Negative cyclic AMP response elements in the promoter of the L-type pyruvate kinase gene

Laurence Gourdon[1], Dan Qing Lou[1], Michel Raymondjean, Mireille Vasseur-Cognet, Axel Kahn*

Unité de Recherches en Physiologie et Pathologie Génétiques et Moléculaires, Institut Cochin de Génétique Moléculaire, INSERM Unité 129, 24 rue du Faubourg Saint Jacques, 75014 Paris, France

Received 1 September 1999

Abstract L-type pyruvate kinase gene expression is modulated by hormonal and nutritional conditions. Here, we show by transient transfections in hepatocytes in primary culture that both the glucose response element and the contiguous hepatocyte nuclear factor 4 (HNF4) binding site (L3) of the promoter were negative cyclic AMP (cAMP) response elements and that cAMP-dependent inhibition through L3 requires HNF4 binding. Another HNF4 binding site-dependent construct was also inhibited by cAMP. However, HNF4 mutants whose putative PKA-dependent phosphorylation sites have been mutated still conferred cAMP-sensitive transactivation of a L3-dependent reporter gene. Overexpression of the CREB binding protein (CBP) increased the HNF4-dependent transactivation but this effect remained sensitive to cAMP inhibition.

Key words: L-type pyruvate kinase gene; Cyclic AMP-dependent inhibition; Hepatocyte nuclear factor 4

1. Introduction

The L-type pyruvate kinase (L-PK) gene encodes a key enzyme of the glycolytic pathway; its expression is transcriptionally activated by glucose and insulin and inhibited by fasting and glucagon. This regulation has been first investigated in animals; while transcriptional activation by a glucose-rich diet is a slow phenomenon, requiring an active protein synthesis, inhibition by glucagon or cyclic AMP (cAMP) analogue injection is very rapid (less than 10 min) and insensitive to cycloheximide [1]. Inhibition of the L-PK gene by glucagon has been reproduced in hepatocytes in primary culture and is mimicked by an adenylate cyclase activator and cAMP analogues [2].

Elevation of intracellular cAMP, as a consequence of adenylate cyclase activation, has been shown to regulate a variety of cellular events, including activation and repression of gene transcription [3]. Several positive *cis*-acting cAMP response elements (CRE) have been described [4]. Cyclic AMP stimulates gene expression by activating protein kinase A (PKA), which, in turn, phosphorylates mainly members of the cAMP response element binding protein/activating transcription factor (CREB/ATF) family of transcription factors, thereby increasing their transactivating efficacy, in particular through interaction with coactivators [5–7]. Much less is known about negative *cis*-acting cAMP-response elements.

Cyclic AMP inhibits transcription of the genes for interleukin-2 (IL-2) and interleukin-2 receptor (IL-2R) in EL4 cells; the inhibition requires an activator protein 1 (AP1) site. In this case, cAMP increases the binding of Jun/Fos heterodimers to the AP1 site and alters the composition of Jun proteins that participate in the AP1 complex [8]. Cyclic AMP also inhibits insulin-induced transcription of the gene for fatty acid synthase in the liver [9]. The cAMP negative *cis*-acting element seems in this case to be an inverted CAAT box [10] binding a factor which could be a member of the nuclear factor Y (NFY) family [11]. The 5′-flanking DNA of the gene for malic enzyme contains at least four *cis*-acting DNA sequences that are involved in the negative action of cAMP acting through the classical PKA signaling pathway. The major negative cAMP response element is similar to the consensus binding site for AP1 and binds c-Fos and ATF2 in presence of cAMP [12]. A fourth example of cAMP-dependent transcription inhibition involves the rat aldehyde dehydrogenase class-3 gene in which a 66-bp promoter region has been shown to confer the negative cAMP response. However, neither the precise *cis*-acting element nor the cognate *trans*-acting factor has been characterized [13].

For the L-PK gene, we have previously demonstrated that the glucose response element (GlRE) located in the L-PK promoter, built around two non-canonical E boxes (L4 element), functions in close cooperation with a contiguous HNF4 binding site (L3 element) to assure, in the L-PK context, a full inhibition by cAMP [14]. In addition, we have more recently shown that the binding activity of the orphan nuclear receptor HNF4 was decreased by PKA-dependent phosphorylation [15]. Therefore, the respective role of the GlRE and the HNF4 binding site in the negative response to cAMP could be questioned.

In order to gain insight into the negative transcriptional control of the L-PK expression by cAMP, we have developed ex vivo analyses by transient transfections in hepatocytes in primary culture. We show that both GlRE and HNF4 binding sites of the L-PK promoter are negative cAMP elements which act synergistically in the natural promoter. PKA-dependent inhibition through HNF4 binding sites requires HNF4 binding but its precise target (HNF4 itself and/or a coactivator) remains unknown.

*Corresponding author. Fax: (33) 1 44-41-24-21.
E-mail: kahn@cochin.inserm.fr

[1] Both authors contributed equally to this work.

0014-5793/99/$20.00
PII: S0014-5793(99)01203-X

2. Materials and methods

2.1. Plasmid constructions

All plasmids were constructed by standard DNA cloning procedures and verified by nucleotide sequencing.

In the Rous sarcoma virus (RSV)/Luci construct, the firefly luciferase gene is driven by the RSV long terminal repeat (LTR). Restriction or oligonucleotidic fragments of the L-PK gene corresponding either to the region −96 or −183 to +11 nucleotides with respect to the transcriptional start site were subcloned into the basic plasmid pGL3 (Promega). The −150PK/Luci construct was a gift from G. Rutter [16]. The (L3)3−54PK/Luci and (L4)3−54PK/Luci constructs comprise three copies of the L3 box or L4 box, respectively, ligated to the −54 to +11 proximal base pairs of the L-PK minimal promoter. The (L4L3)4−54PK/Luci construct consists of four copies of the L4L3 fragment in front of the −54PK minimal promoter. The H4-105TK/Luci plasmid was a gift from G.U. Ryffel and has been described in Drewes et al. [17]. It consists of four HNF4 binding sites of the human α1-anti-trypsin gene promoter in front of the −105 minimal promoter of the thymidine kinase gene.

The different HNF4 cDNAs were cloned into a eukaryotic expression vector driven by the cytomegalovirus immediate-early promoter region. The expression vectors for HNF4 wild-type and HNF4 Ala have been previously described [15]. In the HNF4 Ala mutant, the two Ser at positions 133 and 134 were replaced by Ala and Gly. Site-directed mutagenesis of the HNF4 wild-type or HNF4 Ala sequences was performed by fusion of a two-part PCR amplification with outside primers containing engineered *Bam*HI 5′ and *Eco*RI 3′ sites and internal primers containing the engineered *Hin*dIII site (AAG CTT [Lys Leu]) resulting in the mutants termed HNF4 Leu or HNF4 Leu-Ala, with respect to the initial matrix, HNF4 wild-type or HNF4 Ala. In the HNF4 Leu mutant the Arg-Ser residues at positions 303 and 304 are replaced by Lys and Leu. The HNF4 Leu-Ala mutant combines mutations of residues 133, 134 and 303, 304. All mutations and ligation junctions were confirmed by sequencing. The vector coding for DN-HNF4 was a gift from T. Leff [18]. DN-HNF4 is a selective dominant negative mutant which forms defective heterodimers with wild-type HNF4, thereby preventing DNA binding and subsequent transcriptional activation by HNF4.

The expression vector pSVPKA Cα, coding for the catalytic Cα subunit of protein kinase A (PKA), was a gift from P. Sassone-Corsi. The CREB binding protein (CBP) expression vector was a gift from C.K. Glass and M.G. Rosenfeld. This plasmid contains the complete coding sequence of the murine CREB binding protein under the control of the CMV promoter.

2.2. Hepatocyte isolation, cell culture conditions and transfection

Hepatocytes were isolated from male Sprague-Dawley rats (180–200g) fed with a normal diet by the collagenase perfusion method [2]. One and a half million freshly isolated hepatocytes were plated on 6-cm dishes in a final volume of 3 ml of 199 medium (Gibco) containing 5 mM glucose supplemented with penicillin, streptomycin, 100 nM insulin, 1 μM triiodothyronine, 1 μM dexamethasone, and 3% (V/V) fetal calf serum. After 2 h of attachment, the medium was changed. Hepatocytes were transfected 6 h after isolation. Transfection was performed by the lipofection method using the DOTAP transfection reagent (Boehringer Mannheim), according to the manufacturer's instructions. Four microgram of the reporter construct, alone or with various amounts of expression vectors, or 1 μg of the reference RSV/Luci construct were transfected. The pKS Bluescript vector was used to adjust total amounts of DNA to 5 μg in each experiment. The concentrations of expression vectors used were determined in preliminary experiments as the maximal concentrations before occurrence of non-specific squelching phenomena. The medium containing the liposome-DNA complex was replaced 16 h after transfection with hormone-supplemented fresh 199 medium containing 5 mM glucose or 25 mM glucose with or without 0.5 mM 8-bromoadenosine 3′, 5′-cyclic monophosphate (cAMP, SIGMA). Hepatocytes were harvested 24 h later.

2.3. Luciferase assay

Cellular protein extraction was performed as previously [19]. Luciferase activity was determined as described [20]. Results were normalized with the cellular content determined in each cell extract by a Bradford assay.

2.4. Data analysis

Statistical analysis was performed by the Student's *t* test for unpaired data using the StatView software. The significance has been considered at $*P < 0.05$, $**P < 0.01$ or $***P < 0.001$.

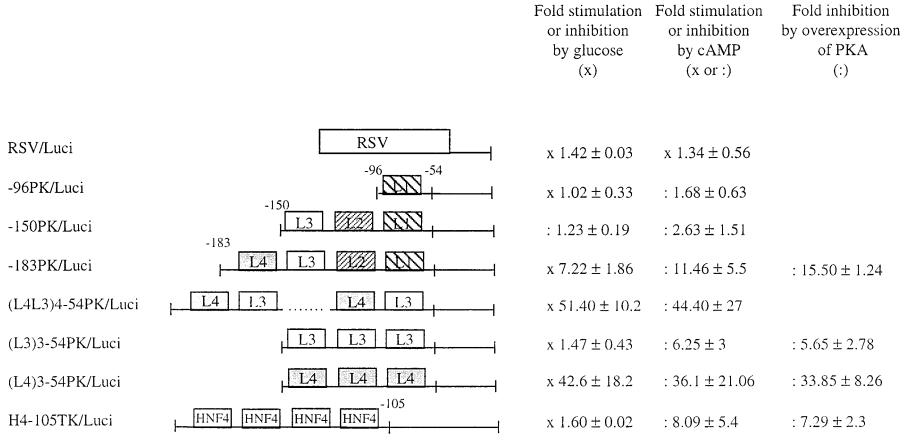

Fig. 1. Response to glucose, cAMP and PKA catalytic subunit overexpression of various luciferase constructs in hepatocytes in primary culture. The RSV/Luci construct corresponds to the LTR of RSV in front of the luciferase reporter gene. Boxes L1 to L4 represent the various binding sites for different proteins identified on the L-PK promoter [51]: from 3′ to 5′, L1 binds hepatocyte nuclear factor 1, L2 binds nuclear factor 1, L3 binds mainly hepatocyte nuclear factor 4 and also nuclear factor 1, L4 is the GlRE, binding basic/helix-loop-helix/leucine zipper factors, in particular USFs 1 and 2; other binding activities are currently being characterized. The H4-105TK/Luci construct consists of four HNF4 binding sites of the human α1-anti-trypsin promoter in front of the −105 minimal promoter of the thymidine kinase gene. The extent of induction, or inhibition in the case of −150PK/Luci, by glucose is the ratio between luciferase activities with 25 mM and 5 mM glucose. The extent of inhibition, or induction in the case of RSV/Luci, by cAMP is the ratio between luciferase activities with 25 mM glucose and 25 mM glucose plus cAMP. The extent of inhibition by PKA is the ratio between luciferase activities with 25 mM glucose and 25 mM glucose with overexpression of the catalytic subunit of the PKA (1 μg). This concentration of expression vector for the catalytic subunit of the PKA (1μg) increased the activity of a reporter driven by the rat somatostatin cAMP-response element (CRE) 15-fold (data not shown). Each value represents the mean of at least five independent experiments, and are represented ± S.D.

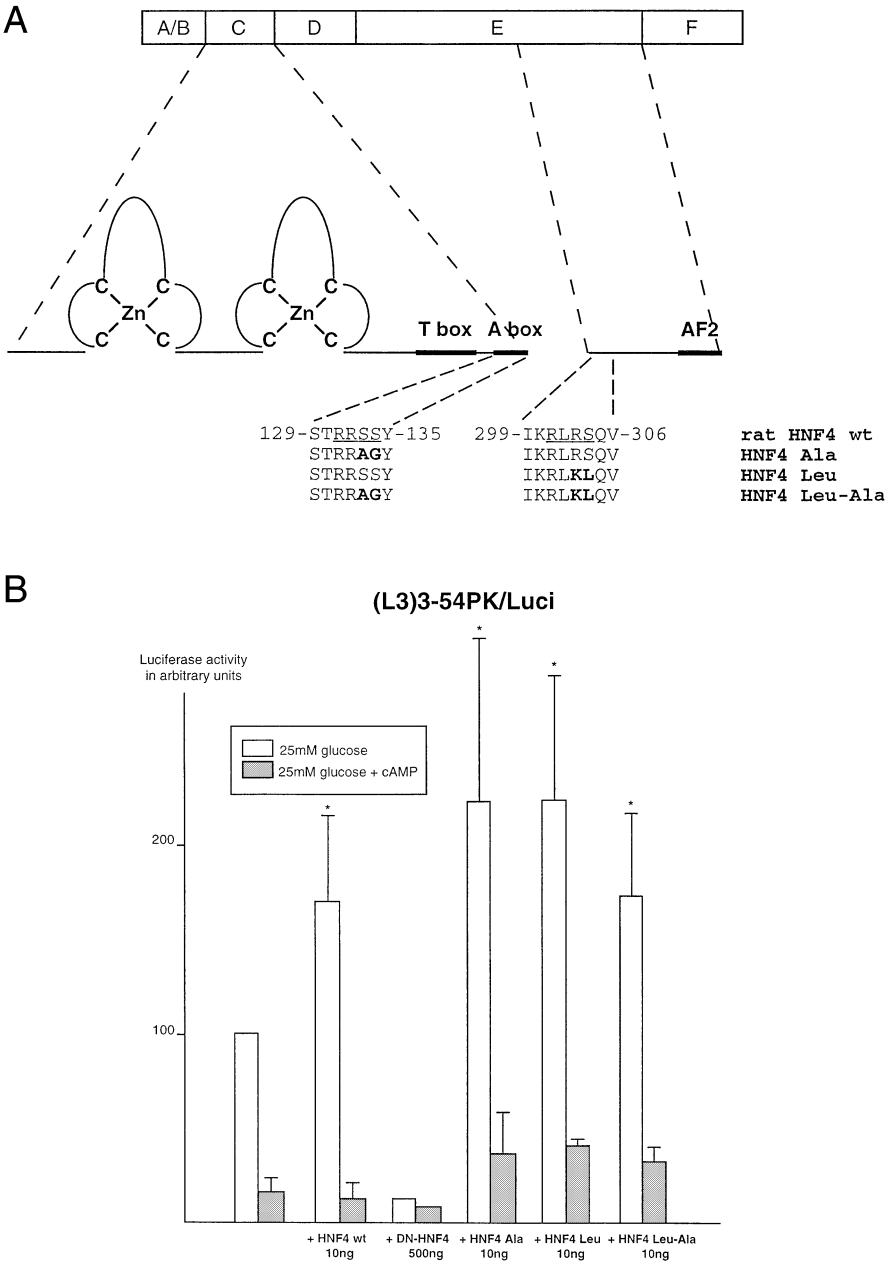

Fig. 2. *Trans*-acting effects of various wild-type and mutant HNF4 proteins on the activity of the (L3)3−54PK/Luci construct in presence and absence of cAMP. A: Letters in boxes represent the functional domains of nuclear receptors family. The two zinc finger motifs are shown with the downstream T and A boxes and the activation function 2 domain (AF2). Amino acid sequences around the potential PKA phosphorylation sites are presented. Numbers indicate amino acids in rat HNF4. Potential PKA phosphorylation sites are underlined. Sequences of the mutated HNF4 proteins at positions 133- 134 and 303-304 are also presented. Ser133-Ser134 and Arg303-Ser304 were changed to neutral residues and mutant proteins were termed HNF4 Ala and HNF4 Leu, respectively. The double mutant was termed HNF4 Leu-Ala. Mutated amino acids are represented with bold letters. wt, wild-type. B: The (L3)3−54PK/Luci construct was transfected alone or cotransfected with the indicated amounts of HNF4 wt, DN-HNF4, HNF4 Ala, HNF4 Leu or HNF4 Leu-Ala expression vectors. The amount of the transfected expression plasmids was determined from dose-effect experiments and was checked not to result in squelching (data not shown). Hepatocytes were cultured in the presence of 25 mM glucose with (gray bars) or without (open bars) 0.5 mM 8-bromoadenosine-cAMP. Each value is 'the mean of at least three independent experiments, represented ± S.D. * The values obtained in absence of cAMP after overexpression of HNF4wt, HNF4 Ala, HNF4 Leu or HNF4 Leu-Ala, are statistically different from those without any overexpression ($P = 0.0457$, $P = 0.0404$, $P = 0.0296$ and $P = 0.0363$, respectively).

3. Results

3.1. Both L4 and L3 are negative cAMP response elements

The different constructs studied were transfected in hepatocytes in primary culture, at low (5 mM) or high (25 mM) glucose, with or without cAMP or PKA catalytic subunit overexpression. Under these conditions, a control RSV/Luci plasmid was insensitive to glucose and cAMP (Fig. 1). However, we verified that a reporter gene directed by the positive cAMP response element of the rat somatostatin gene was activated 10- and 15-fold by cAMP and overexpression of the PKA catalytic subunit, respectively.

As previously reported [14–21], the −183PK construct was stimulated by glucose about seven-fold and inhibited by cAMP about 11-fold. The glucose-dependent stimulation was also totally abolished by cotransfection with the PKA expression vector (Fig. 1). Constructs devoid of element L4 (the GlRE) were insensitive to glucose, and seemed slightly inhibited by cAMP, especially the −150PK/Luci construct (2.63 ± 1.51 fold inhibition). However, this inhibition was not statistically significant compared to the −96PK/Luci construct (1.68 ± 0.63, $P = 0.1353$). This latter result is in discrepancy with results previously reported by Bergot et al. [14] who found that −96PK and −150PK/CAT constructs transfected in hepatocytes, isolated from a rat that has fasted, and cultured in the absence of glucose and in a serum-free-medium were stimulated by cAMP instead of being inhibited. The different experimental conditions most likely account for these discordant results. In particular, it could be that serum and low glucose present in our experimental conditions mimic a non-specific positive effect of cAMP in serum-free and glucose-free conditions on basal transcription.

We have previously shown that in the context of the natural L-PK promoter, the presence of both L4 and L3 elements was required for the positive response to glucose, and therefore for a negative response to cAMP since this one consists mainly of inhibition of the glucose-dependent stimulation [14]. However, a positive response to glucose and partial negative response to cAMP was conferred on a CAT gene by oligomerized L4 boxes [14]. We now confirmed that the (L4)3−54PK/Luci construct was indeed strongly stimulated by glucose (43-fold) and inhibited by cAMP (36-fold). The only partial cAMP-dependent inhibition reported by Bergot et al. [14] was probably due to interference with the non-specific cAMP stimulatory effect observed in the experimental conditions. In contrast, the (L3)3−54PK/Luci construct was almost insensitive to glucose, which confirmed that L4, but not L3, is the GlRE (Fig. 1). However, this L3-dependent construct was inhibited about six-fold by both cAMP and PKA overexpression. The construct directed by oligomerized L4L3 elements was stimulated by glucose and inhibited by cAMP in the same proportions (about 50-fold) as the construct directed only by oligomerized L4 elements (Fig. 1).

3.2. HNF4-dependent transactivation is inhibited by cAMP and PKA

To confirm that oligomerized HNF4 binding sites constitute intrinsically negative cAMP response elements, we used a reporter gene directed by four HNF4 binding sites from the α1-anti-trypsin gene promoter (H4-105TK/Luci construct) [17]. This construct was inhibited about eight-fold by both cAMP and overexpression of the PKA catalytic subunit (Fig. 1). In order to verify that the inhibited activity required HNF4 binding on its cognate sites, we looked at the effect of HNF4 overexpression on cAMP-dependent inhibition. While the (L3)3−54PK/Luci construct was only slightly (but significantly for $P < 0.05$) stimulated by HNF4 overexpression, probably because hepatocytes already contain a high endogenous HNF4 level, it was still inhibited by cAMP to the same extent as in the absence of HNF4 expression vector (Fig. 2B). Overexpression of a HNF4 negative transdominant mutant, DN-HNF4, which prevents formation of active HNF4 homodimers, resulted in inhibition of the basal promoter activity and suppressed any further negative effect of cAMP (Fig. 2B).

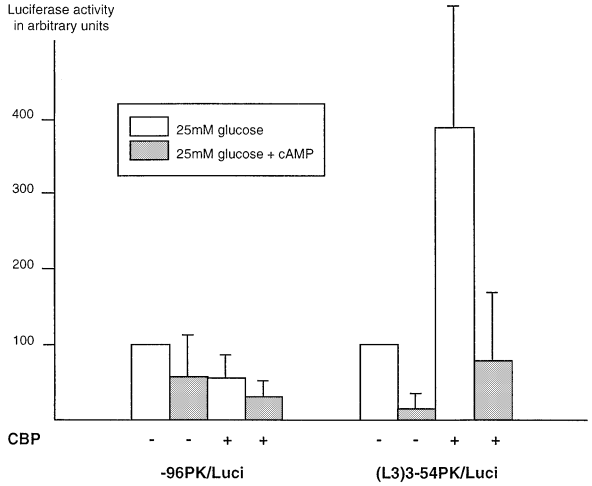

Fig. 3. Effect of a CBP overexpression on the −96PK/Luci and (L3)3−54PK/Luci constructs activities. The −96PK/Luci and (L3)3−54PK/Luci constructs were either transfected alone or cotransfected with 250 ng of the CBP expression vector. The amount of the transfected CBP expression plasmid was determined from dose-effect experiments (data not shown). Hepatocytes were cultured in the presence of 25 mM glucose with (gray bars) or without (open bars) 0.5 mM 8-bromoadenosine-cAMP. Each value is the mean of three experiments, shown ± S.D.

These results indicate that the transcriptional activity inhibited by cAMP requires binding of HNF4 to its cognate sites.

3.3. Mutant HNF4 variants whose PKA phosphorylation sites have been mutated still confer HNF4-dependent cAMP-mediated inhibition of the (L3)3−54PK/Luci construct

We have recently shown that the binding of HNF4 was decreased by PKA-dependent phosphorylation. Therefore, to test the hypothesis that inhibition of the (L3)3−54PK construct by cAMP could be due to inhibition of the HNF4 binding activity by PKA-dependent phosphorylation, we investigated activity and response to cAMP of the (L3)3−54PK reporter cotransfected with an expression vector for the previously described HNF4 Ala mutant [15] whose consensus PKA-dependent phosphorylation site at position 133–134 has been mutated (Fig. 2A). This mutant is insensitive to inhibition of its DNA binding activity after in vitro phosphorylation by the PKA catalytic subunit [15]. We also tested the effect of a mutation of another putative PKA-dependent phosphorylation site (301-RLRS-304) conserved among mammals, alone (HNF4 Leu), or in association with the former mutation (HNF4 Leu-Ala) (Fig. 2A). In fact, Fig. 2B shows that all three HNF4 variants transactivated the (L3)3−54PK/Luci construct to the same extent as the wild-type and remained sensitive to the negative action of cAMP. Thus, these results indicate that the putative PKA-dependent phosphorylation sites of HNF4 do not play a crucial role in the negative regulation of the HNF4 activity by cAMP in hepatocytes in primary culture.

3.4. CREB binding protein (CBP) is a coactivator of HNF4

CBP has been demonstrated to be a coactivator of HNF4 [22]. In order to determine whether CBP could be implicated in the inhibition of the (L3)3−54PK/Luci construct by cAMP, we have cotransfected this reporter plasmid with a CBP expression vector. Overexpression of CBP produced a four-fold

stimulation of the HNF4-dependent construct while it had no effect on a construct devoid of HNF4 binding site, the 96PK/Luci plasmid, demonstrating the specificity of the CBP action. However, the CBP effect remained sensitive to cAMP inhibition (Fig. 3). Thus, in hepatocytes in primary culture, we confirm that CBP is a HNF4 coactivator but is unable to reverse cAMP-dependent inhibition.

4. Discussion

While the stimulation of gene transcription by cAMP is very well documented, much less is known about negative regulation of gene expression by this second messenger. Transcription of the L-PK gene is stimulated by glucose and inhibited by glucagon acting through its second messenger, cAMP. The inhibition of the L-PK gene by cAMP was shown to involve the classical PKA signaling pathway since the cAMP effect could be reproduced by overexpression of the PKA catalytic subunit [21]. We have previously shown that the in vivo transcriptional inhibition by glucagon of glucose responsive genes in the liver was a very rapid phenomenon, detectable in only a few minutes by run-on transcription assays [1], contrasting with the activation by glucose which is delayed for several hours [23]. In addition, Tanaka's group has reported that transcriptional activation of the L-PK gene by glucose was inhibited by cycloheximide while cAMP-mediated inhibition was insensitive to this translational inhibitor [24]. Consequently, the cAMP action is most likely explained by a post-translational event mediated by PKA, tentatively a direct or indirect phosphorylation of transcription factors. Indeed, indirect transcriptional effects, for instance inhibition of the glucokinase gene by cAMP [25], would not be so rapid in inhibiting transcription of downstream genes and would be expected to be sensitive to cycloheximide. The goal of the present study was to firmly identify the negative cAMP response elements in the L-PK gene promoter, in particular to determine the respective role of the L4 and L3 elements in this response.

While, as previously reported [14], the L4-dependent construct, but not the L3-dependent one, was positively regulated by glucose, both constructs were inhibited by cAMP or overexpression of the PKA catalytic subunit. Therefore, the cAMP/PKA signaling pathway seems to be able to down-regulate transactivation mediated by both the glucose-response complex assembled on L4, i.e. the GlRE and the L3 complex. The fact that the (L4L3) oligomeric construct does not exhibit a higher response to both agents than the L4 oligomeric construct suggests that L4 is the main target of the two signaling pathways.

The glucose response complex is able to bind members of the basic-helix-loop-helix-leucine zipper family, especially upstream stimulatory factors (USFs). While we provided evidence in vivo and ex vivo for the role of the USFs in the glucose response [16,19,26,27], this point is still disputed by others [28]. In addition, we [29] and others [30] are currently studying the putative role of other partners of the complex, such that the exact targets of the cAMP pathway in this complex still can not be determined.

For the L3 complex, HNF4 is an obvious candidate target for cAMP/PKA-dependent inhibition. Indeed, we show in this paper that a reporter gene directed by an oligomerized HNF4 binding site different from L3 (i.e. the α1-anti-trypsin gene promoter site) was also inhibited by cAMP. Overexpression of HNF4, which prevents that the element L3 becomes occupied by a different DNA binding factor, does not impair cAMP-dependent inhibition, while the very low activity of the L3-dependent construct co-transfected with an expression vector for a negative transdominant HNF4 mutant appears to be insensitive to cAMP. Viollet et al. have recently shown that HNF4 can be phosphorylated on the A-box by cAMP-dependent protein kinase A resulting in decrease of its DNA-binding activity [15]. However, surprisingly, neither a A-box HNF4 mutant (HNF4 Ala), nor other mutants whose other putative PKA phosphorylation site located more downstream has been mutated, alone (HNF4 Leu) or in conjunction with the Ala A-box mutation (HNF4 Leu-Ala), were able, when overexpressed, to impair the cAMP negative action on the L3-dependent construct in transfected hepatocytes. This suggests that the decreased binding activity of HNF4 phosphorylated on the A-box PKA-phosphorylation site (and perhaps on the more downstream putative site) plays no detectable role when tested on oligomerized L3 sites in hepatocytes. It may be that cooperative binding on oligomerized sites compensates for decreased binding activity. In addition, our results indicate that cAMP and PKA can decrease HNF4-mediated transactivation by another means than direct phosphorylation of consensus PKA phosphorylation sites. It is conceivable that PKA acts indirectly through a cascade of phosphorylation events modifying other sites known to be multiple in HNF4 [31] or through HNF4 partners, coactivators or corepressors [32–34]. In the present work, we focused on a possible implication of CBP. Indeed, the well known CBP/p300 factor is a coactivator integrating many different transduction pathways and is able to synergize HNF4 transcriptional action through two physically separated domains [22]. Our experiments show that CBP overexpression stimulates the L3-dependent construct four-fold, confirming the crucial role played by CBP in the transcriptional activity of HNF4 in hepatocytes. An hypothesis could be that the PKA signaling pathway may inhibit HNF4-mediated transcription by phosphorylating proteins, such as CREB, which compete for limiting amounts of the coactivator CBP, as it has been demonstrated for cAMP inhibition of NF-κB-mediated transcription [35]. However, in our system, the induction by CBP of the L3-dependent construct remains sensitive to cAMP inhibition, therefore precluding the hypothesis of nuclear competition for limiting amounts of CBP. Phosphorylation of CBP/p300 by PKA is well documented [36] but has generally been involved in the transcriptional activation by cAMP [36–38]. Indeed, cAMP-dependent phosphorylation of both CREB and CBP increases their interaction and resulting transactivation [39–41]. However, it cannot be ruled out that interaction between HNF4 and CBP is inhibited by CBP phosphorylation.

It is also noteworthy to recall that HNF4 has also been demonstrated to be a *trans*-acting factor of genes transcriptionally stimulated by cAMP, such as the phosphoenolpyruvate carboxykinase and tyrosine aminotransferase [42–44]. In both cases the cAMP effect is mediated by the CREB activator, associated with C/EBPα formerly [45–47]. Therefore, it could be that HNF4 is by itself insufficient, when bound to a single site, to impose on a promoter a negative response to cAMP; it could rather act synergistically with other regulatory complexes, e.g. the GlRE complex in the L-PK gene.

In conclusion, the identification in the L-PK gene promoter

of two negative cAMP response elements binding different transcriptional complexes, confirms the multiplicity of the possible mechanisms of the cAMP-dependent transcriptional controls, activation [5,6,45–50] as well as inhibition [8–13].

Acknowledgements: We are grateful to Dr. M. Casado, Dr. C. Lenzner and Dr. A.L. Pichard for critically reading the manuscript. We thank G. Rutter, G.U. Ryffel, T. Leff, P. Sassone-Corsi and C.K. Glass for providing −150PK/Luci and H4−105TK/Luci constructs and DN-HNF4, PKA and CBP expression vectors. This work was supported by Institut National de la Santé et de la Recherche Médicale, le Ministère de l'Enseignement Supérieur et de la Recherche, la Fondation pour la Recherche Médicale et l'Institut Danone.

References

[1] Vaulont, S. et al. (1984) Biochem. Biophys. Res. Commun. 125, 135–141.
[2] Decaux, J.F., Antoine, B. and Kahn, A. (1989) J. Biol. Chem. 264, 11584–11590.
[3] Mellon, P.L., Clegg, C.H., Correll, L.A. and McKnight, G.S. (1989) Proc. Natl. Acad. Sci. USA 86, 4887–4891.
[4] Delmas, V., Molina, C.A., Lalli, E., de Groot, R., Foulkes, N.S., Masquilier, D. and Sassone-Corsi, P. (1994) Rev. Physiol. Biochem. Pharmacol. 124, 1–28.
[5] Richards, J.P., Bachinger, H.P., Goodman, R.H. and Brennan, R.G. (1996) J. Biol. Chem. 271, 13716–13723.
[6] Montminy, M. (1997) Annu. Rev. Biochem. 66, 807–822.
[7] Daniel, P.B., Walker, W.H. and Habener, J.F. (1998) Annu. Rev. Nutr. 18, 353–383.
[8] Tamir, A. and Isakov, N. (1994) J. Immunol. 152, 3391–3399.
[9] Paulauskis, J.D. and Sul, H.S. (1989) J. Biol. Chem. 264, 574–577.
[10] Rangan, V.S., Oskouian, B. and Smith, S. (1996) J. Biol. Chem. 271, 2307–2312.
[11] Roder, K., Wolf, S.S., Beck, K.F., Sickinger, S. and Schweizer, M. (1997) Gene 184, 21–26.
[12] Mounier, C., Chen, W., Klautky, S.A. and Goodridge, A.G. (1997) J. Biol. Chem. 272, 23606–23615.
[13] Xiao, G., Falkner, K.C., Xie, Y., Lindahl, R.G. and Prough, R.A. (1997) J. Biol. Chem. 272, 3238–3245.
[14] Bergot, M.O., Diaz-Guerra, M.J., Puzenat, N., Raymondjean, M. and Kahn, A. (1992) Nucleic Acids Res. 20, 1871–1877.
[15] Viollet, B., Kahn, A. and Raymondjean, M. (1997) Mol. Cell. Biol. 17, 4208–4219.
[16] Kennedy, H.J., Viollet, B., Rafiq, I., Kahn, A. and Rutter, G.A. (1997) J. Biol. Chem. 272, 20636–20640.
[17] Drewes, T., Clairmont, A., Klein-Hitpass, L. and Ryffel, G.U. (1994) Eur. J. Biochem. 225, 441–448.
[18] Taylor, D.G., Haubenwallner, S. and Leff, T. (1996) Nucleic Acids Res. 24, 2930–2935.
[19] Lefrancois-Martinez, A.M., Martinez, A., Antoine, B., Raymondjean, M. and Kahn, A. (1995) J. Biol. Chem. 270, 2640–2643.
[20] de Wet, J.R., Wood, K.V., De Luca, M., Helinski, D.R. and Subramani, S. (1987) Mol. Cell. Biol. 7, 725–737.
[21] Cuif, M.H., Doiron, B. and Kahn, A. (1997) FEBS Lett. 417, 81–84.
[22] Yoshida, E., Aratani, S., Itou, H., Miyagishi, M., Takiguchi, M., Osumu, T., Murakami, K. and Fukamizu, A. (1997) Biochem. Biophys. Res. Commun. 241, 664–669.
[23] Vaulont, S., Munnich, A., Decaux, J.F. and Kahn, A. (1986) J. Biol. Chem. 261, 7621–7625.
[24] Noguchi, T., Inoue, H. and Tanaka, T. (1985) J. Biol. Chem. 260, 14393–14397.
[25] Iynedjian, P.B., Jotterand, D., Nouspikel, T., Asfari, M. and Pilot, P.R. (1989) J. Biol. Chem. 264, 21824–21829.
[26] Vallet, V.S., Henrion, A.A., Bucchini, D., Casado, M., Raymondjean, M., Kahn, A. and Vaulont, S. (1997) J. Biol. Chem. 272, 21944–21949.
[27] Vallet, V.S., Casado, M., Henrion, A.A., Bucchini, D., Raymondjean, M., Kahn, A. and Vaulont, S. (1998) J. Biol. Chem. 273, 20175–20179.
[28] Kaytor, E.N., Shih, H. and Towle, H.C. (1997) J. Biol. Chem. 272, 7525–7531.
[29] Lou, D.Q., Tannour, M., Selig, L., Thomas, D., Kahn, A. and Vasseur-Cognet, M. (in press).
[30] Hasegawa, J.-i., Osatomi, K., Wu, R.-F. and Uyeda, K. (1999) J. Biol. Chem. 274, 1100–1107.
[31] Jiang, G.Q., Nepomuceno, L., Yang, Q. and Sladek, F.M. (1997) Arch. Biochem. Biophys. 340, 1–9.
[32] Chen, J.D. and Li, H. (1998) Crit. Rev. Eukaryot. Gene Expr. 8, 169–190.
[33] Hong, H., Kohli, K., Trivedi, A., Johnson, D.L. and Stallcup, M.R. (1996) Proc. Natl. Acad. Sci. USA 93, 4948–4952.
[34] Hong, H., Kohli, K., Garabedian, M.J. and Stallcup, M.R. (1997) Mol. Cell. Biol. 17, 2735–2744.
[35] Parry, G.C. and Mackman, N. (1997) J. Immunol. 159, 5450–5456.
[36] Chrivia, J.C., Kwok, R.P., Lamb, N., Hagiwara, M., Montminy, M.R. and Goodman, R.H. (1993) Nature 365, 855–859.
[37] Janknecht, R. and Nordheim, A. (1996) Oncogene 12, 1961–1969.
[38] Swope, D.L., Mueller, C.L. and Chrivia, J.C. (1996) J. Biol. Chem. 271, 28138–28145.
[39] Kwok, R.P. et al. (1994) Nature 370, 223–226.
[40] Parker, D., Ferreri, K., Nakajima, T., La Morte, V.J., Evans, R., Koerber, S.C., Hoeger, C. and Montminy, M.R. (1996) Mol. Cell. Biol. 16, 694–703.
[41] Arias, J., Alberts, A.S., Brindle, P., Claret, F.X., Smeal, T., Karin, M., Feramisco, J. and Montminy, M. (1994) Nature 370, 226–229.
[42] Hall, R.K., Sladek, F.M. and Granner, D.K. (1995) Proc. Natl. Acad. Sci. USA 92, 412–416.
[43] Lemaigre, F.P. and Rousseau, G.G. (1994) Biochem. J. 303, 1–14.
[44] Nitsch, D., Boshart, M. and Schutz, G. (1993) Proc. Natl. Acad. Sci. USA 90, 5479–5483.
[45] Croniger, C., Leahy, P., Reshef, L. and Hanson, R.W. (1998) J. Biol. Chem. 273, 31629–31632.
[46] Liu, J.S., Park, E.A., Gurney, A.L., Roesler, W.J. and Hanson, R.W. (1991) J. Biol. Chem. 266, 19095–19102.
[47] Roesler, W.J., Crosson, S.M., Vinson, C. and McFie, P.J. (1996) J. Biol. Chem. 271, 8086.
[48] Foulkes, N.S., Borrelli, E. and Sassone-Corsi, P. (1991) Cell 64, 739–749.
[49] Zanger, K., Cohen, L.E., Hashimoto, K., Radovick, S. and Wondisford, F.E. (1999) Mol. Endocrinol. 13, 268–275.
[50] Edelman, A.M., Blumenthal, D.K. and Krebs, E.G. (1987) Annu. Rev. Biochem. 56, 567–613.
[51] Vaulont, S., Puzenat, N., Levrat, F., Cognet, M., Kahn, A. and Raymondjean, M. (1989) J. Mol. Biol. 209, 205–219.