REVIEW ARTICLE

New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c

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The regulation of hepatic glucose metabolism has a key role in whole-body energy metabolism, since the liver is able to store (glycogen synthesis, lipogenesis) and to produce (glycogenolysis, gluconeogenesis) glucose. These pathways are regulated at several levels, including a transcriptional level, since many of the metabolism-related genes are expressed according to the quantity and quality of nutrients. Recent advances have been made in the understanding of the regulation of hepatic glycolytic, lipogenic and gluconeogenic gene expression by pancreatic hormones, insulin and glucagon and glucose. Here we review the role of the transcription factors forkhead and sterol regulatory element binding protein-1c in the inductive and repressive effects of insulin on hepatic gene expression, and the pathway that leads from glucose to gene regulation with the recently discovered carbohydrate response element binding protein. We discuss how these transcription factors are integrated in a regulatory network that allows a fine tuning of hepatic glucose storage or production, and their potential importance in metabolic diseases.

Key words: carbohydrate response element binding protein, forkhead, glucokinase, gluconeogenesis, SREBP.

INTRODUCTION

Living organisms must continuously adapt their metabolism to the nutritional environment, since the quality and quantity of available nutrients does not always temporally match their energetic needs. This is especially true for glucose, since this substrate is used continuously at a high rate in mammals by organs such as the brain (120 g/day in humans), red blood cells and renal medulla. When a meal that contains carbohydrate is absorbed, it induces several metabolic events aimed at decreasing endogenous glucose production by the liver (glycogenolysis and gluconeogenesis) and increasing glucose uptake and storage in the form of glycogen in the liver and muscle. If glucose is delivered into the portal vein in large quantities, and once the hepatic glycogen stores are repleted, glucose can be converted in the liver into lipids (lipogenesis) which are exported as very-low-density lipoproteins and stored ultimately as triacylglycerols in adipose tissue. If the liver is overloaded with glucose, a hepatic steatosis can develop together with a high glycogen content, and this characteristic is exploited by farmers in ducks and geese to provide fatty liver ('foie gras'). Conversely, if glucose availability in the diet is reduced, glucose-utilizing pathways are inhibited and glucose-producing pathways are activated. The regulation of metabolic pathways involves the rapid modulation of the activity of specific proteins (enzymes, transporters), but also, on a longer-term basis, changes in their quantities. This can be achieved by modulating their transcription rate or post-transcriptional steps such as mRNA half-life and translation efficiency.

In the present review we address the transcriptional regulation of hepatic glycolytic and lipogenic genes by insulin and glucose. Indeed, at the present time this class of genes allows the most comprehensive view of the response of the genetic program to the nutritional environment, since it involves both hormone- and nutrient-dependent mechanisms. In addition, important progress has recently been made in the understanding of the cellular and molecular mechanisms involved.

GLUCOSE UTILIZATION AND PRODUCTION IN THE LIVER

Glucose entry into the hepatocyte is mediated by a glucose transporter, GLUT2 (facilitated diffusion), which has a K_m in the 10 mM range and is present constitutively in the plasma membrane [1]. Thus, after a meal rich in carbohydrates, the increase in the portal vein glucose concentration (10–15 mM) will result in a proportional increase in glucose influx into hepatocytes. Glucose will be then phosphorylated by glucokinase (GK; hexokinase IV), which, in contrast with other hexokinases, is not inhibited by glucose 6-phosphate, the product of the reaction. Hepatic GK is regulated by a 68 kDa regulatory protein, glucokinase regulatory protein (GKRP) [2,3]. GKRP is located mainly in the nucleus of hepatocytes, whereas GK translocates between the nucleus and the cytoplasm. GK binds GKRP in the nucleus at low glucose concentrations, and is translocated to the cytoplasm at elevated plasma glucose or fructose concentrations [4]. The K_m for glucose in this system (GK/GKRP) is in

Abbreviations used: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; bHLH-LZ, basic domain helix–loop–helix leucine zipper; CHO, Chinese-hamster ovary; ChoRE, carbohydrate response element; ChREBP, carbohydrate response element binding protein; DN-SREBP-1c, dominant negative form of SREBP-1c; DP-SREBP-1c, dominant positive form of SREBP-1c; EMSA, electrophoretic mobility shift assay; ER, endoplasmic reticulum; FAS, fatty acid synthase; FKHR, forkhead-related protein; GK, glucokinase; GKRP, glucokinase regulatory protein; Glc-6-Pase, glucose-6-phosphatase; GlRE, glucose response element; IGFBP-1, insulin-like growth factor binding protein-1; IRE, insulin response element; IRS, insulin receptor substrate; L-PK, liver pyruvate kinase; LXR, liver X receptor; MAP kinase, mitogen-activated protein kinase; PEPCK, phosphoenolpyruvate carboxykinase; PI 3-kinase, phosphoinositide 3-kinase; PKA, protein kinase A; PKB, protein kinase B; SCAP, SREBP cleavage activating protein; Snf1, sucrose non-fermenting; S1P/S2P, site-1/site-2 protease; SRE, sterol regulatory element; SREBP, sterol regulatory element binding protein; TAT, tyrosine aminotransferase; USF, upstream stimulatory factor.

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the 15–20 mM range. The kinetic characteristics of the glucose transport and phosphorylation steps in hepatocytes imply that the rate of phosphorylation of glucose is proportional to its plasma concentration. Glucose 6-phosphate can then enter several pathways: glycogen synthesis, glycolysis and the pentose-phosphate pathway. Interestingly enough, in the liver, the major function of glycolysis may be to provide pyruvate not for oxidative purposes, but rather for *de novo* lipogenesis. When glucose is the main substrate used for fatty acid synthesis, the enzymes of the glycolytic pathway can then be considered as an extended part of the lipogenic pathway. The pentose-phosphate pathway is also directly related to lipogenesis, since it can provide the NADPH necessary for the final synthesis of acyl-CoA in the reaction catalysed by fatty acid synthase (FAS).

During fasting, or if the carbohydrate content of the diet is low, glucose will be produced by the liver and glucose utilization is then inhibited. Glucose production arises first from the glycogen stores (a maximum of 70–80 g in a human individual) through a pathway called glycogenolysis, which is tightly regulated through successive cascades of enzyme phosphorylation [5]. Then, when the glycogen stores are depleted, glucose is produced *de novo* from precursors such as lactate, alanine or glycerol through a pathway called gluconeogenesis, which utilizes some of the reversible enzymic steps of glycolysis, but also has specific steps catalysed by pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), frucose-1,6-bisphosphatase and glucose-6-phosphatase (Glc-6-Pase) [6].

Regulation of the activity of the enzymes of glucose utilization and production involves both short-term (e.g. dissociation in the presence of glucose of GK from its nuclear docking protein and translocation into the cytoplasm) and long-term mechanisms. We will consider here only the latter. The mechanisms involved in the short-term regulation of liver metabolism have been described in detail in several reviews, e.g. [5–8].

EXPRESSION OF GLYCOLYTIC/LIPOGENIC AND GLUCONEOGENIC GENES IS REGULATED BY CARBOHYDRATE AVAILABILITY IN THE DIET

The expression of several key glycolytic and lipogenic enzymes in the liver is induced by a high-carbohydrate diet (Scheme 1): GK [9], 6-phosphofructo-1-kinase [10], 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase [11], aldolase B [12] and liver pyruvate kinase (L-PK) [13] for glycolysis; ATP citrate-lyase [14], acetyl-CoA carboxylase (ACC) [15], FAS [16,17] and stearoyl-CoA desaturase [18] for lipogenesis; and glucose-6-phosphate dehydrogenase [19,20] and 6-phosphogluconate dehydrogenase [21] for the pentose-phosphate pathway. We should also mention here the induction of the *S14* gene, which encodes a small acidic polypeptide that seems to be related to lipogenesis. S14 is expressed in lipogenic tissues (white and brown adipose tissue, liver, lactating mammary gland) and shares a similar regulation with genes involved in the lipogenic pathway [22–24]. The specific function of the S14 protein remains unclear, although some studies have suggested that S14 may be involved itself in the machinery that regulates lipogenic enzyme expression [25,26]. For most of these genes involved in glucose carbon utilization, the induction of their mRNA expression by a carbohydrate-rich diet is powerful (4–25-fold), rapid (in the 1–2 h range) and involves a transcriptional mechanism.

Conversely, a high-carbohydrate diet inhibits the expression of gluconeogenic enzymes. This is especially obvious for PEPCK, and one must emphasize that this is the only means of regulating the activity of this important enzyme, since there is no post-translational modification of the protein [27]. The inhibition is rapid, but also easily reversible once carbohydrate availability decreases.

Absorption of carbohydrate in the diet is concomitant with increases in the concentrations of substrates such as glucose and lactate, but also with changes in the concentrations of the pancreatic hormones insulin and glucagon. Differentiating between the respective roles of hormones and substrates with regard to the regulation of gene expression is not easy in *in vivo* experiments. *In vivo*, the classical experimental protocol for investigating the involvement of insulin is to study diabetic animals injected with insulin. However, since insulin can also increase substrate metabolism independently of its potential effects on genes, even this experiment is not totally conclusive. Unravelling the mechanisms by which hormones and substrates can modulate gene transcription has thus benefited greatly from *in vitro* studies on either primary cultured hepatocytes or cell lines. From these studies, different kinds of gene regulation have emerged (Scheme 1); for example, the transcription of the GK gene can be up-regulated in the presence of a high insulin concentration [28]. The genes encoding L-PK, FAS, ACC, S14 and stearoyl-CoA desaturase require both increased insulin and glucose concentrations in order to be induced [29–33]. Finally, PEPCK expression can be down-regulated independently by insulin [34] or glucose [35,36]. Interestingly, the transcription of these genes is also modulated by glucagon in the opposite direction compared with insulin. We will address successively the mechanisms by which insulin and glucose can control gene expression.

INSULIN REGULATION OF HEPATIC GENE EXPRESSION

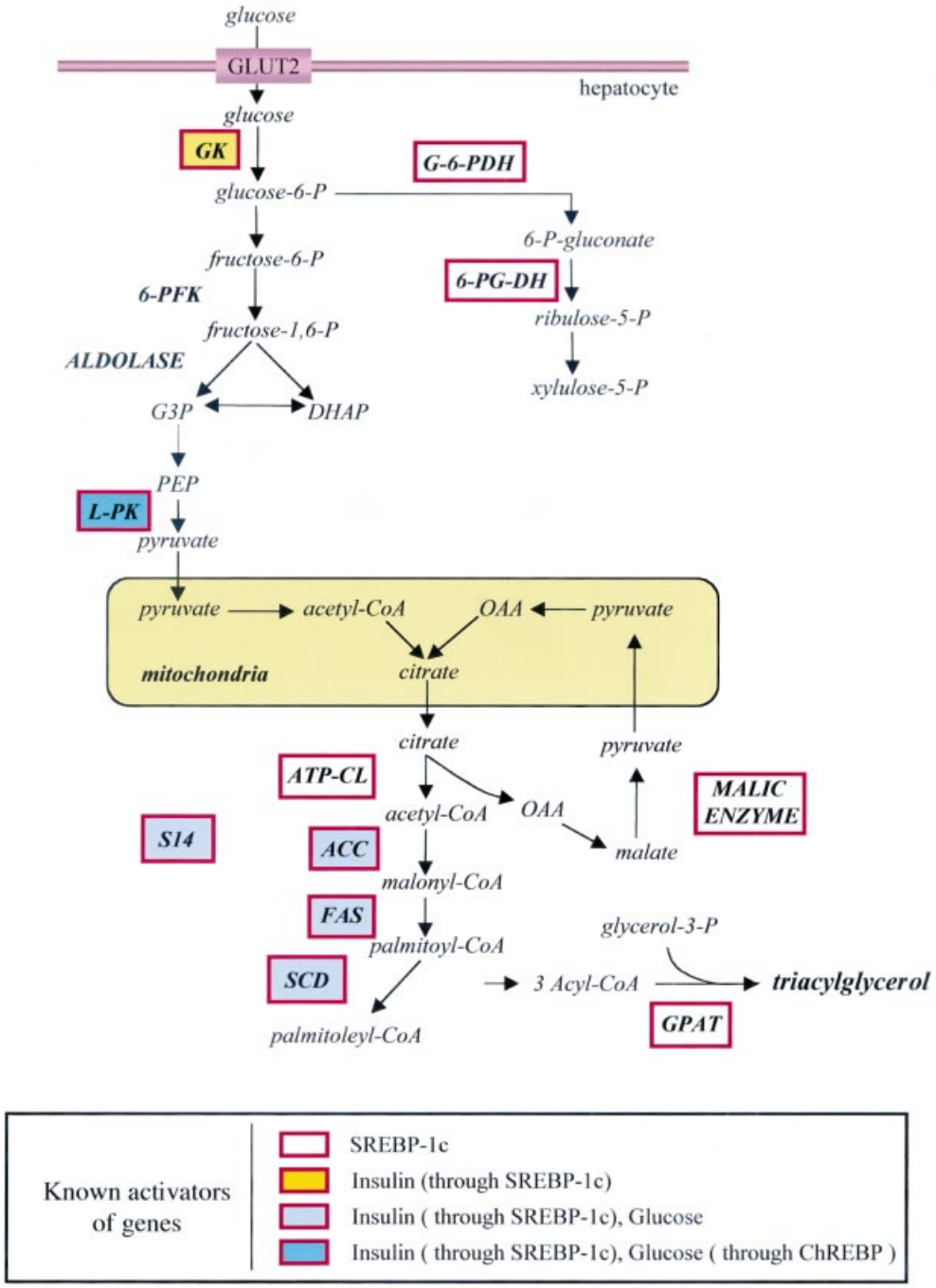
Insulin is known to modulate the expression of over 100 genes at the transcriptional level in mammals. The transcriptional effects of insulin are widespread and concern multiple biological phenomena [37,38]. In the liver, the transcription of most of the genes encoding metabolic enzymes is induced by insulin. The genes that are inhibited by insulin are limited, and encode mainly enzymes involved in hepatic glucose production [37,38].

In the last few years, important progress has been made in the identification of the partners involved in the events following insulin binding to its receptor. In contrast, the factors involved in the transcriptional effects of insulin were, until recently, largely unknown [although insulin response elements (IREs) have been identified in some genes], despite intensive studies on the two well known insulin-responsive genes, namely those encoding GK and PEPCK.

Here we will review recent findings concerning the roles of two members of different classes of transcription factors that have been shown to be involved in the regulation of hepatic metabolic genes by insulin. The first is a member of the forkhead-related protein (FKHR) family of transcription factors and has been implicated in the inhibition of hepatic gene transcription by insulin. The second factor belongs to the family of sterol regulatory element binding proteins (SREBPs; SREBP-1c isoform), and has been mainly associated with the induction of glycolytic and lipogenic gene transcription by insulin, although recent studies indicate that it can also inhibit gluconeogenic gene expression in the liver.

FKHR family of transcription factors

In the liver, insulin inhibits the transcription of PEPCK, Glc-6-Pase, insulin-like growth factor binding protein-1 (IGFBP-1), aspartate aminotransferase, tyrosine aminotransferase (TAT) and apolipoprotein CIII [37,38]. A consensus IRE containing the core motif T(G/A)TTT(TG)(GT) has been identified and associ-



Scheme 1 Glycolytic and lipogenic pathways in the liver

All enzymes indicated in this Scheme are induced at a transcriptional level by a high-carbohydrate diet. Known activators of their transcription are shown at the bottom of the Scheme. Abbreviations used: ATP-CL, ATP citrate-lyase; DHAP, dihydroxyacetone 3-phosphate; G-6-PDH, glucose-6-phosphate dehydrogenase; GPAT, glycerol-phosphate acyltransferase; G3P, glyceraldehyde 3-phosphate; OAA, oxaloacetate; 6-PG-DH, 6-phosphogluconate dehydrogenase; PEP, phosphoenolpyruvate; P, phosphate; 6-PFK, 6-phosphofructo-1-kinase; SCD, stearoyl-CoA desaturase.

ated with the repression by insulin of these genes [39–44]. For the IGFBP-1, PEPCK and TAT genes, the IRE is co-localized with a sequence conferring activation by glucocorticoids [39,41,42]. For most of these genes, the inhibition of transcription by insulin is mediated through a phosphoinositide 3-kinase (PI 3-kinase) pathway [45–47]. The downstream target of PI 3-kinase has been the subject of conflicting reports. Several groups have proposed that protein kinase B (PKB) mediates the inhibition of transcription by insulin [47–49], whereas other authors found that PKB is not required [46,50,51]. A recent study has suggested a role for glycogen synthase kinase 3 in the inhibition by insulin of the PEPCK and Glc-6-Pase genes [52].

The interest in the role of FKHR in the inhibition of gene expression by insulin results from the findings that an insulin-like pathway exists in the nematode *Caenorhabtidis elegans*. This pathway involves the proteins DAF-2, AGE-1, PDK-1 and AKT-1/AKT-2, which represent homologues of, respectively, the insulin receptor, the catalytic subunit of PI 3-kinase, 3-phosphoinositide-dependent kinase-1 and PKB. Genetic studies in *C. elegans* have shown that activation of the PKB pathway leads to the inactivation of transcription factors of the forkhead family named DAF-16 [53]. Three DAF-16 isoforms are produced by differential splicing of the *daf*-16 gene: DAF-16a1, DAF-16a2 and DAF-16b. DAF-16a and DAF-16b proteins possess distinct (although highly related) forkhead-type DNA binding domains, suggesting that they may bind to different DNA response elements. The proteins most closely related to DAF-16 in humans are the three members of the FKHR subclass of the Fox family of transcription factors, namely FKHR, FKHRL1 and AFX. Members of the Fox family are characterized by the presence of a conserved 100-amino-acid DNA binding domain or forkhead domain. FKHR shares the highest identity with DAF-16 (76% in the DNA binding domain). This led to the hypothesis that FKHR might be the target of insulin for the inhibitory effects of the hormone on gene transcription through a PI 3-kinase/PKB pathway.

Numerous studies have shown that FKHR can bind *in vitro* to the IGFBP-1, PEPCK, TAT and Glc-6-Pase IREs [49,54,55]. In cells, overexpression of FKHR proteins leads to the activation of transfected reporter constructs containing the IREs of IGFBP-1, Glc-6-Pase and PEPCK [47,54,56]. The transactivation by FKHR is blocked when the cells are treated with insulin [54,55,57].

The use of chemical inhibitors of the activation of PI-3 kinase (wortmannin and LY 294002) has revealed that insulin phosphorylates FKHR via a PI 3-kinase-dependent pathway. Subsequent studies have demonstrated that FKHR is phosphorylated *in vivo* by insulin on three different phosphorylation sites (Thr-24, Ser-253 and Ser-319) via a PKB-dependent pathway [58–60]. Once FKHR is phosphorylated by PKB, it is dissociated from its DNA binding site and exported outside the nucleus, leading to the inhibition of its target genes [59]. Mutants of FKHR in which Thr-24 and/or Ser-253 have been mutated into an alanine residue are resistant to both phosphorylation by PKB and nuclear export [59].

From these results, a general model has been developed in which insulin activates PKB, resulting in the phosphorylation and nuclear exclusion of the transcriptional activator FKHR, leading in turn to a decrease in gene transcription. However, several workers, using different approaches, have reported that FKHR is probably not implicated in the regulation of all genes that are inhibited by insulin. In a kidney epithelial cell line, expression of PEPCK and Glc-6-Pase is normally induced by glucocorticoids and cAMP, but inhibition by insulin does not occur. Adenoviral expression of one FKHR isoform (FOXO 1) in these cells is sufficient to restore insulin inhibition of Glc-6-Pase, but has no effect on the regulation of the PEPCK promoter by insulin [61]. Granner and colleagues [56] have shown that FKHR mediates the transactivation of the PEPCK and IGFBP-1 promoters and inhibition by insulin, but only when it is overexpressed in cells. At physiological levels, FKHR cannot account for this inhibition, suggesting that other proteins are involved in the response to insulin of these two genes [56]. In the same way, Yeagley and colleagues [62] have investigated the contribution of FKHR and the IREs of the PEPCK and IGFBP-1 promoters to the inhibition by insulin. They showed that FKHR can interact with the IRE in the IGFBP-1 gene and that insulin can inhibit basal expression by antagonizing this interaction. In contrast, the PEPCK IRE can bind FKHR, but this is not essential for insulin inhibition of the PEPCK promoter [62].

In conclusion, studies involving FKHR as a mediator of the inhibition of gene transcription by insulin have led to promising but conflicting results. It appears that FKHR may be involved in the inhibition of IGFBP-1 and Glc-6-Pase transcriptional activity by insulin, but cannot by itself account for the inhibition of PEPCK transcriptional activity. As described in the next subsections, the factor SREBP-1c has been proposed as a mediator of insulin action for inhibition of the PEPCK promoter.

SREBP family of transcription factors

Different isoforms: structure and tissue distribution

SREBP-1c belongs to a family of transcription factors originally found to be involved in the regulation of genes by the cellular availability of cholesterol [63]. Three members of the SREBP family have been described in several mammalian species (mouse, rat, hamster and human). SREBP-1a and SREBP-1c (also named ADD1, for adipocyte determination and differentiation [64]) are encoded by a single gene through the use of alternative transcription start sites and differ in their first exon [65]. The other exons are common to both isoforms. In humans, alternative coding sequences in the 3′ end have also been described, but the functional significance of this splicing remains obscure [65]. In mice, the first exon of SREBP-1a is composed of 29 amino acids, of which eight are acidic, whereas the first exon of SREBP-1c is composed of five amino acids, of which only one is acidic. *In vitro* and *in vivo* experiments expressing identical levels of the two isoforms have shown that SREBP-1a is a much more potent activator than SREBP-1c [66]. This can be explained by the fact that SREBP-1a interacts with transcriptional activators such as cAMP binding protein/p300 [67,68], whereas the interactions of SREBP-1c may be less efficient, due to the short acidic domain. The third member of the family, SREBP-2, is derived from a different gene and shows 50% similarity with the SREBP-1 amino acid sequence. Another major difference between the 1a and 1c isoforms is in their tissue distributions. SREBP-1c is expressed in most of the tissues of mice and humans, with especially high levels in the liver, white adipose tissue, adrenal gland and brain [69]. SREBP-1c is also expressed in various muscles in adult rats and humans at appreciable levels [70,71]. In contrast, SREBP-1a is expressed mainly in cell lines and tissues with a high capacity for cell proliferation, such as spleen and intestine [69].

SREBPs are synthesized in a precursor form that is bound to the endoplasmic reticulum (ER) and nuclear membranes. They all have a common tripartite structure: (i) an N-terminal fragment of $\approx$480 amino acids, which is in fact a transcription factor of the basic domain helix-loop-helix leucine zipper (bHLH-LZ) family; (ii) a central domain of 80 amino acids containing two transmembrane sequences separated by 31 amino acids that are in the lumen of the ER; and (iii) a regulatory C-terminal domain of $\approx$590 amino acids. The transcriptional part of SREBPs is composed of a stretch of acidic amino acids comprising the transactivation domain, a domain rich in proline residues, and a bHLH-LZ domain allowing dimerization, nuclear import and DNA binding. Unlike other bHLH-LZ transcription factors, which contain a well conserved arginine residue in their basic domain, SREBPs have a tyrosine residue. This amino acid substitution allows SREBPs to bind on both E-boxes (5′-CANNTG-3′, where N represents any base), like all bHLH proteins, and also SRE (sterol regulatory element) sequences (5′-TCACNCCAC-3′) [72]. The importance of this tyrosine residue

for binding on SRE sequences was demonstrated by Kim et al. [72], who changed it to an arginine residue, as is present in other bHLH transcription factors. The resulting protein bound only on E-boxes. Conversely, mutation of the arginine residue of upstream stimulatory factor (USF), a classic bHLH-LZ protein, into a tyrosine allows its binding on both E-boxes and SRE sequences [72].

Proteolytic cleavage of SREBPs

Brown and Goldstein [73,74] have elegantly unravelled the mechanisms by which the transcriptionally active fragments of SREBP-2 and -1a are liberated in response to cholesterol depletion. When the concentration of cholesterol decreases in the membranes, the N-terminal part (called the mature form) of SREBP-2 or SREBP-1a is released from the membranes via a complex mechanism involving two sequential proteolytic cleavages [73,74]. The mature form is then translocated into the nucleus via a nuclear transport pathway involving an interaction between the HLH-LZ domain of SREBPs and importin β [75]. It has been shown that SREBP-2 proteins are transported as a dimer inside the nucleus [76]. Then SREBPs transactivate, through SRE sequences, the promoters of genes involved in cholesterol biosynthesis (hydroxymethylglutaryl-CoA synthase and hydroxymethylglutaryl-CoA reductase) and cholesterol uptake (low-density lipoprotein receptor), allowing the replenishment of cholesterol stores [74].

Intensive studies in mutant Chinese-hamster ovary (CHO) cells with defects in feedback regulation by cholesterol have allowed the characterization of the components involved in the cleavage of SREBPs [74]. The first proteolytic cleavage involves a site-1 protease (S1P), which cleaves the luminal loop of SREBPs, separating it in two parts. S1P is a membrane-bound serine protease belonging to the subtilisin family [77]. Analysis of the tissue distribution of S1P revealed that this protease is expressed in most human tissues [77]. S1P is itself activated by several proteolytic cleavages in order to produce the active enzyme [78]. The most important cleavage is an autocatalytic cleavage, which liberates an inactive form, S1P-B. S1P-B is cleaved further to yield the active form, S1P-C [78]. Immunocytochemical studies indicate that the active S1P-C is found in or near the Golgi apparatus [79].

The second cleavage of the SREBPs is completely dependent on the first one, and occurs only when S1P has cut SREBP. The site-2 protease (S2P) is a zinc metalloprotease that cleaves SREBP within transmembranes segments [80].

The key protein in the cleavage of SREBPs is a polytopic membrane protein designated SCAP (SREBP cleavage activating protein) [81]. SCAP is composed of two distinct domains: an N-terminal domain that anchors SCAP to the membranes of the ER, and a C-terminal domain which contains tryptophan–aspartic acid repeat sequences allowing protein–protein interactions. Within the cells SCAP is associated via its C-terminal domain to the C-terminal regulatory domain of SREBP. In sterol-depleted cells, SCAP escorts SREBPs to a post-ER compartment containing the active S1P-C. In contrast, in the absence of sterols, the SCAP–SREBP complex remains in the ER, and SREBPs are not cleaved [79].

SCAP is the sterol sensor protein. The sterol-sensing domain of SCAP is located in a segment of the N-terminal part comprising five membrane-spanning sequences [82]. A similar motif is found in other proteins that interact with sterols, namely hydroxymethylglutaryl-CoA reductase, the Niemann–Pick type C1 protein and the patched protein [74]. Point mutations in the sterol-sensing domain of SCAP prevent repression by sterol of SREBP cleavage, and lead to an overproduction of cholesterol in CHO cells [81]. Direct evidence for a sterol sensor role for SCAP in the liver was obtained by the production of SCAP-deficient mice [83]. In these animals, a profound decrease in the nuclear amounts of SREBP-1 and SREBP-2 is observed. This leads to a dramatic decrease in the basal levels of mRNAs encoding enzymes of cholesterol and fatty acid biosynthesis. In addition, these mRNAs fail to increase normally in response to cholesterol deprivation [83].

SREBP-1c expression is controlled by the nutritional environment in the liver

The first evidence that the SREBP-1c isoform is not induced and processed under conditions of low cholesterol availability came from dietary experiments in the hamster. A nutritional protocol aimed at increasing the demand for cholesterol induced in the hamster liver a clear-cut increase in the expression and nuclear abundance of SREBP-2, as expected, whereas SREBP-1 expression and nuclear abundance were somewhat decreased [84].

In fact, the expression and transcriptional effects of SREBP-1c seem to be related to carbohydrate and lipid metabolism. In mouse and rat liver, the expression and the presence of the mature form of SREBP-1c in nuclei are increased when starved animals are fed with a high-carbohydrate diet [85–87]. In contrast, this experimental protocol has only a modest effect on SREBP-2 expression [85].

The decisive evidence for the involvement of SREBP-1c in insulin signalling came from studies performed in primary cultured hepatocytes, in which insulin is able to strongly activate SREBP-1c expression at concentrations compatible with an effect through the insulin receptor [87]. This effect was transcriptional, as shown by run-on assays. Glucagon, which antagonizes the action of insulin on many metabolic hepatic processes, opposes the effects of insulin on SREBP-1c expression via its second messenger cAMP [87]. At the protein level, the induction of SREBP-1c expression by insulin is followed by an increase in the precursor form of SREBP-1c and then by a concomitant increase in the nuclear mature form, which is detectable 2 h after insulin addition [88]. The effect of insulin on SREBP-1c was corroborated by *in vivo* studies showing that SREBP-1c expression and nuclear abundance were low in the livers of diabetic rats, and increased markedly after insulin treatment [89]. The effect of insulin on the SREBP-1c transcript was highly specific, in that no changes in the levels of the mRNAs encoding SREBP-1a or SREBP-2 were detected [89].

A stimulatory effect of glucose on SREBP-1c expression and transcription was described in an hepatoma cell line (H2-35) transformed with a temperature-sensitive strain of simian virus 40 [90]. We have never observed such an effect of glucose on SREBP-1c in primary cultured hepatocytes, whereas other well-known glucose-responsive genes were perfectly well induced in this model [91]. This absence of an effect of glucose on SREBP-1c mRNA levels in primary rat hepatocytes was confirmed recently [92].

An inducing effect of insulin on SREBP-1c expression has also been reported in other insulin-responsive tissues. Thus insulin stimulates SREBP-1c expression in mouse and human adipose tissue [70,86], as well as in muscles in rats and humans [70,71].

How does insulin stimulate SREBP-1c expression in the liver? As described above, the main effect of insulin is to stimulate the transcription of SREBP-1c in cultured hepatocytes [87]. This effect could be mediated through insulin receptor substrate-1 (IRS-1), since animal models of insulin resistance presenting

reduced or absent expression of IRS-2 (*ob*/*ob* mice, lipodystrophic mice and IRS-2$^{-/-}$ mice) are still able to strongly express SREBP-1c and its target genes [93,94]. In addition, inhibition of insulin-induced IRS-1 phosphorylation abolishes the induction of SREBP-1c gene expression by insulin in cultured rat hepatocytes [95]. Studies using inhibitors of various branches of the insulin signalling pathway have shown that the effect of insulin on SREBP-1c expression and synthesis involves mainly the PI 3-kinase pathway [88,95,96]. The identity of the downstream effector of PI 3-kinase is more controversial. On the one hand, it was shown that acute activation of PKB/Akt is sufficient to induce SREBP-1c mRNA accumulation in primary hepatocytes [96]; on the other hand, a dominant negative form of PKB/Akt promotes the accumulation of SREBP-1c mRNA [95].

The fact that SREBP-1c is induced mainly at a transcriptional level has prompted several groups to analyse the SREBP-1c promoter. A cluster of putative binding sites for several transcription factors (NF-Y, SRE, E-box and Sp1 site) is located at position −90 of the mouse SREBP-1c promoter [97]. The SRE region is necessary for basal expression of the promoter and can be activated by nuclear SREBPs [97]. In a recent study performed in primary cultured hepatocytes, Deng et al. [92] demonstrated that the 1.5 kb segment of SREBP-1c promoter upstream of the transcription start site contains regulatory elements conferring responsiveness to insulin, cAMP and polyunsaturated fatty acids. Whether SREBP-1c is responsible for its own synthesis in response to insulin through a feed-forward mechanism remains unresolved. The presence of an SRE sequence in a region that seems responsive to insulin argues in favour of this hypothesis. Another piece of evidence was from SCAP- and S1P-deficient mice, in which the expression of SREBP-1c in the liver is strongly reduced, suggesting that SREBPs can control their own expression [83,98]. However, in knockout mice in which the wild-type SREBP-1 gene has been replaced by a gene that encodes a truncated non-functional form of SREBP-1, the SREBP-1c promoter is still able to respond to carbohydrate refeeding [99]. This might indicate that a factor other than SREBP-1c itself is responsible for the effect of insulin on the SREBP-1c promoter.

Liver X receptor (LXR) is a member of the nuclear hormone receptor superfamily of transcription factors. The LXRα isoform is highly expressed in the liver, kidney and adipose tissue, whereas the expression of LXRβ is ubiquitous [100]. The endogenous LXR ligands are oxysterols, which are metabolic derivatives of cholesterol [101]. The expression and the amount of the nuclear, active form of SREBP-1c are significantly decreased in LXRα knockout mice, leading to decreases in the levels of mRNAs for lipogenic enzymes [102]. Moreover, treatment of mice with selective agonists of LXR or RXR (retinoid X receptor; the dimerization partner of LXR) induces a 4-fold increase in the expression of SREBP-1 mRNA, as well as in the co-ordinate expression of major lipogenic enzymes in the liver [102]. Recently it was observed that the induction of SREBP-1c expression by insulin, as well as that of its target genes, is blunted in the liver of LXR$\alpha/\beta^{-/-}$ mice, leading to the conclusion that LXR is an intermediate in the insulin-stimulated transcription of SREBP-1c [103]. In contrast, the induction of SREBP-1c in a fasting/refeeding experiment is not modified in the livers of LXR$\alpha/\beta^{-/-}$ mice, suggesting that the effect of insulin on SREBP-1c expression is independent of the LXR pathway [102]. Obviously further work is required to resolve these discrepancies.

As described above, insulin acts principally at a transcriptional level to induce SREBP-1c expression. However, insulin could also increase rapidly the availability of the active form of SREBP-1c in the nucleus. An obvious question is thus whether insulin, like cholesterol for SREBP-2 and SREBP-1a, is inducing not only the synthesis, but also the proteolytic cleavage, of SREBP-1c. The experiments we have performed tend to suggest that this is not the case, and that the proteolytic cleavage of SREBP-1c may be a constitutive, non-regulated process [88]. However, their interpretation is not straightforward, due to the fact that insulin also increases the synthesis of SREBP-1c. Definitive proof would require the insulin-independent expression of a tagged SREBP-1c in primary cultured hepatocytes. Nevertheless, what we can conclude is that the activation of SREBP-1c gene transcription is a major means by which insulin regulates the activity of SREBP-1c.

Another important question concerns the roles of SCAP and the S1P/S2P proteases in the cleavage of the SREBP-1c isoform in the liver. Although SCAP is described as a sterol sensor, it could also transport SREBP-1c to the S1P protease in the Golgi in response to insulin, as described previously for cholesterol. Experiments of fasting/refeeding in SCAP-deficient mice seem to indicate that the presence of an active SCAP is necessary for a functional SREBP-1c. Indeed, in these animals the basal expression of lipogenic and cholesterogenic enzymes is very low, and is only slightly induced during refeeding [83]. However, the expression of SREBP-1c itself is also quite low in these animals, suggesting that the absence of regulation may be due to the absence of the SREBPs rather than to the absence of SCAP. It is thus difficult to draw decisive conclusions concerning the role of SCAP in the activation of SREBP-1c by insulin.

Concerning the S1P protease, its role in the cleavage of SREBP-1c implies that the SREBP-1c precursor can be transported to the Golgi compartment, where the active form of S1P is present. *In vivo* experiments in S1P-deficient mice tend to demonstrate that S1P is required for the processing of all SREBPs. The expression of both precursor and nuclear forms of SREBP-1 is strongly reduced in these animals, suggesting that (1) S1P is necessary for the cleavage of SREBP-1c, and (2) SREBPs can control their own expression [98].

A number of arguments suggest that insulin may also stimulate the mature form of SREBP-1c. When the mature form of SREBP-1c is overexpressed in 3T3-L1 adipocytes, its transcriptional activity is enhanced further by insulin [86]. It has been shown that, in HepG2 cells, insulin stimulates the transcriptional activity of SREBP-2 and SREBP-1a via a mitogen-activated protein kinase (MAP kinase) pathway [104]. In SREBP-1a, Ser-117 (which is also present in the SREBP-1c isoform) has been identified as the major phosphorylation site for MAP kinases [105]. Mutation of this serine to an alanine residue abolishes MAP kinase-dependent transcriptional activation of SREBP-1a by insulin [105]. Using transfection in a cell line, Kotzka et al. [106] reported that SREBP-1c is phosphorylated on the N-terminal part of the protein through the MAP kinase pathway. However, in cultured hepatocytes, inhibitors of the MAP kinase pathway do not antagonize the effect of insulin on SREBP-1c expression [88] or the effect of insulin on SREBP-1c-target genes [107,108], suggesting that SREBP-1c could instead be phosphorylated by kinases downstream of PI-3 kinase.

Thus, although the main activating action of insulin on SREBP-1c is at the transcriptional level, additional regulatory steps may exist at the post-translational level.

SREBP-1c is the mediator of insulin action on hepatic glycolytic and lipogenic genes

A key role of SREBP-1c in the action of insulin was first suggested by the studies of Kim et al. [86] in adipocytes, demonstrating that the overexpression of SREBP-1c in these cells mimicked the effects of insulin on the FAS and leptin

promoters. In addition, the fact that insulin strongly activates SREBP-1c gene expression [87,89], whereas glucagon opposes this effect of insulin, is consistent with the known effects of insulin and glucagon on the regulation of gene expression in the liver.

In order to demonstrate the involvement of SREBP-1c in mediating the effects of insulin on hepatic gene expression, two different forms of SREBP-1c were overexpressed in hepatocytes using an adenoviral vector: (i) a dominant positive version of SREBP-1c (DP-SREBP-1c), which corresponds to the mature nuclear form of SREBP-1c, directly imported into the nucleus; and (ii) a dominant negative form of SREBP-1c (DN-SREBP-1c), which is the mature form of SREBP-1c containing an alanine mutation at amino acid 320 in the basic domain. This mutation abolishes the binding of SREBP-1c to both the SRE and E-boxes, but still allows dimerization, leading to decreased availability of endogenous SREBP-1c [72]. Overexpression of DN-SREBP-1c in hepatocytes counteracts the stimulatory effect of insulin on GK expression [109]. Conversely, overexpression of DP-SREBP-1c bypasses the insulin requirement for GK expression [109]. Similar results were obtained for genes that require for their full expression both the presence of insulin and a high glucose concentration, namely those encoding L-PK, FAS, ACC and S14 [87,109]. The bulk of these studies demonstrate that SREBP-1c is the mediator of positive insulin action on glycolytic and lipogenic genes.

As described above, the genes encoding gluconeogenic enzymes are negatively controlled by insulin in the liver. In order to study whether SREBP-1c can account for the inhibitory effects of insulin on this class of genes, the two mutated forms of SREBP-1c were overexpressed in primary cultured hepatocytes. Expression of DP-SREBP-1c inhibited basal and glucocorticoid- or cAMP-induced PEPCK expression. In contrast, DN-SREBP-1c stimulated the accumulation of PEPCK mRNA [110]. In HepG2 hepatoma cells, transfection with DP-SREBP-1c completely blocked the induction of PEPCK gene transcription by the catalytic subunit of protein kinase A (PKA), and DN-SREBP-1c markedly induced both the basal and PKA-stimulated transcription of PEPCK [110]. Further experiments [110] suggested an interaction between SREBP-1c and cAMP-binding protein, the transcriptional co-activator that is critical in coordinating the cAMP stimulation of PEPCK gene transcription [111]. It must be emphasized that, in cultured primary hepatocytes, we have been unable to show an effect of DP- or DN-SREBP on Glc-6-Pase gene expression (F. Foufelle and P. Ferré, unpublished work), suggesting that the action of insulin on this gene is mediated by another factor.

The importance of SREBP-1c for the expression of glycolytic/lipogenic genes was also demonstrated *in vivo* by showing that their induction by a high-carbohydrate diet was precluded in SREBP-1 knockout mice [99]. In streptozotocin-diabetic mice, adenovirus-mediated overexpression of SREBP-1c in the liver resulted in increases in the expression of glycolytic (GK) and lipogenic (FAS, S14) enzymes and a dramatic decrease in PEPCK expression [112], mimicking perfectly the effect of an insulin injection. The physiological consequences of the hepatic overexpression of SREBP-1c were an increase in the hepatic content of glycogen and triacylglycerol and a marked decrease in the hyperglycaemia of diabetic mice [112].

The involvement of SREBP-1c in the inhibition of PEPCK expression by insulin was recently challenged using a model of mice lacking the SREBP-1c isoform [113]. In these animals under post-absorptive conditions, the mRNA levels of PEPCK are 3-fold higher than in control mice, suggesting the removal of an inhibitory factor, a result consistent with the SREBP-1c hypothesis. However, PEPCK expression was suppressed normally by refeeding the starved animals with a high-carbohydrate diet, despite the absence of SREBP-1c [113]. The authors thus concluded that SREBP-1c is not involved in the inhibition of PEPCK expression. It must be emphasized that the inibitory effect of refeeding on PEPCK expression does not involve solely insulin. Refeeding in rodents is also concomitant with a decrease in elevated plasma glucagon and corticosterone levels, thus decreasing the induction of PEPCK. In addition, one role of insulin, totally independent of SREBP-1c, is to accelerate the degradation of cAMP (a stimulus for PEPCK expression) by activation of an insulin-sensitive phosphodiesterase [114].

SREBP binding sites and responses to insulin

As shown in Scheme 1, the consumption of a high-carbohydrate diet increases the expression of many enzymes involved in glycolytic and lipogenic pathways in the livers of rodents. Studies performed in animal models and in cultured cells over- or under-expressing SREBP-1c have demonstrated that this factor regulates the expression of most of them [66,87,99,109,115]. SREBP binding sites have been characterized further in the promoters of the enzymes FAS [116], ACC [117], ATP citrate-lyase [118,119], S14 [32], glycerol-phosphate acyltransferase [120] and stearoyl-CoA desaturase [121,122]. However, whether these SREBP binding sites mediate the responses of these genes to insulin remains unknown. The co-localization of SREBP binding sites and IREs has been well characterized for the FAS and S14 promoters. In the FAS gene, two SREs are found overlapping an E-box site at approx. -65 bp in the promoter [123]. This site has been shown previously to mediate responses to insulin in adipocytes and hepatocytes [86,124,125]. The presence of both insulin and glucose is required for the full expression of the S14 gene in hepatocytes [32]. Analysis of the S14 promoter has revealed that the region -139 to -131 supports responsiveness to insulin [32] as well as containing a binding site for SREBP-1 [126]. This region was described as an E-box-like element. Mutation in this sequence abrogates the response to insulin of the S14 promoter [32]. Thus, at least for the FAS and S14 genes, there is a clear co-localization of an insulin response region and a functional SREBP-1c binding sequence.

Unfortunately, despite numerous efforts, it has never proved possible to characterize a precise insulin-responsive region in the GK hepatic promoter, thus precluding a comparative study of the transactivating effects of insulin and SREBP-1c.

Integration of the role of SREBP-1c in energy metabolism

Among the various effectors of insulin, the SREBP-1c pathway can be considered as a system that signals to the liver and prepares it for a period of carbohydrate availability. First, by increasing the glucose-phosphorylating activity in the liver (GK activity), it will contribute to replenishment of the hepatic glycogen stores [127–129], which can then, during the next interprandial interval, provide glucose to organs such as the brain. This is an important role, since defects in glycogen metabolism are concomitant with dramatic alterations in glucose homoeostasis in humans [130].

The ability of SREBP-1c to maintain a lipogenic capacity in the liver (in conjunction with a carbohydrate-sensitive transcription factor; see below) extends its role further, since glucose carbons will be transformed into lipids ultimately stored in adipose tissue, allowing survival during even longer periods of energy deprivation. It is probably not a coincidence if SREBP-1c is also an adipocyte differentiation factor [64]. SREBP-1c can

thus be considered as a real 'thrifty' gene, i.e. a gene that has allowed species survival in the context of successive episodes of food availability and restriction.

SREBP-1c and insulin sensitivity

In terms of relationships with metabolic diseases, SREBP-1c can be considered from two opposite perspectives. First, when acting as a transcription factor central to the genomic actions of insulin on both carbohydrate and lipid metabolism, a loss of function should lead ultimately to the phenomenon of 'insulin resistance'. Interestingly enough, in SREBP-1c knockout mice there is a tendency for a higher basal plasma glucose when compared with wild-type mice, and a higher glycaemia during a carbohydrate refeeding period [113]. On another hand, SREBP-1c promotes fatty acid synthesis and lipid deposition. Since lipids have been largely implicated in the development of insulin resistance by mechanisms involving substrate competition, antagonism of insulin signalling or lipotoxicity [131], SREBP-1c could also be considered as a factor responsible for insulin resistance. Liver overexpression of SREBP-1c has indeed been described in several models of insulin resistance, such as lipodystrophic and *ob*/*ob* mice [93], IRS-2 knockout mice [94] and Zucker obese *fa*/*fa* rats [132]. Thus a mutation inducing a gain of function for SREBP-1c could also be responsible for insulin resistance. Whatever the underlying mechanism, the effects of this factor should be considered in pathologies such as Type II diabetes, obesity, dyslipidaemia and other states of insulin resistance.

GLUCOSE REGULATION OF HEPATIC GENE EXPRESSION

As stated above, a subset of glycolytic/lipogenic genes in the liver requires both increased insulin and glucose concentrations to be fully expressed [133–135]. *In vivo* and *in vitro* experiments have demonstrated that glucose up-regulates ACC, FAS and stearoyl-CoA desaturase expression in adipose tissue [136,137], L-PK, FAS, S14 and ACC expression in the liver, [29,30,138], and ACC and L-PK expression in a pancreatic β-cell line [139,140]. This regulation involves stimulation of their transcription rates. In the absence of glucose, insulin by itself is unable to induce expression. In the absence of insulin, the effect of glucose is greatly reduced in adipose tissue and nearly absent in cultured hepatocytes. One potential reason why, in contrast with GK, these genes require both a high insulin and a high glucose concentration could be linked to the fact that, after a meal, the metabolic priority is to replenish glycogen stores, and it is only if glucose is particularly abundant that the glucose carbon atoms are orientated towards lipid synthesis.

Glucose must be metabolized in order to exert its transcriptional effect. For instance, in the liver, the glucose effect requires the presence of GK, the enzyme responsible for glucose phosphorylation into glucose 6-phosphate [30,141]. Since GK expression is strongly activated by insulin, the insulin dependency of these genes was first explained by the necessity for GK induction by the hormone in order to allow glucose metabolism. However, several lines of evidence also argue for a direct effect of insulin, as discussed above. The questions which then arise concern the nature of the glucose signal (which metabolite?), the transduction mechanism from the glucose metabolite to the transcriptional machinery, the glucose response element (GlRE) on the gene promoter, and the transcription factor involved.

Glucose metabolism is required: the signal metabolite

For a metabolite to qualify as a signal, its concentration must vary in proportion to the extracellular glucose concentration (since the glucose effect on gene expression is concentration-dependent), and must change before changes in mRNA levels. In previous works, glucose 6-phosphate was proposed as the metabolite acting as a signal for FAS transcription [30,136], and hence for genes belonging to the same class, i.e. L-PK and S14. This proposal was based on the facts that: (i) in adipose tissue and a β-cell line (INS1), the effect of glucose is mimicked by 2-deoxyglucose, a glucose analogue whose metabolism stops after its phosphorylation into 2-deoxyglucose 6-phosphate, which accumulates in the cell [136,139,140]; (ii) the intracellular glucose 6-phosphate concentration varies in parallel with the expression of these genes [30,91,136]; and (iii) *in vivo*, the kinetics of hexose-phosphate concentration fit with the time-related pattern of gene induction [142]. It must be also emphasized that glucose 6-phosphate is used as a signal of glucose availability in hepatic glycogen metabolism, since it is an activator of glycogen synthase activity [5]. Interestingly, in GLUT2-null mice, fasted animals have paradoxical increases in L-PK expression and glycogen content, and this mirrors the concentrations of glucose 6-phosphate, which remain high [143]. Another interesting situation is found when rats are injected with an inhibitor of the glucose 6-phosphate translocator activity, a component of the Glc-6-Pase system. This induces a large increase in hepatic concentrations of glucose 6-phosphate, glycogen and triacylglycerol, and an activation of FAS and ACC gene expression [144].

However, xylulose 5-phosphate, an intermediate of the non-oxidative branch of the metabolic pathway, has also been proposed as the signal metabolite [145]. This is based on the following evidence. (i) In some cells, 2-deoxyglucose 6-phosphate can be metabolized further, particularly into pentose-phosphate-pathway intermediates. (ii) Xylitol, a precursor of xylulose 5-phosphate, is able to stimulate the transcription of a reporter gene driven by the L-PK promoter in a hepatocyte cell line (AT3F) at a low concentration (0.5 mM) without detectable changes in the glucose 6-phosphate concentration. (iii) In primary cultured hepatocytes, xylitol (5–10 mM) is able to induce a 6-fold increase in the L-PK mRNA concentration [145]. (iv) Xylulose 5-phosphate has been shown to activate phosphatase 2A-mediated dephosphorylation [146], and this phosphatase is involved in the dephosphorylation of transcription factors. Similarly, *in vivo* effects of xylitol on the induction of the Glc-6-Pase gene have been described. This was concomitant with a doubling of the concentration of xylulose 5-phosphate, but no change in that of glucose 6-phosphate [147].

In hepatocytes cultured in the presence of various concentrations of glucose, xylitol and dihydroxyacetone, which enter at different levels of the glycolytic/pentose-phosphate pathways, it was found that, in contrast with glucose 6-phosphate, there was no parallelism between the concentration of xylulose 5-phosphate and the extent of induction of FAS and S14 gene expression [91]. Interestingly, xylitol induces dose-dependent increases in the glucose 6-phosphate concentration. This latter finding is not unexpected, since metabolites from the pentose-phosphate pathway can be recycled back to glycolysis. Thus, in these experiments, xylulose 5-phosphate does not qualify as a signal metabolite for FAS and S14 induction. It must be also underlined that the first two steps in the pentose-phosphate pathway are catalysed by glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, two enzymes that are also induced by glucose/insulin availability.

In view of these conflicting results, it is difficult to reach a definite conclusion at present concerning the identity of the signal metabolite. Definitive evidence will be obtained only when the full transduction machinery and the exact role of the signal metabolite in this context are elucidated (see below).

Cellular cascade from glucose to the transcription machinery: a role for AMP-activated protein kinase (AMPK)?

In order to account for glucose responsiveness in specific tissues, several hypothesis can be considered. (i) The presence of glucose could modify the nuclear amount of the transcription factor (including by a modification of its cellular localization). (ii) In glucose-responsive tissues, a transcription factor may undergo a post-translational modification in the presence of glucose, which modifies its interaction with the basic transcription machinery or with a co-activator protein. Post-translational modifications of a transcription factor could involve two types of mechanisms: (a) a direct allosteric modification of the protein by binding of the glucose-related signal metabolite, or (b) modifications through phosphorylation/dephosphorylation mechanisms by modulation of the activity of a nuclear protein kinase/phosphatase. A combination of mechanisms (a) and (b) is also possible: binding of the glucose metabolite to the transcription factor could modify its conformation and increase its affinity for a protein kinase/phosphatase.

The possible involvement of phosphorylation/dephosphorylation mechanisms has been addressed for the S14 gene [148]. The effects of okadaic acid, an inhibitor of protein phosphatases 1 and 2A, have been tested in cultured hepatocytes transfected with a construct S14 promoter/reporter gene or with the S14 carbohydrate response element (ChoRE) inserted into an otherwise glucose-unresponsive promoter. It has been shown that, in the presence of okadaic acid, the effect of glucose on both constructs was abolished, whereas glucose utilization was not modified. Further experiments have pointed to a role for a protein phosphatase 2A in the glucose effect. Finally, since the calcium ionophore A23187, an activator of calmodulin-dependent kinase, inhibited the glucose effects, it was concluded that calmodulin-dependent kinase and protein phosphatase 2A were implicated [148].

Similar studies for the FAS gene have been performed in cultured hepatocytes. Glucose-induced activation of FAS gene transcription is markedly reduced by incubation of the cells with okadaic acid, suggesting that the stimulatory effect of glucose could involve a dephosphorylation mechanism [149].

AMPK provides a potential candidate for a protein kinase that may be involved in the regulation of glucose-activated genes. AMPK is a heterotrimeric complex formed of a catalytic subunit (α) and two regulatory subunits (β and γ) [150]. Several isoforms with tissue-specific expression have been described for each subunit. A significant clue regarding a possible role for AMPK in the regulation of gene transcription came from the finding that it is structurally and functionally related to the yeast protein kinase complex Snf1 ('sucrose non-fermenting 1') [151]. In yeast, the transcription of a number of genes is repressed by high concentrations of glucose [152]. The kinase activity of Snf1 is essential for the derepression of these genes in yeast grown under conditions of glucose limitation. AMPK and Snf1 both form heterotrimeric complexes consisting of a catalytic subunit and two regulatory subunits [150,153]. The amino acid sequences of the mammalian AMPK subunits are highly related to those of their counterparts in Snf1, and the kinases show functional similarities. Taken together, these findings led us and others to speculate that AMPK may be involved in regulating gene transcription in mammals. Evidence that this may be the case came from studies in which AMPK in hepatocytes was activated by incubation with 5-amino-imidazolecarboxamide ('AICA') riboside, leading to the inhibition of the glucose-induced gene expression of FAS, L-PK and S14 [149,154]. A similar inhibition of FAS, L-PK and S14 gene expression was obtained when AMPK activity was increased in cultured hepatocytes by the overexpression of a constitutively active form of AMPK [155]. These results imply that AMPK is involved in the repression of insulin/glucose-activated genes. Interestingly, the inhibitory effect of AMPK activation on lipogenic enzyme gene expression could be secondary to decreased expression of SREBP-1c, as suggested recently [156].

Since an increase in AMPK activity inhibits insulin/glucose-activated gene transcription, it follows that a decrease in AMPK activity could be part of a mechanism involved in the stimulation of gene transcription by glucose. In order to test this hypothesis, a dominant negative form of the kinase was expressed in hepatocytes grown in 5 mM glucose, leading to the disappearance of the AMPK complex and a marked decrease in the activity of AMPK [155]. Inhibition of AMPK activity had no detectable effect on any of the aforementioned genes. In addition, changing the glucose concentration in the medium of cultured hepatocytes from 5 to 25 mM had no inhibitory effects on AMPK activity [149]. Taken together, these results rule out the possibility that, in the liver, glucose exerts its effects on gene expression by inhibiting AMPK directly.

It must be pointed out that, in a β-cell line, raising the glucose concentration from 3 to 30 mM induced a 30–40 % decrease in AMPK activity, and this was concomitant with increased activity of a transfected L-PK promoter. In these cells, inhibiting the AMPK activity at 3 mM glucose by an injection of a specific anti-AMPK antibody mimics the effect of a high glucose concentration on L-PK promoter activity [157]. It is thus possible that, in cells which are especially sensitive to a low glucose concentration, such as β-cells, inhibition of AMPK activity when the glucose concentration increases is part of the mechanism of activation of gene transcription.

In conclusion, in the liver, AMPK is involved in the inhibition of insulin/glucose-activated gene expression, but not in the induction pathway.

cis-Acting DNA sequences involved in responses to glucose

Two groups have made decisive contributions to the identification of GlREs, using L-PK and S14 as model genes. The first GlRE (also called ChoRE) to be identified was that of the L-PK gene [158,159]. It was found in the region up to position −183 of the L-PK promoter, a region that is sufficient for the specificity of tissue expression as well as for responses to nutrients and hormones, as shown in transgenic mice [160]. The region was further delineated as a fragment between −183 and −96 bp using transfection experiments in isolated hepatocytes [158,159, 161]. The glucose responsiveness is conferred through the close co-operation of L3 (−126 to −144) and L4 (−145 to −168) elements. In fact, a multimer of the L4 element, but not of the L3 element, is able to confer glucose responsiveness to a glucose-unresponsive heterologous promoter [159]. Nevertheless, the full glucose response is conferred only when both L3 and L4 elements are present. A second GlRE was then characterized in the S14 gene. The region from −1439 to −1423 of the rat S14 promoter was found to be essential for the glucose responsiveness of this gene in isolated hepatocytes [162]. More recently, a ChoRE/GlRE has also been found in the rat FAS promoter between positions −7214 and −7190 [163], in the ACC αPI promoter (this promoter is expressed mainly in adipose tissue and liver) between −126 and −102 [31], and in the mouse S14 promoter between −1450 and −1425 [164]. A comparison of the sequences of these various ChoREs/GlREs is shown in Table 1. The ChoRE is described as two E-box or E-box-like sequences of the form CANNTG, separated by a defined distance. The first

Table 1 Comparison of the sequences of the different ChoRE/GlREs

Gene	Position	Consensus sequence	References
Rat L-PK	−166	**CACGGG**GCACT**CCCGTG**	[159,179]
Rat S14	−1439	**CACGTG**GTGGC**CCTGTG**	[166]
Mouse S14	−1442	**CACGCT**GGAGT**CAGCCC**	[164]
Rat FAS	−7210	**CATGTG**CCACA**GGCGTG**	[163]
Rat ACC PI	−122	**CATGTG**AAAAC**GTCGTG**	[31]

4 bp of each E-box is critical for the glucose response, as is the length of the spacing sequence [165,166]. The E-box described in the L-PK and S14 ChoRE/GlRE is a binding site for the bHLH class of transcription factors.

trans-Acting factors

Once the GlREs had been characterized, a logical approach was then to identify the proteins that are able to bind to these elements, using electrophoretic mobility shift assays (EMSAs). From these studies, the transcription factor USF (belonging to the bHLH-LZ family) was presented as a potential candidate for a carbohydrate-responsive factor, since it was the main protein bound on the ChoRE/GlRE. USF in fact has two isoforms, USF1 and USF2; these are encoded by different genes and differ only in their N-terminal domain. USF binds DNA as a dimer, most probably as a USF1/USF2 heterodimer, in the liver [167]. A series of studies was then designed to test this hypothesis, including overexpression in cultured cells of wild-type and dominant negative forms of USF [168,169], mice deleted for the USF1 and USF2 genes [170,171], studies of USF synthesis and binding activity in response to diet, and studies of USF binding activity on wild-type and mutated forms of GlRE/ChoRE in parallel with their glucose responsiveness. From the bulk of these studies, it was concluded that these factors cannot on their own explain the the responsiveness to glucose mediated through GlRE/ChoRE [169].

By varying the conditions of the EMSA, newly identified proteins were then shown to bind on GlRE/ChoRE, and some of these were presented as novel candidates for a glucose-responsive transcription factor, although they were not characterized further [164,172,173]. It is only recently that one of the proteins that binds to GlRE/ChoRE has been identified, and it emerges as a potential glucose-responsive transcription factor [174]. Uyeda and co-workers [174] have purified a transcription factor from rat liver, based on its capacity to bind to the ChoRE of the L-PK promoter, and named it ChREBP (carbohydrate response element binding protein). This factor fulfils a number of criteria for a *bona fide* ChoRE binding protein. It is indeed a bHLH-LZ protein with a bipartite nuclear localization signal, and its binding capacity to mutated ChoREs parallels the capacity of these ChoREs to respond to a high glucose concentration in cultured hepatocytes. When transfected in hepatocytes, ChREBP is able to stimulate the L-PK promoter which contains the ChoRE, and this effect is increased dramatically in the presence of glucose. Finally, although the mRNA for this factor is found in tissues such as the cerebellum, intestine, kidney and liver, DNA binding to the ChoRE is found only in liver [174].

The mechanism suggested by the authors to explain the effect of glucose on target gene transcription is translocation of ChREBP between the cytoplasm and the nucleus: ChREBP is located in the cytoplasm when the glucose concentration is low, and enters into the nucleus under conditions of high glucose [175]. This would be secondary to dephosphorylation of a specific serine residue (Ser-166) that is otherwise phosphorylated by PKA in conditions of high cAMP concentrations, e.g. the fasting state. A second PKA phosphorylation site near the DNA binding domain, and which precludes ChREBP binding, would also be dephosphorylated in the presence of high glucose [175]. Thus the main effect of glucose would be to activate a phosphatase that counteracts the effect of cAMP, inducing the translocation of ChREBP into the nucleus and stimulating its DNA binding activity. This would be consistent with the antagonistic effects of glucagon and insulin/glucose on L-PK and lipogenic genes.

This is obviously a great advance in the field which, if confirmed, should allow us in the near future to identify the entire glucose signalling pathway and the glucose metabolite involved. However, a number of points await confirmation or raise questions. It must first be shown that ChREBP accounts for the complex observed in EMSAs on the GlREs of L-PK and other glucose-responsive genes. Insulin is known to oppose glucagon through a decrease in the cAMP concentration. Thus, in hepatocytes cultured in the absence of glucagon and in the presence of maximal insulin concentrations, PKA activity should be minimal. Despite this, glucose is still able to strongly activate gene transcription in the presence of insulin and in the absence of glucagon. This has to be reconciled with the proposed hypothesis implying opposite effects of glucose and PKA on ChREBP. Finally, ChREBP has been cloned previously as WBSCR14, a gene mapping to the DNA region that is deleted in Williams–Beuren syndrome [176]. In these studies, this protein has been described as forming heterodimers with another bHLH-LZ factor named Mlx, a member of the c-Myc/Max family of transcription factors, and as inhibiting E-box-dependent transcription. Another member of this family, named MondoA, has been cloned, and like WBSCR14/ChREBP it heterodimerizes *in vivo* with Mlx [177]. More importantly, MondoA is also found predominantly in the cytoplasm of cells, and can shuttle between the cytoplasm and the nucleus (although the factors responsible for the shuttling are unknown). Interestingly, although included in a sequence with high similarity between MondoA and WBSCR14/ChREBP, Ser-196 is not conserved in the two sequences. Whether glucose is also responsible for MondoA shuttling needs to be determined, as does the potential presence of Mlx in the complex observed on the ChoRE.

INTEGRATION OF INSULIN/GLUCOSE REGULATION OF GLYCOLYTIC AND LIPOGENIC GENES

When a carbohydrate-rich meal is absorbed, the plasma glucose concentration is increased, which induces the secretion of insulin by the pancreatic β-cell. High concentrations of both glucose and insulin then reach the liver through the portal vein. Insulin will induce SREBP-1c transcription by a PI 3-kinase-dependent mechanism, and hence the synthesis of the precursor form in the ER. This precursor form will be then cleaved by a mechanism which may or may not depend upon insulin. The mature form of SREBP-1c will translocate into the nucleus, activate GK transcription and inhibit PEPCK transcription. It is not clear whether the mature form of SREBP-1c is activated further by an insulin-dependent mechanism. Synthesis of GK increases glucose phosphorylation and glycogen repletion. A signal metabolite downstream of the GK step (glucose 6-phosphate or xylulose 5-phosphate) activates a transcription factor (ChREBP), possibly by a dephosphorylation-mediated mechanism. ChREBP then translocates into the nucleus where, together with SREBP-1c, it activates genes of the glycolytic/lipogenic pathway, such as L-PK, ACC, FAS and S14. This will lead finally to the synthesis

of fatty acyl-CoA from glucose carbon atoms. It should be emphasized (i) that the activation of both SREBP-1c and ChREBP requires their translocation into the nucleus, and (ii) that their activity is decreased by cAMP-generating hormones. The SREBP-1c and ChREBP transcription factors are thus functioning in a co-ordinated manner in order to regulate hepatic glucose metabolism. As underlined in [178], this dual. system provides a means of using glucose carbons for lipid storage only when all the substrate conditions are met and once the glycogen stores have been repleted. In addition, it can also integrate multiple metabolic and hormonal signals (e.g. glucose, insulin, LXR agonists, glucagon, catecholamines, poly-unsaturated fatty acids, cell energetic state), allowing a fine tuning of glucose utilization and lipid synthesis.

GENERAL CONCLUSION

Our understanding of the long-term regulation of the fate of glucose in the liver has made considerable progress in the past few years. Two important actors in the system, SREBP-1c and ChREBP, have been discovered, and potential insulin and glucose signalling pathways leading to their effects have been described. We have described here the system that prevails in the liver. It would be interesting to analyse whether these signalling cascades also exist in other tissues that are insulin-sensitive and crucial for glucose and lipid metabolism, such as muscle, adipose tissue and the β-cell. Finally, if the importance of SREBP-1c and ChREBP is confirmed in humans in processes related to glucose and lipid metabolism, their involvement in pathophysiologies such as diabetes and obesity and their potential as pharmaceutical targets should be thoroughly analysed.

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