 90:8454–58

45. Jump DB, Narayan P, Towle HC, Oppenheimer JH. 1984. Rapid effects of triiodothyronine on hepatic gene expression. Hybridization analysis of tissue-specific triiodothyronine regulation of $mRNA_{S14}$. *J. Biol. Chem.* 259:2789–97
46. Jump DB, Oppenheimer JH. 1985. High basal expression and 3,5,3′-triiodothyronine regulation of messenger ribonucleic acid S14 in lipogenic tissues. *Endocrinology* 117:2259–66
47. Kadesch T. 1993. Consequences of heteromeric interactions among helix-loop-helix proteins. *Cell Growth Differ.* 4:49–55
48. Kahn CR, Lauris V, Koch S, Crettaz M, Granner DK. 1989. Acute and chronic regulation of phosphoenolpyruvate carboxykinase mRNA by insulin and glucose. *Mol. Endocrinol.* 3:840–45
49. Katsurada A, Iritani N, Fukuda H, Matsumura Y, Nishimoto N, et al. 1990. Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of acetyl-CoA carboxylase in rat liver. *Eur. J. Biochem.* 190:435–41
50. Katsurada A, Iritani N, Fukuda H, Matsumura Y, Noguchi T, Tanaka T. 1989. Effects of insulin and fructose on transcriptional and post-transcriptional regulation of malic enzyme synthesis in diabetic rat liver. *Biochem. Biophys. Acta* 1004:103–7
51. Katsurada A, Iritani N, Fukuda H, Matsumura Y, Nishimoto N, et al. 1990. Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of fatty acid synthase in rat liver. *Eur. J. Biochem.* 190:427–33
52. Katz NR, Giffhorn S. 1983. Glucose- and insulin-dependent induction of ATP citrate lyase in primary cultures of rat hepatocytes. *Biochem. J.* 212:65–71

52a. Kaytor EN, Shih H-M, Towle HC. 1997. Carbohydrate regulation of hepatic gene expression: evidence against a role for the upstream stimulatory factor. *J. Biol. Chem.* 272:7525–31

53. Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, Wahli W. 1993. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc. Natl. Acad. Sci. USA* 90:2160–64
54. Keller H, Mahfoudi A, Dreyer C, Hihi AK, Medin J, et al. 1993. Peroxisome proliferator-activated receptors and lipid metabolism. *Ann. NY Acad. Sci.* 684:157–73
55. Kim K-H, Lopez-Casillas F, Bai D-H, Luo X, Pape ME. 1989. Role of reversible phosphorylation of acetyl-CoA carboxylase in long chain fatty acid synthesis. *FASEB J.* 3:2250–56
56. Kim K-H, Tae H-J. 1994. Pattern and regulation of acetyl-CoA carboxylase gene expression. *J. Nutr.* 124:1273–83S
57. Kim K-S, Park S-W, Kim Y-S. 1992. Regulation of ATP-citrate lyase at transcriptional and post-transcriptional levels in rat liver. *Biochem. Biophys. Res. Commun.* 189:264–71
58. Kinlaw WB, Church JL, Harmon J, Mariash CN. 1995. Direct evidence for a role of the "spot 14" protein in the regulation of lipid synthesis. *J. Biol. Chem.* 270:16615–18
59. Kinlaw WB, Schwartz HL, Towle HC, Oppenheimer JH. 1986. Opposing effects of glucagon and triiodothyronine on the hepatic levels of messenger ribonucleic acid S14 and the dependence of such effects on circadian factors. *J. Clin. Invest.* 78:1091–96
60. Kinlaw WB, Tron P, Friedmann AS. 1992. Nuclear localization and hepatic zonation of rat spot 14 protein: immunohistochemical investigation employing antifusion protein antibodies. *Endocrinology* 131:3120–22
61. Kinlaw WB, Tron P, Witters LA. 1993. Thyroid hormone and dietary carbohydrate induce different hepatic zonation of both "spot 14" and acetyl-coenzyme-A carboxylase: a novel mechanism of coregulation. *Endocrinology* 133:645–50
62. Landschulz KT, Jump DB, MacDougald OA, Lane MD. 1994. Transcriptional control of the stearoyl-CoA desaturase-1 gene by polyunsaturated fatty acids. *Biochem. Biophys. Res. Commun.* 200:763–68
63. Lefrancois-Martinez A-M, Diaz-Guerra M-JM, Vallet V, Kahn A, Antoine B. 1994. Glucose-dependent regulation of the L-pyruvate kinase gene in a hepatoma cell line is independent of insulin and cyclic AMP. *FASEB J.* 8:89–96
64. Lefrancois-Martinez A-M, Martinez A, Antoine B, Raymondjean M, Kahn A. 1995. Upstream stimulatory factor proteins are major components of the glucose response complex of the L-type pyruvate kinase gene promoter. *J. Biol. Chem.* 270:2640–43
65. Lemaigre FP, Ace CI, Green MR. 1993. The cAMP response element binding protein, CREB, is a potent inhibitor of

diverse transcriptional activators. *Nucl. Acids Res.* 21:2907–11
66. Lemaigre FP, Rousseau GG. 1994. Transcriptional control of genes that regulate glycolysis and gluconeogenesis in adult liver. *Biochem. J.* 303:1–14
67. Liaw C, Seelig S, Mariash CN, Oppenheimer JH, Towle HC. 1983. Interactions of thyroid hormone, growth hormone, and high carbohydrate, fat-free diet in regulating several rat liver messenger ribonucleic acid species. *Biochemistry* 22:213–21
68. Liimatta M, Towle HC, Clarke S, Jump DB. 1994. Dietary polyunsaturated fatty acids interfere with the insulin/glucose activation of L-type pyruvate kinase gene transcription. *Mol. Endocrinol.* 8:1147–53
69. Liu H-C, Towle HC. 1994. Functional synergism between multiple thyroid hormone response elements regulates hepatic expression of the rat S14 gene. *Mol. Endocrinol.* 8:1021–37
70. Liu Z, Thompson KS, Towle HC. 1993. Carbohydrate regulation of the rat L-type pyruvate kinase gene requires two nuclear factors: LF-A1 and a member of the c-myc family. *J. Biol. Chem.* 268:12787–95
71. Luo X, Park K, Lopez-Casillas F, Kim K-H. 1989. Structural features of the acetyl-CoA carboxylase gene: mechanisms for the generation of mRNAs with 5′ end heterogeneity. *Proc. Natl. Acad. Sci. USA* 86:4042–46
72. MacDonald MJ, Kaysen JH, Moran SM, Pomije CE. 1991. Pyruvate dehydrogenase and pyruvate carboxylase. Sites of pretranslational regulation by glucose of glucose-induced insulin release in pancreatic islets. *J. Biol. Chem.* 266:22393–97
73. MacFarlane WM, Read ML, Gilligan M, Bujalska I, Docherty K. 1994. Glucose modulates the binding activity of the β-cell transcription factor IUF1 in a phosphorylation-dependent manner. *Biochem. J.* 303:625–31
74. Mariash CN, Jump DB, Oppenheimer JH. 1984. T3 stimulates the synthesis of a specific mRNA in primary hepatocyte culture. *Biochem. Biophys. Res. Commun.* 123:1122–29
75. Mariash CN, Kaiser FE, Schwartz HL, Towle HC, Oppenheimer JH. 1980. Synergism of thyroid hormone and high carbohydrate diet in the induction of lipogenic enzymes in the rat. *J. Clin. Invest.* 65:1126–34
76. Mariash CN, McSwigan CR, Towle HC, Schwartz HL, Oppenheimer JH. 1981. Glucose and triiodothyronine both induce malic enzyme in the rat hepatocyte culture. *J. Clin. Invest.* 68:1485–90
77. Mariash CN, Oppenheimer JH. 1983. Stimulation of malic enzyme formation in hepatocyte culture by metabolites: evidence favoring a nonglycolytic metabolite as the proximate induction signal. *Metabolism* 33:545–52
78. Mariash CN, Seelig S, Schwartz HL, Oppenheimer JH. 1986. Rapid synergistic interaction between thyroid hormone and carbohydrate on $mRNA_{S14}$ induction. *J. Biol. Chem.* 261:9583–86
79. Marie S, Diaz-Guerra M-J, Miquerol L, Kahn A, Iynedjian PB. 1993. The pyruvate kinase gene as a model for studies of glucose-dependent regulation of gene expression in the endocrine pancreatic beta-cell type. *J. Biol. Chem.* 268:23881–90
80. Massillon D, Barzilai N, Chen W, Hu M, Rossetti L. 1996. Glucose regulates in vivo glucose-6-phosphatase gene expression in the liver of diabetic rats. *J. Biol. Chem.* 271:9871–74
81. Matschinsky FM. 1996. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes* 45:223–41
82. Melloul D, Ben-Neriah Y, Cerasi E. 1993. Glucose modulates the binding of an islet-specific factor to a conserved sequence within the rat I and the human promoters. *Proc. Natl. Acad. Sci. USA* 90:3865–69
83. Miksicek RJ, Towle HC. 1983. Use of a cloned cDNA sequence to measure changes in 6-phosphogluconate dehydrogenase mRNA levels caused by thyroid hormone and dietary carbohydrate. *J. Biol. Chem.* 258:9575–79
84. Narayan P, Liaw CW, Towle HC. 1984. Rapid induction of a specific nuclear mRNA precursor by thyroid hormone. *Proc. Natl. Acad. Sci. USA* 81:4687–91
85. Naya FJ, Stellrecht CM, Tsai M-J. 1995. Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. *Genes Dev.* 9:1009–19
86. Nelson C, Shen L-P, Meister A, Fodor E, Rutter WJ. 1990. Pan: a transcriptional regulator that binds chymotrypsin, insulin, and AP-4 enhancer motifs. *Genes Dev.* 4:1035–43
87. Newgard CB, McGarry JD. 1995. Metabolic coupling factors in pancreatic β-cell signal transduction. *Annu. Rev. Biochem.* 64:689–719
88. Nielsen SA, Welsh M, Casadaban MJ, Steiner DF. 1985. Control of insulin gene expression in pancreatic β cells and in an insulin-producing cell line, RIN 5F

cells. I. Effects of glucose and cyclic AMP on the transcription of insulin mRNA. *J. Biol. Chem.* 260:13585–89
89. Nishimura M, Fedorov S, Uyeda K. 1994. Glucose-stimulated synthesis of fructose 2,6-bisphosphate in rat liver. *J. Biol. Chem.* 269:26100–6
90. Nishimura M, Uyeda K. 1995. Purification and characterization of a novel xylulose 5-phosphate-activated protein phosphatase catalyzing dephosphorylation of fructose-6-phosphate, 2-kinase: fructose-2,6-bisphosphatase. *J. Biol. Chem.* 270:26341–46
91. Noguchi T, Inoue H, Chen H-L, Matsubara K, Tanaka T. 1983. Molecular cloning of DNA complementary to rat L-type pyruvate kinase mRNA. *J. Biol. Chem.* 258:15220–23
92. Noguchi T, Inoue H, Tanaka T. 1985. Transcriptional and post-transcriptional regulation of L-type pyruvate kinase in diabetic rat liver by insulin and dietary fructose. *J. Biol. Chem.* 260:14393–97
93. Ntambi J. 1992. Dietary regulation of stearoyl-CoA desaturase 1 gene expression in mouse liver. *J. Biol. Chem.* 267:10925–30
94. O'Brien RM, Granner DK. 1991. Regulation of gene expression by insulin. *Biochem. J.* 278:609–19
95. Ohlsson H, Karlsson K, Edlund T. 1993. IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO J.* 12:4251–59
96. Pape ME, Lopez-Casillas F, Kim K-H. 1988. Physiological regulation of acetyl-CoA carboxylase gene expression: effects of diet, diabetes, and lactation on acetyl-CoA carboxylase mRNA. *Arch. Biochem. Biophys.* 267:104–9
97. Paulauskis JD, Sul HS. 1988. Cloning and expression of mouse fatty acid synthase and other specific mRNAs: developmental and hormonal regulation in 3T3-L1 cells. *J. Biol. Chem.* 263:7049–54
98. Paulauskis JD, Sul HS. 1989. Hormonal regulation of mouse fatty acid synthase gene transcription in liver. *J. Biol. Chem.* 264:574–77
99. Perez-Castillo A, Schwartz HL, Oppenheimer JH. 1987. Rat hepatic mRNA-S14 and lipogenic enzymes during weaning: role of S14 in lipogenesis. *Am. J. Physiol.* 253:E536-42
100. Pilkis SJ, Claus TH. 1991. Hepatic gluconeogenesis/glycolysis: regulation and structure/function relationships of substate cycle enzymes. *Annu. Rev. Nutr.* 11:465–515
101. Potter JJ, Cheneval D, Dang CV, Resar LMS, Mezey E, Yang VW. 1991. The upstream stimulatory factor binds to and activates the promoter of the rat class I alcohol dehydrogenase gene. *J. Biol. Chem.* 266:15457–63
102. Prostko CR, Fritz RS, Kletzian RF. 1989. Nutritional regulation of hepatic glucose-6-phosphate dehydrogenase. Transient activation of transcription. *Biochem. J.* 258:295–99
103. Ren B, Thelen A, Jump DB. 1996. Peroxisome proliferator-activated receptor α inhibits hepatic S14 gene transcription. Evidence against the peroxisome proliferator-activated receptor α as the mediator of polyunsaturated fatty acid regulation of S14 gene transcription. *J. Biol. Chem.* 271:17167–73
104. Rencurel F, Waeber G, Antoine B, Rocchiccioli F, Maulard P, Girard J. 1996. Requirement of glucose metabolism for regulation of glucose transporter type 2 (GLUT2) gene expression in liver. *Biochem. J.* 314:903–9
105. Rongnoparut P, Verdon CP, Gehnrich SC, Sul HS. 1991. Isolation and characterization of the transcriptionally regulated mouse liver (B-type) phosphofructokinase gene and its promoter. *J. Biol. Chem.* 266:8086–91
106. Sanchez HB, Yieh L, Osborne TF. 1995. Cooperation by sterol regulatory element-binding protein and Sp1 in sterol regulation of low density lipoprotein receptor gene. *J. Biol. Chem.* 270:1161–69
107. Semenkovich CF, Coleman T, Gofarth R. 1993. Physiologic concentrations of glucose regulate fatty acid synthase mRNA stability. *J. Biol. Chem.* 268:6961–70
108. Sharma A, Stein R. 1994. Glucose-induced transcription of the insulin gene is mediated by factors required for β-cell-type-specific expression. *Mol. Cell. Biol.* 14:871–79
109. Shih H-M, Liu Z, Towle HC. 1995. Two CACGTG motifs with proper spacing dictate the carbohydrate regulation of hepatic gene transcription. *J. Biol. Chem.* 270:21991–97
110. Shih H-M, Towle HC. 1992. Definition of the carbohydrate response element of the rat S14 gene: evidence for a common factor required for carbohydrate regulation of hepatic genes. *J. Biol. Chem.* 267:13222–28
111. Shih H-M, Towle HC. 1994. Definition of the carbohydrate response element of the rat S14 gene: context of the CACGTG motif determines the specificity of carbohydrate regulation. *J. Biol. Chem.* 269:9380–87

112. Shin D-H, Paulauskis JD, Moustaid N, Sul HS. 1991. Transcriptional regulation of p90 with sequence homology to *Escherichia coli* glycerol-3-phosphate acyltransferase. *J. Biol. Chem.* 266:23834–39
113. Sirito M, Lin Q, Maity T, Sawadogo M. 1994. Ubiquitous expression of the 43- and 44-kDa forms of transcription factor USF in mammalian cells. *Nucleic Acids Res.* 22:427–33
114. Sniderman AD, Cianflone K. 1993. Substrate delivery as a determinant of hepatic apoB secretion. *Arterioscler. Thromb.* 13:629–36
115. Spence J, Pitot HC. 1982. Induction of lipogenic enzymes in primary cultures of rat hepatocytes. *Eur. J. Biochem.* 128:15–20
116. Spence JT. 1983. Levels of translatable mRNA coding for rat liver glucokinase. *J. Biol. Chem.* 258:9143–46
117. Stabile LP, Hodge DL, Klautky SA, Salati LM. 1996. Posttranscriptional regulation of glucose-6-phosphate dehydrogenase by dietary polyunsaturated fat. *Arch. Biochem. Biophys.* 332:269–79
118. Sudo Y, Mariash CN. 1993. The thyroid hormone receptor can unmask a glucose response in the S14 gene. *Endocrinology* 133:129–34
119. Sudo Y, Mariash CN. 1994. Two glucose-signaling pathways in S14 gene transcription in primary hepatocytes: a common role of protein phosphorylation. *Endocrinology* 134:2532–40
120. Suzuki H, Kawarabayasi Y, Kondo J, Abe T, Nishikawa K, et al. 1990. Structure and regulation of rat long-chain acyl-CoA synthetase. *J. Biol. Chem.* 265:8681–85
121. Takeda Y, Inoue H, Honjo K, Tanioka H, Daikuhara Y. 1967. Dietary response of various key enzymes related to glucose metabolism in normal and diabetic rat liver. *Biochim. Biophys. Acta* 136:214–22
122. Thiede MA, Strittmatter P. 1985. The induction and characterization of rat liver stearyl-CoA desaturase mRNA. *J. Biol. Chem.* 260:14459–63
123. Thompson KS, Towle HC. 1991. Localization of the carbohydrate response element of the rat L-type pyruvate kinase gene. *J. Biol. Chem.* 266:8679–82
124. Thorens B, Flier JS, Lodish HF, Kahn BB. 1990. Differential regulation of two glucose transporters in rat liver by fasting and refeeding and by diabetes and insulin treatment. *Diabetes* 39:712–19
125. Towle HC. 1995. Metabolic regulation of gene transcription in mammals. *J. Biol. Chem.* 270:23235–38
126. Towle HC, Mariash CN. 1986. Regulation of hepatic gene expression by lipogenic diet and thyroid hormone. *Fed. Proc.* 45:2406–11
127. Van den Berghe G. 1978. In *Metabolic Effects of Fructose in the Liver*, ed. BL Horecker, ER Stadtman, pp. 97–135. New York: Academic
128. Van Schaftingen E. 1994. Short-term regulation of glucokinase. *Diabetologia* 37(Suppl. 2):S43–47
129. Vaulont S, Kahn A. 1994. Transcriptional control of metabolic regulation genes by carbohydrates. *FASEB J.* 8:28–35
130. Vaulont S, Munnich A, Decaux JF, Kahn A. 1986. Transcriptional and post-transcriptional regulation of L-type pyruvate kinase gene expression in rat liver. *J. Biol. Chem.* 261:7621–25
131. Vaulont S, Munnich A, Marie J, Reach G, Pichard A-L, et al. 1984. Cyclic AMP as a transcriptional inhibitor of upper eukaryotic gene transcription. *Biochem. Biophys. Res. Commun.* 125:135–41
132. Vaulont S, Puzenat N, Levrat F, Cognet M, Kahn A, Raymondjean M. 1989. Proteins binding to the liver-specific pyruvate kinase gene promoter: a unique combination of known factors. *J. Mol. Biol.* 209:205–19
133. Volpe JJ, Vagelos PR. 1974. Regulation of mammalian fatty-acid synthetase: the roles of carbohydrate and insulin. *Proc. Natl. Acad. Sci. USA* 71:889–93
134. Wang D, Sul HS. 1995. Upstream stimulatory factors bind to insulin response sequence of the fatty acid synthase promoter. *J. Biol. Chem.* 270:28716–22
135. Weber A, Marie J, Cottreau C, Simon M-P, Besmond C, et al. 1984. Dietary control of aldolase B and L-type pyruvate kinase mRNAs in rat. *J. Biol. Chem.* 259:1798–802