The proximal element of the human apolipoprotein A-II promoter increases the enhancer activity of the distal region

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We have previously shown that human apolipoprotein A-II (apoA-II) transcription is controlled by a complex set of regulatory elements. In this study, we demonstrate that the distal region of the apoA-II promoter (–911/–614) acts as an enhancer and results in a 6-fold increase in activity when the proximal AB element is inserted between this enhancer and a TATA box. The AB element alone does not display any transcriptional activity. The combination of the proximal AB element and the enhancer is sufficient to activate transcription to the same level as that achieved with the full-length promoter. DNA binding and competition assays, and binding interference experiments allowed us to identify two adjacent binding sites

within the AB element. These binding activities designated CIIB1 and AIIAB3/4, respectively. Mutation on each of these sites showed that the binding site of CIIB1 within the AB element played a major role in the interaction with the enhancer. Whereas transcriptional activation of other apolipoprotein genes involves the binding of the liver-enriched hepatocyte nuclear factor 4 (HNF4) on their proximal promoter, the present study demonstrates that neither HNF4 nor ApoA-I regulatory protein 1, its antagonistic orphan receptor, was able to bind the AB element. Instead, apoA-II transcription was driven by the interaction of apoA-II enhancer with proximal AB element, which involves an unidentified activity, CIIB1.

INTRODUCTION

Human apolipoprotein A-II (apoA-II) is a major protein component of high-density lipoprotein (HDL) and is synthesized by the liver and to a much lesser extent by the intestine [1–3]. Recent genetic studies in mice and humans have pointed out that apoA-II is a risk factor for atherosclerosis [4–6]. Overexpression of mouse apoA-II in transgenic mice results in elevated HDL cholesterol and the development of fatty streak lesions as compared with normal mice [7]. Transgenic mice overexpressing both human apoA-I and apoA-II are less protected against atherosclerosis in response to an atherogenic diet than transgenic mice overexpressing apoA-I alone [8]. These *in vivo* observations are further supported by *in vitro* experiments. ApoA-II-containing HDL does not stimulate lecithin-cholesterol acyltransferase [9,10] and behaves as a poor cholesteryl ester substrate donor for the cholesteryl ester transfer protein [11,12] compared with apoA-I-containing HDL. Altogether, these data are consistent with the hypothesis that apoA-II counteracts the stimulatory effect of apoA-I on reverse cholesterol transport. Furthermore, Staels and co-workers have reported that apoA-I and apoA-II gene expression was regulated in an opposite way in rat liver under various conditions such as hyperthyroidism [13], ethinyl oestradiol treatment of ovariectomized rats [14], and treatment with different corticosteroids [15].

Gene transcription is controlled by a complex array of regulatory domains binding transcriptional factors, the combination of which determines a specific pattern of gene expression. At the present time, the liver phenotype has been shown to be controlled by four liver-enriched transcriptional factor families, i.e. CCAAT/enhancer binding protein (C/EBP), hepatocyte nuclear factors 1, 3 and 4 (HNF1, HNF3 and HNF4) (see [16] for a review). Apolipoproteins are a multigenic family with an expression restricted to the liver and the intestine, except for

apoE whose expression is more ubiquitous. As is the case for other genes, apolipoprotein gene expression is controlled by various negative and positive, distal and proximal regulatory elements (see [17] for a review). These elements bind transcriptional factors already known to be implicated in either basal transcription (NFY, NF1, AP1), or hepatic specificity (C/EBP, HNF1, HNF4), or inducibility (IL6DBP, NFB, various nuclear hormone receptors) as well as unknown binding activities, which have to be characterized.

We and others [18–22] have previously reported that the transcription of the human apoA-II gene is controlled by a complex array of distal and proximal regulatory elements, designated A to N, that have been grouped in three functional regions. Although apoA-II expression is restricted to the liver, elements J, H and C of the apoA-II promoter which bind HNF4, HNF1 and C/EBP, respectively, do not play an essential role in transcription in HepG2 cells [20]. Conversely, the functional analysis of the apoA-II promoter demonstrated that four domains are important for transcription: distal elements N, K and L, and the proximal AB element. These domains bind transcriptional factors unknown up to now.

In this study, we have demonstrated that: (1) the proximal AB element and the distal enhancer synergistically activate human apoA-II transcription; (2) this effect involves the binding site of CIIB1, on the proximal AB element; and (3) CIIB1 is distinct from members of the nuclear receptor family.

MATERIALS AND METHODS

Materials

The Klenow fragment of DNA polymerase I, T4 DNA ligase and restriction enzymes were purchased from New England Biolabs. Polynucleotide kinase and double-stranded poly(dI-dC) were

Abbreviations used: AdML, adenovirus major late promoter; apoA-II, apolipoprotein A-II; ARP1, ApoA-I regulatory protein 1; C/EBP, CCAAT/enhancer binding protein; CAT, chloramphenicol acetyltransferase; HDL, high-density lipoprotein; HNF, hepatocyte nuclear factor; TK, thymidine kinase; TR, thyroid receptor; RXR, retinoid X receptor.

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Figure 1 Plasmid FL map

The plasmid FL comprises the CAT gene under the control of the -911 to $+29$ human apoA-II full-length promoter [18]. The 14 elements of the human apoA-II promoter are indicated by bold boxes. The arrows are oligonucleotides used for PCR strategy to design mutations in AB element.

purchased from Pharmacia. $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$, $[\alpha\text{-}^{32}\text{P}]\text{dGTP}$ (3000 Ci/mmol), $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (> 5000 Ci/mmol) and $[\text{H}]\text{acetyl-CoA}$ (6.1 Ci/mmol) were purchased from ICN. Acrylamide, N,N' -methylenebisacrylamide (electrophoresis grade) and protein markers were obtained from Bio-Rad. Sequenase sequencing kit was purchased from Amersham. Acetyl-CoA and chloramphenicol were obtained from Sigma.

Synthetic oligonucleotides used for plasmid construction

The following were used for plasmid construction:

PCRxb: ATCTCTAGATTC $^{-618}$ GAACATACCCTACCCCCAGTAAAC $^{-642}$

TATAc: ATCTCTAGA $^{-32}$ AGGTATATAGCCCCTTCCTC $^{-13}$

AII2: ATCCTGCTCGAG $^{+29}$ CAGCGTCTCTGTCCTTGGTG $^{+10}$

AII80c: CTAGTCTAGAG $^{-81}$ CTGGGCCCATCCATAG $^{-66}$

ABM1c: $^{-73}$ CCATAGTCAGAGTACCTGACAGGGGGTGGGTAAACAGACA $^{-32}$

ABM1nc: $^{-32}$ TGTCTGTTTACCCACCCCCTGTCAGGTACTCTGACTAT $^{-69}$

ABM6c: $^{-55}$ TGACAGGGGGTGGGTAACTCCAGGTATATAGCCC $^{-20}$

ABM6nc: $^{-23}$ CTATATACCTGGAGTGTTACCCACCCCCTGTCAGG $^{-57}$

ABM1P: $^{-33}$ GGAGTGTTACCCACCCCCTGTCAGGTACTCTGACTATGGATG $^{-74}$

R26: TCACACAGGAAACAGCTATAGCCATG.

Synthetic oligonucleotides used for gel-shift assays and binding-interference assays

The following were used for gel-shift and binding interference assays:

ABc: CCC $^{-68}$ TAGTCCTGTCACCTGACAGGGGGTGGGTAAACAGACA $^{-32}$

ABnc: CCC $^{-32}$ TGTCTGTTTACCCACCCCCTGTCAGGTGACAGGACTA $^{-68}$

Ac: GGG $^{-52}$ CAGGGGGTGGGTAAACAGACAGGTAT $^{-27}$

Anc: GGG $^{-27}$ ATACCTGTCTGTTTACCCACCCCCTG $^{-52}$.

Plasmid construction

The plasmid FL, comprising the chloramphenicol acetyltransferase (CAT) gene under the control of the $-911/+29$ human apoA-II full-length promoter (Figure 1), has been described previously [18]. To place the distal region N to I directly upstream of the AB element, the sequence $-911/-614$ was excised by *Hind*III digestion from the AII-HL plasmid [23], purified by electroelution and cloned in sense and antisense orientations into the AB_{AII} plasmid previously described as AII($-80/+29$)-CAT [18], resulting in plasmids E-AB_{AII} and E_{inv}-AB_{AII}, respectively. The E-TATA_{AII} plasmid, comprising the distal region directly upstream of the apoA-II TATA box, was generated by inverse PCR [24] using oligonucleotides TATAc and PCRxb as primers and the plasmid E-AB_{AII} as a template. The E-TATA_{AII} plasmid was digested using *Xba*I to generate the plasmid TATA_{AII}, which only comprises the apoA-II ($-30/+29$) region upstream of the CAT gene. Plasmids comprising mutations in the AB element were generated from the FL plasmid by successive PCR. The first PCR, using oligonucleotides AII2 and ABM1c or ABM6c as primers and FL plasmid as a template (Figure 1), generated PCR product I comprising, respectively, mutation M1 or M6 on the coding strand. A second PCR, using oligonucleotides R26 and ABM1nc or ABM6nc as primers and FL plasmid as a template, generated PCR product II, comprising respectively mutation M1 or M6 on the non-coding strand. Finally, a third PCR, using oligonucleotides AII2 and R26 as primers and PCR products I and II as templates, generated PCR product III, which was substituted to the wild-type corresponding sequence in the FL plasmid, leading to plasmids FL-ABM1 and FL-ABM6, respectively. The double mutant FL-ABM7 was generated by the same method from the plasmid FL-ABM6 as template using the appropriate oligonucleotides ABM6c and AII2, and R26 and ABM1P as primers. Plasmids FL-ABM1, FL-ABM6 and FL-ABM7 were used as templates to produce by PCR the corresponding mutated $-80/+29$ sequences. The resulting PCR products were digested, electroeluted and inserted at the site of the AB_{AII} plasmid, resulting in AB_{AII}M1, AB_{AII}M6, and AB_{AII}M7 plasmids, respectively. Mutated plasmids E-AB_{AII}M1 and E-AB_{AII}M6 were generated by inserting the $-911/-614$ fragment from AII-HL [23] at the *Xba*I site of

plasmids AB_{AII}M1 and AB_{AII}M6, respectively. To place the apoA-II distal region in front of the TATA box of the adenovirus major late promoter (AdML), the -911/-614 fragment was inserted in sense or antisense orientations at the *Hind*III site of plasmid pML44 [25], resulting in plasmids E-TATA_{AdML} and E_{inv}-TATA_{AdML}, respectively. Double-stranded oligonucleotides AB, ABM1 and ABM6 were synthesized and inserted at the *Pst*I site of plasmid E-TATA_{AdML} to generate plasmids E-AB_{AdML}, E-ABM1_{AdML} and E-AB_{AdML}M6, respectively. Plasmid AB_{AdML} was generated by *Hind*III digestion of E-AB_{AdML}. All of these plasmids were sequenced.

Cell transfection and CAT assay

These different plasmids were co-transfected with the plasmid RSV-gal in HepG2 cells using the calcium phosphate-DNA coprecipitation method [26]. β -Galactosidase activity was determined to normalize the variability in transfection efficiency [27]. CAT assays were performed in a liquid phase as described in [28]. The initial velocity of the enzymic reaction was estimated by measuring the amount of labelled acetylchloramphenicol which diffused directly into the liquid phase in the scintillation counting vial.

Preparation of extracts from COS-transfected cells

HNF4 and ApoA-I regulatory protein 1 (ARPI) cDNA were subcloned into the eukaryotic expression vector pMT2X and transfected into COS cells using the calcium phosphate coprecipitation method. Whole-cell extracts were prepared as previously described [29] to be used in gel-retardation assays.

DNA binding gel electrophoresis and competition assays

Annealing and labelling of synthetic oligonucleotides were performed as previously described [30]. Rat liver nuclear extracts [31] were preincubated for 15 min at 4 °C in a 20 μ l reaction solution containing 25 mM Hepes, pH 7.6, 8% Ficoll, 40 mM KCl, 1 mM dithiothreitol (DTT), 3 μ g of double-stranded poly(dI-dC), and 5 mM MgCl₂ in the presence or absence of competitor oligonucleotides in 25- to 500-fold excess [32]. Labelled double-stranded oligonucleotides (60000 c.p.m.), corresponding to the AB element of apoA-II promoter (-32 to -68), or to the element A (-27 to -52) containing CCC or GGG 5' protruding ends, or to the mutated sequences, were then added and the incubation was carried out for 30 min at 4 °C. Free DNA and DNA-protein complexes were resolved on a 4% polyacrylamide gel in 1 $\times$ TAE (1 $\times$ TAE = 6.7 mM Tris, 3.3 mM sodium acetate, 1 mM EDTA, pH 7.9). The gel was dried and exposed to an X-ray film.

Binding-interference assays

Purine residues (G residues preferentially) were methylated by DMSO [30], and thymidine DNA modifications by KMnO₄ were performed as described [33]. Each strand of the synthetic oligonucleotide used for band-shift assays, corresponding to the regulatory AB element (-32 to -68) or A element (-27 to -52) of the apoA-II promoter, was first labelled with [γ -³²P]ATP, using T4 polynucleotide kinase, and then chemically modified. Each modified and labelled strand was annealed with its unlabelled complementary strand. Methylated and modified-thymidine probes were incubated with rat liver nuclear extracts [31] and analysed by preparative DNA binding gel electrophoresis. Complexed and free oligonucleotides were detected by autoradiography, excised from the gel, purified by electroelution, and

treated with 1 M piperidine at 90 °C for 30 min [34]. Samples were subsequently analysed by 20% polyacrylamide/urea electrophoresis and autoradiography. A nucleotide is considered strongly affected by chemical modification when the corresponding band disappears, and weakly affected when the intensity decreases. The intensity of the non-affected nucleotides remains unmodified as compared with control.

RESULTS

1. Distal enhancer and proximal AB element activate synergistically apoA-II transcription

Figure 2 demonstrates that the -911/-614 distal region of the human apoA-II promoter acts as an enhancer. CAT activity rose 15.5- and 18-fold when the distal region was placed in front of the homologous TATA_{AII} box as well as the heterologous TATA_{AdML} box, respectively (Figures 2A and 2B). The distal

Figure 2 Distal enhancer and proximal AB element activate synergistically apoA-II transcription

CAT assays were performed after transient transfection of HepG2 cells as described in the Materials and methods section. Experiments were done in triplicate and repeated three to five times. For each experiment, CAT activity was calculated by linear regression as the slope of the reaction kinetics and expressed as c.p.m./min. CAT activities were expressed as the percentage of the activity of a plasmid arbitrarily chosen as a function of the experiment. Results are means $\pm$ S.D.

Band-shift experiments were carried out as described in the Materials and methods section using crude rat liver nuclear extracts and a labelled double-stranded oligonucleotide. Competitors were used at a 100-fold excess. **(A)** Binding to element AIIAB. **(B)** Binding to element AIIA. **(C)** Element AB and designed mutation sequences.

Finally, the comparison of CAT activities obtained with plasmids E-AB_{ATI} or E-AB_{AdML} with that of plasmid FL (Figure 2C) shows that the combination of the enhancer and the proximal AB element was sufficient to reproduce the transcriptional activity of the full-length promoter. Moreover CAT activity of plasmid E-AB_{ATI} was 120 % of that corresponding to the full-length promoter.

Interference binding of crude rat liver nuclear extracts to AB element and A element by DMSO and by KMnO_4 modifications of G and T residues, respectively, were performed as described in the Materials and methods section with a labelled coding or non-coding strand, as indicated. AIIAB probe (−32 to −69) was used for CIIIB1 and AIIAB1 binding interference and AIIA probe (−27 to −52) for AIIAB3/4 binding interference. Abbreviations: F, free probe; B, probe recovered from the DNA–protein complexes with AIIAB1, CIIIB1, AIIAB3 and AIIAB4. The position of G and T residues which strongly or weakly interfere with the binding are indicated, respectively, by * and by ○. Some A residues which interfere with binding are indicated by ●. (A) Methylation of G residues with DMS. (B) Modification of T residues with KMnO_4 . (C) Summary of the chemical modification interference patterns with CIIIB1, AIIAB3/4 and AIIAB1.

Figure 5 Mutations on AB are designed to abolish specifically either CIIB1 binding or AIIAB3/4 binding

Band-shift experiments were performed with rat liver nuclear extracts as described in the Materials and methods section and in the legend to Figure 2. (A) Binding to AIIABM1 probe. (B) Binding to AIIABM6.

2. Proximal AB element comprises two adjacent binding sites

Gel-retardation assays performed with rat liver nuclear extracts and a labelled oligonucleotide corresponding to the AB element showed that this element bound four proteic activities, AIIAB1, AIIAB2 or CIIB1, AIIAB3 and AIIAB4 (Figure 3A). Systematic mutagenesis of the AB element (Figure 3C) and competition experiments allowed us to determine the binding site of each activity. Mutants ABM3 and ABM4 were able to compete out any complex, indicating that the nucleotide sequence $-44/-54$ was not involved in the binding of any of the four activities. The binding of CIIB1 on the AB element was competed out by mutants ABM5 and ABM6 and by oligonucleotide CIIB, corresponding to the binding site of CIIB1 on the element B of the apoCIII promoter [30], but not by ABM1 and ABM2 (Figure 3A). On the contrary, the complexes AIIAB3 and AIIAB4 were not competed out by mutants ABM5 and ABM6 and oligonucleotide CIIB. Mutants ABM1 and ABM2 only affected the complex AIIAB4. To better characterize the complexes AIIAB3 and AIIAB4, gel-retardation assays were performed using oligonucleotide AIIA, corresponding to the sequence $-52/-32$, as a probe (Figure 3B). Only complexes AIIAB3 and AIIAB4 were generated with the AIIA probe. As expected, these complexes were competed out by mutant ABM1 and by AIIAB, but not by mutants ABM5 and ABM6. These experiments allowed us to characterize two distinct binding domains on the AB element: the first was defined by mutants ABM1 and ABM2, which affected CIIB1 activity, whereas the second was defined by mutants ABM5 and ABM6, which affected AIIAB3 and AIIAB4 activities. AIIAB1 binding activity was competed out by every mutant, and was not generated when oligonucleotide AIIA was used as a probe. This indicates that AIIAB1 activity required at least a binding site within the sequence $-65/-52$ but that the binding was not abolished by any 5-bp-long mutation.

In order to determine the nucleotides involved in the formation of the different complexes, binding interference analysis was performed with rat liver nuclear extracts. The following modifications interfered strongly with the CIIB1 binding: (a) modification of G residues at positions -61 and -54 on the coding strand and at positions -59 , -57 , -56 and -52 on the non-

Figure 6 CIIB1 binding site plays a major role in the communication enhancer/AB element

CAT assays were performed as described in the Materials and methods section and in the legend to Figure 1.

coding strand (Figure 4A); (b) modification of T residues at position -55 on the coding strand and at positions -58 , -53 and -51 on the non-coding strand (Figure 4B). The binding of activities AIIAB3 and AIIAB4 involved the same nucleotides, i.e. G residues at positions -44 , -43 and -42 on the coding strand and -37 , -33 on the non-coding strand, and T residues at positions -40 , -39 , -38 and -36 on the non-coding strand. The only exception was the G-44 on the coding strand which only interfered with the binding of AIIAB4. The contact points of AIIAB1 activity corresponded to the sum of the contact points of CIIB1 and AIIAB3/4 activities (Figure 4C). Furthermore, AIIABM1 and AIIABM6 probes were not able to bind the AIIAB1 complex (Figure 5). This indicated that AIIAB1 requires two binding sites, one within $-65/-52$ and one within $-52/-33$. These results could suggest that AIIAB1 is a heterodimeric form of CIIB1/AIIAB3/4, in agreement with the competition experiments of Figure 2.

3. CIIB1 site plays a major role in the synergistic effect of the proximal AB element on the transcriptional activity of the enhancer

In order to determine the factors bound to AB which were involved in the synergistic effect between the AB element and the enhancer, specific mutants were designed. Gel-retardation competition experiments (Figure 3A) gave indirect evidence that mutant ABM1 was not able to bind CIIB1 activity, whereas

Figure 7 CIIB1 is a new activity, distinct from members of the nuclear receptor family

Band-shift experiments were carried out as described in the Materials and methods section and in the legend to Figure 2, using nuclear extracts from transfected COS cells and labelled double-stranded CIIB or AIIAB. Competitors were used at a 100-fold excess.

mutants ABM6 were not able to bind activities AIIAB3/4. This was confirmed by a gel-retardation assay using mutants ABM1 and ABM6 as probes (Figure 5). Probe ABM1 only generated complexes AIIAB3 and AIIAB4 (Figure 5A), whereas probe ABM6 only generated CIIB1 activity (Figure 5B). Both probes gave the expected pattern of competition with the different mutants.

The effect of these mutations on the transcriptional activity of the apoA-II promoter was then measured by CAT assay in HepG2 cells (Figure 6). When mutation ABM1 was introduced in the full-length promoter, CAT activity was 6.5-fold lower than with the wild-type promoter, mutation ABM6 only inducing a 1.7-fold decrease (Figure 6A). Furthermore, when the two binding sites of the AB element were mutated simultaneously (mutant ABM7) the transcription was reduced 10-fold. Similar results were obtained when HepG2 cells were transfected with constructs into which the enhancer was placed directly upstream of mutated AB element in front of the homologous apoA-II or heterologous AdML TATA boxes (Figures 6B and 6C respectively). In both cases mutation ABM1 resulted in a 4.5-fold decrease in CAT activity as compared with wild-type plasmids. Furthermore, by abolishing CIIB1 binding, this mutation reduced CAT activity to the same range as those obtained with the enhancer placed directly in front of the TATA box. However, the transcriptional activity of the different constructs was only reduced 1.8-fold by the suppression of the binding of AIIAB3/4 (mutation ABM6). Thus, these results demonstrate that the CIIB1 binding site of element AIIAB plays the major role in the communication between the enhancer and the proximal AB element.

4. CIIB1 is a new activity, distinct from members of the nuclear receptor superfamily

It has been shown that the B element of the apoCIII promoter was able to bind transcriptional factors HNF4 and ARP1 on a

site overlapping with the CIIB1 binding site [29,35]. Figure 7 investigates the binding of HNF4 and ARP1 to the AB element of the apoA-II promoter. Neither HNF4 nor ARP1 over-expressed in COS cells was able to bind directly to the AB element. In addition, the binding of HNF4 and ARP1 on CIIB1 was poorly competed out by an excess of AIIAB oligonucleotide when compared with an excess of CIIB1 oligonucleotide. We furthermore showed that the heterodimer thyroid receptor (TR)/retinoid X receptor (RXR) bound to the AB element. However, the electrophoretic mobility of the complex DNA/heterodimer was different from the CIIB1/DNA one (results not shown). These results strongly suggest that CIIB1 is not identical to these factors.

DISCUSSION

Recent studies have suggested that the balance between plasma levels of apoA-I and apoA-II is involved in the control of reverse transport of cholesterol [4–12] and that plasma apoA-II levels are controlled at the level of hepatic synthesis [36]. Besides, it has been reported that liver expression of apoA-I and apoA-II genes is controlled in an opposite way by different hormones and effectors [13–15]. These effects may modulate reverse transport of cholesterol. Therefore, understanding the mechanisms involved in the regulation of apoA-II gene expression is of physiological relevance. The fact that apoA-II expression is exclusively restricted to the liver [2] makes apoA-II a good model to study mechanisms controlling liver-specific gene expression. Indeed, the functional analysis of the apoA-II promoter which we previously reported [18–20] indicated that the domains binding known liver-enriched factors HNF1, HNF4 and C/EBP do not play an essential role in apoA-II gene transcription in HepG2 cells. On the contrary, apoA-II gene transcription is most highly regulated by three distal domains (K, L and N) of the enhancer and by the proximal domain AB [20]. This raises the question of which combination of transcriptional factors drives apoA-II expression in the liver.

Our present results demonstrate that the distal region of the apoA-II promoter (–614/–911) displays enhancer properties, since it was able to activate transcription, in sense or anti-sense orientation, from its homologous TATA box as well as from heterologous TATA boxes from AdML or TK genes. Furthermore, we show that the activity of the enhancer was 6-fold enhanced when the proximal AB element was inserted between the enhancer and the TATA box. This enhancement occurred in the presence of the apoA-II TATA box and of the heterologous AdML TATA box as well, suggesting that a specific interaction between AB element and the enhancer may be involved. A position effect is unlikely since the transcriptional activation we measured was dramatically reduced when the AB element was substituted by the mutated sequence ABM7, which was unable to bind any factor in a gel-retardation assay.

In addition, the combination of the enhancer and the proximal AB element proved to be sufficient to reproduce the transcriptional activity of the full-length promoter. This further supports the assumption that the middle region (–614/–80) of the apoA-II promoter is not necessary for apoA-II transcription in HepG2 cells [18,20]. Indeed, we have recently shown that, within this middle region, the domain D even acted as a slightly negative regulatory element [37].

Synergism between distal and proximal regulatory elements of various promoters has been described to be involved in tissue-specific and inducible transcriptional control of numerous genes. For example, the high level of tissue-specific transcription of the rat platelet factor 4 gene in megakaryocytes [38], the transcription

of the ϵ -globin gene in erythroid cells [39] or the induction of human serum amyloid A gene transcription by interleukin 1 and interleukin 6 [40] require distal and proximal elements. Moreover, tissue-specificity of hormonal control is based on the interaction between responsive elements and tissue-specific elements. Thus, the hormonal activation of the tyrosine aminotransferase gene is restricted to the liver by the interaction of a GRE (Glucocorticoid Responsive Element) with an HNF3 binding site and by the interaction of an HNF4 binding site with an essential element in the cAMP-responsive enhancer [41]. Therefore, the specific pattern of gene transcription under different hormonal conditions appears to be determined by particular combinations of responsive and tissue-specific regulatory elements and transcriptional factors.

The A, C and E apolipoproteins belong to a multigene family sharing a conserved gene structural organization [42]. Furthermore, apolipoproteins As, B and Cs are expressed only in the liver and/or the intestine, suggesting similar mechanisms of gene activation. It has recently been shown that apoC-III and apoA-IV gene transcription in HepG2 cells involves interactions between an HNF4 site within the proximal promoter and upstream elements [43,44]. Although an interaction with distal elements has not yet been reported for apoB or apoA-I, the transcription of both genes depends on the binding of HNF4 on a proximal element. This interaction is suppressed by the antagonistic binding of ARP1, which leads to repression of transcription [29]. Similarly, apoA-I and apoC-III transcription is controlled by a balance in the binding of HNF4 and ARP1, which allows or suppresses interactions with the other factors involved in the activation complex [29,45]. In contrast, the present data show that the activation of apoA-II transcription does not involve the binding of HNF4 on the proximal region of the apoA-II promoter. Rather, the enhancement of transcription induced by the AB element involves the binding site of CIIB1 within the AB element. Actually a mutation of the binding site for CIIB1 within the AB element abolishes this enhanced activity.

Our results suggest that CIIB1 activity plays a crucial role in the interaction of the enhancer with the proximal AB element. In support to this hypothesis, we have previously reported that, in addition to the AB element, two elements, L and K, bound CIIB1 activity within the enhancer, and that the substitution or the deletion of each of these distal elements resulted in a dramatic decrease in transcriptional activity [19]. The interaction of CIIB1 bound on the two distal elements and on the AB element could induce DNA bending and/or stabilize the transcriptional complex.

Several indirect arguments have questioned the relationship between CIIB1 activity and orphan receptors. CIIB1 binds to elements which bind equally HNF4 and other orphan receptors [29,30,35]. Furthermore, the CIIB1 binding site in element AGCAGGTGAC partially overlapped the consensus site of HNF4 (TGGACTT/CC) [45]. To solve this question, purification of CIIB1 activity was carried out but did not lead to the identification of the transcriptional factor [35]. In this study, we have demonstrated that neither HNF4 nor ARP1 was able to bind the AB element. Though this element was able to bind the heterodimer TR/USP, comprising the thyroid hormone receptor and the *Drosophila* homologue of RXR [46], the electrophoretic mobility of this complex was different from that of the CIIB1 complex (results not shown). These data clearly indicate that CIIB1 is not identical to one of these nuclear receptors. Although CIIB1 activity is thermostable [20,30], we cannot exclude the possibility that CIIB1 activity could be a new member of the nuclear receptor superfamily. Partial sequencing of the CIIB1

protein, purified from nuclear extracts of rat liver, will allow us to determine the nature of this transcriptional factor and to develop the tools required to characterize the role it plays in apoA-II transcriptional activation.

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