A CYP7A promoter binding factor site and Alu repeat in the distal promoter region are implicated in regulation of human CETP gene expression

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Abstract The cholesteryl ester transfer protein (CETP) plays a key role in reverse cholesterol transport in mediating the transfer of cholesteryl ester from HDL to atherogenic apolipoprotein B-containing lipoproteins (VLDL, IDL, and LDL). Variation in plasma CETP mass in both normolipidemic and dyslipidemic individuals may reflect differences in CETP gene expression. As the 5′ flanking sequence up to 3.4 kb of the human CETP gene contributes to transcriptional activity and tissue-specific gene expression, we evaluated the role of the distal promoter region in the modulation of CETP gene expression. In transfection experiments in HepG2 cells, we presently demonstrate that an Alu repeat (−2,153/−2,414) acts as a repressive element, whereas a binding site for the orphan nuclear receptor CYP7A promoter binding factor (CPF), at position −1,042, facilitates activation of human CETP promoter activity. Cotransfection of liver receptor homolog, the mouse homologue of CPF in HEK293 cells that lack CPF, indicated that the −1,042 CPF site is sufficient to induce CPF-mediated activation of CETP promoter activity. Taken together, our results indicate that the distal-promoter region is a major component in the modulation of human CETP promoter activity, and that it may contribute to the liver-specific expression of the CETP gene.—Le Goff, W., M. Guerin, M. J. Chapman, and J. Thillet. A CYP7A promoter binding factor site and Alu repeat in the distal promoter region are implicated in regulation of human CETP gene expression. J. Lipid Res. 2003. 44: 902–910.

Supplementary key words cholesteryl esters • cholesteryl ester transfer protein • liver receptor homolog-1

In mediating the intravascular transfer of cholesteryl esters (CEs) from atheroprotective HDL to atherogenic apolipoprotein B-containing lipoproteins (VLDL, VLDL remnants, IDL, and LDL), the CE transfer protein (CETP) plays a key role in reverse cholesterol transport (1). The relevance of CETP to atherosclerosis risk remains controversial. Indeed, human CETP deficiency arising from mutations in the CETP gene is frequently associated with high HDL-C levels (2, 3), but potentially with elevated cardiovascular risk (4, 5). By contrast, transgenic mice overexpressing CETP tend to display reduction in HDL-C levels (6, 7) and to develop premature atherosclerosis (8).

In humans, CETP is expressed in a wide variety of tissues, among which the liver and adipose tissue are major sources (9, 10). Significant variation in both plasma CETP mass and activity has been demonstrated, however, in both normolipidemic individuals and in dyslipidemic subjects (11), possibly reflecting differences in CETP gene expression.

The human CETP gene contains about 25 kb and is composed of 16 exons (9, 12). The promoter region displays several regulatory elements implicated in control of its transcriptional activity (13–19). Among the trans-acting factors that contribute to regulation of human CETP promoter activity, nuclear receptors appear to play a key role. Indeed, induction of CETP gene transcription by dietary cholesterol (20, 21) was recently shown to require the liver X receptor (LXR) and involves its binding to a proximal direct repeat of a nuclear receptor site separated by 4 nucleotides (DR4 element, −384 to −399) (18). In addition, the liver receptor homolog-1 (LRH-1) was found to transactivate the human CETP promoter and potentiate sterol-mediated induction by LXR (16). LRH-1 is the mouse homolog of the human CYP7A promoter binding factor (CPF), and these two nuclear factors belong to the orphan nuclear receptor fushi tarazu Fi family (Ftz-F1) from Drosophila.

The proximal region of the human CETP promoter, up to position −629, has been extensively analyzed. Several studies indicate, however, that the distal promoter region

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equally contributes to the transcriptional activity of the human CETP gene (16, 19, 22, 23). Indeed, Oliveira et al. (23) reported that the human CETP promoter region upstream of position −570 contains positive elements determining the level of gene expression in the liver and spleen of transgenic mice expressing the human CETP gene. In addition, the expression of LXR-RXR in HEK293 cells led to a more robust sterol induction with the 3.4 kb promoter than that seen with the −570 bp construct (16), accompanied by a further increase of this induction by LRH. These data strongly suggest the presence of sterol-responsive element(s) upstream of position −570. Finally, whereas the proximal −138 bp construct in vitro induced maximal CETP promoter activity, it has been clearly demonstrated that extension of the promoter up to −4.7 kb results in marked repression of promoter activity (19), thereby indicating that repressive regulatory elements are present in the distal promoter region.

We presently evaluated the contribution of the distal region of the human CETP gene promoter to transcriptional activity and searched for potential regulatory elements. Two regulating regions were identified that acted either as an activator (−1,012/−1,398) or a repressor (−2,146/−2,680) of CETP promoter activity. This latter region is composed of numerous repeat elements, i.e., SINE and LINE (Short and Long interspersed sequence). Here we identified a full-length Ahu repeat sequence (−2,153/−2,414) as the repressive element and a functional −1,042 CPF site in the enhancer region that may be involved in liver-specific expression of the CETP gene.

**EXPERIMENTAL PROCEDURES**

**Plasmid constructs**

A plasmid containing a 3,488 bp fragment [nt −3,459/+29, relative to the transcription start site (27)] of the human CETP promoter was generously provided by Bayer Pharma (Germany). A 3,101 bp SacI-XbaI fragment (nt −3,238/−137) of the human CETP promoter was cut out from this plasmid and cloned directionally into the previously described (24) SacI-XbaI digested −971GG construct, which contains a 1,077 bp fragment of the human CETP promoter, to generate the p3242 construct. Deletions of this 3.2 kb promoter segment were performed by digestion between the SacI restriction site at the 5′ end and the PmlI, Apal, BstGI, ThrI11I, and EcoRIII restriction sites at the 3′ end in order to generate constructs that contain a 1,012, 1,398, 1,707, 2,146, and 2,680 bp fragment of the human CETP promoter (p1012, p1398, p1707, p2146, and p2680, respectively) after blunt-ending and ligation.

Constructs p138-Alu and p138-Rep were obtained as follows: 282 bp and 544 bp fragments were amplified by PCR from the p3242 construct and subcloned into a pGEM®-T vector (Promega, Charbonnieres, France). The sequence of the downstream primer was 5′-ACAGAGGTAGCTGCTGCG-3′; the sequences of the upstream primers were 5′-GACATAGGTACCCCATGCTG-3′ and 5′-CCGAGAGGTCTACATCGTG-3′, with the underlined letters indicating the restriction site. These primers contain sequences for the generation of a NheI restriction site in the downstream primer and KpnI and SacI restriction sites in the upstream primers, respectively. Subsequently, NheI-KpnI 282 bp and NheI-SalI 544 bp fragments were cut out from the pGEM®-T vectors and directionally cloned into the earlier described p138 constructs that contain a 138 bp fragment of the human CETP promoter (15), previously digested by NheI-KpnI and NheI-XbaI, to generate the p138-Alu and p138-Rep constructs.

For mutational analysis, one or two point mutations were introduced either at the −1,042 site or at the −74 LRH site, or both, using the GeneEditor™ in vitro Site-Directed Mutagenesis System kit (Promega) according to the manufacturer’s protocol in order to generate p1398M1 (−1,042 mutated site), p1398M2 (−74 mutated site), and p1398M1M2 (both −1,042 and −74 mutated sites). Oligonucleotides used to create mutations in the −1,042 and −74 binding sites were 5′-TGCTCTGAGCCAAGAACAGT-3′ and 5′-GGCCAGGAGAtGCAGTCG-CCGG-3′ (16), respectively, with the lowercase letters indicating the mutation site.

The integrity of inserts and the presence of the mutations were verified by sequencing using the DNA Big Dye™ Terminator Cycle Sequencing kit (ABI Prism, Applera, France) and a Perkin Elmer 377 DNA sequencer.

The pCMX-mLRH1 expression vector was a generous gift from Dr. Kristina Schoonjans (UMR-CNRS 7034, Université Louis Pasteur de Strasbourg, Illkirch, France).

**Cell culture and transfection experiments**

The human hepatocellular carcinoma cell line HepG2 and the transformed human embryonic kidney cell line HEK293 (American Type Culture Collection, Rockville, MD) were grown at 37°C in 5% CO₂ in Dulbecco’s Modified Eagle’s Medium containing 10% and 8% fetal calf serum, respectively (Invitrogen, Cergy Pontoise, France), and 2 mM l-glutamine and 40 μg/ml gentamicin. Cells were seeded on 6-well plates at 3 × 10⁵ cells per well. After 48 h growth, 3 μg of each CETP promoter construct was cotransfected with 0.5 μg of a β-galactosidase expression vector (pSV-βgal; Promega) using the Lipofectin Liposomal reagent (Invitrogen) according to the manufacturer’s instructions. In cotransfection experiments, HEK293 cells were transfected with 1 μg of each CETP promoter construct, 30 ng of pCMV-Sport-βgal (Invitrogen), and 0.5 μg of pCMX-mLRH1 expression vector. Twenty-four hours after transfection, the medium was replaced by fresh medium and the cells were incubated for an additional period of 16 h. Cells were harvested with 150 μl of Cell Culture Lysis Reagent (Promega). The lysate was centrifuged for 10 min at 14,000 rpm in order to remove cellular fragments. Luciferase activity was measured on the supernatant using the Luciferase Assay System kit (Promega) in a 1420 VICTOR Multilabel counter (Wallac, EG and G Co.), and β-galactosidase activity was measured using the β-galactosidase Enzyme Assay System kit (Promega). Protein concentrations were determined using the bicinchoninic acid assay reagent (BCA:Pierce, Bezos, France). Transcriptional activity was expressed in relative luciferase units (RLUs) or as x-fold induction after normalization for β-galactosidase activity. Experiments were performed in triplicate (duplicate in cotransfection experiments), and values correspond to the mean from at least three independent experiments.

**Electrophoretic mobility shift assays**

HepG2 nuclear extracts were prepared from confluent 150 mm dishes as previously described by Dignam et al. (25), and stored at −80°C before use. The protein concentration of nuclear extracts was determined using the bicinchoninic acid assay reagent (BCA:Pierce). The translated LRH-1 protein was obtained using the in vitro TNT Quick-Coupled Transcription/Translation System (Promega). The electrophoretic mobility shift assay (EMSA) was performed as follows: 24 bp synthetic oligonucleotides (Invitrogen) were used to generate the probe. The probe was then incubated with nuclear extracts, and then electrophoresed on a native 5% polyacrylamide gel in 0.5× TBE buffer. The gel was exposed to a phosphor screen, and the images were quantified using ImageQuant™ (Amersham).
rrogen) (CPF, 5’-TGCTCTGACCTTGGAGAACAGT-3’ and
CPFmut, 5’-TGCTCTGACCCAAAGGAAACAGT-3’; the
underlined and the lowercase letters indicating the CPF site and the
mutation site, respectively) were annealed with their respective
complementary strand at 100°C for 3 min in a solution containing
100 mM Tris-HCl (pH 7.5), 100 mM MgCl₂, 13 mM EDTA, 13
mM spermidine, and 20 mM dithiothreitol (DTT). Double-
strand probes were radiolabeled with 20 μCi of [γ²P]ATP (5
mCi/ml, 3,000 Ci/mmol; NEN Life, Paris, France) by T4 polynu-
cleotide kinase (Promega) at 37°C for 30 min. Radiolabeled double-
strand probes (0.25 pmol) were incubated for 15 min on ice
in a final volume of 20 μl in the presence of 10 mM Tris-HCl (pH
7.5), 100 mM NaCl, 3 mM MgCl₂, 5 mM EDTA, 1 mM DTT, 5%
glycerol, 2 μg poly(dI-dC), 4 mM spermidine, 1 μg BSA, and 8
μg of nuclear extracts or 10 μl of in vitro translated LRH-1 lysate.
In experiments that required the presence of an excess of unla-
beled competitor (100-fold excess), the latter was added to the
mixture before the addition of radiolabeled probe. After incuba-
tion, samples were loaded onto a 6% acrylamide gel (acrylamide-
bis acrylamide, 29:1). Electrophoresis was performed at room
temperature at 200 V for 3 h, and the gels were transferred onto
3MM paper (Whatman, Ivry sur seine, France), dried, and ex-
posed to Hyperfilm MP (Amer sham Biosciences, Saclay, France)
at −20°C overnight.

RESULTS

The distal promoter region of the human CETP gene contains sequences that contribute to promoter activity

In order to determine whether the distal region of the human CETP promoter contributes to control of pro-
moter activity, a set of constructs containing fragments of 1 kb, 1.4 kb, 1.7 kb, 2.1 kb, 2.7, and 3.2 kb of the human
CETP promoter (p1012, p1398, p1707, p2146, p2680, and p3242, respectively) were made, and their transcriptional
activities were compared in transient transfection experi-
ments in HepG2 cells. As shown in Fig. 1, the p1398 con-
struct displayed a 2-fold higher level of luciferase expres-
sion (P < 0.05; Fig. 3) as compared with that of the
p1012 construct. In addition, the promoter activity of
both the p2680 and p3242 constructs was significantly
lower than that of the p2146 construct (−26% and −21%,
respectively, P < 0.05). There was no significant differ-
ence in luciferase activity, however, between the both the
p2146 and the p1707 constructs as compared with the
1,398 construct. Therefore, our results demonstrate that
the promoter region from −1,012 to −1,398 induces
marked activation of CETP promoter activity whereas that
from −2,146 to 2,680 is responsible for repression.

Analysis of the human CETP promoter sequence from
−1,012 to −3,242 using the Repeat Masker program,
which screens DNA sequence against a library of repetitive
elements, revealed that this promoter region contains
multiple sequences of type Sine and Line as illustrated in
Fig. 2. Thus, three L1 sequences (305 bp, −1,635/−1,941;
66 bp, −2,491/−2,424; 87 bp, −2,908/−2,996), two pre-
viously described Alu sequences (290 bp, −2,129/−2,420;
250 bp, −2,996/−3,242) (22), and one Mir sequence
(107 bp, −2,549/−2,657) were identified on the human
CETP promoter and accounted for 46% of the promoter
sequence from −1,012 to −3,242. Interestingly, the pres-
ently identified repressive region (−2,146/−2,680) is
composed of a full-length Alu sequence and a fragment of
both Mir and L1 sequences that represent almost the
totality of this region.

An Alu sequence is responsible for the repressive action of
the −2,146/−2,680 promoter region

The Alu repetitive sequence is the most abundant of the
human Sine and represents about 3–6% of the human ge-
one, being present in about 500,000 to 1 million copies.
Previous studies reported that interspersed sequences such as
Alu repeats may be involved in regulation of the
transcriptional activity of human genes (26–30). It was
thus of interest to study the potential implication of the
Alu sequence (−2,129/−2,420) in the repression exerted
by the −2,146/−2,689 promoter region. To this end, we
made two constructs containing either the complete repre-
sessor region (−2,153/−2,680) or the Alu repeat (−2,153/−2,414)
upstream to the proximal 138 bp (relative to the translation start site) of the human CETP
promoter (p138-Rep and p138-Alu, respectively). Indeed, an
earlier study demonstrated that this 138 bp fragment is
sufficient to promote maximal activity of the CETP pro-
moter (19). In transient transfections in HepG2 cells, the
presence of the Alu sequence (p138-Alu) led to significant
repression (−36%, P < 0.05; Fig. 3) of luciferase activity
as compared with the construct containing only the prox-
imal 138 bp promoter fragment (p138). Such repression is
similar to that observed with the full-length repressive
region (p138-Rep; −41%, P < 0.05; Fig. 3). This result con-
firmed that the region from −2,146 bp to −2,698 bp in-
duces repression of CETP promoter activity and indicated that
the Alu sequence −2,146/−2,414 accounts for this ef-
fect.

The 10 bp insertion in the −2,153/−2,414 bp Alu/Sx
repeat of the human CETP promoter facilitates specific
binding of nuclear factors

The −2,146/−2,414 Alu sequence belongs to the class
II early Alu subfamily (type Sx) (31), which represents
the majority of Alu repeats. The fact that this −2,146/
−2,414 Alu/Sx element represses the promoter activity of
the human CETP gene led us to suggest that this specific
Alu/Sx sequence facilitates the binding of specific tran-
scription factors that might be responsible for the repres-
sion of CETP promoter activity. As illustrated in Fig. 4,
the comparison of the −2,153/−2,414 bp Alu/Sx with
the human Alu/Sx consensus sequence (32) indicated that
the two sequences were not identical. Interestingly, a
10 bp insertion was observed in the hormone-responsive
element (HRE)-rich sequence generally present in such
Alu/Sx repeat elements (27). To determine whether such
nucleotide insertion permits the binding of nuclear fac-
tors, we carried out EMSAs (Fig. 5) using two radiola-
beled probes, Alu/Sx and Alu-CETP (−2,357/−2,381),
which differ only by the insertion of those 10 bp. In the
presence of nuclear extracts of HepG2 cells, we observed
that the AluSx probe, corresponding to the 50/64 bp region of the human Alu/Sx consensus sequence, did not lead to the formation of a DNA-complex (Fig. 5, lanes 1 to 4). By contrast, two specific DNA complexes (Fig. 5, Complex I and Complex II, lane 5) were observed with the Alu-CETP probe. The specificity of Complexes I and II was confirmed by the fact that an excess of nonradiolabeled probe for Alu-CETP (Fig. 5, lane 7), but not for AluSx (Fig. 5, lane 6) or a nonspecific competitor (Fig. 5, lane 8), abolished their formation. In conclusion, the 10 bp insertion in the 11002/2,153/11002/2,414 bp Alu/Sx repeat of the human CETP promoter permits the specific binding of nuclear factors that might be implicated in the specific repressive action of this element.

Identification of a CPF binding site in the −1,012/−1,398 activating promoter region

Analysis of the sequence of the −1,012/−1,398 enhancer promoter region allowed the identification of a motif corresponding to the consensus DNA recognition sequence for the CPF (33) between positions −1,042 and −1,050 bp (5'-GAGCCTTGG-3'). CPF is the human homolog of the orphan nuclear receptor Ftz-F1 from Drosophila and of LRH-1 from the mouse. To verify whether this region binds the transcription factor CPF, we performed EMSAs (Fig. 6) using a radiolabeled synthetic probe (CPF) spanning the sequence of the CETP gene promoter from −1,034 to −1,058 bp. Incubation of the radiolabeled CPF probe with in vitro translated LRH-1 protein resulted in the formation of a specific DNA protein complex (Fig. 6, Complex I, lane 2) that is not observed with the control lysate (Fig. 6, lane 1). The Complex I is also obtained with the radiolabeled CL1 probe (33) corresponding to the CPF binding site of the cholesterol 7α-hydroxylase promoter gene (Fig. 6, Complex I, lane 2) that is not observed with the control lysate (Fig. 6, lane 1). The Complex I is also obtained with the radiolabeled CL1 probe (33) corresponding to the CPF binding site of the cholesterol 7α-hydroxylase promoter gene (Fig. 6, Complex I, lane 2) that is not observed with the control lysate (Fig. 6, lane 1). The Complex I is also obtained with the radiolabeled CL1 probe (33) corresponding to the CPF binding site of the cholesterol 7α-hydroxylase promoter gene (Fig. 6, Complex I, lane 2) that is not observed with the control lysate (Fig. 6, lane 1).
of nonradiolabeled CPF probe (Fig. 6, lane 3) or CL1 probe (Fig. 6, lanes 5 and 10), but not with an excess of CPFmut (Fig. 6, lane 4). Taken together, our results indicated that the Complex I was formed as a result of the interaction with LRH-1. In conclusion, we demonstrate that the DNA sequence from $\text{H}_110021,042$ to $\text{H}_110021,050$ bp permits the binding of the transcription factor LRH-1, the mouse homolog of CPF.

![Fig. 3](image3.png) The $\text{H}_11002\text{-}2,153/-2,414$ Alu repeat represses human CETP promoter activity. HepG2 cells were transiently transfected with constructs containing the proximal 138 bp (relative to the transcription start site) of the human CETP promoter alone or downstream to either the $\text{H}_11002\text{-}2,153/-2,680$ or the $\text{H}_11002\text{-}2,153/-2,414$ distal promoter region (p138, p138-Rep, and p138-Alu, respectively). Luciferase activity is expressed in RLUs after standardization for $\beta$-galactosidase activity. Experiments were performed in triplicate and values correspond to the mean $\pm$ SD from three independent experiments. **$P < 0.005$ versus p138.

![Fig. 4](image4.png) Comparison between the nucleotide sequence of the Alu/Sx repeat of the human CETP promoter (GenBank™ accession number U71187) (23) and the human Alu/Sx consensus sequence (GenBank™ accession number U14574) (32) using the Clustal X Windows® interface (37). Filled boxes indicate the nucleotide differences between the two sequences. Frameworks correspond to potential hormone-responsive elements (HREs) that match the consensus hexamer half sites (A/G)(G/T)(C/G)(A/G). An insertion of 36 bp due to the previously described tetranucleotide repeat polymorphism (38) generates a shift in the position of the Alu/Sx repeat in the human CETP promoter sequence. Thus, the Alu/Sx repeat is located herein at positions $\text{H}_11002\text{-}2,153/-2,414$ bp, whereas the positions are $\text{H}_11002\text{-}2,117/-2,378$ bp in the sequence reported by Oliveira et al. (23).
The CPF site participates in the transcriptional activation mediated by the −1,012/−1,398 CETP promoter region

To determine whether the −1,042 CPF site is implicated in the transcriptional activation mediated by the 1,012/−1,398 promoter region, we performed transient transfection experiments in both HepG2 cells, a cell line which expresses CPF, and HEK293 cells that lack CPF (33). As shown in Fig. 7, the −1,398 construct displayed a significant 2-fold higher luciferase activity (P < 0.0005) than the p1012 construct in HepG2 cells, as reported in Fig. 1; by contrast, the difference in luciferase activity between these two constructs in HEK293 cells was only 26% (P < 0.005). In addition, mutation of the CPF site (p1398M1) significantly reduced promoter activity (−24%, P < 0.05) in HepG2 cells, as compared with the construct containing the intact CPF site (p1398), whereas it induced no effect in HEK293 cells. We conclude that the −1,042 site accounts for a significant proportion (up to 50%) of the activation mediated by the −1,012/−1,398 promoter region in HepG2 cells.

The transcription factor CPF activates CETP promoter activity at the −1,042 site

The orphan nuclear receptor LRH-1, the mouse homolog of CPF, is known to transactivate the human CETP promoter by binding to a proximal promoter element at position −75 (16). To test the implication of the distal −1,042 CPF site in the LRH-1-mediated activation of the human CETP promoter, transient cotransfection experiments were performed in the HEK293 cell line with a pCMX-mLRH1 expression vector and a set of constructs described in Experimental Procedures. Briefly, the p1398 and p1398M1 constructs, and two additional constructs (pM2 and pM1M2) in which the proximal CPF site at position −75 was mutated, were used.

As shown in Fig. 8, cotransfection of the p1398 construct, which contains the two intact CPF sites, with a pCMX-mLRH1 expression vector led to significant induction of luciferase expression (2-fold, P < 0.05) as compared with the p1398M1M2 construct mutated at both sites. In addition, whereas mutation at both CPF sites repressed the LRH-1-mediated activation of the human CETP promoter, mutation of either the proximal −75 or the −1,042 site alone (p1398M1 or p1398M2, respectively) did not affect this induction. These results indicated that the transcriptional factor CPF activates CETP promoter activity at the −1,042 site to the same extent as the −75 CPF site.

DISCUSSION

We report that the distal promoter region of the human CETP gene plays a key role in the regulation of promoter activity. Analysis of nucleotide sequences from −1,012 bp to −3,242 bp revealed that this promoter region is composed of multiple repeat elements, among which an Alu repeat sequence acts as a repressive regulatory element of CETP promoter activity. We equally describe a functional CPF site at position −1,042 bp, which may contribute to the hepatic expression of the human CETP gene.

We report that a full-length Alu repeat, located at positions −2,153/−2,414 bp in the distal promoter region,
acts as a repressor of human CETP promoter activity. A transcriptional repressive role of the Alu element has already been demonstrated in several studies (29, 30). Thus, the Wilms’ tumor gene WT1 contains a repressive regulatory element composed of a full-length Alu repeat in the third intron, located at 12 kb from the promoter, that represses WT1 gene expression (30). In addition, the Alu repetitive element found in the distal promoter region (1,007/1,330) of the human glycoprotein hormone subunit acts as a negative transcriptional regulatory element (29). In these studies, transcription factor(s) involved in such an Alu-associated repression were not identified. Vansant and Reynolds (27) reported that the consensus sequence of Alu repeats contains a functional retinoic acid response element that is implicated in the regulation of expression of the keratin K18 gene expression. Indeed, the K18-associated Alu is a member of the evolutionarily more recent subfamilies (classes III and IV) and has four HREs (AGGTCA) separated by 2 bp (DR2), consistent with the binding specificities of retinoic acid receptors (RARs). The repressive Alu repeat found in the CETP promoter belongs to the class II early Alu subfamily (type Sx) (31), which represents the majority of Alu repeats. As illustrated in Fig. 4, this latter contains four HREs, separated

Fig. 7. The −1,042 CPF site contributes to the transcriptional activation mediated by the −1,012/−1,398 CETP promoter region in a hepatocyte-specific manner. HepG2 (left panel) and HEK293 cells (right panel) were transiently transfected with constructs containing either 1,012 bp or 1,398 bp of the human CETP gene promoter (p1012 and p1398, respectively). Two point mutations were introduced in the p1398 construct in the CPF binding site located at position −1,042, thereby generating the p1398M1 construct. Luciferase activity is expressed in RLUs after standardization for β-galactosidase activity. Experiments were performed in triplicate and values correspond to the mean ± SD from at least three independent experiments. * P < 0.05, ** P < 0.005, *** P < 0.0005 versus p1398; † P < 0.05, †† P < 0.005 versus p1398M1.

Fig. 8. The −1,042 CPF site is sufficient to induce the CPF-mediated activation of CETP promoter activity. One microgram of each CETP promoter construct was transiently cotransfected with 0.5 µg of pCMX-mLRH1 expression vector in HEK293 cells. Results were expressed as fold induction relative to luciferase activities normalized for β-galactosidase activity obtained without pCMX-mLRH1 expression vector. The marginal increment of promoter activity of the p1398M1M2 construct (mutated in both the −75 and −1,042 CPF sites) in response to CPF was subtracted from the promoter activity of all the constructs. Experiments were performed in duplicate, and values correspond to the mean ± SEM from three independent experiments. * P < 0.05 versus p1398M1M2.
by 2 bp and 4 bp, thereby generating two potential DR2 and one DR4 binding sites. A recent study reported that the DR4 found in this class of Alu repeat exhibits selective binding to the heterodimer complex of nuclear receptors LXRα-RXR, which is implicated in LXR induction by LXR ligands (35); however, the implication of RARs and/or LXRs in the repressive effect of the −2,153/−2,414 bp Alu/Sx region appears unlikely, since the transcriptional activity of a 3.4 kb promoter fragment of the human CETP gene was stimulated by trans retinoic acids (36) and LXR nuclear receptors (18).

It is unlikely that all Alu repeats function as regulatory elements due to their abundance; however, it is very interesting to note that the −2,153/−2,414 bp Alu repeat significantly decreases transcription activity from the CETP promoter when located upstream of the 138 bp minimal promoter region, indicating that the repressive action of this element is not a function of its position in the CETP promoter. We observed that the −2,153/−2,414 bp Alu/Sx element displayed nucleotide differences with the Alu/Sx consensus sequence (Fig. 4). We speculate that these nucleotide differences might be responsible for the specific Alu-mediated repression of the CETP promoter. Among them, we report that a 10 bp insertion located in the HRE-rich region, which led to the creation of a potential DR2 element, permits the specific binding of nuclear factors. Nevertheless, further investigations are required to identify these factors and to determine whether they may account for the specific repressive action of this element.

We identified a binding site for the orphan nuclear receptor CPF at position −1,042 bp in the promoter region, which transactivates the human CETP promoter. However, this site accounts for only half of the activation mediated by the −1,012/−1,398 promoter region in HepG2 cells, suggesting that other(s) positive regulatory element(s) are present in this promoter region. The presence of a positive regulatory element in the distal CETP promoter is consistent with a previous study, which reported that the 5.7 kb promoter region is responsible for a significant transcripional activation in a conditionally transformed mouse hepatocyte line as compared with the proximal 137 bp promoter fragment (22). The orphan nuclear receptor CPF has already been described as being able to transactivate the CETP promoter by binding to a proximal promoter element at position −75 (16). We demonstrated that the new −1,042 CPF site is sufficient to induce the CPF-mediated activation of the human CETP promoter to the same extent as the proximal −75 CPF site. The liver-specific expression of this nuclear receptor has been reported to be a key regulator of human CYP7A1 gene expression in the liver (33). In agreement with the liver-specific expression of CPF, we observed that the −1,042 site participates in CETP promoter activity in HepG2 cells, whereas this site is not functional in nonhepatic HEK293 cells. Interestingly, Oliveira et al. (23) reported that the CETP promoter region between −570 bp and −3,400 bp confers predominant expression of the CETP gene in liver. The fact that the presently described CPF site is located in this promoter region led us to suggest that the binding of the orphan nuclear receptor CPF on the −1,042 site may contribute to the hepato-specific expression of the CETP gene.

In conclusion, our study provides evidence that the distal region of the human CETP promoter contains regulatory elements that contribute to the transcriptional activity of the CETP gene. Furthermore, it appears that, in the same manner as in the proximal region, nuclear receptors are intimately involved in the contribution of the distal promoter region to regulation of the transcriptional activity of the CETP gene, thereby confirming the crucial role of this family of transcription factors in the control of genes of lipid metabolism.

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