Transcriptional regulation of the human carboxyl ester lipase gene in THP-1 monocytes: an E-box required for activation binds upstream stimulatory factors 1 and 2

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The bile salt-stimulated carboxyl ester lipase (CEL) is important for the digestion and absorption of dietary lipids, and is expressed at high levels by the exocrine pancreas and the lactating mammary gland. However, the presence of CEL in human plasma suggests that the role of CEL in lipid metabolism may stretch beyond its function in the intestinal lumen, and possibly include interactions with cholesterol and oxidized lipoproteins to modulate the progression of atherosclerosis. We have used the CEL-expressing human monocytic cell line THP-1 to investigate the transcriptional regulation of the human CEL in monocytes. Analyses of the promoter region revealed that an E-box located at $-47/-52$ is necessary for CEL expression. Point mutations in the E-box

almost completely abolish the transcriptional activity. Electrophoretic mobility-shift assay analyses reveal that the E-box binds the upstream stimulatory factors 1 and 2, and the binding of an upstream stimulatory factor-containing complex in THP-1 cells also requires the presence of a putative nuclear receptor-binding site at $-60/-66$. Furthermore, we demonstrate that the E-box is also necessary for CEL expression in the pancreas and the mammary gland, although there are tissue-specific requirements for additional activating elements.

Key words: atherosclerosis, gene expression, nuclear receptors, promoter elements.

INTRODUCTION

The susceptibility to develop atherosclerosis and coronary heart disease involves an important genetic component, which is currently only understood to a limited extent. Elevated plasma total cholesterol, which is one of the most important risk factors for atherosclerosis, the low plasma high-density lipoprotein (HDL) cholesterol and the elevated plasma triglycerides are generally due to a complex interaction between genetic and environmental factors, which in most cases cannot be explained by a variation in a single gene [1]. The bile salt-stimulated carboxyl ester lipase (CEL) is an important enzyme in the digestion and absorption of dietary lipids and fat-soluble vitamins [2], and in particular, in the uptake of cholesterol esters [3]. The enzyme is expressed at high levels in the lactating mammary gland and in the exocrine pancreas. A high-fat/high-cholesterol diet induces increased pancreatic CEL mRNA levels in rats and rabbits [4,5], and cationized low-density lipoprotein (LDL) can induce a 2-fold increase in the CEL mRNA level in the pancreatic cell line AR4-2J [6]. A relationship between serum lipid levels and CEL genotype has been described by Aleman-Gomez et al. [7], suggesting that CEL may influence the lipid phenotype in humans. Furthermore, the human CEL gene maps to locus q34.3 on chromosome 9 [8], close to the ABO blood group antigen locus, which is linked to lipid phenotype and cardiovascular disease in humans [9].

In terms of tissue distribution, Shamir et al. [10] proposed a wider role for CEL in lipid metabolism when they showed that CEL is present in human plasma and that it has the ability to hydrolyse the core lipids of human LDL and HDL using bile salt concentrations that occur outside the intestinal lumen. It is possible that the plasma CEL corresponds to a fraction of

pancreatic CEL that has transcytosed through the intestinal epithelium [11], or it may be locally synthesized. To discover whether the plasma CEL could originate from local synthesis, macrophages and vessel-wall endothelial cells were investigated and found to express CEL [12,13]. Although the physiological function of CEL in these cell types is yet unclear, it has been suggested that CEL may interact with cholesterol and oxidized lipoproteins to modulate the progression of atherosclerosis [10,12,13]. To examine further this possible new role for CEL, we have used the human monocytic cell line THP-1 to investigate the transcriptional regulation of the CEL gene in human monocytes. We identify a region of the human CEL gene promoter containing two putative nuclear receptor-binding sites and an E-box. The E-box, which we found to be crucial for expression, can bind the upstream stimulatory factors USF1 and USF2.

Members of the orphan nuclear receptor family are implicated in numerous aspects of cholesterol homeostasis [14]. The accumulation of cholesterol by macrophage foam cells in the atherosclerotic lesion is believed to involve the uptake of modified, presumably oxidized, LDL (oxLDL), and this activates peroxisome proliferator-activated receptor γ (PPAR γ)-dependent transcription in the foam cells [15]. PPAR γ forms a DNA-binding dimer with the 9-*cis*-retinoic receptor, is expressed at high levels in atherosclerotic lesions, and has been found to regulate monocytic gene expression [16]. PPAR γ has also been found to influence the macrophage differentiation as well as cholesterol uptake and removal from foam cells [17,18]. The presence of putative binding sites for nuclear receptors in the human CEL promoter region around the E-box, and the involvement of PPAR γ and other nuclear receptors in the regulation of lipid metabolism, makes them possible candidates for CEL gene regulation. We report that the formation of a complex

Abbreviations used: CAT, chloramphenicol acetyltransferase; CEL, carboxyl ester lipase; CMV, cytomegalovirus; EMSA, electrophoretic mobility-shift assay; FBS, foetal bovine serum; HDL, high-density lipoprotein; LDL, low-density lipoprotein; oxLDL, oxidized LDL; PPAR, peroxisome proliferator-activated receptor; PPARE, PPAR response element; RXR, retinoid X receptor; USF, upstream stimulatory factor.

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containing USF requires both an E-box and a putative nuclear receptor-binding site.

EXPERIMENTAL

Construction of deletion and point mutations of the human CEL promoter in luciferase reporter plasmids

Reporter plasmids were constructed as described previously [19]. Point mutations were inserted using the Quick Change™ site-directed mutagenesis kit (Stratagene, La Jolla, CA, U.S.A.). The primers used for point-mutation generation were the oligonucleotides used in the electrophoretic mobility-shift assay (EMSA) (SK2mut1, SK2mut2 and SK2bmutEbox; see Table 1). The integrity and fidelity of all promoter-reporter constructs were verified by sequencing.

Cell-culture transfections and reporter-gene assays

THP-1 monocyte cells (kindly provided by Dr B. Ohlsson, Wallenberg Laboratory, Gothenburg, Sweden) were cultured in RPMI-1640 supplemented with 10 % foetal bovine serum (FBS), 0.5 % (v/v) penicillin-streptomycin, 2 mM L-glutamine, 2 mM sodium pyruvate, 0.05 mM β-mercaptoethanol and 1×non-essential amino acids (Life Technologies, Täby, Sweden). The mouse mammary epithelial cell line HC11 (kindly provided by Dr R. Ball, Friedrich Miescher-Institute, Basel, Switzerland) was grown in RPMI-1640 supplemented with 10 % FBS, 1 % (v/v) penicillin-streptomycin, 5 µg/ml insulin and 10 ng/ml epidermal growth factor. The rat pancreatoma cell line AR4-2J (obtained from A.T.C.C.) was grown in Dulbecco's modified Eagle's medium supplemented with 10 % FBS, 1 % (v/v) penicillin-streptomycin. All cells were maintained at 37 °C in a humidified 5 % CO₂ atmosphere.

THP-1 cells were transfected with 1 µg of promoter construct vector and 0.1 µg of cytomegalovirus (CMV)-Renilla control vector using 0.5 mg/ml DEAE-dextran (Promega, Madison, WI, U.S.A.). For every transfection, 1 × 10⁷ cells were used and the DNA was preincubated with DEAE-dextran for 30 min before the addition of cells. The transfection mixture was then incubated for 2.5 h and DMSO shock treatment given for 2 min before the addition of fresh medium. The cells were harvested after 72 h by freeze-thawing. HC11 cells were transfected with 7 µg of promoter construct vector and 0.5 µg of CMV-β-gal control vector and AR4-2J cells were transfected with 10 µg of promoter construct vector and 1 µg of CMV-chloramphenicol acetyltransferase (CAT) control vector using lipofectin (Invitrogen Life Technologies, Palo Alto, CA, U.S.A.) and harvested after 48 h by freeze-thawing. The luciferase activity was normalized to

Figure 1 Transcriptional activity of consecutive 5' deletions of the human CEL gene promoter in THP-1 cells

THP-1 cells were transfected with various 5'-deletion constructs as described in the Experimental section. (A) 5'-Deletion constructs of the human CEL gene promoter coupled with the luciferase reporter gene. (B) Transcriptional activity of 5'-deletion constructs. Bars represent the average of at least three independent transfections ± S.E.M.

Renilla luciferase, β-gal or CAT, and the luciferase activity was verified by repeated transfection assays using at least three independent preparations of the promoter construct. Firefly luciferase and Renilla luciferase were measured in a luminometer using the Promega kits. CAT activity was measured in an automatic scintillation counter (Beckman, Fullerton, CA, U.S.A.) and β-gal activity was measured using the Clontech (Palo Alto, CA, U.S.A.) method.

EMSA

Nuclear extracts were prepared from harvested cells as described previously [19]. Oligonucleotides for EMSA were end-labelled with [³²P]dCTP using Klenow fill-in and purified using Bio-Spin 30 Chromatography Columns (Bio-Rad, Hercules, CA, U.S.A.). An approximately 20000 c.p.m. probe and 4–8 µg of nuclear extract were routinely used in the binding reactions. Binding

Table 1 Oligonucleotides used in EMSA

Nucleotides shown in lower case have been mutated compared with the corresponding wild-type oligonucleotide.

Oligonucleotides used in EMSA	Name
— 81 AGGGAGTGCCTGAGAGCTCATGAACA — 54	SK1
— 76 AGTGCCTGAGAGCTCATGAACAAGCATGTGACCTTGGATCCAGCT — 31	SK2
— 70 TGAGAGCTCATGAACAAGCATGTGACCTTGG — 39	SK2b
— 54 AGCATGTGACCTTGGATCCAGCTCCA — 28	SK3
— 76 AGTGCCTGAGAGTaTATGAACAAGCATGTGACCTTGGATCCAGCT — 31	SK2mut1
— 76 AGTGCCTGAGAGCTCATGAACAAGCATGTaAtaTTGGATCCAGCT — 31	SK2mut2
— 70 TGAGAGCTCATGAACAAGhTGTGACCTTGG — 39	SK2bmutEbox
TCGAGACGTGACCTTTGCTCGGTC	AOxPPRE [20]
TCGAGACGTaAtaTTTaTtTGGTC	AOxPPREmut

Figure 2 Overview of the $-30/-80$ region of the human CEL gene promoter

Possible nuclear receptor half-sites and an E-box are enclosed by boxes and consensus sequences are indicated below. Arrows indicate that the two nuclear receptor half-sites appear in the opposite orientation.

Figure 3 EMSA analysis of the $-30/-80$ region of the human CEL gene promoter using THP-1 nuclear extracts

Nuclear extracts from THP-1 cells were prepared as described in the Experimental section. **(A)** Schematic overview of the oligonucleotides used in the EMSA. All oligonucleotides are listed in Table 1. SK2 contains both the putative nuclear receptor half-sites and the E-box, whereas the other oligonucleotides either lack one site or have a mutated site. The mutations in SK2mut2 alter both the E-box and the nuclear receptor half-site. **(B)** [γ - 32 P]dCTP-labelled SK2 gives rise to a shift indicated by an arrow, not obtained with any of the other oligonucleotides. **(C)** In the competition assay, the EMSA reactions containing labelled SK2 oligonucleotides were incubated with 200-fold molar excess of unlabelled oligonucleotides. The arrow indicates the position of the band corresponding to the band in Figure 3(B).

reactions were incubated at room temperature for 20 min in a 20 μ l reaction volume containing 20 mM Hepes, 50 mM KCl, 10 % (v/v) glycerol, 2 mM MgCl₂, 0.5 mM EDTA, 0.1 mg/ml BSA and 1.5 μ g of poly(dI/dC). For supershift assays, antibodies were added 15 min before the addition of the probe. In competition experiments, unlabelled probes were added in 200-fold molar excess. DNA–protein complexes were separated on a non-denaturing 6 % polyacrylamide gel (Tris/glycine) at 4 °C in 1 $\times$ Tris/glycine buffer, and the gel was dried and exposed to an X-ray film at –80 °C. Oligonucleotides used as EMSA probes are listed in Table 1. Anti-USF1 (sc-229x), anti-USF2 (sc-861x), anti-retinoid X receptor (RXR) α (sc-774x), anti-PPAR γ (sc-7273x), anti-ROR α (sc-6062x), anti-SF-1 (sc-10975x), anti-CPF (sc-5995x) and anti-sp1 (sc-59x) were all obtained from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

RESULTS

Transcriptional activity of the human CEL gene promoter in human THP-1 monocyte cells

Analyses of the transcriptional activity of transiently transfected consecutive 5'-deletion constructs of the human CEL promoter, coupled with the luciferase reporter gene, in the monocytic cell line THP-1 reveal a sharp increase in luciferase activity between the –53 construct and the –80 construct (Figure 1). This indicates the presence of positive regulatory elements in this region. No further increase in luciferase activity is observed by increasing the length of the 5' region beyond –138 (Figure 1). In contrast, a slow decline occurs, indicating that several negative elements are present throughout the promoter. Examination of the promoter region between –40 and –80 reveals an AGCTCA sequence, closely resembling a nuclear receptor half-site, at position –60/–66. The consensus sequence of a nuclear receptor half-site is AGGTCA, and another putative site, which matches the consensus perfectly, is located closer to the transcription start at –43/–48. Overlapping this proximal nuclear receptor-binding site is an E-box motif CANNTG (Figure 2). For simplicity, the –60/–66 putative nuclear receptor half-site is hereafter referred to as half-site 1 and the –43/–48 site as half-site 2.

USF-1 and USF-2 bind to the E-box motif at –47/–52

Mutation analysis of the promoter region between –40 and –80 by EMSA showed that an oligonucleotide (SK2), spanning from –31 to –76 of the promoter and containing both the putative nuclear receptor-binding sites and the E-box, gives rise to a slowly migrating band indicated by an arrow in Figures 3(B) and 3(C). This shift is not obtained with any of the oligonucleotides SK1, SK3, SK2mut1 or SK2mut2, which contain only the half-site 1 or half-site 2/E-box region, or are mutated in either of these sites (Figures 3A and 3B). A faint band obtained with SK3 may be explained by weak binding of the complex even in the absence of half-site 1, whereas the slightly lower, very faint band obtained with SK2mut1 may be a consequence of the mutation enabling binding of a different protein. The slowly migrating shift can be abolished by competition with excess of unlabelled SK2 oligonucleotide, but not by unlabelled SK1, SK3 or SK2mut2 (Figure 3C). Although the labelled SK2mut1 oligonucleotide does not give rise to the shift, excess of unlabelled SK2mut1 can nevertheless reduce the intensity of the shift obtained with SK2 to some extent (Figure 3C). This suggests that a mutation in half-site 1 weakens the binding, but does not altogether prevent the complex from binding. Hence, although both half-site 1, and the region containing half-site 2 and the overlapping E-box seem to be required for formation of the

Figure 4 EMSA (competition assay) with a consensus PPRE oligonucleotide

Nuclear extracts from THP-1 cells were prepared and EMSA reactions were set up as described in the Experimental section. (A) Overview of the oligonucleotides used in this EMSA (see Table 1). (B) EMSA showing [γ -³²P]dCTP-labelled SK2b and 200-fold molar excess of unlabelled SK2b, SK2mut2, AOxPPRE, AOxPPREmut and non-specific oligonucleotide. The arrow indicates the position of the band corresponding to the band in Figure 3(B).

observed complex, binding to the latter region appears to be stronger.

To test if the factors in the slowly migrating complex could also bind to a consensus PPAR response element (PPRE), we performed a competition assay using a consensus PPRE oligonucleotide (AOxPPRE; [20]) and the corresponding oligonucleotide mutated in both half-sites (AOxPPREmut; see Table 1). Unlabelled AOxPPRE oligonucleotide could not inhibit the shift entirely, but was more efficient than AOxPPREmut (Figure 4). One interpretation of this is that elements resembling the nuclear receptor-binding sites are involved only to some extent, but other sites are also needed in the formation of the complex. This raised the possibility that a nuclear receptor may be involved in the complex, but when antibodies directed towards different members of the nuclear receptor family (RXR α , PPAR γ , ROR α , SF-1, CPF-1) were included in the binding reactions, none of them gave rise to any supershifts (results not shown).

To distinguish between half-site 2 and the overlapping E-box, an E-box-specific mutation, which does not alter half-site 2, was introduced into the SK2b oligonucleotide (SK2bmutEbox; Table 1). The SK2b oligonucleotide is a slightly shorter version of SK2 (see Table 1) and it gives rise to essentially the same shifts except for some quickly migrating bands. A labelled SK2bmutEbox does not give rise to the slowly migrating shift, and consistent

Figure 5 USF-1 and -2 bind to the E-box element at $-47/-52$

Nuclear extracts from THP-1 cells were prepared and EMSA reactions were set up as described in the Experimental section. **(A)** Overview of the oligonucleotides used in this EMSA. The SK2b oligonucleotide corresponds to the SK2 oligonucleotide used in Figure 3, but is slightly shorter. The SK2mutEbox contains an E-box-specific mutation, which does not alter the putative nuclear receptor site (for a list of all oligonucleotides, see Table 1). **(B)** EMSA showing [γ - 32 P]dCTP-labelled SK2mutEbox and competition assay with labelled SK2b and 200-fold molar excess of unlabelled SK2b and SK2mutEbox. **(C)** Supershift assays show that anti-USF-1 and anti-USF-2 can supershift the slow-migrating complex. A white arrow indicates the supershifts. Anti-Sp1 was included as a control. The weakening of the band is a common feature observed with several other non-specific antibodies (results not shown) and is therefore presumably a general effect caused by addition of extra protein. Anti-USF-1 (Santa Cruz; sc-229x), anti-USF-2 (Santa Cruz; sc-861x) and anti-Sp1 (Santa Cruz; sc-59x) were added 15 min before the addition of the oligonucleotide.

with this, an unlabelled SK2bmutEbox cannot efficiently inhibit the shift obtained with a labelled SK2b oligonucleotide (Figure 5B). Supershift assays show that both anti-USF-1 and anti-USF-2 can supershift the complex, although the USF-1 antibody seems to supershift the complex more efficiently than USF-2 (Figure 5C). This is indicative of the presence of an USF-1/USF-2 heterodimer. Hence, we conclude that USF-1 and USF-2 bind to the E-box motif, and that half-site 1 is also required for the formation of the USF-1/USF-2 containing EMSA shift, although what binds to this site is as yet unknown.

The E-box motif at $-47/-52$ is necessary for transcriptional activity, but needs to co-operate with distal elements to induce transcription

To investigate whether the mutations identified using EMSA had any transcriptional relevance, a series of transfection assays were performed with the mutated promoter constructs represented in

Figure 6(A). Point mutations in the E-box element (corresponding to the SK2mut2 or SK2bmutEbox oligonucleotides; Table 1) almost completely abolish the transcriptional activity of the promoter constructs (Figure 6B). Mutation in half-site 1 also decreases the activity, but not to the same extent as the E-box mutations (Figure 6B). This correlates with the EMSA data, which suggests that the E-box is the more important element for complex formation. Hence, we conclude that the $-47/-52$ E-box motif is absolutely necessary for transcriptional activity, but cannot independently induce transcription, as evidenced by the low transcriptional level of the -53 construct (Figure 1B).

Analysis of the proximal promoter region in the mouse mammary gland-derived cell line HC11 and the rat pancreatic cell line AR4-2J

To analyse if the E-box and half-site 1 participate in the CEL gene regulation in mammary gland and pancreas, we repeated

Figure 6 Mutation in the E-box and in the $-60/-66$ half-site 1 element reduces transcriptional activity

THP-1 cells were transfected with point-mutated CEL promoter constructs as described in the Experimental section. (A) Schematic overview of the constructs used in the transfection assay. The $-138\text{mut}2\text{Luc}$ construct contains a mutation corresponding to the SK2mut2 EMSA oligonucleotide and -138mutE-boxLuc contains an E-box-specific mutation corresponding to the SK2bmutEbox EMSA oligonucleotide (see Table 1). The $-80\text{mut}1\text{Luc}$ construct contains a mutation in the half-site 1 corresponding to the SK2mut1 EMSA oligonucleotide (see Table 1). (B) Bars represent the luciferase activity determined by the average of at least three independent transfections $\pm$ S.E.M.

the transfection assays in the mouse mammary gland cell line HC11 and in the rat pancreatoma cell line AR4-2J. Both these cell lines have been used in previous transfection studies [19,21,22]. In contrast with the slow decline in THP-1 cells, the transcriptional activity increases with increasing promoter length in both HC11 (M. Kannius-Janson, personal communication) and AR4-2J [19]. The HC11 cells show a pattern similar to THP-1 cells up to -138 , but have additional positive regions further upstream. Mutation in the E-box decreases the transcriptional activity of the -138 construct to the same level as the -45 construct, similar to the situation in THP-1 (Figure 7B). However, mutation in half-site 1 does not seem to have any effect in HC11 cells (Figure 7B), indicating a difference between the two cell types with respect to this site.

In AR4-2J cells, the transcriptional activity is low in the absence of the pancreas-specific enhancer element at $-632/-672$, but the -759 construct with an E-box mutation introduced shows a 5-fold decrease in luciferase activity (results not shown). In THP-1 and HC11 cells, the transcriptional activity of the -759 Eboxmut construct is as low as the -138 Eboxmut construct, which was expected since the pancreas-specific enhancer does not give any additional increase in transcriptional

Figure 7 Mutation in the E-box reduces transcriptional activity in HC11 cells, but the $-60/-66$ element does not have any effect

HC11 cells were transfected with 5' deletions and point-mutated CEL promoter constructs as described in the Experimental section. (A) Schematic overview of the constructs used in the transfection assays. The $-138\text{mut}2\text{Luc}$ construct contains a mutation corresponding to the SK2mut2 EMSA oligonucleotide (see Table 1). The $-80\text{mut}1\text{Luc}$ construct contains a mutation corresponding to the SK2mut1 EMSA oligonucleotide (see Table 1). (B) Bars represent the luciferase activity determined by the average of at least three independent transfections $\pm$ S.E.M.

activity in these cell lines (see Figure 1 for THP-1 and see [19] for HC11).

A similar pattern is observed in EMSA for THP-1, HC11 and AR4-2J, with a slow migrating band, which can be supershifted, or with AR4-2J cells abolished, by an USF-1-specific antibody (Figure 8). This indicates that a USF-containing complex is present in all these cell types, although at the transcriptional level the importance of the complex differs. Taken together, these data show that the E-box, which was found to bind USF-1/USF-2 in the EMSA assays, is indeed crucial for transcription, although it cannot by itself confer any activation potential and needs to co-operate with more distal sites. All three cell types which were investigated needed an intact E-box, but there are tissue-specific requirements for additional activating elements.

DISCUSSION

Previous findings that CEL is present in the human aorta and that it has the ability to modify the LDL and HDL composition and to reduce the atherogenicity of oxLDL by decreasing its lysophosphatidylcholine (lysoPC) content, invoked a potential new role for CEL as a protective factor in the development of

Figure 8 USF-1 is present in the E-box-binding complex in THP-1, HC11 and AR4-2J cells

Nuclear extracts from THP-1, HC11 and AR4-2J cells were prepared and EMSA reactions were set up as described in the Experimental section. The black arrow indicates the position of the USF-containing complex; the white arrow indicates the supershifts obtained with the anti-USF-1 antibody (Santa Cruz; sc-229x). Antibody was added 15 min before the addition of oligonucleotide.

atherosclerosis [10]. LysoPC acts as a chemoattractant for monocytes, induces monocyte adhesion to the vascular endothelium and promotes macrophage proliferation, which eventually leads to foam-cell formation (reviewed in [23]). Similar to pancreatic and mammary gland-derived CEL, the macrophage-expressed CEL is secretory as evidenced by bile salt-dependent cholesterol hydrolysis in conditioned medium from PMA-stimulated THP-1 cells and monocyte-derived macrophages [12]. However, there have also been suggestions that CEL may have an intracellular role and may be involved in the cellular cholesterol homeostasis [24].

The potential role of CEL in relation to atherosclerosis calls for further investigation into the role of the CEL expressed from endothelial cells and monocytes/macrophages. Whether the enzyme has a positive or negative effect on the pathological process of atherosclerosis remains to be elucidated, and it cannot be ruled out that CEL may exert both positive and negative effects, depending on the stage of disease progression. Species-specific differences are important to bear in mind when using cell- or animal-model systems. Li and Hui [12] compared the expression of CEL in mouse and human macrophages and suggested that there may be a species-specific difference, as no CEL mRNA could be detected in mouse macrophages.

In the present study, we investigate the transcriptional regulation of the human CEL gene in human monocytes. We have previously studied the transcriptional regulation of CEL in the pancreas and the mammary gland and found several differences between these two tissues. In the pancreas, there is an absolute

requirement for a pancreas-specific enhancer to be located between -632 and -672 [19]. This enhancer element is not needed for expression in the mouse mammary gland-derived cell line HC11 [19] or in the human monocytic cell line THP-1, as evidenced by the present study. In THP-1 cells, there is a slow decline in transcription activity with increasing promoter length beyond -138 , which indicates the presence of negative elements throughout the promoter. In contrast, the activity increases with increasing promoter length both in HC11 cells and in the rat pancreatoma cell line AR4-2J. This may be a reflection of the fact that the overall expression of CEL in the latter two cell types is much higher than in monocytes.

From this perspective, it seems as though there are several differences in the regulation of the CEL gene between the different tissues and that factors, which are quite different, act to promote or suppress transcription. However, the identification of the E-box at $-47/-52$, reported here, revealed a common element for the different tissues. Although the E-box alone is not sufficient to drive high-level expression in any of the cell lines investigated, it is nevertheless essential. We provide evidence that USF-1 and USF-2 bind to this E-box, and EMSA experiments suggest that a region containing a putative nuclear receptor half-site is also involved in the formation of the USF-containing complex in THP-1 cells. In AR4-2J cells, both the E-box and the pancreas-specific element must be present to achieve a high-level expression. Neither the pancreas-specific enhancer [19] nor the putative nuclear receptor site (half-site 1; the present study) have any effect on HC11 cells, and hence the co-operative element in HC11 cells remains to be identified.

USF binds DNA as a dimer at sites with the consensus CANNTG (CACGTG). In many different cell types, homodimers and heterodimers of USF-1 and USF-2 are present in different ratios. The major species of USF in most tissues are the heterodimers; USF-1 dimers are less common, and USF-2 dimers are usually quite scarce [25]. We have not attempted to determine the relative quantities of the different USF types in THP-1 cells, but both USF-1 and USF-2 are present in the THP-1 nuclear extract, although the USF-1 antibody appears to supershift the complex more efficiently than the USF-2 antibody. USFs do not appear to transactivate effectively in highly purified *in vitro* systems, presumably due to the requirement for additional interacting proteins [26], and it has been suggested that USF may be associated with the basal transcription machinery [27]. This is consistent with our data, which suggest that USF does not by itself confer any tissue specificity or high-level expression, but is important for basal transcription in all three cell types investigated.

CEL, like several other genes involved in lipid metabolism, is regulated by dietary lipids [4–6], and the orphan nuclear receptor family is an important group of regulators of genes involved in numerous aspects of cholesterol homeostasis [14]. The data presented in the present study suggest that a putative nuclear receptor-binding site may co-operate with the E-box element in the formation of the USF-containing complex in THP-1 cells. Our observation that the USF-containing shift can be inhibited more efficiently by an oligonucleotide containing a consensus PPRE [20] than one mutated in both half-sites of the PPRE suggests that protein(s) involved in the USF complex can also bind to a PPRE. We speculate that it is the binding to half-site 1 that is prevented by the PPRE oligonucleotide. However, neither of the antibodies directed towards RXR, the heterodimeric partner of several factors including PPAR, liver X receptor, farnesoid X receptor, pregnane X receptor, constitutive androstane receptor and nerve growth factor inducible gene B [28], nor antibodies directed towards three different monomeric

nuclear receptors, ROR α , SF-1 and CPF-1, had any effect on the complex. We can either draw the conclusion that none of the above-mentioned nuclear receptors is involved in the formation of the complex, or alternatively, that the antibodies cannot access the factor due to some conformational aspect of the complex. Hence, future studies are required to identify the factor(s) binding to the region containing the PPRE-like element.

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