REVIEW ARTICLE

Transcriptional control of genes that regulate glycolysis and gluconeogenesis in adult liver

Frédéric P. LEMAIGRE and Guy G. ROUSSEAU*

Hormone and Metabolic Research Unit, University of Louvain Medical School and International Institute of Cellular and Molecular Pathology, 75 Avenue Hippocrate, B-1200 Brussels, Belgium

INTRODUCTION

Glycolysis is the metabolic pathway through which glucose is converted into pyruvate, with a net yield of 2 mol of ATP and NADH per mol of glucose used. In liver, where the glycolytic flux is low except when glucose concentrations are high or during anoxia, the main function of glycolysis is to provide substrates for anabolic processes. While glycolysis occurs in every tissue, gluconeogenesis is specific for the liver, the kidney and the small intestine. Gluconeogenesis supplies glucose to the body and provides a way to dispose of amino acids and lactate produced by erythrocytes and during muscle contraction. It is also a means of disposal for glycerol produced during lipolysis.

Liver glycolysis and gluconeogenesis share several enzymes which catalyse reactions that are close to equilibrium (reversible) under physiological conditions. On the other hand, three substrate cycles, i.e. the glucose/glucose 6-phosphate, fructose 6-phosphate/fructose 1,6-bisphosphate and phosphoenolpyruvate/pyruvate cycles, involve exergonic reactions that regulate the pace of glycolysis and gluconeogenesis. These reactions, which are maintained far from equilibrium, are catalysed by different enzymes in the glycolytic and gluconeogenic pathways. It is therefore not surprising that these key enzymes are the main targets of regulatory mechanisms. Short-term regulation involves both the supply of glycolytic or gluconeogenic substrates and the control of the catalytic properties of the enzymes through allosteric changes and phosphorylation [1,2]. Long-term regulation in liver involves changes in gene expression and protein synthesis [3,4] and is the subject of the present review.

Indeed, several mechanisms that restrict gene transcription to the liver and modulate it upon hormonal stimulation and nutrient supply have been unravelled. These mechanisms rely upon the interaction of nuclear proteins, called *trans*-acting factors, with *cis*-acting DNA sequences that belong to the gene considered. These sequences usually lie upstream from the transcription initiation (cap) site, in the promoter proper or at a distance that may reach several kilobases, in which case they are called enhancers (or silencers). The latter are sometimes found downstream from the cap site. *Trans*-acting factors are ubiquitous or 'tissue-enriched', and are distinct from the general transcription factors that directly assist RNA polymerase II through binding in the vicinity of the cap site. Some of them are hormone receptors, the activity of which is triggered by ligand binding. The five, well-characterized, classes of liver-enriched factors are listed in Table 1. The ubiquitous factors that control the genes discussed here are listed in Table 2. These genes code for glucokinase, 6-phosphofructo-2-kinase (PFK-2)/fructose-2,6-bisphosphatase (FBPase-2), pyruvate kinase and phosphoenolpyruvate carboxykinase (PEPCK), namely enzymes that control key steps, and for aldolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which are enzymes controlling reversible steps.

In the present paper, we will review the data pertaining to the transcriptional control of these genes in liver. We will describe first the glycolytic enzymes and then the gluconeogenic enzyme PEPCK. Aldolase B and GAPDH are discussed with the glycolytic enzymes, as they are hormonally controlled like the latter. The genes that code for the other enzymes of glycolysis and gluconeogenesis have not been studied in sufficient detail at the transcriptional level to warrant consideration here. We will focus on the relevant *cis*-acting regions and *trans*-acting factors in an attempt to assign them a role in the liver-specific transcription and (or) in the response to hormones and carbohydrates.

GLUCOKINASE

Hexokinases (EC 2.7.1.1.) catalyse the phosphorylation of glucose to glucose 6-phosphate. The liver contains four hexokinases (A to D or I to IV), glucokinase (hexokinase D or IV) being by far the predominant isoenzyme. Pancreatic β cells also contain a glucokinase activity. However, the regulation of hepatic and β-cell glucokinases differs in a way related to the functions of the two cell types. Whereas hepatic glucokinase modulates glucose uptake, β-cell glucokinase may play the role of a glucose sensor (reviewed in [5]). By controlling the rate of the glycolytic flux in β cells, glucokinase determines the ATP:ADP ratio, which is postulated to affect insulin secretion. Hepatic and β-cell glucokinases therefore play a complementary role in glucose homoeostasis. In liver, the regulatory protein of glucokinase binds to and inhibits glucokinase in the presence of fructose 6-phosphate (reviewed in [6]).

Gene organization

The differential regulation of liver and β-cell glucokinases relies on a genic basis (reviewed in [7]). The liver and β-cell mRNAs are identical with the exception of their 5′ end that codes for a different 15-amino-acid stretch. In the rat [8,9] and in the human [10,11], there is a single glucokinase gene containing 11 exons

Abbreviations used: AF, accessory factor; b-HLH, basic region/helix–loop–helix; C/EBP, CCAAT/enhancer-binding protein; COUP-TF, chicken ovalbumin upstream promoter-transcription factor; CRE, cyclic AMP response element; CREB, cyclic AMP response element-binding protein; DBP, D-binding protein; FBPase-1, fructose-1,6-bisphosphatase; FBPase-2, fructose-2,6-bisphosphatase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GIRE, glucose/insulin response element; GRU, glucocorticoid-responsive unit; HNF, hepatocyte nuclear factor; IRE, insulin response element; IRS, insulin response sequence; NF-I, nuclear factor-I; NFY, nuclear factor Y; Oct-1, octamer factor-1; PEPCK, phosphoenolpyruvate carboxykinase; PFK-1, 6-phosphofructo-1-kinase; PFK-2, 6-phosphofructo-2-kinase; PKA, cyclic AMP-dependent protein kinase; PKC, protein kinase C; RAR, retinoic acid receptor; RARE, retinoic acid response element; STAT, signal transducer and activator of transcription; T_3, tri-iodothyronine; T_3R, T_3 receptor; USF, upstream stimulating factor.

* To whom correspondence should be addressed.

Table 1 Classification of liver-enriched factors

Family	Consensus	Structure	Function	Members (synonyms)	Notes
HNF-1	GTTAATTNATTAAC	POU-homeodomain Homo- or heterodimer	Transcription activation	HNF-1α (HNF-1, LF-B1, APF, HP-1) HNF-1β (vHNF-1, LF-B3)	Glycosylated
HNF-3	TATTGA$^{C}/_{T}$TT$^{A}/_{T}$G and T$^{A}/_{G}$TTTG$^{C}/_{T}$ (HNF-5 site)	Fork-head domain Monomer	Transcription activation	HNF-3α HNF-3β HNF-3γ	Regulated by phosphorylation
HNF-4	TGGAC$^{T}/_{C}$$^{T}/_{C}(N)_{1-4}$TGGCCC	Zinc finger Related to steroid receptors Homodimer	Transcription regulation	HNF-4 (HNF-2, LF-A1, Tf-LF1) ARP-1	Transcription activator Transcription inhibitor
C/EBP	No consensus	Basic region/leucine zipper Homo- or hetero-dimer	Transcription regulation	C/EBPα 40 (C/EBP, Tf-LF2) C/EBPα 32 C/EBPβ (NF-IL6, IL6-DBP, LAP, AGP-EBP, CRP-2, NF-M) C/EBPγ (Ig/EBP-1) C/EBPδ (CRP-3, NF-IL6 β) C/EBPε (CRP-1) CHOP-10 LIP	Transcription activator Transcription activator Transcription activator Regulated by phosphorylation Mediator of IL6, cyclic AMP Mediator of IL6, IL1 Dominant negative modulator of C/EBPα, β, γ Dominant negative modulator of C/EBPβ
PAR	No consensus	Proline-acid-rich region Homo- or hetero-dimer	Transcription activation	DBP Hlf	Circadian rhythm

Table 2 Ubiquitous factors that regulate expression of genes coding for glycolytic and gluconeogenic enzymes

Family	Consensus	Structure	Function	Members (synonyms)	Notes
CCAAT-box factors	TGG$^{C}/_{A}$(N)$_{5}$GCCAA	Proline-rich activation domain Homo- or hetero-dimer	Transcription activation	CTF/NF-I NFY	
Octamer factors	TNATTTGCATA	POU-homeodomain Homodimer	Transcription activation	Oct-1 (OTF-1)	
Sp1	$^{G}/_{T}$$^{G}/_{A}GGC^{G}/_{T}$$^{G}/_{A}$$^{G}/_{A}$$^{G}/_{T}$	Zinc finger Monomer	Transcription activation	Sp1	
CREB/ATF	TGACG$^{C}/_{T}$$^{C}/_{A}$$^{G}/_{A}$	Basic region/leucine zipper Homo- or hetero-dimer	Transcription activation	CREB	Mediator of cyclic AMP
Activator protein 1	TGA$^{G}/_{G}$T$^{C}/_{A}$A	Basic region/leucine zipper Homo- or hetero-dimer	Transcription regulation	c-Jun c-Fos	Regulated by phosphorylation
b-HLH	No consensus	Basic region/helix–loop–helix	Transcription activation	USF (MLTF)	
Nuclear hormone receptors	AGAACA AGGTCA	Zinc finger Homo- or hetero-dimer	Transcription regulation	Steroid hormone receptors Thyroid hormone receptors Retinoic acid receptors COUP-TFI COUP-TFII	

and two promoters. The upstream promoter is functional in β cells and in the pituitary [8,12], while the downstream promoter functions only in liver [9]. Tissue-specific promoter activity produces a liver-type primary transcript that is spliced to yield an mRNA containing exons 1h and 2 to 10, and a β-cell primary transcript that is spliced to yield an mRNA containing exons 1β and 2 to 10. The organization of the gene therefore explains the tissue-specific structure of the glucokinase mRNAs. Several minor forms are generated by alternative splicing (reviewed in [7,13]) and some of them code for proteins devoid of glucose phosphorylating activity.

Tissue-specific control

Magnuson et al. [9] described the liver promoter and identified sequences that are potential binding sites for basal and liver-specific transcription factors. A poor TATA consensus is detected from −29 to −25, but a perfect Sp1 consensus is located from −442 to −432. We do not think the potential hepatocyte nuclear factor (HNF)-1 site they pointed out at −170 is a target for HNF-1, since it does not display the required palindromic pattern found in the GTTAATTNATTAAC consensus [14]. On the contrary, the three potential HNF-4 sites all fit well with the bipartite consensus which contains the TGGACT/CT/C and TGGCCC motifs [15]. One report [16] showed promoter activity between −87 and +17 upon transfection of hepatocytes in culture; deletion of nucleotides −87 to −48 reduced promoter activity. This coincided with the removal of a potential HNF-4 site. A role for regulation by the CCAAT/enhancer-binding protein (C/EBP) has been proposed [17]. The regulatory elements of the β-cell promoter have been characterized recently using promoter–reporter gene constructs transfected in β-cell-derived HIT M2.2.2 cells. Maximal promoter activity was obtained with sequences located between −280 and the cap site [18]. This region contains binding sites for a rat β-cell-specific factor IPF-1, known to bind also the insulin-I gene promoter.

Nutritional and hormonal control

Modulation of glucokinase activity in liver relies on changes in the amount of glucose and of enzyme, as well as on the activity of the regulatory protein of glucokinase. Insulin stimulates transcription approx. 20-fold [8,19–21]. This stimulation does not depend on extracellular glucose [20,21] and occurs within 30 min [22]. No insulin response element (IRE) has been experimentally localized on the liver promoter, but O'Brien and Granner [23] pointed to the existence of a TGGTTCTTTG motif at −83 that resembles the negative insulin response sequence (IRS) TGGTGTTTTG of the PEPCK gene. Glucagon, acting via cyclic AMP, inhibits the stimulation by insulin [20]. The mechanism is unknown, but the dominance of glucagon over insulin suggests that insulin stimulation may consist of derepression. Indeed, Nouspikel and Iynedjian [22] showed that phosphodiesterase inhibitors, which are expected to increase cyclic AMP concentrations, abolish insulin stimulation of transcription. However, it is unlikely that insulin acts by stimulating phosphodiesterase as insulin treatment does not change cyclic AMP concentrations [22]. In addition, okadaic acid, an inhibitor of protein phosphatases PP1 and PP2A, abolishes the response to insulin. The protein synthesis inhibitor cycloheximide also blocks the response to insulin [21,22] but, given the rapidity of the insulin effect, it is unlikely that insulin action is a secondary response. In fact, cycloheximide could act through a mechanism that does not involve protein synthesis inhibition [22]. Alternatively, cycloheximide could block the synthesis of a factor that acts co-operatively on the glucokinase gene with the mediator of insulin action. A similar mechanism regulates the glucocorticoid-inducibility of the α1-acid glycoprotein gene [24]. The effect of insulin might also involve protein kinase C (PKC), since a PKC inhibitor blocks insulin stimulation [21]. However, phorbol esters do not mimic the effects of insulin [7]. In cultured hepatocytes, dexamethasone and tri-iodothyronine (T_3) enhance the stimulation by insulin [21,25].

β-Cell glucokinase is not sensitive to insulin and its mRNA levels are not affected by variations in extracellular glucose concentration. Rather, glucose-dependent changes in β-cell glucokinase activity are regulated translationally or post-translationally [26]. Thus the way in which glucagon and insulin modulate glucokinase gene transcription in liver and the gene sequences involved remain to be identified.

6-PHOSPHOFRUCTO-2-KINASE/FRUCTOSE-2,6-BISPHOSPHATASE

Fructose 2,6-bisphosphate strongly stimulates 6-phosphofructo-1-kinase (PFK-1), which catalyses the first committed step in glycolysis, and it inhibits fructose-1,6-bisphosphatase (FBPase-1), which catalyses the reverse reaction. Its synthesis and degradation are catalysed by PFK-2 (EC 2.7.1.105) and FBPase-2 (EC 3.1.3.46), two catalytic activities borne by the same peptide. The properties of PFK-2/FBPase-2 have been reviewed [27,28] and we therefore focus on the molecular biology of this bifunctional enzyme.

Gene organization

There are several PFK-2/FBPase-2 isoenzymes which differ in tissue distribution, molecular mass, catalytic properties and response to protein kinases (reviewed in [28,29]). The best characterized are the liver (L), skeletal muscle (M), heart (H), testis (T) and brain isoenzymes. The sequence of the L mRNA of several species is known [30–32] as well as that of the rat M-, T- and H-type mRNAs [33–36]. In addition, a fetal (F)-type mRNA was recently identified [37]. Two rat genes have been characterized, one coding for the F-, L- and M-type mRNAs (gene A) and one for the H-type mRNAs (gene B) [38,39].

Gene A (55 kb) contains three promoters (F, M and L promoters) and 17 exons (1aF, 1bF, 1M, 1L and exons 2 to 14). The F-, M- and L-type mRNAs share exons 2 to 14 but differ at the 5′ end. The F-type mRNA contains exons 1aF, 1bF and part of 1M, the M-type mRNA contains exon 1M, and the L-type mRNA contains exon 1L. The M-type mRNA codes for a protein identical with the L isoenzyme, except that the 32 N-terminal residues coded by exon 1L are replaced by an unrelated sequence of nine amino acids coded by exon 1M. As a consequence of this alternative promoter usage, the M isoenzyme is not controlled by the cyclic AMP-dependent protein kinase (PKA), which phosphorylates Ser-32 in the L isoenzyme, thereby activating FBPase-2 and inactivating PFK-2. Exons 1aF and 1bF are non-coding, so that translation of the F-type mRNA is expected to yield a protein identical with the M isoenzyme.

Tissue-specific control

The L promoter is functionally much more tissue-restricted than the F and M promoters. RNAase protection and S1 nuclease mapping experiments allowed us to detect L promoter activity in liver and adipose tissue, and to a much lower degree in skeletal muscle. These three tissues do not originate from the same primary germ layer. No signal was detected in intestine, heart, brain, lung, spleen, placenta, kidney, thymus or testis ([40], F. P.

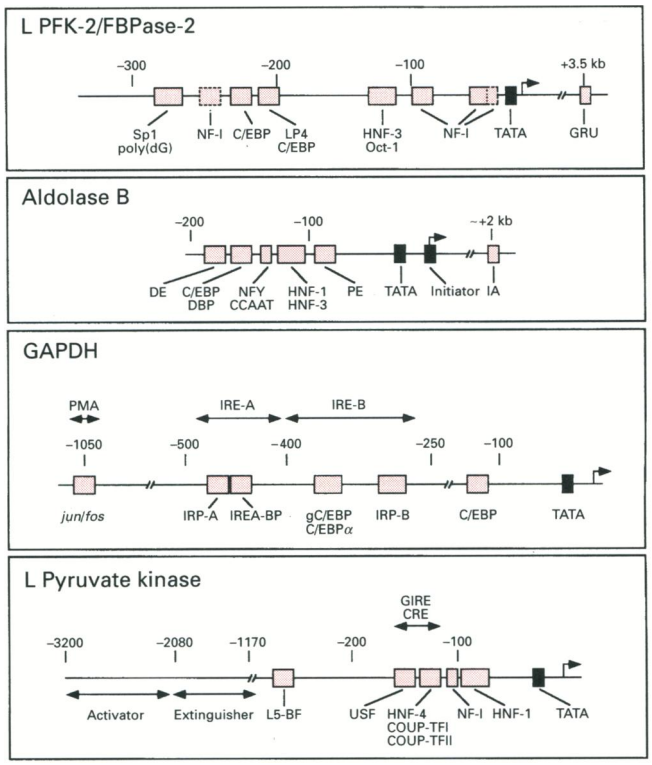

Figure 1 Map of the promoter of genes coding for glycolytic enzymes

Abbreviations: PMA, phorbol 12-myristate 13-acetate response element; DE, distal element; PE, proximal element. For other abbreviations, see the text.

Lemaigre and G. G. Rousseau, unpublished work). The tissue distribution of L promoter activity does not correlate with the expression pattern of the transcription factors that bind to the L promoter (see below). This implies that other effectors are involved in its tissue-specificity.

Transient transfections of mouse or rat hepatoma cells with L promoter–luciferase constructs and 5′ deletants thereof localized the promoter between −360 and +1 relative to the L initiation site [41]. Several DNAase I footprints that involve ubiquitous and liver-specific proteins (Figure 1) were detected [41,42]. Ubiquitous proteins were identified as nuclear factor-I (NF-I) (four sites), octamer factor-1 (Oct-1) (one site) and poly(dG)-tract binding proteins. The latter correspond to Sp1 and to uncharacterized proteins (F. P. Lemaigre, C. Pierreux and G. G. Rousseau, unpublished work). The liver-specific HNF-3 α, β and γ bind in a mutually exclusive way to the same site as Oct-1. This site is the most potent stimulator of L promoter activity. Another liver-specific footprint located upstream of the HNF-3/Oct-1 site participates in L promoter regulation. We have shown that it corresponds to the binding of C/EBP-related proteins and of a novel liver-specific factor that we purified to near homogeneity and called LP4 [42]. A third liver-specific footprint was detected on the L promoter. Its sequence contains a potential binding site for C/EBP-related proteins.

Based on detection of the M-type mRNA, the M promoter is functional in all tissues tested, namely skeletal muscle, heart, adipose tissue, liver and testis (M. Darville and G. G. Rousseau, unpublished work), but its activity is predominant in muscle. Transient transfection experiments delineated the M promoter within 200 bp upstream of the cap site and three sites for DNA–protein interactions were visualized on this region by DNAase I footprinting [43]. The two proximal sites were assigned to NF-I. A third site involves uncharacterized proteins. Its sequence contains an E-box (known to bind muscle-specific transcription factors), a hemipalindrome homologous to a nuclear-hormone-receptor-binding site, and a consensus for C/EBP-related proteins. In addition, a footprint extending over the TATA box was seen with liver and hepatoma nuclear extracts. Further upstream, i.e. between −1600 and −800 relative to the M initiation site, Darville et al. [43] located a region that behaves as an enhancer. Interestingly, this region corresponds to the F promoter.

The F-type mRNA is present in established rat cell lines (FTO-2B and Fa32 hepatomas, L6 myoblasts but not myocytes, rat-1 fibroblasts), fetal liver and fetal muscle, pre-term placenta, lung and thymus, but occurs at very low levels or is undetectable in other adult tissues. Thus, F promoter function correlates with cell proliferation. DNA–protein interaction studies and transient transfection experiments demonstrated that Sp1 and the *ets*-related proto-oncogene GABP regulate the activity of the F promoter [37]. It is noteworthy that the F promoter was the first known non-viral DNA target for GABP.

Hormonal and nutritional regulation

Glucocorticoids stimulate L-type mRNA transcription in rat liver *in vivo* [44] and in cultured hepatocytes [45] as well as in FAO rat hepatoma cells [46]. The M-type mRNA level increases *in vivo* in skeletal muscle when adrenalectomized rats are treated with triamcinolone [47]. By transient transfection, Lange et al. [48] localized a glucocorticoid-responsive unit (GRU) in the intron located 3′ of exon 1L. This GRU contains two glucocorticoid-receptor-binding sites and a potential NF-I site. The GRU behaved as an enhancer when put upstream of the L or M promoter. This suggests that this GRU modulates *in vivo* the activity of both promoters. In FTO-2B cells, dexamethasone stimulates the synthesis of an mRNA [49] that appears to correspond to the F-type mRNA. We therefore believe that glucocorticoids also stimulate transcription from the F promoter.

Liver PFK-2/FBPase-2 mRNA levels increase in hypothyroid rats upon treatment with T_3 [50]. In cultured rat hepatocytes, thyroid hormone has no effect on its own but potentiates the glucocorticoid induction [45]. Whether this reflects transcriptional or post-transcriptional regulation is unknown.

Glucagon, acting via cyclic AMP, and insulin exert opposite short-term effects on PFK-2/FBPase-2 activity (reviewed in [28]); however, long-term regulation has been documented as well. Glucagon inhibits transcription from the L promoter and destabilizes the L mRNA *in vivo* [51]. In fasted or diabetic rats, refeeding or insulin treatment increases PFK-2/FBPase-2 mRNA concentration in liver after 24–48 h [52–54]. Cifuentes et al. [49] and Espinet et al. [46] analysed the effects of insulin in hepatoma cell lines. Upon insulin treatment the F-type mRNA and L-type mRNA accumulate in FTO-2B and FAO cells respectively as a result of an increased rate of transcription. This insulin effect on the F-type mRNA is glucose-dependent. The induction by insulin or dexamethasone of the F-type mRNA in FTO-2B cells and of the L-type mRNA in FAO cells is inhibited by cyclic AMP [46,49], but cyclic AMP does not modify basal L-type mRNA levels in unstimulated FAO cells. The effects of insulin on L promoter activity depend on the hormonal context. Indeed, we recently showed that insulin inhibits and reverses the glucocorticoid-induced stimulation of transcription of the L-type mRNA. This inhibitory effect of insulin is independent of extracellular glucose and does not require ongoing protein

synthesis [55]. This represents an exception to the rule, in the regulation of glycolytic and gluconeogenic genes, that cyclic AMP and insulin exert effects that are opposite to each other (see below).

Zimmermann and Rousseau [40] identified in liver chromatin five DNAase I-hypersensitive sites, two of which could correspond to other regulatory regions of gene A. Indeed, one site points to the M promoter, another to the L promoter, and a third co-localizes with the GRU described by Lange et al. [48]. A fourth site is centred on the −1000 region of the L promoter and the fifth in the intron located 3′ of exon 1L, 500 bp upstream of the GRU.

ALDOLASE

This enzyme (EC 4.1.2.13) converts fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate. It participates in glycolysis and gluconeogenesis and is also an enzyme of fructose metabolism. Aldolase is a tetramer for which three subunit types, encoded by three different genes, have been described. The aldolase A subunit is ubiquitously distributed but is very abundant in muscle. Aldolase C is present in brain, fetal tissues and cancer cells. Aldolase B is detected only in liver, in kidney proximal tubular cells and in enterocytes. We limit ourselves to aldolase B expression since this isoenzyme accounts for 98 % of liver aldolase activity.

Gene organization and tissue-specific control

The structures of the rat, human and chicken aldolase B genes have been determined [56–58]. In the three species the gene spans 15 kb and contains nine exons downstream of a single promoter. The regulatory elements and the transcription-factor-binding sites of the rat aldolase B promoter have been investigated and their function was analysed in isolated hepatocytes and in hepatoma cells [59–62]. Figure 1 shows the topographical organization of the regulatory elements. The distal element (DE in Figure 1) behaves as a negative regulatory sequence since its deletion increases reporter gene activity in transfection experiments. The proteins that bind to this element have not been characterized. The proximal element (PE in Figure 1), whose function is unknown, corresponds to a region protected against DNAase I in footprinting experiments. Recombinant C/EBPα binds to the C/EBP site (referred to as 'C' box) and competing oligonucleotides known to bind C/EBP reduce transcription activity in experiments *in vitro*. Overexpression of C/EBPα or of the D-binding protein (DBP) in transfection stimulates promoter activity, demonstrating that C/EBP-related proteins might regulate the aldolase B promoter. Nuclear factor Y (NFY) and other CCAAT-binding factors bind to the so-called 'B' box, as assessed by oligonucleotide competition experiments. The function of this box is unclear. The liver-specific transcription activators HNF-1 and HNF-3 bind in a mutually exclusive way in a region referred to as the 'A' box. While the two proteins are detected in band-shift experiments, preferential HNF-3 binding is seen in footprinting. The respective function of the two proteins is not clear. As to initiation of aldolase B transcription, it might be regulated by both a TATA box and an initiator (Figure 1). Finally, Gregori et al. [60] showed that aldolase B promoter activity is strongly stimulated by the addition of sequences (IA in Figure 1) from the first intron.

Analysis of the chromatin structure revealed two DNAase I-hypersensitive sites upstream (−2.6 kb and −0.3 kb) of the cap site, two in the middle of the first intron, one in the eighth intron and two located 2.7 kb and 3.4 kb downstream of the polyadenylation site [60,63,64]. Only the site at −0.3 kb and the second intronic site are restricted to the tissues where the gene is expressed. These two sites correspond to the promoter and to the intronic regulatory element. Daimon et al. [63] investigated the methylation status of the gene and found a correlation between the expression of the gene and demethylation of Cyt-129 and of several cytosines in the first intron. The −129 nucleotide is located in the 'B' box described above.

Nutritional and hormonal control

This was investigated by Munnich et al. [65] and shown to differ in the three tissues expressing the enzyme. Starvation leads to a 10-fold decrease in mRNA concentration in liver and small intestine, but not in kidney. Refeeding the rats with a carbohydrate-rich diet restores normal mRNA levels, provided that the animals secrete glucocorticoid and thyroid hormones and insulin. The three hormones act in a permissive way since they are all inactive alone. Re-induction of liver mRNA is blocked by glucagon or cyclic AMP. In the small intestine, only insulin and thyroid hormones play a permissive role, glucocorticoids being dispensable. In addition, glucagon cannot inhibit re-induction of small intestine mRNA by carbohydrates. In the kidney, no effect of hormones could be monitored. Interestingly, refeeding rats with a fructose diet increases kidney mRNA levels and allows restoration of normal liver mRNA levels in diabetic rats. This suggests a role for fructose in the control of aldolase B expression. In cultured hepatocytes isolated from fasted rats, the transcription rate of aldolase B is stimulated by glucose together with insulin, but not by glucose or insulin alone [66]. The gene sequences that mediate this hormonal and nutritional control are unknown.

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

GAPDH (EC 1.2.2.12) converts glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate. This ubiquitous enzyme is a tetramer of four identical subunits. Only one gene for GAPDH is functional in chicken, man, mouse and rat. Whereas chicken has a single gene, man, hare, guinea-pig and hamster have 10–30 copies of GAPDH pseudogenes also. In mouse and rat there are more than 200 pseudogenes [67].

Gene organization and tissue-specific control

The human gene contains nine exons and is very similar to the chicken gene [68]. The promoter contains a TATA box and binding sites for several transcription factors [69] (Figure 1). A phorbol ester-response element (PMA in Figure 1), which binds a c-*jun*/c-*fos* heterodimer, is located at −1050 relative to the cap site. A C/EBP site is detected around −100 as well as upstream of the IRP-B site (see below), around −350. The latter C/EBP site was shown to bind purified C/EBPα in DNAase I footprinting experiments. However, in band-shift experiments with adipocyte extracts, this site binds a heat-labile protein distinct from C/EBPα, which is heat-stable. This protein is induced during differentiation from pre-adipocytes to adipocytes, and is referred to as gC/EBP. The proteins that bind to the IREs (IRE-A and IRE-B) are best described in the context of the insulin regulation of GAPDH gene transcription.

Nutritional and hormonal control

GAPDH mRNA accumulates in adipocytes upon exposure to insulin and mRNA levels also increase in liver upon refeeding

after fasting [70]. The induction of GAPDH mRNA by insulin is tissue-specific, since it occurs only in lipogenic tissues. Secondly, it is differentiation-dependent since it occurs in mature adipocytes but not in pre-adipocytes [69]. Thirdly, both stable and transient transfection experiments in 3T3 adipocytes and hepatoma cells demonstrated that insulin stimulates transcription from the GAPDH promoter [71,72]. Run-on experiments showed that the insulin effect occurs within 30 min and results from an increase in the transcription rate [71]. Deletion analysis located two IREs, from −488 to −408 (IRE-A) and from −408 to −269 (IRE-B). Study of the proteins that bind to IRE-A revealed that a protein, IRP-A, was induced in insulin-treated adipocytes as well as in liver upon refeeding after fasting. IRP-A binds to the 5′ half of IRE-A and recognizes the CCCGCCTC core sequence [72]. By screening an expression library prepared from rat adipocytes with an IRE-A oligonucleotide, Nasrin et al. [73] identified a protein, IREA-BP, that recognizes the 3′ half of IRE-A, in particular the TTCAAAGG motif. IREA-BP is an HMG box-containing protein, structurally related to the product of the testis-determining gene *SRY*. IRE-B binds two proteins present in 3T3 adipocyte nuclear extracts. One is the above-mentioned gC/EBP, the other, called IRP-B, is insulin-induced [69].

PYRUVATE KINASE

This enzyme (EC 2.7.1.40) catalyses the conversion of phosphoenolpyruvate into pyruvate. Four pyruvate kinase isoenzymes have been described. M1 is the major form of adult skeletal muscle, heart and brain. M2 is found in most adult tissues, but it is the only form detected in fetal tissues. The L′ or R isoenzyme is specific for erythrocytes, while the L form predominates in liver but is detectable in kidney and intestine as well. In mammals, two genes have been identified, one coding for the M1 and M2 isoenzymes, the other (*L* gene) coding for the L and L′ isoenzymes (reviewed in [74]).

Gene organization

In rats [75] and in humans [76] the *L* gene (9 kb) contains 12 exons. The first exon codes for the L′-specific amino acids, the second exon for the L-specific ones. A promoter is located upstream of each specific exon. Thus, the L-isoenzyme mRNA has a nucleotide sequence identical with that of the L′ mRNA, except at the 5′ end and for the 3′ untranslated region. The sequence upstream of the fifth codon of the L mRNA is replaced in the L′ mRNA by 98 nucleotides corresponding to an untranslated region followed by coding sequences [75]. The L′ isoenzyme is therefore 31 residues longer than the L-type.

Tissue-specific control

Rat L promoter activity is regulated by sequences within 3.2 kb upstream from the cap site (Figure 1). Experiments with transgenic mice indicated that this region confers tissue-specificity to L promoter activity [77–79]. The promoter (−183 to +11) contains binding sites for HNF-1, NF-I, HNF-4, upstream stimulating factor (USF)-related proteins and L5-binding factors (Figure 1) [80–83]. The HNF-4 site also binds chicken ovalbumin upstream promoter-transcription factor (COUP-TF)I and COUP-TFII [84]. The binding of HNF-4 apparently stabilizes NF-I binding [80]. The promoter corresponds to a liver-specific DNAase I-hypersensitive site HSS-1 [85] and suffices to confer tissue-specificity *in vitro* as well as in transfection and in transgenic mice [79,81,86,87]. The most potent transcriptional stimulators are the liver-specific factors HNF-1 and HNF-4. In transfection experiments COUP-TFI inhibits promoter activity. The role of NF-I and USF in basal transcription is unclear [79,86,88,89]. Yamada et al. [81] transfected hepatocytes in culture and proposed an activating function for USF, while Vaulont et al. [80] could not find any function for USF *in vitro*. The role of USF will be further discussed below. L5-binding factors are as yet uncharacterized proteins. The L5 sequence binds different proteins in liver and intestine [82]. Full understanding of their function awaits their purification since, depending on the experimental conditions, L5-binding factor displays activating [88] or inhibiting [81,82,89] properties.

The activity of the promoter is modulated by upstream sequences. Transient transfection of hepatoma cells [88] and the use of transgenic mice defined, between −2080 and −1170, an extinguisher that is more active in intestine than in liver. This extinguishing activity could not be observed by transfection of hepatocytes in culture [88]. An activating region located around −3 kb is required for full promoter activity [79,88]. This region corresponds to a liver-specific DNAase I-hypersensitive site (HSS-2) [85] and contains Alu-like sequences.

Nutritional and hormonal control

Hormones and diet play an important role in L pyruvate kinase gene expression. Refeeding fasted rats with a carbohydrate-rich diet or treating diabetic rats with insulin induces L-type mRNA in liver [90,91]. Glucose, as well as fructose, regulates the expression of the gene but may act in a different way. Indeed, fructose stimulates the transcription rate early (2–4 h) and transiently, while activation by glucose is detected after 6 h [92]. Work on the L promoter provided important insights on the respective roles of glucose and insulin in stimulating the transcription of genes that require both effectors for stimulation. Indeed, Decaux et al. [66], working on isolated hepatocytes, showed that glucose and insulin are required together, neither of them being active alone. On the other hand, glucose alone stimulated L promoter activity in hepatoma cells [93]. Since Foufelle et al. [94] demonstrated that glucose must be converted into glucose 6-phosphate by a hexokinase to exert its action on gene transcription, Axel Kahn's group proposed that insulin is required for the production of glucokinase in hepatocytes while hepatoma cells may constitutively express hexokinases, thus bypassing the need for insulin [95]. Furthermore, the possibility that insulin action on transcription necessitates ongoing protein synthesis is suggested by Noguchi et al. [90] who showed that this insulin effect is blocked by cycloheximide. While cycloheximide may block the insulin induction of glucokinase, it also inhibits a step downwards in the pathway of glucose stimulation of transcription. Indeed, Lefrançois-Martinez et al. [93] showed that in their hepatoma cells the insulin-independent glucose stimulation of L promoter activity could still be blocked by cycloheximide. Insulin and glucose also stabilize the mRNA [96].

Glucagon, acting via cyclic AMP, inhibits the effect of glucose and insulin in fasted rats and in cultured hepatocytes by decreasing the transcription rate and destabilizing the mRNA [91,96]. Interestingly, Bergot et al. [89] showed that the *cis*-acting sequences that mediate inhibition by cyclic AMP are identical with those conferring stimulation by insulin and glucose. Thyroid and glucocorticoid hormones do not affect the transcription rate but exert a permissive effect on the dietary induction by acting at the post-transcriptional level [91]. Finally, fasting strongly reduces the intensity of the DNAase I-hypersensitive site HSS-1. This probably reflects an effect of diet-dependent hormones or metabolites on chromatin structure [85].

Intense efforts have been devoted to identifying the *cis*-acting elements and *trans*-acting factors that mediate the activity of

insulin and carbohydrates. In transgenic mice, the promoter sufficed to confer glucose and fructose responsiveness [79,97]. Further analysis with transgenic mice and transfected hepatocytes indicated that the USF- and HNF-4-binding sites are required for induction by glucose when these two sites are maintained in their promoter context [83,87,89]. The term insulin/glucose response element (GlRE) was used to qualify the USF site [84]. Indeed, when multimerized, the USF site of the pyruvate kinase gene confers on its own carbohydrate responsiveness to a heterologous promoter. However, in the natural promoter context, any modification of the spatial arrangement of the USF and HNF-4 sites abolishes the response to glucose and insulin. The same holds true for the inhibitory response to cyclic AMP. By extension we therefore consider that the GlRE consists of the USF plus HNF-4 site. Diaz-Guerra et al. [84] demonstrated that the USF site in the pyruvate kinase promoter contains two non-canonical E-boxes, and that the integrity of both is required for the response to glucose and insulin. The protein(s) that bind(s) *in vivo* to the pyruvate kinase USF site in liver has not been identified, but Liu et al. [83] could not distinguish it *in vitro* from the USF protein present in HeLa cells. Therefore, either USF or a USF-related protein or heterodimer in a particular DNA-binding context mediates the response to carbohydrates.

PHOSPHOENOLPYRUVATE CARBOXYKINASE

Gene organization

PEPCK (EC 4.1.1.32) catalyses the synthesis of phosphoenolpyruvate from oxaloacetate. The PEPCK gene is undoubtedly the most intensely studied gene among those that regulate glycolysis and gluconeogenesis. The rat gene spans 6.0 kb and contains 10 exons and a single promoter [98]. It is expressed predominantly in liver, kidney and adipose tissue, and at a lower level in intestinal epithelium, mammary gland, colon, heart, skeletal muscle, ovary, lung, smooth muscle and sublingual gland tissues.

Tissue-specific control

The promoter is rather complex regarding its tissue-specific regulation. Different tissues use different *cis*-acting sequences. Results from experiments with transgenic mice indicated that 2 kb of promoter confers a correct tissue distribution of promoter activity. Sequences from −2088 to −888 are essential for expression in brown and white fat, heart, skeletal muscle, lung and ovary. Expression in kidney requires nucleotides −600 to +69, and elements important for function in mammary glands are located between −460 and −355. Crucial liver-specific elements are located from −460 to +73 [99–102]. The latter elements are not only important for expression in the liver taken as a whole, they also contain the information required for proper spatial expression within the liver. Indeed, according to the metabolic zonation concept, gluconeogenic enzymes are present in higher concentrations in the periportal zone, i.e. around the afferent blood vessels, while glycolytic enzymes are situated predominantly in the perivenous zone [103]. The gluconeogenic enzyme PEPCK is expressed primarily in periportal hepatocytes. Transgenic mice carrying constructs with nucleotides −460 to +73 respect this metabolic zonation [100].

The search for DNAase I-hypersensitive sites in the PEPCK gene, which aims at identifying broad regions involved in the liver-specific regulation, showed hypersensitivity at −6200, −4800, −1300, −400 to −30, +4650 and +6200 when the gene was investigated in rat H4IIE hepatoma cells. The sites located between −4800 and +4650 were observed only in cells expressing PEPCK [104,105]. While the −400 to −30 region obviously corresponds to the promoter, the −4800 site, i.e. from −4902 to −4667, behaves as an enhancer [105]. Results from Faber et al. [106] did not support the possibility that methylation of CpG dinucleotides participates in the tissue-specific regulation.

The map of the PEPCK promoter, with the binding sites for basal, tissue-specific and hormone-dependent transcription factors, is shown in Figure 2. Liver extracts produce eight DNAase I footprints, named cyclic AMP response element (CRE)-1 and -2, and P1 to P6 [107,108]. P3 and P4 are divided into two and three subdomains respectively. Each element binds several proteins and block mutations disrupting the DNAase I footprints indicated that, under basal conditions, CRE-1 and P4 are the most potent stimulatory elements, followed by P1, P3, P5 and P6, and by P2 and CRE-2, which each contribute about 10% of the activity of the promoter *in vitro* [108,109]. Deletion analysis also indicated the presence of basal stimulatory (BSE in Figure 2) and inhibitory (BIE in Figure 2) elements, located close to the CRE-1 site. No protein–DNA interactions were detected on these two regions [108].

Liver-enriched factors that recognize the PEPCK promoter include C/EBPα, C/EBPβ, DBP, HNF-1, HNF-4 and probably HNF-3. Purified C/EBPα binds to a site downstream of the cap site [110] and to the CRE-1, P1, P3(I) and P4 (I-II) sites. The CRE-2 site is also bound by C/EBPα but only when high protein concentrations are used [111]. C/EBPα activates the PEPCK promoter through the P3, P4 and CRE-1 sites, and although it is recognized by C/EBPα, P1 does not mediate its activity. In addition, activation by C/EBPα via CRE-1 requires upstream sequences [111]. DBP binds to the CRE-1 and P3(I), and with a lower affinity to the CRE-2 and P2, sites [110]. Transfection analysis in human hepatoma HepG2 cells showed that DBP might activate the promoter and that the relative levels of C/EBPα and DBP could determine their respective activity [110]. Furthermore, the −117 to −86 region apparently downregulates the activity of DBP [110]. Purified C/EBPβ binds to the same sites as C/EBPα, but displays a much higher affinity for the CRE-1 site [112]. C/EBPβ also activates the promoter essentially through the CRE-1 site, but requires an intact P1 element. Like C/EBPα, C/EBPβ does not therefore activate transcription through all the sites to which it binds [112]. HNF-1 binds to the P2 element [107] and HNF-4 to the P6 element [113]. While both P1 and P6 positively regulate the PEPCK promoter, the individual functions of HNF-1 and HNF-4 were not investigated. A potential HNF-3 site exists in the P3(II) element. The enhancer, located around −4800, binds several proteins including the CRE-binding protein (CREB) and a 49 kDa liver-specific factor called pepA which may correspond to HNF-3 [105,114]. Again, a specific function for CREB or HNF-3/pepA has not been demonstrated.

Ubiquitous factors bind to several sites. CREB binds to the CRE-1 site and serves a dual function; as a cyclic AMP mediator (see below) and as a regulator of basal promoter activity [108,115]. NF-I is involved in the P1 footprint [107,108]. The P1 site is also detected in *in vivo* footprinting experiments, but only in cells expressing PEPCK [106]. The *jun/fos* heterodimer binds avidly to the CRE-1 element and, with lower affinity, to the P2, P3(II) and P4 elements [116]. When HepG2 cells are co-transfected with a *jun* expression vector and a PEPCK promoter–reporter gene construct, *jun* stimulates the PEPCK promoter through the CRE-1, P3(II) and P4 elements. A possible interaction between *jun* and the P3(I)-binding proteins is suggested by the observation that a mutation in P3(I) decreases activation by *jun*. *Fos* plays the role of a dominant negative regulator. In co-transfection experi-

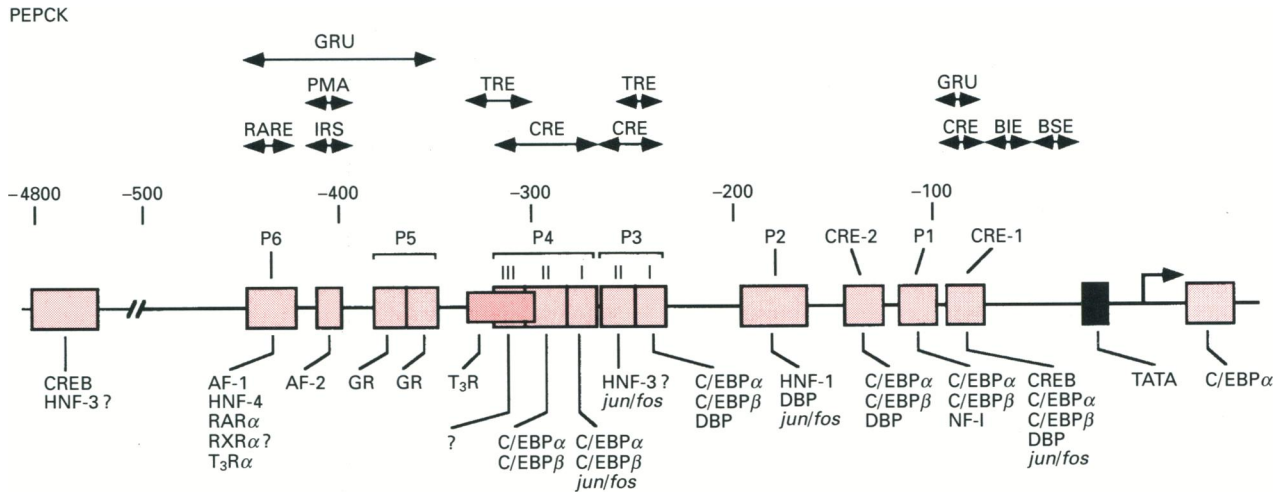

Figure 2 Map of the PEPCK promoter

Abbreviations: BIE and BSE, basal inhibitory and stimulatory response elements; GR, glucocorticoid-receptor-binding site; PMA, phorbol 12-myristate 13-acetate response element; TRE, thyroid hormone response element.

ments it inhibits the activation of the PEPCK promoter by *jun* or by cyclic AMP. The inhibitory action of *fos* requires the integrity of site P3(I) [116]. *Fos* is induced by insulin, and may therefore contribute to the dominant inhibitory effect of insulin. However, as Gurney et al. [116] pointed out, *fos* is also induced by cyclic AMP. This, in turn, could provide an explanation for the biphasic pattern of the cyclic AMP stimulation of the PEPCK promoter (see below). The initial cyclic AMP-mediated stimulation could be attenuated in the second phase by high levels of *fos* protein. The above-mentioned effects of *fos* and *jun* result from the overexpression of the proteins. The experiments therefore only address the question of the basal activity of *fos* and *jun*. In this respect, it is known that activation of PKC, e.g. by phorbol esters, modulates the activity of *fos* and *jun* through dephosphorylation and phosphorylation events. Interestingly, *fos* and *jun* do not seem to be involved in the response of the PEPCK gene to phorbol esters ([117]; see below). Nuclear hormone receptors or hormone action mediators are discussed below.

Nutritional and hormonal control

Fasting increases PEPCK expression, whereas refeeding a carbohydrate-rich diet lowers PEPCK to normal levels. The effects of diet are largely mediated by hormonal changes. It is therefore not surprising that transgenic mice carrying PEPCK constructs that confer hormonal response also show the expected response to dietary changes. In liver, the response to diet is provided by sequences from −402 to +69 [99,102]. Nevertheless, the dietary response may be partly independent from hormones. Indeed, addition of glucose to FAO cells or hepatocytes in culture induces a decrease in PEPCK mRNA levels. The maximal effect of glucose is reached after 2 to 4 h and results from an accelerated rate of mRNA degradation and from a decrease in the transcription rate. Administration of glucose to diabetic rats also decreases PEPCK mRNA levels in liver. Several examples of increased mRNA stability induced by glucose have been reported (reviewed in [118]), but to our knowledge the regulation of the PEPCK mRNA by glucose is the only example of carbohydrate-mediated decrease in mRNA stability and transcription. Taken together, these results show that glucose, independently from insulin, inhibits PEPCK gene expression [119,120].

Insulin decreases the transcription rate of the PEPCK gene [121] and acts as an inhibitor of cyclic AMP and glucocorticoid induction [122,123]. By stable transfection of hepatoma H4IIE cells with PEPCK-reporter gene constructs two IRS were located, one between −271 and +69 and one between −416 and −402 [124,125]. The latter IRS coincides with the accessory factor (AF)-2 binding site (Figure 2) discovered during the study of regulation by glucocorticoids ([126]; see below). Band-shift analyses with the AF-2 site as a probe show several complexes. The proteins involved have not been identified and none is induced by insulin. Since the AF-2 element is crucial both for glucocorticoid induction and insulin repression, O'Brien et al. [124] suggested that insulin could block glucocorticoid activity by disabling the function of AF-2.

Glucagon, acting through cyclic AMP, stimulates PEPCK gene expression. Glucose-refed rats, to which dibutyryl-cyclic AMP is administered, show an increased rate of PEPCK gene transcription in liver [127]. This effect can be reproduced *ex vivo* in H4IIE hepatoma cells, where cyclic AMP produces a biphasic activation pattern. After an initial burst of PEPCK promoter activity culminating after 30 min, the transcription rate decreases but remains 2 to 3-fold higher than the basal level. This effect does not require ongoing protein synthesis [122]. Several *cis*-acting sequences within the PEPCK promoter mediate cyclic AMP activity [128]. The CRE-1, P3(I), and to a lesser extent P3(II) and P4, are involved in the cyclic AMP response. Mutations in the CRE-2, P2, P5 and P6 elements do not affect the activation by cyclic AMP [129]. Each of the sequences that mediate cyclic AMP effects binds several proteins. CRE-1 is the only target for CREB in the PEPCK promoter [111]. C/EBPα, CEBPβ and DBP also bind to CRE-1, but none of them mediates the cyclic AMP response through this sequence [112,130]. The CRE-1 sequence, as well as the P3 and P4 elements, displays a weak cyclic AMP response on its own, but together these elements exert a synergistic activation [130]. The factors that confer cyclic AMP-responsiveness to the P3 and P4 elements are unknown.

However, Roesler et al. [130] showed that administration of dibutyryl-cyclic AMP to rats increases C/EBPβ mRNA levels in liver. This may explain the biphasic pattern of the cyclic AMP response. The sharp rise in promoter activity induced by cyclic AMP may be mediated by CREB, and the second phase of activity may be controlled by C/EBPβ. The way in which CREB mediates cyclic AMP activation results most likely from phosphorylation of the protein by PKA [112,115] and does not imply an increased protein binding to the promoter, as shown *in vitro* and *in vivo* [115,131]. This is consistent with the observation that cyclic AMP does not affect chromatin structure [104]. Cyclic AMP also induces stabilization of the PEPCK mRNA [132]. This effect does not require ongoing protein synthesis [133] but apparently involves a protein whose interaction with PEPCK mRNA is regulated by cyclic AMP-dependent phosphorylation [134].

Glucocorticoids stimulate PEPCK gene transcription in liver and repress it in adipocytes [122,135]. The negative and positive responses to glucocorticoids are mediated through *cis*-acting sequences that are located in different regions of the gene [102]. This mechanism of differential response to glucocorticoids therefore differs from the one known to occur on the proliferin gene. In the latter case, positive or negative glucocorticoid regulation is mediated by a single GRU that binds different combinations of transcription factors depending on the physiological context [136]. The mechanism whereby glucocorticoids activate the PEPCK promoter in liver is complex and involves several proteins in addition to the glucocorticoid receptor. By transfecting hepatoma cells with PEPCK–reporter gene constructs, Imai et al. [126] characterized a GRU between −467 and −349. This region contains two binding sites for the glucocorticoid receptor (−395 to −349; GR in Figure 2) as well as the AF-1 (−455 to −431, i.e. P6 footprint) and AF-2 (−420 to −403) sites. The proteins that bind to the AF-1 and AF-2 sites are uncharacterized. The AF-1, AF-2 and glucocorticoid-receptor-binding sites are individually unable to respond to glucocorticoids. Full activity requires synergism between the four elements. Glucocorticoid regulation also necessitates an intact CRE-1 site, and the unexpected physical interaction [137] between CREB and the glucocorticoid receptor, which bind 400 bp apart, might explain the CRE-1–GRU interaction. However, Xing and Quinn [138] showed that CREB supports the glucocorticoid induction, but through its effect on basal transcription rather than via a CREB–glucocorticoid-receptor interaction. An upstream sequence between −1264 and −1111 may also participate in glucocorticoid regulation [139]. The mode of action of glucocorticoids is unclear. Indeed, in hepatoma cells [122] and *in vivo* [127], glucocorticoids exert a fast (30 min) and direct effect on the transcription rate. In isolated hepatocytes, however, glucocorticoids exert only a permissive effect on cyclic AMP induction. In the latter system, the effect is slow (16 h) and blocked by cycloheximide [140,141]. Finally, glucocorticoids stabilize PEPCK mRNA, an effect mediated by the 3′ non-coding sequence [142].

Loose et al. [143] demonstrated that thyroid hormones increase 6-fold the PEPCK transcription rate in thyroidectomized animals. In transfection of HepG2 cells with PEPCK–reporter gene constructs, T_3 stimulates PEPCK promoter activity 3-fold through binding of the T_3 receptor (T_3R) to the −332 to −308 sequence [144]. Binding of the T_3R to this site probably requires (an) additional factor(s) [145]. Activation by T_3 also necessitates an intact P3(I) site. This enables one to locate the T_3 response element (TRE in Figure 2) at the T3Rα and P3(I) sites. Since cyclic AMP and T_3 synergistically activate the promoter, this synergy could involve the T_3R binding site and the P3(I) element. However, Lucas et al. [146] detected only a 2-fold effect of T_3 in H4II cells and suggested that thyroid hormones may interfere with the effect of retinoic acid. Indeed, at low T_3 concentrations the amounts of $T_3R\alpha$ in liver are high, $T_3R\alpha$ may heterodimerize with and inhibit the retinoic acid receptor (RAR)α and therefore decrease basal activation by RARα (see below). On the contrary, at high T_3 concentrations, $T_3R\alpha$ levels are low and RARα may activate the promoter.

Incubation of H4IIE cells with retinoic acid produces, within 1 h, a 3-fold stimulation of PEPCK gene transcription [147]. Transfection analyses located the retinoic acid response element (RARE) between −451 and −434 [147]. This sequence matches the P6/AF-1 sequence mentioned above. Purified RARα binds as a monomer or dimer to this sequence, but full activity of RARα requires the binding of a co-regulator to the RARE. This co-regulator may be retinoic X receptor α. Deletion of the AF-1 site does not completely prevent retinoic acid stimulation, suggesting the existence of a second RARE in the PEPCK promoter. Both all-*trans* and 9-*cis* retinoic acid activate the promoter [113]. Plasma retinoid levels normally remain constant. It is therefore likely that the RARs regulate basal promoter activity. However, Pan et al. [148] showed that retinoic acid potentiates the effects of glucocorticoids and cyclic AMP.

Phorbol esters, like insulin, inhibit transcription of the PEPCK gene rapidly and without the need for ongoing protein synthesis. This effect is mediated by the −416 to −407 sequence (PMA in Figure 2), which coincides with the insulin-responsive AF-2 site. This region does not resemble any other known phorbol ester response element [117].

Other regulators of PEPCK gene expression include the epidermal growth factor, which decreases mRNA levels in isolated hepatocytes [149], and the tumour necrosis factor, which inhibits its transcription [150]. Finally, Beale et al. [151] established a close correlation between increased transcription rate and cuboidal cell shape, induced by high-density growth conditions.

Faber et al. [152] recently investigated the protein–DNA interactions in intact H4IIE cells by DNAase I protection. They detected not only nearly all the sites seen *in vitro* (Figure 2), but also additional sites between −510 and −500 and between −80 and +29. Importantly, the *in vivo* DNAase I protection pattern was unaffected by treatment with glucocorticoids, cyclic AMP or insulin.

CONCLUSIONS AND PERSPECTIVES

Only four of the eight genes that control, in the liver, key steps in the gluconeogenic and glycolytic pathways (namely those for glucokinase, PFK-2/FBPase-2, pyruvate kinase and PEPCK), and two of the seven genes encoding enzymes that catalyse reversible steps (namely aldolase B and GAPDH) have been characterized in terms of promoter regulation. Despite this limitation, it is interesting to compare the mechanisms involved in their tissue-specific and hormonal control.

All these genes except GAPDH are preferentially transcribed in the liver. For three of them (glucokinase, PFK-2/FBPase-2 and pyruvate kinase) this transcription starts from a so-called liver promoter that co-exists on the gene with (an)other promoter(s). This yields an mRNA whose translation may (PFK-2/FBPase-2, pyruvate kinase) or may not (glucokinase) produce a so-called liver-type isoenzyme. Whether the gene contains one or several promoters, transcription preferentially occurs in liver because this tissue is enriched in *trans*-acting factors that, together with ubiquitous factors, are required to drive these promoters. The liver-enriched transcription factors that bind to the genes

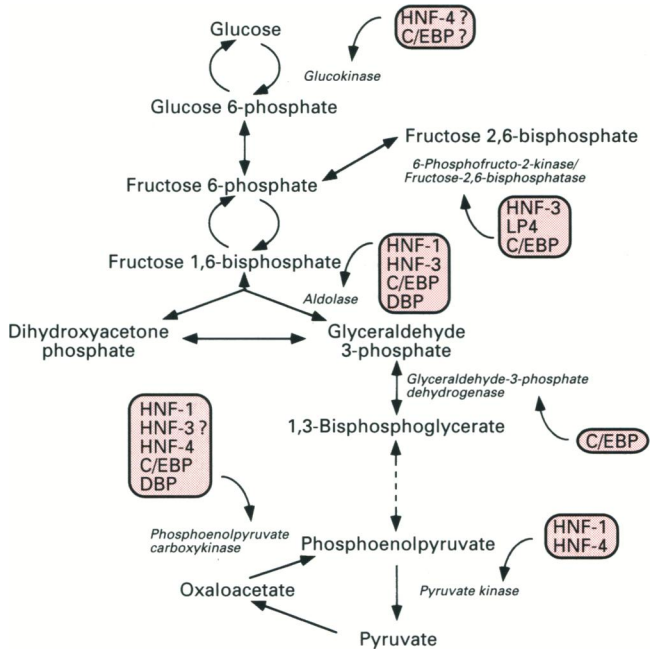

Figure 3 Liver-enriched transcription factors that regulate genes coding for glycolytic and gluconeogenic enzymes

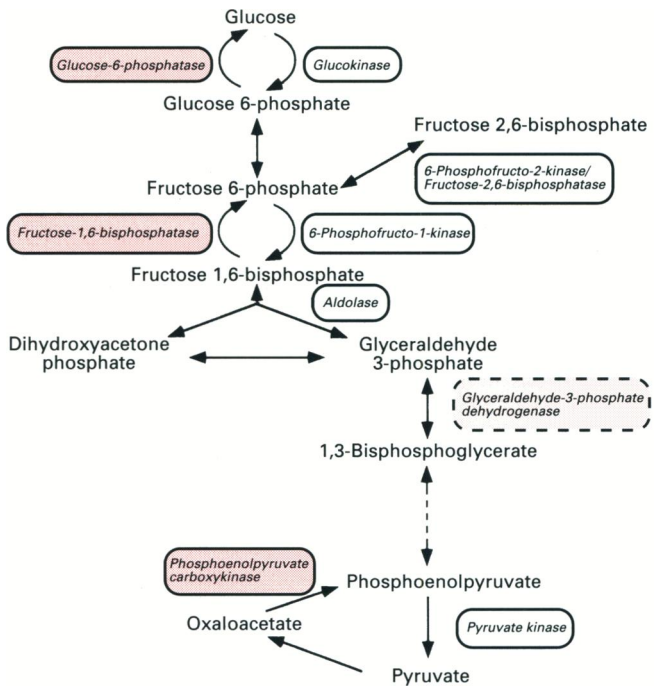

Figure 4 Co-ordinated transcriptional regulation of glycolysis and gluconeogenesis by insulin and cyclic AMP

Darker red blocks, enzymes inhibited by insulin and stimulated by cyclic AMP; white blocks, enzymes stimulated by insulin and/or glucose and inhibited by cyclic AMP; for GAPDH (pale red block) only stimulation by insulin is documented.

reviewed here belong to the five classes described so far (see Table 1 and Figure 3). Members of more than one class bind to each promoter. Moreover, the PEPCK, L pyruvate kinase and aldolase B promoters, which contain a functional HNF-1 site, also bind HNF-3 or HNF-4. Since HNF-3 and HNF-4 stimulate transcription of the HNF-1α gene [153], and therefore occupy a higher position in the hierarchy of transcription factors, one could have expected that they need not bind to genes regulated by HNF-1. The fact that they do suggests a functional redundancy or a requirement *in vivo* for a co-operation between liver-specific factors to promote protein–protein interactions. There is no apparent correlation between the types of factors that interact with each gene and the function of the enzyme it encodes, i.e. glycolytic, gluconeogenic or amphibolic (Figure 3).

A particular case among the liver-enriched transcription factors is the C/EBP family (Table 1). McKnight et al. [154] suggested that C/EBP might be a central regulator of energy metabolism because it regulates genes coding for enzymes that control energetic processes in tissues involved in uptake, metabolism and storage of physiological fuels. Consistent with this hypothesis, we have seen that members of the C/EBP family regulate the PFK-2/FBPase-2, aldolase B, GAPDH, PEPCK, and perhaps glucokinase, promoters. Furthermore, the data on the PEPCK and GAPDH genes suggest that C/EBP proteins are involved in the mediation of cyclic AMP and insulin effects. However, we believe that the conclusions of McKnight et al. [154] were premature. First, other genes (e.g. albumin and transthyretin), not directly involved in energetic processes, are also regulated by C/EBP. Secondly, all C/EBP-family members do not display the same transcriptional properties, and most often the C/EBP isoform was not identified.

Previous reviews [3,4] made the point that insulin stimulates expression of the genes coding for key glycolytic enzymes and inhibits that of genes coding for key gluconeogenic enzymes. Consistent with this view, insulin increases PFK-1 mRNA in mouse liver [155,156] and decreases FBPase-1 mRNA in rat liver [157,158] (Figure 4). We have seen here that the situation is more complex, as insulin stimulates transcription not only of the genes coding for glucokinase, PFK-2/FBPase-2 and pyruvate kinase, but also of the aldolase B and GAPDH genes, which code for enzymes that control reversible steps. Moreover, insulin not only inhibits transcription of the PEPCK gene, it also inhibits the induction of L-type PFK-2/FBPase-2 mRNA by glucocorticoids. The significance of these insulin effects is not clear.

As to the molecular mechanisms of the stimulation of transcription by insulin, we have discussed why it could actually be ascribed to a metabolite of glucose in the case of the PFK-2/FBPase-2, pyruvate kinase and aldolase B genes. The *cis*-acting sequence involved in this indirect effect is therefore called GIRE. It has been identified in the L-type pyruvate kinase promoter and is very similar to the GIRE characterized in the gene coding for liver protein S14, whose function is unknown (Table 3). The pyruvate kinase and S14 GIRE both bind *in vitro* the basic region/helix–loop–helix (b-HLH) protein USF [80,83,159]. Which member of the b-HLH family of transcription factors binds *in vivo* remains to be found. No GIRE has been delineated in the aldolase B or PFK-2/FBPase-2 genes. However, the latter contains, 700 bp downstream from the F promoter, a sequence CCCGTG identical with the pyruvate kinase GIRE.

Insulin can also stimulate transcription independently of glucose, as it does for the glucokinase and GAPDH genes. This genuine effect of insulin mediated by IREs may result from a signalling cascade triggered by the tyrosine-specific protein kinase activity of the insulin receptor. One pathway may involve phosphorylation of IRS-1, which in turn recognizes several downstream effectors perhaps including transcription factors

Table 3 GIREs (a) and IREs (b–d) that mediate glucose and/or insulin responses

	Gene	Sequence	*Trans*-acting factor	Effect	Reference
(a)	L-Pyruvate kinase	CCCGTG	b-HLH protein	Stimulation	[84]
	S14	CACGTG	b-HLH protein	Stimulation	[159]
(b)	GAPDH	TTCCCGCCTC	IRP-A	Stimulation	[72]
	Glucagon	TTCACGCCTG	Unidentified	Inhibition	[172]
(c)	c-fos	CCATATTAGG	Uncharacterized for insulin response	Stimulation	[163]
(d)	PEPCK	TGGTGTTTTG	AF-2	Inhibition	[124]
	Amylase	GTTTATTTTG	Unidentified	Stimulation	[173]

[160]. Another pathway may involve tyrosine phosphorylation of transcription factors called STAT (signal transducers and activators of transcription) proteins. As the insulin receptor can catalyse the tyrosine phosphorylation of the STAT protein p91 *in vitro* [161], insulin could stimulate transcription via this pathway. Indeed, we have noticed that the GAPDH IRE (TTCCCGCCTC) resembles the STAT (TTCCCGTCAA)-binding site of the c-*fos* promoter [162]. Insulin also stimulates transcription via other mechanisms, as the c-*fos* promoter contains an IRE that is identical with the serum response element [163] (Table 3).

Concerning the inhibition of transcription by insulin, a transcription factor (AF-2) and a *cis*-acting sequence (Table 3) have been identified in the PEPCK gene promoter. A similar sequence (TGTGGTTTTG) is present at −300 in the L promoter of the PFK-2/FBPase-2 gene the glucocorticoid-induced transcription of which is also inhibited by insulin. It is puzzling to find that the PEPCK inhibitory sequence resembles the IRE involved in the stimulation of the amylase gene by insulin (Table 3). Likewise, the *cis*-acting sequence involved in the transcriptional inhibition of the glucagon gene by insulin resembles that involved in the stimulation by this hormone of GAPDH gene transcription (Table 3). Since it is known that the same *cis*-acting sequence and *trans*-acting factor can mediate stimulation or inhibition of gene transcription, depending on the promoter and cellular context (e.g. the glucocorticoid receptor [136]), this may apply to the effects of insulin discussed here. Still, it is clear that the molecular mechanism of the transcriptional effects of insulin on the genes reviewed here is not univocal.

Cyclic AMP opposes the action of insulin since insulin-stimulated genes are repressed by cyclic AMP and vice versa (Figure 4). Only the PEPCK gene among those described here has been studied in terms of cyclic AMP action. The cyclic AMP stimulation of this gene is mediated by CREB and members of the C/EBP family. Which member of this family is involved and how its activity is modulated by cyclic AMP is unknown. C/EBPβ may be a good candidate since it mediates the stimulation of the c-*fos* gene by cyclic AMP [164]. The mechanism by which cyclic AMP inhibits transcription of genes coding for glycolytic enzymes has not been elucidated. Still, the GIRE of the pyruvate kinase gene promoter is required for inhibition by cyclic AMP. PKA could inhibit, directly or indirectly, the b-HLH factor that mediates the insulin (i.e. glucose) effect, or it could activate a repressor that binds to the pyruvate kinase GIRE. The work of Burgering et al. [165] is relevant to this issue, as they showed that in fibroblasts cyclic AMP interferes with the insulin signalling pathway at a site downstream of $p21^{ras}$ but upstream of the *raf*-1 kinase.

In liver, glucocorticoids stimulate transcription of the gene coding for PEPCK, a gluconeogenic enzyme, and of the gene coding for glucokinase, a glycolytic enzyme. These paradoxical effects can be reconciled by considering the physiological role of glucocorticoids in carbohydrate metabolism. During fasting, they help to maintain glycaemia by exerting a permissive effect on the glycogenolytic hormones that act via cyclic AMP and they promote gluconeogenesis through increased amino acid uptake in liver and increased activity in this tissue of transaminases, PEPCK and glucose-6-phosphatase. In contrast, in the post-prandial state, glucocorticoids help to store glucose as glycogen and to dispose of the excess through the glycolytic pathway. In this situation, glucocorticoids, together with thyroid hormones, play in liver a permissive role on the action of insulin whose concentration is high. Thus it is not surprising that glucocorticoids stimulate also, like insulin, the glucokinase, aldolase B and PFK-2/FBPase-2 genes. Although the latter codes for a bifunctional enzyme, its stimulation in liver increases fructose 2,6-bisphosphate [44], which is a stimulator of glycolysis.

It is noteworthy that the hormone actions discussed here display a tissue-specific pattern. Proteins that are thought to mediate the insulin response of the GAPDH gene and the glucose response of the pyruvate kinase gene bind in the vicinity of a tissue-specific factor. IRP-B binds close to a C/EBP site on the GAPDH gene, and the USF-related protein requires binding of HNF-4 to the pyruvate kinase promoter. This suggests that a functional interaction between the two proteins confers a tissue-specific response to glucose or insulin. Cyclic AMP also plays a role in the liver-specificity of expression of gluconeogenic genes. Hybrids of different cell types may fail to express the tissue-specific products of either parent. This led to the definition of tissue-specific extinguisher loci (reviewed in [166]). The *Tse-1* locus is responsible for the extinction of the PEPCK, tyrosine aminotransferase and aldolase B genes in hybrid cells [167]. The product of the *Tse-1* locus is the regulatory subunit RIα of PKA [168,169]. RIα is expressed in many cell types but at a 50-fold lower level in the liver. Overexpression of RIα in hybrid cells lowers the activity of the catalytic subunit of PKA and, as a consequence, reduces phosphorylation of the transcription factors that mediate the action of cyclic AMP. Contrary to the PEPCK and tyrosine aminotransferase genes, the aldolase B gene is inhibited by cyclic AMP and yet it is downregulated by *Tse-1*/RIα. This paradox has not been explained. Liver-specific glucocorticoid hormone action on the tyrosine aminotransferase gene has been well documented by Nitsch et al. [170] who demonstrated that HNF-4 may co-operate with the glucocorticoid receptor and so induce liver-specific glucocorticoid stimulation of transcription. Similarly, the proximity of the binding sites for the glucocorticoid receptor and for HNF-4 on the PEPCK gene suggests that these proteins may co-operate in the glucocorticoid response.

Although this review deals primarily with transcriptional control, we have mentioned that the expression of the genes that regulate glycolysis and gluconeogenesis is also regulated through changes in the stability of their mRNAs. Little is known about

the latter mechanism. As T_3 and (or) glucocorticoid hormones stabilize the mRNAs for PEPCK, GAPDH and pyruvate kinase, and cyclic AMP destabilizes PFK-2/FBPase-2 and pyruvate kinase mRNA, these may be excellent models to study the hormonal control of mRNA degradation. Also, glucose exerts opposite effects on the stability of the PEPCK and glucokinase mRNAs. This may indicate the existence of a co-ordinate control of mRNA degradation.

Although our knowledge on the transcriptional control of genes that regulate glycolysis and gluconeogenesis has significantly improved recently, several aspects deserve further investigation. First, the long-term control of the glucose/glucose 6-phosphate substrate cycle remains ill-defined, since few results are available regarding the *cis–trans* regulation of the glucokinase and glucose-6-phosphatase genes despite the recent cloning of the latter [171]. Secondly, while the outlines of the control of the PFK-1/FBPase-1 cycle are known, again data are lacking on its transcriptional regulation. Thirdly, the liver-specific factors that regulate transcription of the genes described here have been fairly well identified *in vitro*. How they stimulate transcription *in vivo* and how they interact with other transcription factors remains to be found. Finally, the transcription factors that mediate insulin and glucose action on these genes are still to be purified and cloned. This is also a prerequisite for understanding how insulin action is facilitated by glucocorticoid and thyroid hormones and is inhibited by cyclic AMP.

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