Human apoC-III (−890/+24) promoter activity is strongly activated by hepatic nuclear factor (HNF)-4 through its binding to the proximal (−87/−72) element B. This site overlaps the binding site for an activity that we identified as the ubiquitously expressed upstream stimulatory factor (USF) (Ribeiro, A., Pastier, D., Kardassis, D., Chambaz, J., and Cardot, P. (1999) J. Biol. Chem. 274, 1216–1225). In the present study, we characterized the relationship between USF and HNF-4 in the activation of human apoC-III transcription. Although USF and HNF-4 binding to element B is mutually exclusive, co-transfection experiments in HepG2 cells surprisingly showed a combined effect of USF and HNF-4 in the transactivation of the (−890/+24) apoC-III promoter. This effect only requires the proximal region (−99/+24) of the apoC-III promoter and depends neither on USF binding to its cognate site in element B nor on a USF-dependent facilitation of HNF-4 binding to its site. By contrast, we found by electrophoretic mobility shift assay and footprinting analysis two USF low affinity binding sites, located within the proximal promoter at positions −58/−31 (element II) and −19/−4 (element I), which are homologous to initiator-like element sequence. Co-transfection experiments in HepG2 cells show that a mutation in element II reduces 2-fold the USF transactivation effect on the proximal promoter of apoC-III and that a mutation in element I inhibits the combined effect of USF and HNF-4. In conclusion, these initiator-like elements are directly involved in the transactivation of the apoC-III promoter by USF and are necessary to the combined effect between USF and HNF-4 for the apoC-III transcription.

The apolipoprotein (apo)1 genes are expressed at different levels in the liver and intestine, according to the apolipoproteins. For example, in humans, apoA-I is equally expressed in the liver and intestine, apoA-IV is expressed mainly in the intestine, and apoC-III is expressed predominantly in the liver (1). By contrast, apoA-II is almost exclusively expressed in the liver. Numerous studies have focused on regulatory mechanisms that control liver-specific expression. Liver-specific gene expression relies on four families of transcription factors that are liver-enriched but not restricted to this tissue: hepatic nuclear factor (HNF)-1, HNF-3, and HNF-4 and CAAT/enhancer-binding protein (C/EBP) (for review, see Ref. 2). As with most eukaryotic genes, liver-specific transcription depends on both liver-enriched and ubiquitous factors, in a particular combination specific to each gene.

Transcriptional regulation of apolipoprotein genes involves the liver-enriched factors C/EBP, HNF-1, HNF-3, and HNF-4 as well as ubiquitous factors such as NF-1, NFY, SP1, and GA binding protein/Ets (for review, see Ref. 3). Proximal promoters of apoA-I, apoC-III, and apoA-IV genes exhibit, as a common feature, an hormone-responsive element that binds transcriptional factors of the nuclear receptor family, and they are transactivated by HNF-4, a member of this family (for review, see Ref. 3). HNF-4 used to be assigned to the orphan receptor family until fatty acyl-CoA thioesters were identified as its ligands (4), a finding of considerable interest in view of the critical role that HNF-4 plays in the expression of genes involved in major metabolic pathways. Furthermore, mutations in HNF-4 gene have been directly associated with maturity onset diabetes of the young (5). HNF-4 is primarily expressed in the liver, gut, kidney, and pancreas (6) and also plays an essential role in embryonic development (7, 8). Adult human and rat liver and kidney contain two main isoforms of HNF-4, generated by differential splicing (9). The longest isoform, HNF-4a2, is the predominant species in liver and kidney, whereas the shortest isoform, HNF-4a1, represents only a minor species (10, 11).

ApoC-III transcription involves both HNF-4 and ubiquitous factors. For instance, multiple SP1-binding sites on the distal apoC-III enhancer allow communication with HNF-4 bound to the proximal promoter of apoC-III (12). The binding site of HNF-4 in element B overlaps the binding site of an activity designated CIIIB1 (13), and it has been hypothesized that CIIIB1 could modulate the HNF-4 effect on apoC-III promoter (14). We recently demonstrated that the CIIIB1 activity corresponds to the ubiquitously expressed upstream stimulatory factor (USF), which plays a major role in apoA-II transcription (15). The CIIIB1 binding site GTCAACCTG contains the CANNTG consensus E-box motif (16, 17). USF belongs to the basic helix-loop-helix/leucine zipper transcription factor family, which contains a basic DNA-binding domain and two contiguous dimerization domains: a helix-loop-helix and a leucine zipper. This group includes a wide variety of transcription factors, such as c-Myc, Max (18), TFE3 (19), TFEB (20), and SREBP1 (21). USF appears to be the predominant basic helix-loop-helix/leucine zipper factor in liver nuclear extracts (22). Three USF isoforms, 1, 2a, and 2b, with apparent molecular
mases of 43, 44, and 38 kDa, respectively, have been described (23–25). USF1 and USF2α and 2b are encoded by two different genes, with USF2α and 2b being generated by differential splicing.

The involvement of two identical factors, HNF-4 and USF, in the transcription of the two liver-expressed genes apoA-II and apoC-III led us to question the relationships between USF and HNF-4 in different promoter contexts. We have previously reported that USF and HNF-4 synergistically transactivate the apoA-II promoter and cooperatively bind to their distal and adjacent cognate sites (15). However, HNF-4 per se is not able to transactivate the apoA-II promoter, in contrast with apoC-III promoter.

In the present study, we found a combined effect of USF and HNF-4 in the transactivation of the human (−890/+24) apoC-III promoter but through a different mechanism than that of the apoA-II promoter. The mutation of the USF binding site in element B does not inhibit this effect, and there is no cooperative binding of USF and HNF-4 to this element. We found that USF binds on two other sites in the proximal promoter that are homologous to an "initiator-like" sequence. Mutagenesis of these elements showed that they account for the combined effect between USF and HNF-4 in the apoC-III transcription.

**EXPERIMENTAL PROCEDURES**

**Synthetic Oligonucleotides**—The following oligonucleotides were used as plasmid construction in this work: 2c, 5'-AGCTTCTCCTACGT-GTACAGATGCTGACCCAGGCGCCCTGTCCTCTCAGTTCATCCCTAGAG-TGGGCAAAGGTCGATATCTGACCAGTGGAGA-3', 3c, 5'-AGCTTCTCCTACGT-GTACAGATGCTGACCCAGGCGCCCTGTCCTCTCAGTTCATCCCTAGAG-TGGGCAAAGGTCGATATCTGACCAGTGGAGA-3', 4c, 5'-ATATAAAACAGGTCAGATATCGACCTTTGCCCAGCGCCCTGGGTCCTCTCAGATAT-

**Cell Transfection and CAT Assay**—The various CAT gene reporter constructs were co-transfected with the plasmid Rous sarcoma virus (-galactosidase in HepG2 cells and COS-1 cells using the calcium phosphate co-precipitation method. Whole cell extracts were prepared as previously described (14) to be used in gel retardation assays.

**Electrophoretic Mobility Shift Assay (EMSA)**—The following double-stranded oligonucleotides were used as gel shift assay as probes of competitors. Oligonucleotides CH1B, CH1B3, and CH1B5 represent the element B of the human apoC-III promoter from −92 to −67, wild type or with mutation M1 or M5, respectively (25). Oligonucleotides from −68 to −31, from −44 to −13, and from −26 to +5 of the apoC-III promoter were used as probes. Annealing and labeling of synthetic oligonucleotides were performed as previously described (13). EMSA was performed according to the protocol of Fried and Crotthers (31) as described by Lacorte et al. (32). Anti-HNF-4 antibody was obtained from Santa Cruz Biotechnology, Inc. and was used according to the manufacturer's instructions. Specific antibody raised against the transactivation domain of USF2α (G domain) was prepared in the INSERM U129 laboratory and was kindly provided by Michel Raymondjean (25, 30). For super-shift assays, anti-USF2α antibody was diluted 10-fold, and 1 μg was added to the reaction mix. Anti-HNF-4 was used according to the manufacturer's instructions.

**RESULTS**

**Mutually Exclusive Binding of USF and HNF-4 on Element B of the apoC-III Promoter**—It has been reported that HNF-4 and the CH1B1 activity that we recently identified as USF (15) had overlapping binding sites on element B of the apoC-III promoter (Fig. 1A, lower panel). An EMSA with the CH1B1 probe (Fig. 1A, upper panel) shows that no slower complex is obtained with USF2α and HNF-4 translated separately (lane 3) or co-translated (lane 4) than with only USF2α (lane 2) or with only HNF-4 (lane 1). Therefore the binding of USF2α and HNF-4 to the CH1B1 probe is mutually exclusive.

**Combined Effect of USF and HNF-4 in the Human apoC-III Promoter Transactivation**—To study the combined effect of USF and HNF-4, co-transfection experiments in HepG2 cells were performed with the CAT reporter gene under the control of the −890/+24 human apoC-III promoter and vectors expressing USF2α and HNF-4 isoforms. This analysis shows (Fig. 1B) that USF2α is able to transactivate 2-fold the −890/+24 apoC-III promoter as compared with the 6-fold increase induced by HNF-4. More strikingly, however, when they were added simultaneously, USF2α and HNF-4 produced a 11-fold increase in apoC-III promoter activity. The specificity of this effect was confirmed by co-transfection experiments with a truncated mutant USF2α protein, TDU2, which lacks the N-terminal activation domain but retains the normal dimerization and binding domains. TDU2 did not display either the transactivation effect of the normal protein or its combined effect with HNF-4 (Fig. 1B).

**The Binding Site of HNF-4 in Element B, but Not That of USF, Is Required for the Combined Effect of USF and HNF-4**—We generated mutations in element B that specifically inhibit the binding of USF or HNF-4, as previously described (13, 15, 26). EMSA analysis of the binding of USF2α or HNF-4 overexpressed in COS-1 cells to wild-type or mutant element B confirms that mutations BM1 or BM5 specifically impair the binding of USF2α or HNF-4, respectively (Fig. 1, lower panel). As expected, the mutation in the HNF-4 binding site (BM5) results in a weaker activation by HNF-4 of the −890/+24 apoC-III BM5 promoter (2-fold) as compared with the wild type (6-fold) (Fig. 1, C versus B). This remaining activation might be because of the binding of HNF-4 to its distal binding site on the apoC-III enhancer (33, 34). By contrast, USF2α exerts a stron-
ger effect on BM5 promoter activity (5-fold; Fig. 1C) than on that of the wild type form (2-fold; Fig. 1B). Nevertheless, co-transfection experiment of HNF-4 and USF2a only resulted in an additive effect on the apoC-III BM5 promoter activity (Fig. 1C).

Conversely, the effects of USF2a and HNF-4 were not affected by the mutation of the USF binding site, designated BM1 (Fig. 1D). USF2a still transactivates 2-fold the −890/+24 apoC-III BM1 promoter. Furthermore, a combined effect of USF2a and HNF-4 is still observed with the mutated BM1 promoter, with transactivation reaching a 14-fold activation versus 2- and 7-fold activation with the individual factors, respectively. TDU2, the truncated USF2a protein, showed no effect either alone or associated with HNF-4 (Fig. 1D). Because the combined effect of USF2a and HNF-4 on the −890/+24 apoC-III promoter was not abolished by the mutation of the USF binding site in element B, it could be hypothesized that USF2a binds to a more distant element of the promoter. Systematic analysis of USF2a binding by competitive EMSA showed that USF2a does not bind to any site in the distal region of the apoC-III promoter (data not shown).

The −99/+24 Proximal Promoter of apoC-III Gene Is Sufficient for a Combined Transactivation by USF2a and HNF-4—Co-transfection experiments of USF2a and HNF-4 expression

![FIG. 1. USF2a and HNF-4 transactivate the human apoC-III promoter, whereas USF2a and HNF-4 binding on element B is mutually exclusive. A, analysis of the binding of USF2a and HNF-4 to the CIIB probe. Whole cell extract of COS-1 cells overexpressing HNF-4 (lane 1), USF2a (lane 2), HNF-4 and USF2a added simultaneously (lane 3), and USF2a and HNF-4 co-transfected (lane 4) were used. The lower panel represents the binding sites of USF2a (box) and HNF-4 (dashed box) on element B of the human apoC-III promoter. EMSA were carried out as described under "Experimental Procedures." B, effect of HNF-4 and USF2a on (−890/+24) apoC-III promoter activity in HepG2 cells. TDU2 is a truncated mutant USF2a protein (lacking the transactivation domain). 500 ng of HNF-4, USF2a, or TDU2 expression vectors were used, and the total amount of DNA was equalized to 1 μg with appropriate amounts of control DNA (pKS plasmid containing no cDNA). The inset represents the binding of USF2a and HNF-4 overexpressed in COS-1 cells to the CIIB probe. The EMSA was performed as described for A. C and D, transfection experiments in HepG2 cells with the (−890/+24) BM5 apoC-III promoter (C) and with the (−890/+24) BM1 apoC-III promoter (D). Mutations are in bold type. The insets represent the binding of USF2a and HNF-4 overexpressed in COS-1 cells to the CIIBM5 (C) and to the CIIBM1 (D) probes. EMSA was performed as described for A. Transient transfections and CAT assays were performed as described under "Experimental Procedures." For each experiment, CAT activity was calculated by linear regression as the slope of the reaction velocity and was expressed in cpm/min. CAT activities are expressed as fold activation of the activity of the reporter in the presence of control DNA, arbitrarily fixed at 1. The experiments were performed in triplicate and repeated three to five times. The results represent the means ± S.E. Statistical significance was determined by Student’s t test. α, p ≤ 0.05 relative to the reporter in presence of HNF-4.]
vectors were then performed in HepG2 cells with the CAT reporter gene under the control of the proximal apoC-III promoter. As expected, because of the lack of the apoC-III enhancer, the HNF-4 transactivation effect on the apoC-III promoter is weaker than on the promoter (4-fold versus 6-fold; Fig. 2A). Fig. 2A also shows a 10-fold transactivation of the proximal apoC-III promoter by USF2a and a combined effect of HNF-4 and USF2a, which are not reproduced by its mutant form, TDU2 (Fig. 2A). The level of expression of USF2a or HNF-4 is similar in cells transfected with the expression vector of USF2a or with the expression vector of HNF-4 and in cells transfected with both vectors. Taken together, our results strongly suggest that USF2a and HNF-4 transactivate the human apoC-III promoter by a mechanism that only requires the −99/+24 proximal region of the promoter and that this potentialized effect does not depend on the USF2a binding to its cognate site in element B.

USF2a Does Not Increase HNF-4 Binding to Element B of the apoC-III Promoter—The combined effect of USF2a and HNF-4 could be explained by an indirect effect of USF on the binding of HNF-4 to element B. It has previously been shown that C/EBPα stimulates the binding of USF to the promoter of the C/EBPα gene and therefore activates its own promoter (35). To test this hypothesis, EMSA with increasing amounts of USF2a and a constant amount of HNF-4 or with increasing amounts of HNF-4 and a constant amount of USF2a were performed with the CHIB probe (Fig. 2B). In any case, the addition of a constant amount (Fig. 2B, lanes 4–6) or an increasing amount...
USF Binds to Two Weak Affinity Sites on the Proximal Region between −63 and −4—To localize the proximal site(s) of USF, we designed three double-stranded oligonucleotides spanning the regions −68/−31, −44/−13, and −26/+5 of the apoC-III promoter. USF2a directly binds to the probes −68/−31 and −26/+5 (Fig. 3A, lanes 2 and 4) but not to the probe −44/−13 (Fig. 3A, lane 3). The binding of USF2a to these probes is weaker than that to the CIIIB probe (compare the band intensity of lane 1 versus lanes 2 and 4 in Fig. 3A). The precise location of USF binding sites in the −99/+24 apoC-III promoter fragment was further determined by footprinting analysis in the presence of bacterially expressed USF1, which has an identical DNA-binding domain and exhibits the same binding properties as USF2a (15, 24, 25) (Fig. 3B). In addition to the previously described (13) USF binding site in element B located at position −85 to −79 and flanked by a hypersensitive site at position −86, Fig. 3B reveals two other footprints at positions −58 to −31 (element II) and −19 to −4 (element I).

These USF binding sites could account for the combined effect observed between HNF-4 and USF2a. These regions do not comprise E-box motifs. However, it has been reported that in addition to the E-box motif, USF recognizes the initiator element (Inr), a sequence rich in pyrimidine nucleotides, near the transcription start site (36). Analysis of the apoC-III regions −58/−31 and −19/−4 reveals pyrimidine-enriched sequences that present homology with Inr of AdML, HIV, and terminal transferase promoters (Table I).

Effect of Mutations in Elements I and II in the Transactivation of the Proximal apoC-III Promoter—To determine whether elements I and II were involved in the activation of the apoC-III promoter, we mutated the elements I or/and II in the proximal (−99/+24) apoC-III promoter (Fig. 4). The mutation BM1 was also inserted to avoid any interference from the USF2a binding to element B with that to elements I and II. Co-transfection experiments with USF2a and HNF-4 expression vectors were performed in HepG2 cells with the CAT reporter gene under the control of the proximal (−99/+24) apoC-III promoter comprising the different mutations described in Fig. 4. Fig. 4A shows that USF2a by itself transactivates 5-fold the −99/+24 C-IIIBM1 promoter, as does HNF-4 for a total of 10-fold additive transactivation. However, the combination of both factors, HNF-4 and USF2a, results in a 15-fold activation of this reporter, which is more than additive.

Introduction in the −99/+24 C-IIIBM1 reporter of the mutation Im (Table II), which avoids USF2a binding to element II, results in a 2-fold reduction in the transactivation capacity of USF2a but without altering its capacity to potentize the effect of HNF-4 (Fig. 4, B versus A). The element I has also been mutated to a “classic Inr,” which supports TFII-I binding but not USF2a (mutation Im, described in Table II) (36, 37). This mutation does not alter the capacity of USF2a to transactivate the −99/+24 C-IIIBM1Im reporter but results in a 2-fold reduction in the transactivation capacity of HNF-4, and the combined effect between HNF-4 and USF2a led to a transactivation that is not significantly different from the transactivation by USF2a alone (Fig. 4C). Finally, when elements I and II are
mutated, the combined effect of USF2a and HNF-4 led similarly to a nonsignificant transactivation of the hapC-III reporter activity (Fig. 4D).

**DISCUSSION**

Eukaryotic gene expression involves a combination of transcription factors interacting with specific binding sites clustered within an enhancer and the proximal promoter of a given gene. Such a mechanism combines tissue-specific and ubiquitously expressed transcription factors. Altogether these elements, with chromatin structure, yield a great diversity of

**TABLE I**

*Comparison of the sequences of Inr and the (−68/−31) and (−15/+5) C-III promoter regions*

Pyrimidines are indicated in bold type. +1 refers to the position of the A residue in the consensus sequence (40). Sequence comparison of Inr among the HIV, AdML, and terminal transferase (TdT) promoters were described by Du et al. (36). Py, pyrimidine.

| Inr | Tdt (−5/+9) | C | C | T | C | A | T | T | C | G | G | A | G |
|-----|-------------|---|---|---|---|---|---|---|---|---|---|---|---|
| HIV | (−5/+9)     | T | A | C | T | T | G | G | G | A | G | T | T |
| HIV | (+28/40)    | C | T | C | C | A | C | C | C | T | C | C | C |
| AdML (−5/+9) | T | C | C | T | C | A | C | C | C | C | C | C | C |
| AdML (−43/+56) | T | G | A | G | T | A | C | T | C | C | C | T | T |
| C-III (−59/−47) | C | T | C | T | C | A | G | G | T | C | C | T | G |
| C-III (−43/−31) | C | A | C | T | G | G | G | A | G | T | A | T | T |
| C-III (−15/−2) | C | C | C | T | G | C | C | T | G | C | T | G | T |
| C-III (−9/−5) | C | T | G | C | C | T | G | T | C | G | C | T | C |

**TABLE II**

*Mutations in elements B, I, and II of the apo C-III promoter*

Sequences of element B, element II, and element I and their associated mutations are described. Mutated nucleotides are indicated in bold type.

| Element B | −90TCAGCAGTGACCTTTGCC−72 |
| Element BM1 | −90TCAGATATCGACCCCTTGGC−72 |
| Element II | −58CCCTAGTCTGCTGCCTGCTGGAGATGAT−31 |
| Element IIm | −58CCCTCAGATATCCGACCCCTGGAGATGAT−31 |
| Element I | −110CAGAACCCTCCTGCTC−4 |
| Element IIm | −110CAGAATCCTACTCTCTCTC−4 |
specific patterns of gene expression, although the actual mechanism controlling this specificity is still elusive. In the liver, this regulation is even more complex because none of the liver-specific factors are restricted to hepatocytes, and a unique combination of regulators defines a particular phenotype. It might be expected that liver-specific genes involved in the same metabolic pathway could be expressed under the same combinatorial control. The apolipoprotein gene family represents an accurate model to test such a hypothesis.

Our results show that liver-specific expression of both apoA-II (15) and apoC-III genes relies on a combination of the liver-enriched HNF-4 factor and USF. USF has been found to regulate a wide variety of genes involved in different specialized functions, such as tissue specificity, development, and metabolic regulation (38). In the case of apoA-II promoter, USF and HNF-4 bind cooperatively to the distal enhancer and communicate synergistically with USF bound to the proximal promoter (15). This is not the case for apoC-III, where the USF cognate site overlaps that of HNF-4 within the proximal promoter of apoC-III. This overlapping binding could suggest that USF may negatively modulate HNF-4 activation of apoC-III transcription. Surprisingly, despite a mutually exclusive binding of USF and HNF-4 on element B, we observed a combined effect of these transcription factors in the transactivation of the human apoC-III promoter. This combined effect is not because of a distal USF binding site nor to a facilitated binding of HNF-4 in the presence of USF. This mechanism differs from that we previously elucidated for apoA-II gene transcription (15), and this reinforces the idea that a unique arrangement of regulatory elements and factors bound to promoter allows the formation of a unique DNA-protein complex that finally promotes the transcription of a particular gene.

The second finding of our study is that USF binds two new sites in the proximal promoter, different from element B. By EMSA and footprinting analysis in vitro, we localized two regions, element II and element I, which bind USF, at positions (−58/−31) and (−19/−4) on both sides of the TATA box, respectively. Sequence analysis of the −71/+24 apoC-III region reveals that this region does not comprise an E-box motif, is pyrimidine-enriched, and presents homology with the Inr sequences of AdML, HIV, and terminal transferase promoters (Table I). It has been reported that in addition to the E-box motif, USF recognizes Inr (36). Inr was first described as a promoter element that allows direct transcription from a TATA-less promoter. Various studies have demonstrated that Inr can also be present in promoters that contain a TATA box and can act independently or in concert with the TATA box to enhance the transcription. Generally, Inr is located approximately between −5 and +5 around the initiation transcription start site (+1), but pyrimidine-rich Inr-like elements have also been found distal to the initiation site (37, 39). A weak consensus sequence has been defined by Javahevy et al. (40): YYA⁻¹NWYY. The pyrimidine content surrounding the ANT has to be assessed with particular attention. In general, the greater the number of pyrimidines at this location, the greater the activity of the Inr. Furthermore, in the absence of optimal sequences surrounding the −1 to +3 position, the CANT sequence is insufficient for Inr activity.

The functional analysis of element I and element II by direct mutagenesis shows that the potentialized transactivation of the apoC-III promoter by HNF-4 and USF is dependent on the element I (Fig. 4). This effect may be because of the stabilization of the basal transcriptional machinery or to a better accessibility of the preinitiation complex to the promoter. USF presents a weak binding to both Inr-like sites of the apoC-III promoter. Such a low affinity has been described for the binding of USF to AdML Inr, which is markedly enhanced by the general transcription factor TFII-I (41). TFII-I binds to Inr of the AdML promoter and may serve as a co-regulator that can integrate regulatory responses of USF to the basal machinery (41). USF may play a similar role in the transactivation of the apoC-III promoter by HNF-4. A direct interaction between these two factors has never been reported, and co-immunoprecipitation assays reveal the absence of physical interactions between USF and HNF-4 (data not shown). An alternative hypothesis could involve a co-activator that bridges transcription factors to the basal transcriptional machinery and allows recruitment and/or stabilization of the preinitiation complex. A recent study suggests the existence of a specialized co-activator that is not, in contrast to USF itself, ubiquitously expressed (42). The existence of specific co-activators might explain the different functions of distinct basic helix-loop-helix/leucine zipper proteins through common E-box in different promoters. It may be hypothesized that a specific co-activator, common to HNF-4 and USF, allows an enhancement of transcription by the TATA-box through the binding of HNF-4 to the hormone-responsive element of element B and USF to the Inr-like element I. Indeed, HNF-4 interacts with co-regulators such as CBP/p300 (43) and SRC-1/GRIP1 (44, 45). Furthermore, the interaction of CBP and HNF-4 enhances apoC-III transcription (46). In the context of the TGFβ2 promoter, USF1 does not interact with p300 (47). However, it has been shown that p300 mediates the transcriptional activation of the F1F0 ATP synthase promoter by USF2 through an Inr element (48). In addition, CBP/p300 and the p300-associated factor display intrinsic acetyltransferase activities. The acetylation of histones is thought to be involved in destabilization of nucleosomes, a crucial event for the access of transcription factors to their DNA templates (49).

In conclusion, the transcription of two liver-expressed genes involved in the same metabolic pathway, the apolipoprotein A-II and C-III genes, requires the same combination of transcription factors: the liver-enriched HNF-4 and the ubiquitously expressed USF. Furthermore, this combination is dependent on the regions responsible for the in vivo liver expression. However, USF and HNF-4 regulate their transcription through different mechanisms: a cooperative binding of USF and HNF-4 to the distal apoA-II promoter, which is involved in the in vivo liver-restricted expression of this gene (15, 32, 50), and a combined effect of USF and HNF-4 dependent on Inr-like elements in the proximal apoC-III promoter, which is sufficient for the liver specificity of the apoC-III expression in vivo (51). These results underline the fact that the understanding of the actual role of transcription factors in regulating gene transcription requires the elucidation of their mechanisms of action.

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