Cooperative Binding of Upstream Stimulatory Factor and Hepatic Nuclear Factor 4 Drives the Transcription of the Human Apolipoprotein A-II Gene*

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The activity of the human apoA-II promoter is controlled by a synergistic interaction of the distal enhancer and the proximal promoter. An important role in apoA-II promoter activity is exerted by a transcription factor, designated CIIIB1, which binds to the proximal element AB and the distal elements of the enhancer, K and L. In the present communication we establish that CIIIB1 corresponds to the previously described factor, upstream stimulatory factor (USF) using the following criteria. (a) Purification of CIIIB1 by affinity chromatography provided a heat-stable protein with an apparent molecular mass of 45 kDa that cross-reacted with anti-USF1 and -USF2a antibodies; (b) CIIIB1 bound to the elements AB, K, and L was supershifted by these antibodies; (c) the heterodimer USF1/2a is the predominant form that corresponds to CIIIB1. Cotransfection experiments in HepG2 cells established the functional significance of USF in apoA-II transcription. It was found that the minimal promoter AB was transactivated by USF2a. In addition, all three E-box motifs present in elements AB, K, and L are necessary for maximum transactivation by USF2a. A dominant negative form of USF2a inhibits the activity of apoA-II promoter. The USF1/2a heterodimer, which is naturally expressed in the liver, is as efficient as the USF2a homodimer in the transactivation of apoA-II promoter/enhancer constructs. Cotransfection experiments in COS-1 cells showed that hepatic nuclear factor 4 (HNF-4) synergized with USF2a in the transactivation of the apoA-II promoter. In addition, we showed that HNF-4 and USF2a bind to the enhancer cooperatively. This may account for the transcriptional synergism observed between USF and HNF-4 in the transactivation of the apoA-II promoter.

Although apolipoprotein A-II (apoA-II) is the second most abundant apolipoprotein in high density lipoprotein (1), its physiological functions are still unclear. Inactivation of the apoA-II gene in mice suggests a complex role for apoA-II in atherosclerosis, with both antiatherogenic and proatherogenic properties (2). Clinical observations and tissue culture studies suggest that an increase in apoA-II production may be less protective against atherosclerosis by raising the proportion of a high density lipoprotein subclass containing both apoA-I and apoA-II (LpAI:AII), thereby decreasing the proportion of the antiatherogenic subclass of high density lipoprotein containing only apoA-I (LpAI) (3, 4). ApoA-II is expressed in the liver and, to a much lesser extent, in the human fetal intestine (5). Recent kinetic data in normolipemic humans indicate that human apoA-II production rate is the major factor determining the distribution of apoA-I between LpAI and LpAI:AII (6, 7). Understanding the molecular mechanisms involved in hepatic transcription of apoA-II may, therefore, lead to the development of new tools to control the ratio between LpAI and LpAI:AII.

We have already reported that the human (−911/+29) apoA-II promoter is sufficient for liver-restricted expression of a reporter gene in transgenic mice (8). The −911/−614 base pair distal region exhibits enhancer properties and potentiates the strength of the homologous proximal promoter AB (−65/−33 base pairs) (9–12) as well as the strength of heterologous promoters (13). The element AB is important for the function of the apoA-II promoter and binds an activity designated CIIIB1 (12). CIIIB1 was first described as a heat-stable factor that binds to element B of the apoC-III promoter (14, 15). The CIIIB1 binding site GTCACTTG contains the CANNTG consensus E-box motif (16, 17). E-box motifs are recognized by basic helix/loop/helix transcription factors, which contain a basic (b) DNA binding domain and a helix/loop/helix (HLH) dimerization domain. In the case of bHLH/ZIP-related proteins, the bHLH domain is contiguous with a second dimerization domain, a leucine zipper (ZIP). The latter group includes a wide variety of transcription factors, such as c-Myc, Max (18), TFE3 (19), TFE2 (20), SREBP1 (21), and upstream stimulatory factor (USF) (22). USF appears to be the predominant bHLH/ZIP factor in liver nuclear extracts (22). Human USF was first designated as major late transcription factor (16). Three USF isoforms have been described, USF1, 2a, and 2b, with apparent molecular masses of 43, 44, and 38 kDa, respectively (23–25). USF1 and USF2a and 2b are encoded by two different genes, and USF2a and 2b are generated by differential splicing. Although ubiquitously expressed, USF has been involved in transcription of genes with tissue specificity (25, 26).

In the present study, we purified CIIIB1 from rat liver nuclear extracts by DNA sequence-specific affinity chromatography. We found that CIIIB1 is immunologically related to USF using anti-USF1 and anti-USF2a. DNA binding and competi-

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§ The abbreviations used are: apo, apolipoprotein; b, basic; C/EBP, CAAT enhance-binding protein; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assay; HLH, helix/loop/helix; HNF-4, hepatic nuclear factor 4; NonO, non-POU domain-containing, octamer-binding protein; PK, pyruvate kinase; FSP, poly(ADP-ribose) polymerase (NADP)-dependent transcription factor; SREBP, sterol regulatory element-binding protein; USF, upstream stimulatory factor; ZIP, zipper; PAGE, polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; RSV, Rous sarcoma virus; CMV, cytomegalovirus; NFY, nuclear factor Y; MLP, major late promoter.
tion experiments showed that USF binds to the regulatory elements AB, K, and L. We have established in HepG2 cells the functional significance of USF in apoA-II promoter activity. Finally, cotransfection experiments in COS-1 cells and DNA binding assays demonstrated synergism between USF2a and HNF-4 in the transactivation of the apoA-II promoter and a cooperative binding of the two factors to their cognate sites of the apoA-II promoter.

**Fig. 1.** Affinity purification and immunological relationship of rat CIIIB1 complex to USF. CIIIB1 is predominantly a USF1/2a heterodimer. A, analysis of CIIIB1 eluted from affinity chromatography by 12% SDS-PAGE. Proteins were stained with Coomassie Brilliant Blue. N.E., crude rat liver nuclear extracts. N.E. 85°C, crude rat liver nuclear extract heated at 85 °C for 5 min. 1st to 3rd AFF., fractions eluted in 0.5 M KCl from the first, second, and third DNA-specific affinity chromatographies. M.W., molecular mass markers are as noted. Arrows indicate the two main proteins, with apparent molecular masses of 97 and 45 kDa, respectively. B, analysis of purified CIIIB1 by Western blotting after two affinity chromatographies (2nd AFF.). FT, flow-through of the second affinity column. USF2a, whole-cell extract of COS-1 cells overexpressing USF2a. Proteins were electrophoresed on a 10% SDS-polyacrylamide gel and analyzed by Western blotting, as described under “Experimental Procedures.” Anti-USF1 and anti-USF2a were diluted 200- and 1,000-fold, respectively. The position of USF1 and USF2a polypeptides are indicated as a closed circle and open triangle, respectively. C and D, analysis of CIIIB1 by EMSA using element B (−92 to −33) of the human apoC-III promoter (CIIIB probe) (B) or the element AB (−68 to −33) of the human apoA-II promoter (AIIAB probe) (C) as probes, in the presence or absence of anti-USF1 or anti-USF2a, as indicated in the figure. CIIIB1 activity was obtained from rat liver nuclear extract (N.E.), from rat liver nuclear extract heated 5 min at 85 °C (N.E. 85°C), from the first affinity column fraction (1st AFF.), and from the second affinity column fraction (2nd AFF.). USF1 and USF2a contain whole-cell extract of COS-1 cells overexpressing USF1 or USF2a, respectively. Oligo CIIIB and oligo AIIAB, corresponding, respectively, to CIIIB and AIIAB probes, are competitors added in 100-fold molar excess. The asterisk indicates USF1/2a heterodimer, the open triangle indicates the USF2a/2a homodimer, and the closed circle indicates the USF1/1 homodimer. EMSA were carried out as described under “Experimental Procedures.”
Comparison of the amino sequence of the peptides obtained from the 97-kDa purified protein with PSF and NonO (38, 39)

| Peptide 1: GIVEFASK | PSF: 434 | NonO: -GIVEFSEK- |
|---------------------|---------|------------------|
| Peptide 2: YGEPGEVINK | PSF: 320 | NonO: -YGKAGEVINK- |
| Peptide 3: LESSLAEIK | PSF: 339 | NonO: -LEGELAEIK- |
| Peptide 4: TYTQRCRLVGNPLAPIDETEFK | PSF: 292 | NonO: -TETQRCRLVGNPLAPIDETEFK |
| Peptide 5: ANLSSLRRFGEK | PSF: 280 | NonO: -ANLSSLRRFGEK- |

EXPERIMENTAL PROCEDURES

Synthetic Oligonucleotides—The following oligonucleotides were used for plasmid construction in this work. KJ Kpn Hind c contains the sequence of the domains K and J from position 769 to 772 (9) with two restriction sites, Kpn and HindIII. KJ Kpn Hind nc is the complementary strand. L Sal Kpn, contains the domain L from position 807 to 769 (9) with SalI and KpnI restriction sites. L Sal Kpnnc represents the complementary strand. For gel-shift assays, the used double-stranded oligonucleotides of element AB (−65 to −32) and mutated element AB have been previously described (12) (see Fig. 2A). Oligonucleotides J (−738 to −712), K (−762 to −741), K/J (−764 to −712) and L (−805 to −771) represent the J, K, K/J, and L regions of the apoA-II promoter (9). Oligonucleotide CIIIB represents the element B of the human apoC-III promoter from −92 to −67 (14). Oligonucleotides NFY, MLP, and HNF-4 contain the binding site for NFY proteins (27), a consensus binding site for HNF-4, and a binding site for NFY proteins (25), respectively (27). Partial Amino Acid Sequencing—After the third affinity chromatography, the various CAT gene reporter plasmids were cotransfected with the plasmid RSV-βgal in HepG2 cells and COS-1 cells using the calcium phosphate-DNA coprecipitation method (32). β-Galactosidase activity was determined to normalize the variability in transfection efficiency (33). CAT assays were performed in a liquid phase, as described previously (34). The initial velocity of the enzymatic reaction was estimated by measuring the amount of labeled acetylchlamrophenil, which diffused directly into the liquid phase in the scintillation counting vial.

Cell Transfection and CAT Assay—The various CAT gene reporter plasmids were cotransfected with the plasmid RSV-βgal in HepG2 cells and COS-1 cells using the calcium phosphate-DNA coprecipitation method (32). β-Galactosidase activity was determined to normalize the variability in transfection efficiency (33). CAT assays were performed in a liquid phase, as described previously (34). The initial velocity of the enzymatic reaction was estimated by measuring the amount of labeled acetylchlamrophenil, which diffused directly into the liquid phase in the scintillation counting vial.

RESULTS

CIIIB1 Is Immunologically Related to USF—To further characterize CIIIB1, which is essential for apoA-II promoter activity, the protein was purified to near homogeneity from rat liver nuclear extracts by heat treatment at 85 °C for 5 min followed by three cycles of DNA sequence-specific affinity chromatography with the element B of the human apoCIII promoter (Fig. 1). The presence of CIIIB1 in purified fractions was assessed by EMSA analysis using the CIIIB1 probe (Fig. 1C, lanes 1–4). SDS-PAGE analysis of the affinity-purified CIIIB1 revealed two predominant proteins, with molecular masses of 45 and 97 kDa, respectively (Fig. 1A). The 97-kDa protein was not a dimer of the 45-kDa protein, since affinity samples were loaded on SDS-PAGE under reducing conditions. Digestion with lysyl endopeptidase of the 97-kDa protein generated five peptides, which were microsequenced. The sequences obtained matched perfectly those of the human polypyrimidine tract binding protein-associated splicing factor (PSF) (Table 1). Peptide sequences also shared significant homology with two Pou domain-containing, octamer-binding proteins (NonO) (30, 39, 40).

The 45-kDa polypeptide might correspond to the 41-kDa protein observed by Ogami et al. (15). We investigated whether this protein corresponds to USF because the CIIIB1 binding site, GTTCAGCTG, contains an E-box motif, CANNTG. This motif bends heat-stable bZIP/ZIP proteins such as USF, which is expressed abundantly in the liver. An immunoblot...
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**Fig. 2.** USF binding to element AB; analysis of specific DNA binding pattern. A shows the sequence of the wild type and mutated oligonucleotides of the element AB that were used as probes or competitors in B and C. The CIIIB1 binding site is represented by an opened square. The mutant sequences are underlined. B, binding of USF1 and USF2a overexpressed in COS-1 cells to AIIAB probe. C, competition assays of the binding of USF2a to AIIAB probe using mutated oligonucleotides as competitors. ABM1 and ABM2 are specific mutations in the E-box motif. Competitors were added in 10- and 100-fold molar excess. D, DNA binding assays of USF1 and USF2a overexpressed in COS-1 cells to the wild type AIIAB probe or mutated ABM1 and ABM6 probes. EMSA and supershift assays were performed as described under “Experimental Procedures” and in Fig. 1.

analysis was performed with specific antibodies raised against the transactivation domain of USF1 (M domain) or USF2a (G domain) (25) (Fig. 1B). Anti-USF1 or anti-USF2a (Fig. 1B, left and right panels) cross-reacted with a 43-44-kDa polypeptide in the purified affinity fraction (Fig. 1B, 2nd AFF.). When both antibodies were used (Fig. 1B, middle panel), two polypeptides were observed in the affinity-purified fraction (Fig. 2B, 2nd AFF.). The upper polypeptide comigrates with the 44-kDa isoform USF2a in the USF2a control (Fig. 2B, USF2a), and the lower polypeptide corresponds to the 43-kDa isoform USF1. To further confirm the identity of CIIIB1, EMSA and supershift assays were performed with anti-USF1 and anti-USF2a (Fig. 1, C and D). The CIIIB1 complex is competed out by a 100-fold excess of unlabeled oligonucleotides CIIB1 and AIIAB (Fig. 1C, lanes 5 and 6, and Fig. 1D, lanes 15 and 16). When both antibodies were added to the reaction, the CIIB1 complex was totally supershifted (Fig. 1C, lane 9, and Fig. 1D, lane 19). A residual complex remained after addition of each antibody. The mobility of the remaining complex corresponds to the homodimer USF1/1 (Fig. 1C, lanes 8 and 10, and Fig. 1D, lanes 18 and 20) or to the homodimer USF2a/2a (Fig. 1C, lanes 7 and 12, and Fig. 1D, lanes 17 and 22). These findings provide strong evidence that CIIB1 is mostly the heterodimer USF1/2a.

**USF Binds to Element AB and Transactivates the Proximal Apoa-II Promoter**—USF1 and USF2a overexpressed in COS-1 cells formed specific complexes with the AIIAB probe (Fig. 2B).
Oligonucleotides containing specific mutations in the element AB were used as probes that either avoid or do not affect the binding of CIIIB1 (12) (Fig. 2A). Fig. 2C shows that binding of USF2a to element AB was not competed out by oligonucleotides ABM1 and ABM2 containing mutations in the E-box motif, which abolish the binding of CIIIB1 (12). In contrast, mutations ABM3 to ABM6, which do not affect CIIIB1 binding (12), competed out USF2a binding. Furthermore, as expected, USF1 and USF2a bound to oligonucleotide ABM6 but not to oligonucleotide ABM1 (Fig. 2D).

To evaluate the potential role of USF in the transactivation of the human apoA-II promoter, cotransfection experiments were performed in HepG2 cells using USF1 or USF2a expression vectors and a vector expressing the CAT gene under the control of the wild-type proximal apoA-II promoter (AB plasmid) shown in Fig. 3A, as well as AB plasmid derivatives containing the mutated forms ABM1 and ABM6 (Fig. 3B). This analysis showed that USF2a transactivated 4.5-fold and 13-fold the proximal AB promoter with 50 ng and 500 ng of plasmid, respectively (Fig. 3B). Similar results were obtained by cotransfection with USF1 (data not shown). Cotransfection with a truncated mutant USF2a protein, TDU2, that lacks the NH2-terminal activation domain but contains normal DNA binding and dimerization domains had no effect on the minimal apoA-II promoter activity. Mutation within the E-box motif (ABM1 plasmid) diminished the USF2a-mediated transactivation, whereas mutation outside the E-box motif (ABM6 plasmid) resulted in transactivation by USF2a similar to that obtained.
with the wild type construct (Fig. 3B). The combined data of Figs. 2 and 3 demonstrate that USF binds to element AB and transactivates the proximal apoA-II promoter.

Synergistic Interaction of USF Bound to Proximal and Distal Elements Containing E-box Motifs—Similarly to element AB, elements K and L bind CIIIB1 (10). The addition of anti-USF1 or -USF2a antibodies supershifted the CIIIB1 complex formed with the AIJK probe and crude nuclear extracts obtained from HepG2 cells or affinity-purified CIIIB1 fractions (Fig. 4, N.E. and 2nd AFF.). The AIIL probe formed with HepG2 nuclear extract multiple DNA-protein complexes related to NFY and C/EBP family, giving a smeary pattern (Fig. 4B, lanes 7–9). After competition with an oligonucleotide containing the binding site of NFY, CIIIB1 became the predominant activity (Fig. 4B, lane 10) and was specifically supershifted by anti-USF1 and -USF2a (Fig. 4B, lanes 11 and 12). Fig. 4B (lanes 13–16) shows that affinity-purified CIIIB1 binds to the AIIL probe and is supershifted by both antibodies. In addition, EMSA with USF1 and USF2a, overexpressed in COS-1 cells, gave direct evidence of the binding of USF1 or USF2a to K and L elements (Fig. 4C).

To evaluate the respective contribution of distal E-box motifs within elements K and L in the apoA-II promoter activity, vectors placing the distal elements L or K/J upstream of the proximal AB promoter were generated (Fig. 3A). The addition of elements L or K/J to the proximal AB promoter did not modify the transcriptional activity of the proximal apoA-II promoter and the level of transactivation by USF2a (Fig. 3C). However, the addition of both distal elements L and K/J (Fig. 3A) increased 8-fold the activity of the proximal promoter and resulted in an 8.5-fold transactivation by USF2a. Moreover, mutation within the proximal E-box motif diminished the activity of the promoter (L-K/J-ABM1 promoter) as well as its transactivation by USF2a. Given that CIIIB1 mostly consists of a heterodimer USF1/2a (Fig. 1), we analyzed the role of the different isoforms of USF in the transactivation of the apoA-II promoter. Cotransfection of HepG2 cells with USF1 and USF2a expression vectors led to a similar transactivation of L-K/J-AB promoter as cotransfection with USF2a (Fig. 3C). These findings suggest that distal elements L and K/J and proximal element AB are all required for the transactivation of the apoA-II promoter by USF homo- or heterodimers. The role of USF in the transactivation of the human apoA-II promoter was further evaluated using a dominant negative form of USF2a, TDU2, that lacks the transactivation domain. This analysis showed that the activity of the L-K/J-AB promoter is decreased by 40 and 67% by 50 and 500 ng of TDU2 expression vector, respectively, as compared with 50 and 500 ng of the control expression vector, pCMV (Fig. 3D, left panel). The right panel of the Fig. 3D shows a similar 20, 60, and 53% inhibition of the E-AB promoter activity by 50 ng, 500 ng, and 1 μg of TDU2,
Supershift assays were performed as described in Fig. 1. 100-fold molar excess of oligonucleotide AIIL \textsuperscript{(a)} performed cotransfection experiments of HNF-4, C/EBP \textsuperscript{a} and HNF-4, we observed a synergistic 36-fold transactivation of the apoA-II promoter activity (Fig. 5). This transactivation was specific because deletion of the activation domain of USF2a (in the mutant TDU2) abolished the USF2a-mediated transactivation, and mutation of the proximal E-box motif (in the E-ABM1 reporter) was not specific to HNF-4 and USF2a increased, whereas the band corresponding to USF2a binding decreased. When the USF2a/HNF-4 ratio increased (Fig. 6A), the slower migrating band corresponding to the simultaneous binding of USF2a and HNF-4 decreased. When the USF2a/HNF-4 ratio decreased (lanes 7–9), the slower migrating band corresponding to the simultaneous binding of HNF-4 and USF2a increased, whereas the band corresponding to USF2a binding decreased. These results demonstrate a cooperative binding of HNF-4 and USF2a on the element K/J of the apoA-II promoter.

**DISCUSSION**

The distal region (−911/−614) of the human apoA-II promoter displays an enhancer-type activity (10, 11, 13), which is synergized by the proximal regulatory element AB (12). An important regulatory role is exerted by CIIIB1, a heat-stable transcription factor with a molecular mass of 41 kDa that binds to the elements AB, K, and L (10, 15). Nucleotide substitutions, which prevent the binding of CIIIB1 to element AB, abolished the communication between the proximal region and the enhancer (12). CIIIB1 was purified to homogeneity. However, its relationship with existing factors was not established (15). In the present study, purification of CIIIB1 led to the isolation of two thermolabile proteins with relative molecular masses of 45 and 97 kDa, respectively. The relationship between USF and CIIIB1 was investigated because the CIIIB1 binding site, GTCACCTG, contains
an E-box motif CANNTG, a binding site recognized by bHLH/ZIP proteins. USF family members are thermostable (41) and are expressed predominantly in liver (22). Three major USF isoforms, USF1, 2a, and 2b, have been described, which differ in the NH2-terminal transactivation domain and can form homo- and heterodimers. The USF1/2a heterodimer is predominant in liver and the hepatic cell lines HepG2 and ATF3 (25).

Using specific antibodies against USF1 and USF2a and AB, K, and L probes, we showed by Western blotting and EMSA that the affinity-purified 45-kDa protein was immunologically related to USF1 and USF2a and that the heterodimer USF1/2a is the predominant form. The optimal binding sequence of USF to E-box motifs has been defined as RYCACTG. E-box motifs within elements AB, K, and L mainly contain variations in the central portion of the consensus sequence. This may account for the difference in their relative binding affinities for CIIIB1 reported by Cardot et al. (10).

Sequencing of proteolytic peptides of the 97-kDa protein, co-purified with CIIIB1, shared identity with PSF, another heat-stable protein involved in early spliceosome formation (38). PSF shares significant sequence identity with NonO, a protein that binds to DNA via a helix/turn/helix domain and to RNA via ribonuclear protein-binding motifs (RNP) (39). The sequence homology between these two proteins lies within the adjacent RNP and the helix/turn/helix regions. It is interesting to note that NonO is able to enhance the binding of E47, a bHLH protein, to a noncanonical E-box motif (40). This E-box motif is identical to that of the element AB of the apoA-II promoter. Thus, it is possible that PSF could likewise increase binding of USF to element AB via a mechanism similar to that by which NonO enhances E47 binding. Similarly, the heat-stable protein PC5 has been described as a cofactor that stimulates transcriptional activity of USF (43).

We demonstrated the functional relevance of USF in the control of apoA-II promoter activity by the analysis of the transactivation properties of USF on various apoA-II promoter constructs. The minimal promoter containing the proximal E-box motif (AB) and schematic promoters containing various combinations of distal and proximal E-box motifs were specifically transactivated by USF2a. A negative dominant mutant of USF2a, TDU2, that lacks the transactivation domain but contains the DNA binding domain and the dimerization domain, inhibits apoA-II promoter activity. Mutation in the E-box motif within the element AB, preventing communication between proximal and distal E-box motifs (12), strongly reduces transactivation of the mutant L-K/J-AB promoter by USF2a. It is possible that USF molecules bound to two or more independent sites form multimeric complexes and, thus, promote DNA looping (44). Such a mechanism might be involved in apoA-II promoter activity. Our study shows that CIIIB1 is principally a heterodimer USF1/2a. USF1/2a was shown to transactivate the proximal apoA-II promoter (AB) and the L-K/J-AB promoter with the same efficiency as USF2a.

The ubiquitously expressed USF has been found to regulate a wide variety of genes involved in different specialized functions, such as tissue specificity, development, and metabolic regulation (26). Association of USF with other transcriptional factors has been reported (45). Specifically, C/EBPα promotes USF binding and activation of the C/EBPα promoter (46). Liver-enriched transcriptional factors, C/EBPα and HNF-4, bind
to the enhancer of the apoA-II promoter. We investigated potential synergistic interactions between USF, HNF-4, and C/EBPa. Cotransfection experiments have established synergistic activation of the apoA-II promoter by a combination of USF and HNF-4 but neither by a combination of C/EBPa and HNF-4 nor by a combination of USF and C/EBPa. The K/J region of the apoA-II promoter contains E-box motifs followed by an HNF-4 binding site. We demonstrated by DNA binding experiments a cooperative binding of USF2a and HNF-4 on the element K/J. This cooperative binding might account for the observed synergism between USF and HNF-4 in the transactivation of apoA-II promoter. This is the first time that such cooperative binding between HNF-4 and USF has been reported. It would appear that the optimal transcription of apoA-II gene is determined by the combined action of ubiquitously expressed factor, USF, and a liver-enriched transcription factor, HNF-4.

The glucose response element or carbohydrate response element of the L-type pyruvate kinase (L-PK) promoter contains two E-box motifs adjacent to an HNF-4 binding site located between nucleotides −168 and −144 (47, 48). These cis-elements are responsible for mediating the transcriptional regulation of L-PK by carbohydrate metabolism and glucagon/cAMP (49). USF was shown to bind the glucose response element of the L-PK promoter. However, the role of USF in mediating glucose response is still under discussion (31, 48, 50, 51). Recently, HNF-4 has been shown to be phosphorylated by PKA and is involved in cAMP transcriptional effects on L-PK gene (52). Using transgenic mice expressing the CAT reporter

FIG. 6. Cooperative binding of USF2a and HNF-4 on the element K/J of the apoA-II promoter. A, binding of USF2a and HNF-4 overexpressed in COS-1 cells on the (−762 to −741) AIIK probe (left panel), (−738 to −712) AIID probe (middle panel) and the (−764 to −712) AIIK/J probe (right panel). Oligos AIIAB, AIIK, and AIIK/J correspond to AIIAB, AIIK, and AIIK/J probes, respectively. Oligos MLP and HNF-4 correspond to oligonucleotide sequences containing the binding site of USF and HNF-4, respectively. Competitors are added in 100-fold molar excess. B, binding of USF2a and HNF-4 on the probe K/J. EMSA and supershift assays were performed as described in the Fig. 1.
gene under the control of the (−911/+29) human apoA-II promoter, we demonstrated that the apoA-II gene is transcriptionally regulated at the weaning period (8), during which the high fat diet (milk) shifts to a high carbohydrate (chow) diet, and the insulin/gluca
gen is currently being investigated.

role in the constitutive expression of the liver-restricted extracellular cholesterol levels.
play a role in the regulation of apoA-II promoter (54), suggesting that these elements may also
be involved in recruiting USF or SREBP. It has recently been shown that SREBP-1 and -2
binding to a proximal E-box motif is required for insulin-regulated transcription pathways are probably involved in recruiting USF or SREBP. It has recently been shown that SREBP-1 and -2
and SREBP bind on the fatty acid synthase promoter, independently of their respective binding sites, suggesting that distinct signaling pathways may also play a role in the regulation of apoA-II gene in response to extracellular cholesterol levels.

In conclusion, ubiquitously expressed USF plays a crucial role in the constitutive expression of the liver-restricted apoA-II gene. The binding of USF to proximal and distal E-box motifs of the apoA-II promoter and the synergistic interaction with the liver-enriched factor HNF-4 may be responsible for this synergy. A potential role of USF in the metabolic regulation of the apoA-II gene is currently being investigated.

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REFERENCES

1. Eisenberg, S. (1984) J. Lipid Res. 25, 1017–1058
2. Weng, W. and Breazile, J. L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14788–14794
3. Puchois, P., Kandoussi, A, Fievet, P., Fourrier, J. L., Bertrand, M., Koren, E., and Fruchard, J. C. (1997) Atherosclerosis 68, 35–40
4. Huang, Y. D., von Eckardstein, A., Wu, S., and Assmann, G. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 1412–1418
5. Hussain, M. M., and Zannis, V. I. (1990) Biochemistry 29, 209–217
6. Ikekawa, K., Zech, L. A., Kindt, M., Brewer, H. B. Jr., and Rader, D. J. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 306–312
7. Ikekawa, K., Zech, L., Brewer, H. B. Jr., and Rader, D. J. (1996) J. Lipid Res. 37, 399–407
8. A. Le Beyec, J., Benetollo, C., Chaffeuton, V., Domiar, A., Lefranc, M.-J., Chambaz, J., Cardot, P., and Kandoussi, A. (1997) Atherosclerosis 68, 35–40
9. Ogami, K., Hadzopoulou-Cladaras, M., Cladaras, C., and Zannis, V. I. (1990) J. Biol. Chem. 265, 9640–9646
10. Sawadogo, M., and Rodier, R. G. (1985) Cell 43, 165–175
11. Cartew, R. W., Chodosh, L. A., and Sharp, P. A. (1987) Genes Dev. 1, 973–980
12. Blackwood, E. M., and Eisenman, R. N. (1991) Science 251, 1211–1217
13. Beckman, H., and Kadesch, T. (1991) Genes Dev. 5, 1057–1066
14. Carr, C. S., and Sharp, P. A. (1990) Mol. Cell. Biol. 10, 4384–4388
15. Kim, J. H., Spotts, G. O., Halvorson, Y. D., Shi, H. M., Ellenberger, T., Twel, H. C., and Spiegelman, B. M. (1995) Mol. Cell. Biol. 15, 2585–2588
16. Shi, H., Liu, Z., and Towle, H. C. (1995) J. Biol. Chem. 270, 21991–21997
17. Sawadogo, M. (1988) J. Biol. Chem. 263, 11994–12001
18. Kan, H., Liu, Q., Marity, T., and Sawadogo, M. (1994) Nucleic Acids Res. 22, 427–433
19. Viollet, B., Lefranc-Martin, A.-M., Henrion, A., Khan, A., Raymondjean, M., and Martinez, A. (1990) J. Biol. Chem. 271, 1405–1415
20. Henrion, A. A., Khan, A., and Raymondjean, M. (1995) Genomics 25, 36–43
21. Raymondjean, M., Cereghini, S., and Yaniv, M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5889–5893
22. Cardot, P., Pastier, D., Lacorte, J.-M., Mengagne, M., Zannis, V. I., and Chambaz, J. (1994) Biochemistry 33, 12139–12148
23. Kadunaga, J. T., and Tjian, R. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5889–5893
24. Laemmli, U. (1970) Nature 227, 680–685
25. Lefranc-Martin, A.-M., Martinez, A., Antoine, B., Raymondjean, M., and Khan, A. (1995) J. Biol. Chem. 270, 2640–2643
26. Ogami, K., and van der EB, A. J. (1973) Virology 52, 456–467
27. Edlund, T., Walker, M. D., Barr, P. J., and Rutter, W. J. (1985) Science 230, 912–916
28. Desnoes, C., Massé, T., and Majad, J.-J. (1992) Trends Genet. 8, 300–301
29. Ladis, J. A., Hadzopoulou-Cladaras, M., Kardassis, D., Cardot, P., Cheng, J., Zannis, V., and Cladaras, C. (1992) J. Biol. Chem. 267, 15849–15860
30. Fried, M., and Crothers, D. M. (1981) Nucleic Acids Res. 9, 6505–6525
31. Sladek, F. M., Zhong, W., Lai, E., and Darnell, J. E., Jr. (1990) Genes Dev. 4, 2353–2365
32. Patton, J. G., Porro, E. B., Galceran, J., Tempt, P., and Nadal-Ginard, B. (1993) Trends Genet. 9, 393–406
33. Yang, Y. S., Hanke, J. H., Carchon, C., Kahn, A., and Tucker, P. W. (1993) Mol. Cell. Biol. 13, 5593–5603
34. Yang, Y. S., Yang, M.-S. W., Tucker, P. W., and Capra, J. D. (1997) Nucleic Acids Res. 25, 2284–2292
35. Sawadogo, M., Van Dyke, M. W., Gregor, P. D., and Rodier, R. G. (1988) J. Biol. Chem. 263, 11985–11993
36. Bendall, A. J., and Molloy, P. L. (1994) Nucleic Acids Res. 22, 2801–2810
37. Hale, J. P., Stelzer, G., Gappell, A., and Meistererns, M. (1995) J. Biol. Chem. 270, 21307–21311
38. Ferre-D’Amare, A. R., Pogonozec, P., Roeder, R. G., and Burley, S. K. (1994) EMBO J. 13, 180–189
39. Sieweczke, M. H., Tolke, H., Jaro, U., and Grat, T. (1998) EMBO J. 17, 1728–1739
40. Timchenko, N., Wilson, D. R., Taylor, L. R., Abdelsayed, S., Wilde, M., Sawadogo, M., and Darlington, G. J. (1995) Mol. Cell. Biol. 15, 1199–1202
41. Diaz-Guerra, M. J., M., Berget, M.-O., Martinez, A., Cuf, M.-H, Kahn, A., and Raymondjean, M. (1993) Mol. Cell. Biol. 13, 7725–7733
42. Liu, E., and Towle, H. C. (1995) Biochem. J. 308, 105–111
43. Bergot, M.-O., Diaz-Guerra, M.-J., Mozeti, N., Raymondjean, M., and Khan, A. (1992) Nucleic Acids Res. 20, 1871–1878
44. Kaytor, E. N., Shi, H., and Towle, H. C. (1997) J. Biol. Chem. 272, 7525–7531
45. Vallet, S. V., Heng, A. A., Lucchini, D., Casado, M., Raymondjean, M., Khan, A., and Vallont, S. (1997) J. Biol. Chem. 272, 21944–21949
46. Viollet, B., Khan, A., and Raymondjean, M. (1997) Mol. Cell. Biol. 17, 4288–4219
47. Wang, D., and Sul, H. S. (1997) J. Biol. Chem. 272, 26367–26374
48. Kan, H.-Y., Pissios, P., Chambaz, J., and Zannis, V. I. (1997) Supplement to Circulation, 96, 2708 (abstr.)