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Regulation of liver gene expression by glucose

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Gene expression is modulated when the composition of the external milieu changes. In prokaryotes, there are no hormones to inform the cells that these changes have occurred and nutrients act directly on gene expression. For example, lactose and galactose regulate the expression of gene coding for enzymes involved in their own metabolism (Jacob & Monod, 1961). This phenomenon, largely studied in *Saccharomyces cerevisiae*, facilitates rapid adaptation to environmental changes. When glucose is abundant in the medium, it would be wasteful to express the genes required to metabolize sugars such as lactose, galactose and arabinose. A mechanism termed 'catabolite repression' has evolved to repress genes for the catabolism of lactose, arabinose and other sugars in the presence of glucose, even when these secondary sugars are also present (for review, see Gancedo, 1992). The repressive effect of glucose is mediated by an increase in cell cAMP and a protein known as 'catabolite gene activator protein' (CAP). When glucose is absent from the medium, CAP binds to a specific sequence near the start site of the Lac promoter and stimulates gene expression. CAP is an activator working in concert with a Lac repressor. When the Lac repressor is present and blocks transcription, the dissociation of the repressor from the Lac operator has little effect on gene expression unless CAP is present to facilitate transcription. CAP binding to the Lac promoter occurs when cAMP concentrations are high (in the presence of low glucose concentration) and when the cAMP binding site is occupied. In the presence of high glucose concentrations, cAMP concentrations are low, the CAP-binding site is not occupied, and *Lac* gene transcription is repressed.

In multicellular organisms, hormones inform the cells that nutritional changes have occurred in the external milieu (blood) and then induce changes in gene expression. It has been shown that major (glucose, fatty acids, amino acids) or minor (Fe, vitamins) dietary constituents participate, in concert with many hormones, in the regulation of gene expression in response to nutritional changes (for review, see Clarke & Abraham, 1992).

The liver is crucial to the maintainance of glucose homeostasis

Glucose is an energy substrate for all mammalian cells, and it is the principal fuel for the brain, since neurons are unable to oxidize fatty acids. The blood glucose concentration must be maintained within the range of 800–1200 mg/l to avoid hypo- or hyperglycaemia. The effects of hypoglycaemia occur rapidly (within minutes) and are linked to a decrease in brain glucose consumption. Neuroglucopenic symptoms appear when blood glucose decreases to levels lower than 500 mg/l, and are progressively associated with loss of cognitive function, lethargy, convulsions and irreversible brain damage. Deleterious effects of hyperglycaemia occur only after long-term exposure to high glucose concentrations, in particular in diabetic patients, and are associated with retinopathy, nephropathy and neuropathy. Thus, a strict control of blood glucose levels within the range 800–1200 mg/l is required for the health of individuals.

Whereas all cells of the body are capable of using glucose as a fuel, only the liver and the kidney have the capacity to produce glucose. This is due to the fact that glucose-6-phosphatase (*EC* 3.1.3.9), the enzyme responsible for the hydrolysis of glucose-6-phosphate to glucose, is expressed only in these two organs. Glucose can thus be released into the bloodstream and directed to other organs for further utilization. The liver is quantitatively the main organ involved in glucose production, the kidney contributing 25 % of overall glucose production. The liver also has the capacity to take up glucose in the post-absorptive period, and to store it as glycogen to be released later when blood glucose is reduced. Thus, liver has a function of 'glucostat'.

Glucose enters into and exits from hepatocytes by means of a facilitative glucose transporter, GLUT2 (Thorens *et al.* 1988). GLUT2 has a high K_m for glucose (17–20 mM) by comparison with the other facilitative glucose transporters GLUT1, GLUT3 and GLUT4, which have K_m values between 1 and 5 mM (for review, see Gould & Holman, 1993). This property ensures a high transport capacity and a rate of transport which increases as a direct function of extracellular glucose concentration from normoglycaemic values (5 mM) to hyperglycaemic values (< 10 mM). This means that glucose transport by GLUT2 is never saturated under normal physiological conditions or during the hyperglycaemia observed in moderate diabetes. GLUT2 is expressed in the sinusoidal membranes of hepatocytes, where its kinetic properties allow for rapid trans-membrane flux of glucose. The direction of the glucose flux is dictated by the hormonal and nutritional environment. When the

Abbreviations: ACC, acetyl-CoA carboxylase; CAP, catabolite gene activator protein; CAT, chloramphenicol acetyltransferase; ChoRE, carbohydrate response element; FAS, fatty acid synthase; L-PK, L-type pyruvate kinase; USF, upstream stimulatory factor.

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hormonal and metabolic environment is favourable to hepatic glucose uptake (hyperglycaemia, hyperinsulinaemia, hypoglucagonaemia), glucokinase (*EC* 2.7.1.2) activity is high, glucose is rapidly phosphorylated, and the entry of glucose into the cell is facilitated by the maintainance of an intracellular glucose concentration lower than that in the plasma. When the hormonal and metabolic environment is favourable to hepatic glucose production (hypoglycaemia, hypoinsulinaemia, hyperglucagonaemia), glucokinase activity is low, glucose phosphorylation capacity is decreased, and the exit of glucose from the hepatocyte is facilitated by an intracellular glucose concentration higher than that in the plasma (for review, see Pilkis & Granner, 1992). Since the portal plasma glucose levels do not exceed 10 mM, and since the K_m of GLUT2 for glucose is high (17–20 mmol/l), glucose transport is never rate limiting for the entry or for the exit of glucose into or out of the liver. The high capacity and symmetrical transport properties of GLUT2 appear to be the key for allowing rapid bi-directional fluxes in and out of the hepatocytes.

In vivo studies show that several hepatic genes are regulated by the nutritional state

Fasting–refeeding experiments *in vivo*, designed to modify the direction of glucose flux inside the liver, have shown that the expression of several genes coding for key enzymes involved in glucose and fatty acids metabolism is altered (see Girard *et al.* 1997). Refeeding a high-carbohydrate diet to rats after a 72 h fast, induces a 4–8-fold increase in hepatic concentrations of mRNA coding for aldolase B (*EC* 4.1.2.13) and a 40–100-fold increase in mRNA coding for L-type pyruvate kinase (*EC* 2.7.1.40; L-PK; Weber *et al.* 1984). Starvation, as well as consumption of high-fat or high-protein low-carbohydrate diets provoke a rapid decrease in the concentrations of both mRNA (Weber *et al.* 1984). In diabetic rats, liver L-PK mRNA concentrations are very low and do not increase after feeding a high-carbohydrate diet (Munnich *et al.* 1985*a*). The L-PK mRNA concentration increases if diabetic rats are treated with insulin before feeding a high-carbohydrate diet (Munnich *et al.* 1985*a*). Insulin administration to diabetic rats induces an increase in L-PK mRNA concentration (Noguchi *et al.* 1985). The insulin causes a 3-fold increase in the transcription of the L-PK gene after 6 h. This increase caused by insulin is inhibited by glucagon (Noguchi *et al.* 1985). This suggests that hormonal and nutritional changes occurring during fasting and refeeding a high-carbohydrate diet control liver gene expression, but it was not known whether these effects were due to hormones (insulin, glucagon) and/or nutrients (carbohydrate).

The expression of GLUT2 decreases in the liver of fasted rats and increases after refeeding a high-carbohydrate diet (Thorens *et al.* 1990). Shortly after injection of steptozotocin (6 h) there was a 90 % decrease in liver GLUT2 mRNA concentration in rats, which was correlated with the transient hyperinsulinaemia and hypoglycaemia induced by the destruction of the pancreatic β-cells and the consequent release of insulin (Burcelin *et al.* 1992). After 48 h, liver GLUT2 mRNA levels were 2-fold higher than those of control animals, and a similar increase in GLUT2 protein levels was also observed (Burcelin *et al.* 1992). A similar increase in liver GLUT2 was reported in rats diabetic from 1 week of age (Oka *et al.* 1990), whereas liver GLUT2 was unchanged in rats diabetic from 1 or 2 weeks of age (Thorens *et al.* 1990). This discrepancy could be related to the severity of diabetes in these experiments. Indeed, the diabetes induced by Thorens *et al.* (1990) was more severe (glycaemia 40 mmol/l over 2 weeks) than that induced by Oka *et al.* (1990; glycaemia 25 mmol/l over 1 week). The absence of an effect on GLUT2 expression in the liver of diabetic animals with a glycaemia of 40 mmol/l could be the result of the action of stress hormones such as cortisol, glucagon or catecholamines which can act in opposite ways on hyperglycaemia and GLUT2 expression. The increase in GLUT2 expression observed in the liver of insulinopenic diabetic rats can also be reversed by lowering the blood glucose levels with phlorizin (Brichard *et al.* 1993*b*) or by treatment with vanadate, an insulinomimetic agent (Brichard *et al.* 1993*a*; Valera *et al.* 1993). Hyperinsulinaemic euglycaemic or hyperglycaemic glucose clamps performed in non-diabetic rats have shown that hyperglycaemia partially prevented the inhibitory effect of insulin on liver GLUT2 expression (Postic *et al.* 1993). This suggested that liver GLUT2 expression could be regulated in an opposite manner by insulin and glucose. This was in contrast to the potentiating effects of insulin on the glucose effect described for other liver genes such as aldolase B and L-PK (Decaux *et al.* 1991).

As it is difficult to differentiate *in vivo* between the effects of changes in glucose on L-PK and GLUT2 expression and the associated hormonal changes, it is necessary to use *in vitro* systems.

In vitro studies show that glucose directly stimulates liver gene expression

In vitro experiments allowed the study of the effects of individual nutrients (glucose, fructose etc.) and hormones (insulin, glucagon). Primary cultures of rat hepatocytes must be used since several genes, such as GLUT2 or L-PK, are not expressed or regulated in hepatoma cell lines as they are in hepatocytes (Meienhofer *et al.* 1987; Thorens *et al.* 1988; Gould & Bell, 1990). Studies on primary cultures of rat hepatocytes have shown that carbohydrate metabolism is involved in the transcriptional response of several genes to glucose in the liver. The expression of several genes coding for glycolytic or lipogenic enzymes was stimulated when adult rat hepatocytes were cultured in the presence of both insulin and high glucose concentrations (Decaux *et al.* 1989; Jump *et al.* 1990; Prip-Buus *et al.* 1995). In contrast, insulin was not required for glucose to stimulate GLUT2 expression in hepatocytes (Asano *et al.* 1992; Postic *et al.* 1993).

Insulin is required for glucose to stimulate L-type pyruvate kinase and fatty acid synthase transcription; an effect secondary to the induction of the capacity for glucose phosphorylation

The presence of a high glucokinase activity has been shown to be necessary to support the increased expression of L-PK

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and fatty acid synthase (*EC* 2.3.1.85; FAS) gene expression in response to glucose (Doiron *et al.* 1994; Prip-Buus *et al.* 1995). Glucokinase is the main hexokinase expressed in hepatocytes (Walker & Rao, 1964; Iynedjian *et al.* 1986). Glucokinase is a protein of 50 kDa, which has an high K_m for glucose (10 mmol/l; Salas *et al.* 1965), and is not inhibited by glucose-6-phosphate, its reaction product (for review, see Printz *et al.* 1993).

These kinetic properties allow a rapid and efficient phosphorylation of glucose that is coupled with a high capacity for glucose transport by GLUT2. This system allows the cell to be rapidly informed about changes in extracellular glucose concentration. Although a short-term regulation of hepatic glucokinase involving a regulatory protein has been described (Van Schaftingen *et al.* 1994), the main system regulating glucokinase activity is alteration in its gene expression (for review, see Printz *et al.* 1993).

During fasting or in diabetes, the rate of hepatic glycolysis is low and the rate of gluconeogenesis is high. Transcription of the glucokinase gene is turned off and the concentration of glucokinase mRNA is very low (Sibrowski & Seitz, 1984; Magnusson *et al.* 1989). Oral glucose administration to fasted rats, or insulin injection to diabetic rats, induce a rapid stimulation of glucokinase gene transcription (30–60 min) and an accumulation of glucokinase mRNA (Sibrowski & Seitz, 1984; Magnuson *et al.* 1989). These effects of insulin on glucokinase gene expression are also observed in primary culture of rat hepatocytes (Iynedjian *et al.* 1988; Narkewicz *et al.* 1990). The effect of insulin on glucokinase gene expression does not depend on the concentration of glucose in the culture medium (Iynedjian *et al.* 1989; Narkewicz *et al.* 1990).

Hepatocytes of 14-d-old suckling rats are devoid of glucokinase (Walker & Holland, 1965; Narkewicz *et al.* 1990; Prip-Buus *et al.* 1995). In the absence of insulin, glucose is unable to stimulate FAS gene expression in hepatocytes of 14-d-old suckling rats in primary culture (Prip-Buus *et al.* 1995). After 48 h of culture in the presence of insulin, triiodothyronine and dexamethasone, glucokinase is induced in hepatocytes of 14-d-old suckling rats (Narkewicz *et al.* 1990). Addition of glucose alone is capable of stimulating FAS expression in hepatocytes of 14-d-old suckling rats previously exposed to insulin, triiodothyronine and dexamethasone (Fig. 1; Prip-Buus *et al.* 1995). The presence of glucokinase seems to be crucial for glucose stimulation of FAS gene expression. Similar results have also been obtained for L-PK expression in adult rat hepatocytes (JF Decaux, personal communication). On the other hand, in the well-differentiated hepatoma cell line mhAT3F (Antoine *et al.* 1992), which does not express glucokinase, but has a high hexokinase activity, the stimulatory effect of glucose on L-PK gene expression is observed, despite the absence of insulin in the medium (Lefrançois-Martinez *et al.* 1994).

In order to study whether the effect of insulin on glucose stimulation of L-PK gene expression in primary cultures of adult rat hepatocytes is due to the presence of glucokinase activity, a glucokinase expression vector was co-transfected with a vector containing a fragment of L-PK promoter (containing the glucose response element) linked to a chloramphenicol acetyltransferase (*EC* 2.3.1.28; CAT) reporter gene (Doiron *et al.* 1994). The cells co-transfected with the glucokinase expression vector showed a stimulatory effect of glucose on L-PK CAT promoter activity, independent of insulin. This suggested that insulin stimulated L-PK gene expression in the presence of glucose by inducing the capacity for glucose phosphorylation (secondary to induction of glucokinase) and not by a direct transcriptional effect on the L-PK gene (Doiron *et al.* 1994).

Low concentrations of fructose potentiate the effect of glucose on L-type pyruvate kinase expression

Glucokinase activity is stimulated in presence of low fructose concentrations (0·2 mmol/l) by a mechanism

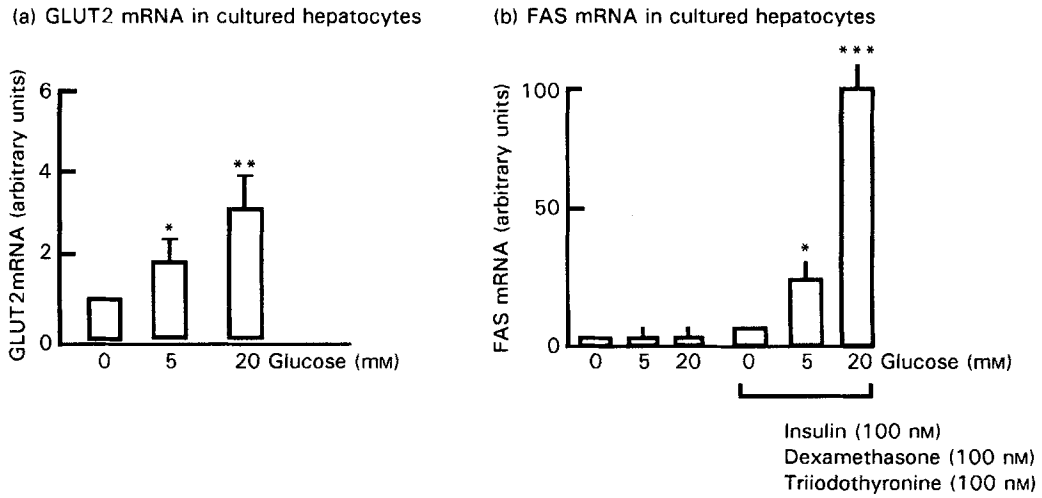

Fig. 1. (a) The effect of glucose on glucose transporter protein GLUT2 gene expression in cultured hepatocytes from 14-d-old suckling rats. (b) The effect of glucose on fatty acid synthase (*EC* 2.3.1.85; FAS) gene expression in cultured hepatocytes from 14-d-old suckling rats devoid of glucokinase (*EC* 2.7.1.2) and when glucokinase is induced by the presence of insulin, triiodothyronine and dexamethasone. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those without glucose: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. For details, see p. 267. (Adapted from Prip-Buus *et al.* 1995 and Rencurel *et al.* 1996.)

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involving a regulatory protein of 62 kDa (Van Schaftingen, 1989). In the absence of fructose, this regulatory protein is bound to glucokinase (Van Schaftingen, 1989). Fructose is phosphorylated in the liver to fructose-1-phosphate by fructokinase (*EC* 2.7.1.4) and the binding of fructose-1-phosphate to glucokinase regulatory protein, liberates glucokinase which has a high activity in this conformation (Van Schaftingen, 1989). Fructose can also induce the translocation of glucokinase (Agius & Peak, 1993). A recent study demonstrated that glucokinase regulatory protein is located in the nucleus and sequesters glucokinase in the absence of fructose; fructose induces the translocation of glucokinase to the cytosol (Brown *et al.* 1997). In the presence of low concentrations of fructose, high glucose concentrations stimulate the activity of the L-PK promoter gene fragment linked to a CAT reporter gene to a similar level to that observed in the presence of glucose and insulin (Doiron *et al.* 1994). This suggests that glucokinase is crucial for the glucose effect on L-PK gene expression, and probably that the glucose effect on gene expression involves a stimulation of glucose metabolism.

Glucokinase is not required for glucose to stimulate GLUT2 gene expression

To test whether the presence of glucokinase was an absolute prerequisite for the stimulatory effect of glucose on GLUT2 expression, we used either hepatocytes from 14-d-old rats or mhAT3F cells, which do not express glucokinase (Walker & Holland, 1965; Lefrançois-Martinez *et al.* 1994). In 14-d-old rat hepatocytes, as in mhAT3F cells, glucose stimulates GLUT2 expression and this effect was correlated with glucose concentration (Fig. 1; Rencurel *et al.* 1996). In contrast, insulin inhibited GLUT2 expression in the presence of low glucose concentrations (5 mmol/l; Postic *et al.* 1993). The stimulatory effect of high glucose concentrations was predominant over the inhibitory effect of insulin (Postic *et al.* 1993). This suggested that insulin and glucose exerted opposite effects on GLUT2 expression in hepatocytes (Postic *et al.* 1993). When insulin was added to the medium, GLUT2 expression was inhibited at low glucose concentrations (5–10 mmol/l; Postic *et al.* 1993). In the absence of insulin in the medium, glucose stimulated GLUT2 gene expression in a dose-dependent manner (Postic *et al.* 1993). Thus, it was apparent that there is a family of glucose-regulated genes in the liver that requires glucokinase (L-PK, FAS) whereas another family of glucose-regulated genes (GLUT2) does not require glucokinase.

Glucose must be metabolized to stimulate liver gene expression

The obvious question which arises when considering the effect of glucose concerns the nature of the molecule which acts as a signal to increase GLUT2 expression. Is it glucose itself or one of its metabolites, and if the latter, which one?

In adipose tissue explants, it was shown that 3-O-methyl glucose, a glucose analogue which is transported into the cell but which is not phosphorylated by hexokinases, is unable to induce expression of FAS and acetyl-CoA carboxylase (*EC* 6.4.1.2; ACC; Foufelle *et al.* 1992). This suggested that glucose metabolism was necessary. Another indication that glucose metabolism is necessary comes from studies of primary cultures of adult rat hepatocytes. In hepatocytes cultured in the presence of N-acetyl-D-glucosamine, a competitive inhibitor of hexokinase and glucokinase (Bontemps *et al.* 1978), the stimulatory effect of glucose on GLUT2 expression was completely abolished (Rencurel *et al.* 1996). Moreover, it was shown that 3-O-methyl glucose was unable to induce the expression of GLUT2 (Rencurel *et al.* 1996). Once it was demonstrated that glucose must be metabolized in order to stimulate liver gene expression, the next question was to determine which metabolite(s) are involved.

Which metabolite(s) are candidates to be the signal for increased liver gene expression?

For a metabolite to qualify as a signal, its concentration must vary in proportion to extracellular glucose concentration, since the effect of glucose on gene expression is concentration-dependent. Usually, in metabolic pathways, one can differentiate between enzymes which are non-equilibrium and near-equilibrium (Foufelle *et al.* 1996).

A large change in flux (such as that produced, for instance, by increasing glucose concentration from 5 to 20 mmol/l) can be accommodated by a near-equilibrium enzyme without a large change in substrate concentration. This implies that substrates of near-equilibrium reactions are unlikely to qualify as the metabolic signal. Conversely, the substrates of non-equilibrium enzymes should vary substantially in response to changes in flux, and thus are more likely candidates.

Starting from glucose-6-phosphate, a number of pathways can be considered: glycolysis and formation of fructose-2,6-bisphosphate, mitochondrial oxidation of CoA arising from glycolysis, pentose phosphate pathway, glycogen synthesis, hexosamine pathway (Girard *et al.* 1997).

Mitochondrial oxidation

In hepatocytes and white-adipose-tissue explants, the expression of FAS, ACC, L-PK and GLUT2 genes remains very low in the absence of glucose (a mixture of lactate and pyruvate was added into the culture medium as energy substrates; Munnich *et al.* 1985*b*; Foufelle *et al.* 1992; Postic *et al.* 1993; Prip-Buus *et al.* 1995). The expression of ACC in pancreatic β-cell line INS-1 was not increased by substrates which are known to increase insulin secretion through their mitochondrial oxidation (leucine, glutamine; Brun *et al.* 1993). These experiments eliminate substrates generated by mitochondrial oxidation as signal molecules for the effect of glucose on liver gene expression.

Glycolysis

The glucose analogue, 2-deoxyglucose, is transported into hepatic cells by the same transporter as glucose and is phosphorylated by glucokinase (K_m 55 mmol/l *v.* 10

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mmol/l for glucose) and hexokinase (K_m 30 μmol/l *v.* 10 μmol/l for glucose). However, in contrast to glucose, 2-deoxyglucose is not isomerized to fructose-6-phosphate and is thus a poor substrate for glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; V_{max} relative to glucose-6-phosphate is 0·05; Bessell & Thomas, 1973). In adipose-tissue explants from suckling rats, a low concentration of 2-deoxyglucose (1 mmol/l) increases FAS expression as do high concentrations of glucose (20 mmol/l; Foufelle *et al.* 1992). The accumulation of FAS mRNA is correlated with intracellular glucose-6-phosphate concentration, and studies with 2-deoxyglucose suggest that glucose-6-phosphate could be the metabolite that transmits the glucose signal to the FAS gene (Foufelle *et al.* 1992). However, 2-deoxyglucose had no effect on L-PK (Bossard *et al.* 1994), FAS (Prip-Buus *et al.* 1995) or GLUT2 (Rencurel *et al.* 1996) gene expression in cultured rat hepatocytes. The absence of a 2-deoxyglucose effect on GLUT2 expression in hepatocytes has been attributed to the fact that glucose-6-phosphatase could dephosphorylate 2-deoxyglucose-6-phosphate and thus prevent its accumulation to the cell. We (Rencurel *et al.* 1996) have estimated the intracellular concentration of 2-deoxyglucose-6-phosphate in cultured hepatocytes by GC–mass spectrometry. After 22 h of culture in the presence of high concentrations (30 mmol/l) of 2-deoxyglucose, the concentration of phosphorylated products was estimated to 30 nmol/mg protein and 66 % of these phosphorylated products were 2-deoxyglucose-6-phosphate. This concentration of 2-deoxyglucose-6-phosphate is similar to the glucose-6-phosphate concentrations measured in hepatocytes cultured in the presence of high glucose concentration (Kang *et al.* 1996). However, under these experimental conditions, the expression of L-PK gene was not stimulated by 2-deoxyglucose (Bossard *et al.* 1994; Kang *et al.* 1996).

In the liver, 2-deoxyglucose-6-phosphate is incorporated into glycogen (Jenkins *et al.* 1986), but the concentrations measured in cells are similar to those of glucose-6-phosphate (Kang *et al.* 1996; Rencurel *et al.* 1996). In transgenic mice which overexpress liver glucokinase (glucokinase gene driven by the phosphoenolpyruvate carboxykinase (*EC* 4.1.1.49) promoter), a significant level of L-PK mRNA was observed during fasting, and was correlated with the glucose-6-phosphate concentrations (Ferre *et al.* 1996). Under the same conditions, liver GLUT2 expression was not altered (Ferre *et al.* 1996). This finding reinforces the possibility that different metabolites could be involved in the stimulation of gene expression in response to glucose. Further experiments are necessary to identify these metabolites. The definitive proof that glucose-6-phosphate could mediate the effect of glucose on FAS or L-PK gene expression would necessitate the determination of the time-course of glucose-6-phosphate accumulation in the presence of glucose. Indeed, in all the studies described previously, the correlation between the glucose-6-phosphate concentration and the mRNA levels (FAS, L-PK etc.) was obtained at only one time interval after the addition of glucose. In addition, it would be interesting to know whether the addition of glucose-6-phosphate to the medium of permeabilized hepatocytes would rapidly increase gene expression.

Mannose, fructose, dihydroxyacetone and sorbitol mimic the effect of glucose on liver gene expression

Several studies have been performed in an attempt to determine if other carbohydrates (fructose, mannose) or other substrates entering glycolysis or the pentose phosphate pathway at a step distal to glucose-6-phosphate can mimic *in vitro* the effect of glucose on liver gene expression. These experiments allow the determination of which metabolite(s) accumulates in the cell and could be a good candidate to be the signal.

Mannose and fructose (which are also transported by GLUT2 into the cell) increase GLUT2 mRNA concentrations (Fig. 2). By contrast, sucrose, a dissacharide, which is not a substrate transported by GLUT2 has no effect (Asano *et al.* 1992). Mannose, an epimer of glucose, is phosphorylated by hexokinase (glucokinase in the liver) and then isomerized to fructose-6-phosphate, which in turn can yield glucose-6-phosphate through phosphoglucose isomerase (*EC* 5.3.1.9). Fructose is phosphorylated by fructokinase to fructose-1-phosphate, which is split into dihydroxyacetone phosphate and glyceraldehyde by aldolase, and these trioses can yield glucose-6-phosphate through gluconeogenesis.

Dihydroxyacetone and sorbitol stimulate GLUT2 gene expression in hepatocytes (Fig. 2; Rencurel *et al.* 1996). In the pancreatic β cell-line INS-1, dihydroxyacetone does not stimulate ACC gene expression (Brun *et al.* 1993), as in the hepatoma cell line FAO in which dihydroxyacetone does not mimic the glucose effect on glucose-6-phosphatase gene expression (Argaud *et al.* 1995). Sorbitol also stimulated glucokinase activity in hepatocytes since sorbitol can be transformed into fructose by sorbitol dehydrogenase (*EC* 1.1.1.14; Agius 1994; Lee *et al.* 1995). In hepatocytes transfected with a plasmid containing the L-PK promoter coupled to the CAT reporter gene, the CAT activity was increased in the presence of low concentrations of xylitol (0·5 mmol/l) and this effect was similar to that observed with 20 mmol glucose/l (Doiron *et al.* 1996). At this low xylitol concentration, there was no

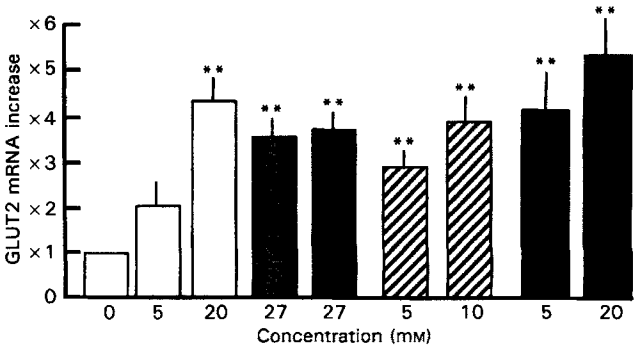

Fig. 2. Mannose (■), fructose (■), dihydroxyacetone (▨) and sorbitol (■) mimic the glucose (□) effect on glucose transporter protein GLUT2 expression in cultured hepatocytes. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from that without glucose: $^{**}P < 0.01$. (Adapted from Asano *et al.* 1992 and Rencurel *et al.* 1996.)

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glucose-6-phosphate accumulation in the cells. It was concluded that the stimulation of the L-PK promoter in response to xylitol was due to the accumulation of xylulose-5-phosphate, a metabolite of the non-oxidative branch of the pentose phosphate pathway (Doiron *et al.* 1996). Unfortunately, xylulose-5-phosphate levels were not determined in this study. However, other studies have shown that xylitol can increase intracellular ribulose-5-phosphate, glycerol-3-phosphate, fructose-6-phosphate and glucose-6-phosphate concentrations in incubated hepatocytes (Cederbaum & Dicker, 1979; Vincent *et al.* 1989; Lindstad *et al.* 1992). It has been shown recently that in hepatocytes cultured in the presence of various concentrations of glucose and xylitol, the expression of FAS or Spot 14 genes was more closely related to glucose-6-phosphate levels than to xylulose-5-phosphate levels (Mourrieras *et al.* 1997). The pentose phosphate pathway is not very active in adipocytes and hepatocytes from 14-d-old suckling rats (Issad *et al.* 1989; Iritani *et al* 1993). Nevertheless, glucose rapidly stimulates the expression of FAS and ACC genes in adipocytes from suckling rats (Foufelle *et al.* 1992) and GLUT2 expression in hepatocytes (Rencurel *et al.* 1996). This reinforces the probability that the pentose phosphate pathway does not play an important role in generating the metabolite which is the signal for the glucose effect on gene expression.

A recent study suggests that fructose-2,6-bisphosphate could play an important role in the stimulatory effect of glucose on glucose-6-phosphatase expression in the liver (Argaud *et al.* 1997). In the hepatoma cell line FAO expressing a high level of glucokinase by the use of an expression vector, the effect of glucose on glucose-6-phosphatase expression was similar to that described in hepatocytes, by comparison with the absence of an effect of glucose in the wild type FAO cell line expressing a low glucokinase level (Argaud *et al.* 1997). Moreover, when the concentration of fructose-2,6-bisphosphate is increased in FAO cells by transfection of an expression vector coding for the bifunctional enzyme 6-phosphofructo-2-kinase (*EC* 2.7.1.105)–fructose-2,6-bisphosphatase (*EC* 3.1.3.46), the concentration of mRNA coding for glucose-6-phosphatase increases (Argaud *et al.* 1997). When the concentration of fructose-2,6-bisphosphate is high, the glycolytic flux is high and when its concentration is low, the gluconeogenic flux is high, since fructose-2,6-bisphosphate is an allosteric activator of 6-phosphofructo-1-kinase and an inhibitor of fructose-1,6-bisphosphatase (for review, see Hers & Van Schaftingen, 1982). The glycolytic flux was estimated from lactate and pyruvate production, but the authors did not obtain a good correlation between lactate production and the concentration of mRNA coding for glucose-6-phosphatase. They argued that dihydroxyacetone does not stimulate glucose-6-phosphatase in FAO cells, while lactate production was increased (Argaud *et al.* 1995, 1997).

Glucose-6-phosphate is a likely candidate to be the signal for insulin-dependent glucose-regulated genes such as L-PK and FAS. The glucose has to be phosphorylated to induce gene expression. The intracellular concentrations of glucose-6-phosphatate vary in parallel with the intensity of FAS and L-PK gene induction. 2-Deoxyglucose mimics glucose totally in adipose tissue or partially in pancreatic β-cells, but not in hepatocytes, despite an accumulation of 2-deoxyglucose-6-phosphate. Additional studies are required to determine if glucose-6-phosphate is really the signal; for example, the metabolites generated from 2-deoxyglucose-6-phosphate could be studied. Fructose-6-phosphate could be a good candidate also, since glucokinase is essential for glucose stimulation of L-PK and FAS genes but not for GLUT2, which introduces another level of complexity.

The effect of glucose on gene expression is transcriptional

To explain the accumulation of mRNA in the presence of glucose, two mechanisms are possible. The first is a stimulation of gene transcription and the second is a post-transcriptional regulation of the level of mRNA (regulation of the half-life of mRNA). By using inhibitors which interact with the intracellular machinery of transcription, many studies have demonstrated that glucose does not stabilize mRNA coding for glucose-stimulated genes. In hepatocytes cultured in presence of a transcriptional inhibitor (actinomycin D), the decay of GLUT2 mRNA concentrations was similar in the presence or in the absence of glucose (Rencurel *et al.* 1996). The half-life was estimated at about 10 h, in accordance with results obtained in insulinoma cells INS-1 in which GLUT2 is also expressed and stimulated by glucose (Waeber *et al.* 1994). In INS-1, glucose stimulates ACC gene expression and does not modify the half-life of mRNA (Brun *et al.* 1993).

Glucose response elements

A glucose response element, also termed carbohydrate response element (ChoRE), was first identified in the L-PK gene (Thompson & Towle, 1991; Cuif *et al.* 1992). The glucose response element is located within the first -183 base pairs (bp) of the L-PK promoter, a region which is responsible for tissue-specific expression, as well as nutrient and hormone regulation in transgenic mice (Cuif *et al.* 1992). By transient transfection in cultured hepatocytes, the region was further characterized as a fragment between -183 and -96 bp (Thompson & Towle, 1991), and DNAase I footprinting experiments allowed the characterization of four elements termed box L1 (-66 -95 bp), L2 (-97 -114 bp), L3 (-126 -144 bp) and L4 (-145 -168 bp). Further experiments have demonstrated that glucose responsiveness was conferred through a cooperation between box L3 and L4 in the L-PK gene (Bergot *et al.* 1992; Liu *et al.* 1993). Nonetheless, a multimer of box L4, but not L3, was able to confer glucose responsiveness to a glucose-unresponsive heterologous promoter (Fig. 3; Bergot *et al.* 1992).

Spot 14 is another well studied gene which is also stimulated by glucose, and a second ChoRE was characterized in this gene (Shih & Towle, 1992). The Spot 14 gene encodes for an unknown protein which is involved in lipogenesis and is expressed in liver and other lipogenic tissues such as white and brown adipose tissues and the lactating mammary gland. The decrease in lipogenic

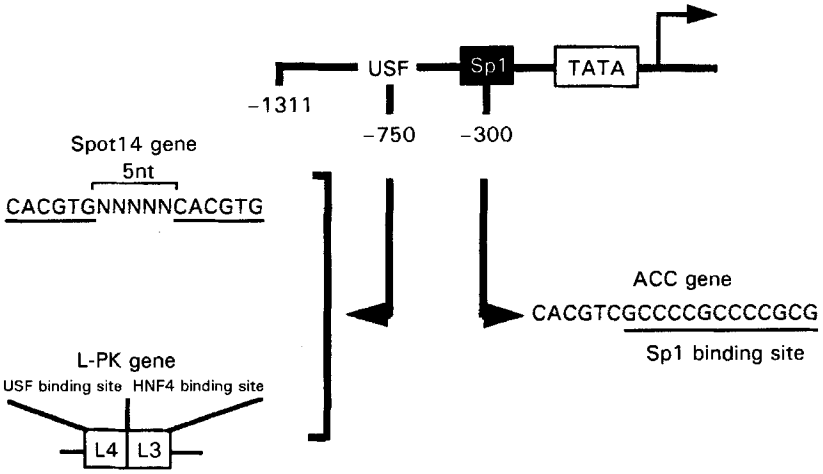

Fig. 3. Elements responsible for the glucose effect on gene expression. By computer analysis, two binding sites for upstream stimulatory factor (USF)–major late transcription factor and Sp1 transcription factors were localized in region –1311 base pairs upstream of the start of the mouse glucose transporter protein GLUT2 promoter. For details, see p. 272. L-PK, L-type pyruvate kinase (*EC* 2.7.1.40). (Adapted from Bergot *et al.* 1992; Waeber *et al.* 1994; Shih *et al.* 1995 and Daniel & Kim 1996.)

enzyme when Spot 14 expression is reduced by an antisense RNA suggests that Spot 14 protein could be involved in long-term regulation of lipogenesis (Kinlaw *et al.* 1995).

In hepatocytes, the region between −1601 and −1395 bp of Spot 14 promoter was found to be essential for glucose responsiveness of this gene (Shih & Towle, 1992). Comparison of the sequence of this region with the box L4 in L-PK promoter revealed a consensus sequence with nine of the ten identical. This sequence is able to confer glucose responsiveness to an heterologous promoter (Shih & Towle, 1994). DNAase I sensitivity revealed a site in the first intron of the FAS gene with elements similar to those described in L-PK and Spot 14 promoters (Foufelle *et al.* 1995), but functional experiments will confirm if this element in the first intron is effectively the ChoRE in the FAS gene.

In three ChoRE defined previously, a common DNA sequence emerges and corresponds to a cannonical (Spot 14 or FAS) or degenerated (L-PK) palindromic sequence 5′-CACGTG-3′ also known as E-box, and previously described as a binding site for a specific class of protein. All these proteins possess a common structure, the 'helix–loop–helix', and a leucine stretch or 'leucine zipper' (Gregor *et al.* 1990). These different structures are involved in the binding to DNA and in protein–protein interactions. Several transcription factors of this class have been characterized, such as c-myc, and its partner Max, also MyoD and upstream stimulatory factor (USF) Two USF (USF1 and USF2) have been characterized with identical DNA-binding properties (Sirito *et al.* 1994); they differ only by their N-terminal domain. USF binds DNA as a dimer. Their expression is ubiquitous and does not seem to be altered by nutritional or hormonal status. For the genes described previously (L-PK, Spot 14), the binding site of USF on the E-box of their ChoRE has been demonstrated (Vaulont *et al.* 1989; Diaz Guerra *et al.* 1993; Shih & Towle, 1994). Both genes contain a common regulatory element that consists of two 5′CACGTG-type E-box motifs separated by 5 bp (Fig. 3; Shih *et al.* 1995). In the context of the natural promoter, each gene contains a distinct accessory-factor site adjacent to the ChoRE that is necessary for the full extent of the response (Diaz Guerra *et al.* 1993; Liu *et al.* 1993). Disruption of the E-box by point mutations abolishes the glucose responsiveness of the L-PK and Spot 14 promoters (Bergot *et al.* 1992; Shih & Towle, 1992). In hepatoma cells, an overexpression of a native USF protein synthesized from expression vectors can act as transactivators of the L-PK promoter. Expression in these cells of a USF mutant deleted at its DNA binding site (it cannot bind ChoRE) but not at its dimerization domain (it dimerizes with and titrates endogenous USF) represses the glucose effect (Lefrançois-Martinez *et al.* 1995). However, the USF-binding site from the adenovirus major late promoter was unable to substitute for the ChoRE in either the L-PK or the Spot 14 gene, indicating that USF alone is not capable of mediating the response (Shih & Towle, 1994). For the L-PK gene, the transcription factor HNF4 is capable of binding immediately 3′ to the ChoRE and enhancing its activity (Diaz Guerra *et al.* 1993).

A recent study suggests that USF is not the factor that binds to ChoRE (Kaytor *et al.* 1997). In this study, the expression in hepatocytes of USF mutants using an overexpression vector does not abolish the glucose responsiveness of the Spot 14 promoter activity (Kaytor *et al.* 1997). This is in contradiction with a previous study (Lefrançois-Martinez *et al.* 1995). As suggested by the authors, this discrepancy could be due to the differences which should exist between the two cellular systems used, hepatocytes and hepatoma cell lines mhAT3F. Differences could be also due to the protocol used in these two studies, one was completed in 24 h (Kaytor *et al.* 1997), the other one continued over 72 h (Lefrançois-Martinez *et al.* 1995). In 72 h the effective suppression of USF could lead to reduced expression of another transcription factor (HNF4) required for the transcription of the L-PK gene. If USF is not the carbohydrate-responsive factor, the reason why the authors do not detect another factor capable of binding the ChoRE could be due to its low concentration in liver

(Kaytor *et al.* 1997). Some other glucose-regulated genes do not contain a ChoRE, but seem to require binding of transcription factors other than USF for glucose responsiveness.

Insulin gene

Although the insulin gene is only expressed in pancreatic β-cells, it is also regulated by glucose. An element of 50 bp, 'Far-FLAT' mini-enhancer, localized in the promoter of the insulin gene, is involved in glucose responsiveness (Melloul *et al.* 1993). This element confers glucose responsiveness to an heterologous promoter (German & Wang, 1994). The Far-FLAT element is situated from −193 to −227 bp of the insulin promoter gene. This element linked to a reporter gene coding for CAT, was transfected in different cell types, and the expression of this plasmid was only detected in cells which have a pancreatic origin such as RIN, HIT and βTC (Melloul *et al.* 1993). This finding demonstrated that this sequence was reponsible for tissue-specific expression of the insulin gene. This sequence contains an AT-rich motif and contains a box A3 which was reported to be important for regulation of insulin gene expression (German *et al.* 1995). In the human insulin promoter construct of −350 bp, a point mutation of the box A3 abolished the glucose responsiveness of this promoter transfected in rat pancreatic β-cells (Marshak *et al.* 1996). Three complexes, C1, C2, C3, were identified with nuclear extracts from β-cells by electromobility shift assay experiments (Melloul *et al.* 1993). A point mutation in the glucose-sensitive factor GSE abolished the binding of complex C1, suggesting an involvement of this complex in the glucose responsiveness of the insulin gene (Marshak *et al.* 1996). Moreover, the binding of C1 is stimulated by glucose in the culture medium. Conversely, binding of complexes C2 and C3 was not modified by the glucose concentration in the culture medium (Melloul *et al.* 1993; Melloul & Cerasi, 1994). The *trans*-factor which binds GSE, is a protein of 45 kDa which has a sequence identical with that of insulin promoter factor 1 which is important for fetal development of the endocrine pancreas (Ohlsson *et al.* 1993; Marshak *et al.* 1996).

The GLUT 2 gene

In the GLUT2 promoter gene there are no consensus sequences like the ChoRE CACGTG-5nt-CACGTG described for the Spot 14 gene (Waeber *et al.* 1994). Experiments in which different fragments (5′ deleted) of the GLUT2 promoter linked to the reporter gene CAT were transfected into insulinoma cells INS-1, hepatocytes and hepatoma cells mhAT3F determined that elements responsible for the glucose-responsiveness of the GLUT2 gene are present in the fragment −338 to +49 bp (Waeber *et al.* 1994; Rencurel *et al.* 1997). This fragment contains not only elements responsible for glucose responsiveness but also elements involved in tissue-specific expression (Fig. 4). The fragment −338 to +49 bp of the GLUT2 promoter gene is expressed only in GLUT2-expressing tissues (liver, pancreatic β-cells, kidney and intestine; Waeber *et al.* 1995). Three regions, GTI (−60 to −41 bp), GTII (−124 to −95 bp) and GTIII (−180 to −171 bp) were determined by DNAase I footprinting experiments in the region −338 bp upstream of the start site of the GLUT2 promoter (Bonny *et al.* 1995). In this study, GTII was defined as an AT-rich region and was specific for GLUT2-expressing tissues (Bonny *et al.* 1995). This result was in accordance with transfection experiments performed in βTC3 cells, demonstrating that a fragment −220 to +1 bp of the GLUT2 promoter could be important for GLUT2 tissue-specific expression (Leibiger & Leibiger, 1995).

The rat gene coding for ACC possesses two promoters (PI and PII) which have different actions in different tissues, and transcribe different mRNA by alternative splicing in the 5′ non-coding region (Lopez-Casillas & Kim, 1989). ACC gene expression is stimulated by glucose in the insulinoma cell line INS-I (Brun *et al.* 1993). The element responsible for glucose responsiveness is contained in the PII promoter which contains a GC box. Mutation in

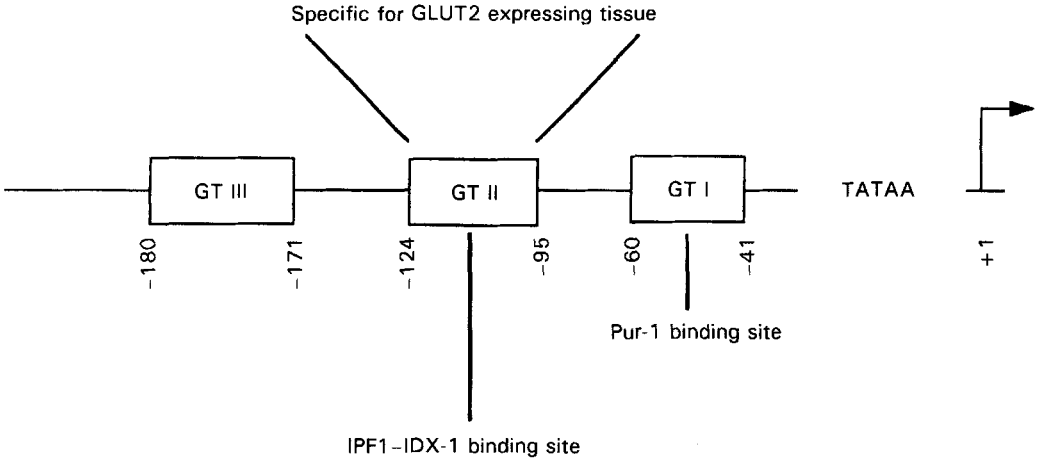

Fig. 4. Localization of three boxes important for regulation of glucose transporter protein GLUT2 gene expression. For details, see p. 272. IPF1, insulin promoter factor 1; IDX1, insulin duodenal homeobox 1. (Adapted from Bonny *et al.* 1995.)

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the GC box abolishes the glucose responsiveness of the ACC promoter (Daniel & Kim, 1996). USF–major late transcription factor cannot stimulate ACC PII promoter, whereas Sp1, which can bind the glucose element in the ACC promoter, can (Daniel & Kim, 1996).

By computer analysis, we have detected a putative binding site for the Sp1 factor in the region −338 to +49 bp of GLUT2 promoter which is responsible for glucose responsiveness of the GLUT2 gene expression. But Sp1 is ubiquitously expressed and raised the question as to how a ubiquitous factor can participate in a specific response such as the glucose effect on gene expression. As suggested by Daniel & Kim (1996), Sp1 could interact with other specific factors.

Conclusion

Future work will determine which metabolites and pathways are involved in the glucose effect on gene expression. Non-invasive techniques, such as NMR, and the development of new glucose analogues could facilitate the monitoring of glucose in the cell, and thus determine if the signal is really a metabolite which accumulates in the cell. The development of techniques in molecular biology could be important to determine the mechanism by which the metabolite signal stimulates gene transcription. The interaction with protein, as described with xylulose-5-phosphate which binds and modulates the activity of the PP2A type protein phosphatase, is a suitable hypothesis (Nishimura *et al.* 1994). The recent experiments demonstrating that USF–major late transcription factor could not be the *trans*-acting factor for glucose responsiveness open a new research field (Kaytor *et al.* 1997). The link between ubiquitous *trans*-acting factors and tissue-specific *cis*-elements in glucose-regulated genes for glucose responsiveness has to be determined. The tissue specificity is important since glucose-regulated genes are expressed mainly in tissues such as liver and pancreatic β-cells which play an important role in maintaining glucose homeostasis.

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