Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase promoter contains a CREB binding site that regulates cAMP action in Caco-2 cells

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cAMP increases transcription of the mitochondrial (mit.) gene for 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase, which encodes an enzyme that has been proposed as a control site of ketogenesis. The incubation of Caco-2 cells with cAMP increased mit.HMG-CoA synthase mRNA levels 4-fold within 24 h. We have identified an active cAMP-response element (CRE) located 546 bp upstream of the mit.HMG-CoA synthase promoter that is necessary for the induction of expression by dibutyl cAMP. Co-transfections of constructs, containing the CRE element of the mit.HMG-CoA synthase promoter fused to the gene for chloramphenicol acetyltransferase, with protein kinase A and a dominant-negative mutant of cAMP-response-element-binding protein (CREB) show that the response to cAMP is mediated by the transcription factor CREB. The CRE element confers re-

sponsiveness of protein kinase A to a heterologous promoter in transfection assays in Caco-2 cells. Gel-retardation assays revealed that the mit.HMG-CoA synthase CRE binds to recombinant CREB. The shifted band obtained with the putative mit.HMG-CoA synthase CRE sequence and nuclear proteins from Caco-2 cells competed with CRE sequences of other genes such as somatostatin and phosphoenolpyruvate carboxykinase. We conclude that the regulation of the expression of the gene for mit.HMG-CoA synthase in Caco-2 cells by cAMP is mediated by a CRE sequence in the promoter.

Key words: cAMP-response element, colon ketogenesis, protein kinase A, transcriptional regulation

INTRODUCTION

Starvation, feeding with fat and sustained exercise stimulate hepatic ketogenesis. Although carnitine palmitoyltransferase I and II are considered to be important in the synthesis of ketones, it is commonly accepted that mitochondrial (mit.) 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase is the main regulatory site in this process [1,2]. This enzyme condenses acetoacetyl-CoA and acetyl-CoA to produce HMG-CoA and free CoA.

Several factors increase both hepatic ketogenesis and the expression of mit.HMG-CoA synthase such as the foetal-to-neonatal and fed-to-starved transitions, feeding with fat, and diabetes. In all these cases there are increases in the mRNA and protein levels for mit.HMG-CoA synthase and in the enzyme activity [3–5]. The *cis* element responsible for the transactivation of fatty acids has been characterized in the promoter of mit.HMG-CoA synthase. This is the nuclear receptor-responsive element ('NRRE'), which recognizes peroxisome-proliferator-activated receptor ('PPAR') [6] and other transcription factors such as chicken ovalbumin upstream promoter transcription factor ('COUP-TF') and hepatocyte nuclear factor 4 ('HNF-4') [7,8].

Mit.HMG-CoA synthase is also expressed in the small intestine of suckling mammals but this expression disappears at weaning [9–13]. In contrast with the small intestine, colon and caecum synthesize ketone bodies in adult animals from the butyrate produced by the colonic bacteria [14]. In these tissues the expression of HMG-CoA synthase is maintained throughout adult life.

cAMP is important in metabolism in the colon. Intracellular signalling via cAMP-mediated pathways regulates intestinal

epithelial differentiation [15]. cAMP levels can be increased by various hormones, including vasoactive intestinal peptide. The presence of the receptor for vasoactive intestinal peptide in Caco-2 cells is well documented [16,17]. Increases in cellular concentration of this second messenger modulate the transcription of several genes in colon cells, among them lactase-phlorizin hydrolase [18], dipeptidyl dipeptidase and alkaline phosphatase [15]. However, no studies on colon cells have been reported concerning the action of transcription factors on the transcriptional regulation of mit.HMG-CoA synthase.

Distinct DNA sequences within gene promoters termed cAMP-response elements (CREs) have been shown to confer such cAMP responsiveness. CREs function by acting as binding sites for transcription factors that mediate the cAMP effect. Two classes of CRE with different affinities for the transcription factor cAMP-response-element-binding protein (CREB) are described. One class containing the symmetrical TGACGTC site shows a high binding affinity for CREB, whereas the other, including the CRE reported here, is characterized by asymmetric and weak binding sites (CGTCA) [19]. Both classes show an increase in binding after phosphorylation of CREB by protein kinase A (PKA). These DNA sequences can be bound by homodimer or heterodimer combinations of a variety of β -ZIP transcription factors including CREB homodimers [20], heterodimers of CREB and activating transcription factor ('ATF') [21] and dimers consisting of other transcription factors in the activating transcription factor family [22].

In the present study we have identified an element located 546 bp upstream of the rat mit.HMG-CoA synthase promoter that confers cAMP responsiveness on homologous and heterologous promoters in Caco-2 cells. Co-transfection experiments with 5' deleted plasmids of the mit.HMG-CoA synthase promoter

Abbreviations used: CAT, chloramphenicol acetyltransferase; CRE, cAMP-response element; CREB, cAMP-response-element-binding protein; db cAMP, *N*⁶,2'-*O*-dibutyl cAMP; EMSA, electrophoretic mobility-shift analysis; KCREB, killing CREB; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; mit., mitochondrial; PEPCK, phosphoenolpyruvate carboxykinase; PKA, protein kinase A; TK, thymidine kinase.

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and a dominant-negative mutant of CREB (killing CREB; KCREB) showed that the effect of cAMP is mediated by the transcription factor CREB. Gel-retardation experiments with recombinant CREB and CRE sequences of somatostatin and mit.HMG-CoA synthase as probes showed the specific binding of CREB to the CRE element described in the mit.HMG-CoA synthase, although with less affinity than somatostatin. All these results show that this CRE sequence is necessary for the induction by cAMP of rat mit.HMG-CoA synthase gene transcription in Caco-2 cells.

EXPERIMENTAL

Materials

Restriction nucleases and modifying enzymes were purchased from Promega. Bluescript was from Stratagene, the chloramphenicol acetyltransferase (CAT) vector pCAT-BASIC reporter gene was from Promega, and the herpes virus thymidine kinase (TK) gene promoter upstream of the CAT reporter gene pBLCAT2 was from [23]. The CREB expression vector RSV-CREB and the dominant-negative CREB expression vector RSV-KCREB were gifts from Dr. Goodman [24]. The PKA expression vector, RSV-PKAc, contained the cDNA for the catalytic subunit of PKA under the control of the RSV promoter [25]. Plasmid isolation kits were from Qiagen. The truncated recombinant protein of CREB (CREB-1 bZIP, a 10–14 kDa protein containing the DNA-binding and dimerization domains, corresponding to residues 254–327) and antibodies against CREB (CREB-1/240) were obtained from Santa Cruz Biotechnology. *N*⁶,2'-*O*-Dibutyryl cAMP (db cAMP) was from Boehringer Mannheim. Dulbecco's modified Eagle's medium was from Gibco Laboratories.

Plasmids

The plasmid pSMPCAT1 contained 1148 bp of the 5' flanking region and the first 28 bp of exon 1 of the rat mit.HMG-CoA synthase gene inserted into the CAT vector pCAT-BASIC reporter gene, as described previously [26]. pSMPCAT3, pSMPCAT4 and pSMPCAT5 contained deleted constructs of the 5' regulatory region and the same 3' deletion end point in exon 1 of the gene, as described previously [6]. For 2xMSCRE-CAT plasmid, synthetic oligonucleotides with 5'-GATC overhangs were used: MS-CREfor (5'-GATCCCTCAGATGTGCGTCAACACCTACTCTCA-3'), corresponding to positions -533 to -562 of the 5' flanking region of the mit.HMG-CoA synthase gene (in which the CRE sequence is underlined) and the complementary oligonucleotide MS-CRErev (5'-GATCTGAGAGTAGGTGTTGACGCACATCTGAGG-3'). For 2xmMSCRE-CAT plasmid, oligonucleotides containing the scrambling mutated mit.HMG-CoA synthase CRE sequence were used: mMS-CREfor (5'-GATCCCTCAGATATCTGTACACACCTACTCTCA-3') and mMS-CRErev (5'-GATCTGAGAGTAGGTGTGTACAGATATCTGAGG-3'). Oligonucleotides were dimerized and subcloned in the forward orientation in the *Bam*HI site of the pBLCAT2. Subcloning and plasmid isolation were performed by standard procedures. All constructs were sequenced by the enzymic method to confirm the identity and the orientation of the inserts.

Cell culture and transfections

Caco-2 cells (from DSM, German Collection of Micro-organisms and Cell Cultures) were cultured in Dulbecco's modified Eagle's

medium supplemented with 20% (v/v) foetal bovine serum, 100 i.u./ml penicillin, 100 µg/ml streptomycin and 1% (w/v) non-essential amino acids as described by the distributors. The cells (passages 10–30) were cultured in 100 mm plates. Cells were transiently transfected with 10 µg of the reporter mit.HMG-CoA synthase-CAT gene construct and, when indicated, with 0.1 µg of RSV-PKAc and 3 µg RSV-CREB and/or 3 µg RSV-KCREB. Plasmid dosage was kept constant by the addition of appropriate amounts of Bluescript. Plasmid pRSVbGAL (Rous sarcoma virus promoter β -galactosidase) (3 µg) was included as an internal control in co-transfections. Transfection experiments were performed by the calcium phosphate method, as described [27,28]. After removal of the calcium phosphate-DNA precipitate, cells were re-fed with the appropriate medium. Cells were stimulated with 0.5 mM db cAMP. After incubation for a further 24 h the cells were harvested and CAT activities were measured as described [27]. The CAT activities were normalized by measuring the β -galactosidase activity in the extracts as described [28]. Each series of experiments was performed at least three times and each transfection was performed in duplicate.

Nuclear extracts and electrophoretic mobility-shift assay (EMSA)

Nuclear extracts were prepared from Caco-2 cells by the method of Dignam et al. [29] with the modification that, after the chromatin extraction step, precipitation with (NH₄)₂SO₄ (0.3 g/ml) was included to concentrate proteins before dialysis. For EMSA, synthetic complementary oligonucleotides for HMG-CoA synthase MS-CREfor and MS-CRErev, mMS-CREfor and mMS-CRErev were used. In addition, oligonucleotides corresponding to somatostatin and phosphoenolpyruvate carboxykinase (PEPCK) were used: SomCREfor (5'-GATCCTCCTTGGCTGACGTCAGAGAGAGAGTA-3') (corresponding to -31 to -58 of the 5' flanking region of the somatostatin gene), SomCRErev (5'-GATCTACTCTCTCTCTGACGTCAGCCAAGGAG-3'), PEPCKCREfor (5'-AGCTCGAGGGCCCCCTTACGTCAGAGGCGAGA-3') (corresponding to -74 to -102 of the 5' flanking region of PEPCK gene) and PEPCKCRErev (5'-AGCTTCTCGCCTCTGACGTAAGGGGCCCTCG-3'). Oligonucleotides were annealed and labelled by a fill-in reaction with [α -³²P]dCTP and Klenow enzyme, then incubated with 15 µg of protein from Caco-2 nuclear extracts, as described in [30]. For the binding reaction with recombinant CREB (CREB-1 bZIP), 2 or 8 ng of protein was incubated, for 10 min on ice, in 20 mM Hepes (pH 7.9)/1 mM EDTA/0.5 mM dithiothreitol/140 mM KCl, 10% (v/v) glycerol/15 µg of BSA with 0.2 µg of poly(dI-dC) as a non-specific competitor in a total volume of 20 µl. The assay was then performed as described [30]. In some binding reactions a specific antiserum against CREB (CREB-1/240) or control antiserum was used. Nuclear extracts (15 µg of protein) were incubated for 20 min at room temperature with 2 µl of an anti-CREB antiserum, or an equal volume of control serum, in 20 µl (total volume) of 20 mM Hepes (pH 7.9)/1 mM EDTA/0.5 mM dithiothreitol/70 mM KCl/10% (v/v) glycerol, and then overnight on ice. After a 10 min incubation on ice with 4 µg of poly(dI-dC), the labelled oligonucleotides were added and the assay was then performed as described [30].

Northern blot analysis

Total RNA from cultured Caco-2 cells incubated with db cAMP or transiently transfected with RSV-PKAc vector was isolated as described [28]. At different times, 15 µg of total RNA samples were fractioned in 1% (w/v) agarose/formaldehyde gels, transferred to Nytran-N membranes (Schleicher and Schuell) and

cross-linked at 80 °C for 2 h. Hybridizations were performed with an *EcoRI*–*EcoRI* fragment of cDNA of mit.HMG-CoA synthase as a probe, in ExpressHyb hybridization solution (Clontech) in accordance with the manufacturer's recommendations. The filters were then subjected to autoradiography.

RESULTS

Stimulation of mit.HMG-CoA synthase gene expression by cAMP and PKA in Caco-2 cells

To investigate the effect of cAMP on the expression of the gene for mit.HMG-CoA synthase in Caco-2 cells, we incubated these cells with 0.5 mM db cAMP for up to 48 h. Figure 1 shows that there was a time-dependent increase in the mRNA levels for mit.HMG-CoA synthase. These results agree with others previously observed for rat liver [3]. The transient transfection with

Figure 1 Effect of cAMP or PKA on the expression of mit.HMG-CoA synthase mRNA levels in Caco-2 cells

Left panel: Caco-2 cells were incubated with 0.5 mM db cAMP for various durations. Right panel: Caco-2 cells were transiently transfected with a vector expressing PKA for several durations. In both cases mit.HMG-CoA synthase (MS) mRNA levels were determined at different times by Northern blot experiments.

Figure 2 KCREB alters cAMP responses of the mit.HMG-CoA synthase promoter in Caco-2 cells

Caco-2 cells were transiently transfected with plasmid pSMPCAT-1 and with the vectors expressing CREB and KCREB as indicated. Cells were incubated with 0.5 mM db cAMP for 24 h. β -Galactosidase-normalized CAT activities (means $\pm$ S.D.) for three independent transfections performed in duplicate are expressed as fold inductions relative to the activity in the absence of expression vectors and activator.

Figure 3 Localization of the CRE element of the mit.HMG-CoA synthase promoter by progressive deletion analysis

CAT reporter constructs containing the indicated position of the 5' flanking region of the mit.HMG-CoA synthase gene were transiently co-transfected in Caco-2 cells with vectors expressing PKA (filled columns) or PKA and KCREB (open columns). β -Galactosidase-normalized CAT activities (means $\pm$ S.D.) for three independent transfections performed in duplicate are expressed as fold inductions relative to the activity to each construct in the absence of expression vectors.

a vector encoding the catalytic subunit of PKA further increased the mRNA levels, suggesting that cAMP produced a transcriptional activation of the mit.HMG-CoA synthase gene in Caco-2 cells.

KCREB abrogates the increased transcriptional activity of cAMP

To test the involvement of CREB in the transcriptional activation of cAMP, Caco-2 cells were co-transfected with plasmid pSMPCAT-1, composed of 1148 bp of the promoter of mit.HMG-CoA synthase and the CAT gene, and with the vector expressing CREB. In addition, cells were co-transfected with a vector encoding KCREB, which abolishes the transcriptional activation of either cAMP or PKA (mediated by CREB). Some cells were incubated with db cAMP for 24 h and the CAT activity was determined. Results in Figure 2 show that cAMP, either alone or in combination with CREB, induced a 2.5-fold increase in transcription rate, which was abolished by KCREB. This was interpreted to indicate that CREB is required for the cAMP response of the mit.HMG-CoA synthase promoter.

Figure 4 mit.HMG-CoA synthase CRE confers PKA responsiveness to TK gene promoter

Caco-2 cells were transiently co-transfected with vectors expressing PKA (filled columns) or PKA and KCREB (open columns), and reporter plasmids containing CAT gene under the control of TK promoter and two fragments containing either the putative CRE element (CRE) or the mutated CRE (mCRE) of mit.HMG-CoA synthase. β -Galactosidase-normalized CAT activities (means $\pm$ S.D.) for at least three independent transfections in duplicate are expressed as fold inductions relative to the activity of plasmid 2xMSCRE-CAT in the absence of the expression vectors.

Localization of the *cis*-acting element in the mit.HMG-CoA synthase promoter responsible for cAMP-induced transcriptional activation

To determine the approximate location of CRE, progressively larger 5' deletions of the 5' flanking region of mit.HMG-CoA synthase from the plasmid pSMACAT1 were produced. These constructs were tested in co-transfection experiments in Caco-2 cells with either PKA or PKA plus KCREB.

Nucleotides up to -760 of the mit.HMG-CoA synthase promoter were deleted without important loss of the response to PKA, which was eliminated by further deletion up to nt -550 . These results identified positions between -760 and -550 as being part of the putative mit.HMG-CoA synthase CRE. This region contains a CRE sequence 5'-GTGCGTCA-3' located between positions -546 and -553 that seems to mediate the response to cAMP (Figure 3). This was confirmed in three ways. First, in transfection experiments a fragment of the putative CRE site was inserted into pBLCAT2, a plasmid containing the CAT gene under the control of the TK promoter. Two copies of the fragment -553 to -546 conferred a PKA response on the otherwise unresponsive TK promoter (Figure 4). Simultaneous co-transfection with the dominant-negative mutant KCREB prevented transcriptional activation by PKA, confirming the involvement of CREB in the interaction with CRE. Secondly, in transfection experiments the putative CRE was altered by a scrambled mutation and inserted into the plasmid of the TK promoter. No PKA activation was produced with respect to the TK promoter taken as a control (Figure 4). Thirdly, several EMSAs (see below) confirmed the binding of CREB protein to

Figure 5 EMSAs of the somatostatin CRE

Nuclear extracts of Caco-2 cells were incubated with labelled somatostatin CRE probe (Som-CRE), and analysed by EMSA. Lane 10 shows the complex without any competition with the unlabelled probe. Lanes 1–3 contained a competition of the complex with 10-fold, 100-fold and 800-fold molar excesses of unlabelled Som-CRE; lanes 4–6 contained a competition of the complex with 10-fold, 100-fold and 800-fold molar excesses of unlabelled mit.HMG-CoA synthase CRE (MS-CRE); lanes 7–9 contained a competition of the complex with 10-fold, 100-fold and 800-fold molar excesses of unlabelled PEPCK CRE (PEPCK-CRE). F indicates free probe.

the CRE elements of the somatostatin and mit.HMG-CoA synthase used as probes.

CREB binds to the CRE sequence of the mit.HMG-CoA synthase gene

Incubation of the somatostatin CRE, as a control, with Caco-2 nuclear proteins resulted in a complex (Figure 5, lane 10). The specificity of the complex was demonstrated by competition with 10-fold, 100-fold and 800-fold molar excesses of unlabelled probes of the somatostatin CRE and also of those of the mit.HMG-CoA synthase and PEPCK. Analogous gel-shift experiments were performed with the putative 32 P-labelled mit.HMG-CoA synthase CRE as the probe. The complex (Figure 6, lane 1) also competed with the unlabelled probes corresponding the CREs of the three genes.

To confirm that CREB bound the mit.HMG-CoA synthase CRE element, a new experiment was performed in which anti-CREB antibodies were added to the incubation mixture. The complex between mit.HMG-CoA synthase CRE and nuclear proteins disappeared (Figure 7, lane 4). The complex between somatostatin and nuclear proteins taken as a control also decreased. A supershifted band corresponding to the ternary antibody–protein–probe complex was not observed in either case. This experiment was also performed with control serum; the complex did not disappear (results not shown).

Incubation of the 32 P labelled CRE elements of somatostatin and mit.HMG-CoA synthase with a truncated recombinant

Figure 6 EMSA of the putative mit.HMG-CoA synthase CRE (MS-CRE)

Nuclear extracts of Caco-2 cells were incubated with labelled MS-CRE and analysed by EMSA. Lane 1 shows the complex without any competition with unlabelled probe; lanes 2–4 contained a competition of the complex with 10-fold, 100-fold and 800-fold molar excesses of unlabelled Som-CRE; lanes 5–7 contained a competition of the complex with 10-fold, 100-fold and 800-fold molar excesses of unlabelled MS-CRE; lanes 8–10 contained a competition of the complex with 10-fold, 100-fold and 800-fold molar excesses of unlabelled PEPCK-CRE. The arrow shows the complex of MS-CRE probe with nuclear proteins. F indicates free probe.

CREB protein resulted in prominent complexes, which were more abundant with somatostatin (Figure 8). When the putative HMG-CoA synthase CRE element was mutated by scrambling, no binding was observed (Figure 8, lanes 5 and 6). The differences in the CRE nucleotide sequence of mit.HMG-CoA synthase and the somatostatin CRE sequence might be responsible for the different affinities for CREB.

DISCUSSION

In the present paper we show that the 5' region of the mit.HMG-CoA synthase contains a CRE sequence (GTGCGTCA) that might determine the response of this gene to cAMP. The influence of cAMP on the expression of this gene in rat liver has been reported previously. The intraperitoneal injection of db cAMP caused a time-dependent increase in mRNA levels of hepatic mit.HMG-CoA synthase [3]. Incubation of Caco-2 (a colonic cell line that expresses mit.HMG-CoA synthase) with cAMP or the transient transfection of cells with PKA also increased the mRNA levels of mit.HMG-CoA synthase.

The sequence responsible for this response to cAMP was mapped in the 5' region of the mit.HMG-CoA synthase gene. We have shown by deletion analysis of the promoter that the CRE is located between –546 and –553 bp. Transfection of plasmids with sequences shorter than –550 of the mit.HMG-CoA synthase promoter in Caco-2 cells abrogates the response to the influence of PKA. This segment contains an asymmetric CRE sequence. It differs from the CRE sequence of human proenkephalin promoter (CTGCGTCA) by only one nucleotide

Figure 7 Effect of anti-CREB antibodies in EMSA of Caco-2 nuclear extracts with mit.HMG-CoA synthase CRE

Labelled Som-CRE (lanes 1 and 2) and MS-CRE probes (lanes 4 and 5) were incubated with samples of nuclear proteins (lanes 1 and 3) or samples of the nuclear proteins that had been incubated previously with anti-CREB antibodies (lanes 2 and 4). All samples were analysed by EMSA. F indicates free probe.

Figure 8 Specific binding of recombinant CREB protein to HMG-CoA synthase CRE, revealed by EMSA

Labelled mit.HMG-CoA synthase CRE (lanes 1 and 2) and Som-CRE probes (lanes 3 and 4) were incubated with truncated recombinant CREB protein [2 ng (lanes 1 and 3) or 8 ng (lanes 2 and 4)] and analysed by EMSA. Lanes 5 and 6 show the results of the incubation of 2 and 8 ng respectively of the recombinant CREB and the mutated mit.HMG-CoA synthase (mMS-CRE) probe. The arrow shows the labelled complex CREB–CREB protein. F indicates free probe.

[31], from rat PEPCK CRE (TTACGTCA) by two nucleotides [32] and from rat somatostatin CRE (TGACGTCA) by three nucleotides [33].

Additional evidence for functional CRE in the mit.HMG-CoA synthase gene is as follows. (1) It confers sensitivity to the TK promoter on the action of PKA when the plasmid that contains the two CRE sequences is transfected to Caco-2 cells. (2) The mutation of this CRE element (GTGCGTCA) by scrambling (ATCTGTAC) abolishes the response in the TK promoter, rendering it insensitive to activation by PKA. (3) Co-transfection of KCREB decreases the response of the HMG-CoA synthase promoter to the activation effect of either cAMP or PKA. The suggested effect is the inhibition of transactivation action of CREB. (4) In gel-retardation experiments *in vitro* a complex is formed after the incubation of the HMG-CoA synthase CRE sequence with either the nuclear extracts of Caco-2 cells or recombinant CREB protein.

The gel-shift experiments merit some comments. Incubation of Caco-2 nuclear extracts with an oligonucleotide containing the CRE sequence of mit.HMG-CoA synthase determined a complex that competed with the unlabelled probe in a simultaneous incubation. Equally, competition with unlabelled heterologous CRE probes from somatostatin and PEPCK indirectly confirmed the CRE sequence. Incubation with anti-CREB antibodies produced a decrease in the complex between Caco-2 nuclear proteins and the CREs of both somatostatin and HMG-CoA synthase. In neither case was a supershift complex observed as reported for other genes [34].

An important complex was also observed after the incubation of mit.HMG-CoA synthase CRE with recombinant CREB. However, this binding is of lower affinity than that of CREB to somatostatin CRE. It has been well established that CREB protein can interact with the CRE sequences with differing affinities. The symmetrical CRE sequences, such as somatostatin [33], α -chorionic gonadotropin [35], fibronectin [36] and PEPCK [32], show a higher binding affinity for CREB than the asymmetric CRE sequences such as urokinase-type plasminogen activator gene [37], pro-enkephalin [31] and tyrosine aminotransferase [19]. These low-affinity binding capacities predict that the asymmetric CRE sequences might show a lower basal transcription level but higher induction by cAMP than the symmetrical CREs [19]. This might have physiological consequences for ketogenesis in the colon.

Starvation in rats produces an increase in plasma glucagon, which in turn increases intracellular cAMP and fatty acid levels in plasma by the lipolysis of adipose tissue. Both effects increase the expression of mit.HMG-CoA synthase. The effect of fatty acids in HepG2 cells is mediated by the peroxisome-proliferator-activated receptor ('PPAR'). Co-transfection of this transcription factor with a construct composed of the HMG-CoA synthase promoter and CAT produces a 15-fold activation in HepG2 cells [6]. A similar value has been observed when co-transfection is performed in Caco-2 cells (C. Caudevilla, G. Asins, F. G. Hegardt and D. Serra, unpublished work). When HepG2 cells [26] and Caco-2 cells are incubated with db cAMP, analogous values of transcriptional activation are observed (approx. 3-fold). It therefore seems that hepatic and colonic cells behave in similar ways in stimulating the transcriptional activation of mit.HMG-CoA synthase both by fatty acids and cAMP, which reflects the high expression of HMG-CoA synthase in liver [3] and colon [14].

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REFERENCES

- 1 Dashti, N. and Ontko, J. A. (1979) *Biochem. Med.* **22**, 365–374
- 2 Hegardt, F. G. (1999) *Biochem. J.* **338**, 569–582
- 3 Casals, N., Roca, N., Guerrero, M., Gil-Gómez, G., Ayté, J., Ciudad, C. J. and Hegardt, F. G. (1992) *Biochem. J.* **283**, 261–264
- 4 Serra, D., Casals, N., Asins, G., Royo, T., Ciudad, C. J. and Hegardt, F. G. (1993) *Arch. Biochem. Biophys.* **307**, 40–45
- 5 Quant, P. A., Tubbs, P. K. and Brand, M. D. (1990) *Eur. J. Biochem.* **187**, 169–174
- 6 Rodríguez, J. C., Gil-Gómez, G., Hegardt, F. G. and Haro, D. (1994) *J. Biol. Chem.* **269**, 18767–18772
- 7 Rodríguez, J. C., Ortiz, J. A., Hegardt, F. G. and Haro, D. (1997) *Biochem. J.* **326**, 587–592
- 8 Rodríguez, J. C., Ortiz, J. A., Hegardt, F. G. and Haro, D. (1998) *Biochem. Biophys. Res. Commun.* **242**, 692–696
- 9 Serra, D., Asins, G. and Hegardt, F. G. (1993) *Arch. Biochem. Biophys.* **301**, 445–448
- 10 Thumelin, S., Esser, V., Charvy, D., Kolodziej, M., Zammit, V. A., McGarry, D., Girard, J. and Pegorier, J. P. (1994) *Biochem. J.* **300**, 583–587
- 11 Serra, D., Bellido, D., Asins, G., Arias, G., Vilaró, S. and Hegardt, F. G. (1996) *Eur. J. Biochem.* **237**, 16–24
- 12 Hahn, P. and Taller, M. (1987) *Life Sci.* **41**, 1525–1528
- 13 Békési, A. and Williamson, D. H. (1990) *Biol. Neonate* **58**, 160–165
- 14 Cherbuy, C., Darcy-Vrillon, B., Morel, M. T., Pegorier, J. P. and Duée, P. H. (1995) *Gastroenterology* **109**, 1890–1899
- 15 Basson, M. D. and Hong, F. (1996) *Cancer Lett.* **99**, 155–160
- 16 Grasset, E., Bernabeu, J. and Pinto, M. (1995) *Am. J. Physiol.* **248**, C410–C418
- 17 Laburthe, M., Rousset, M., Rouyer-Fessard, C., Couvineau, A., Chantret, Y., Chevalier, G. and Zweibaum, A. (1987) *J. Biol. Chem.* **262**, 10180–10184
- 18 Hauri, H. P., Sander, B. and Naim, H. (1994) *Eur. J. Biochem.* **219**, 539–546
- 19 Nichols, M., Weih, F., Schmid, W., DeVack, C., Kowenz-Leutz, E., Luckow, B., Boshart, M. and Schütz, G. (1992) *EMBO J.* **11**, 3337–3346
- 20 Hoeffler, J. P., Meyer, T. E., Yun, Y., Jameson, J. L. and Habener, J. F. (1988) *Science* **242**, 1430–1433
- 21 Meyer, T. E. and Habener, J. F. (1993) *Endocr. Rev.* **14**, 269–290
- 22 Habener, J. F. (1990) *Mol. Endocrinol.* **4**, 1087–1095
- 23 Luckow, B. and Schütz, G. (1987) *Nucleic Acids Res.* **15**, 5490
- 24 Walton, K. M., Rehluss, R. P., Chiriva, J. C., Lochner, J. E. and Goodman, R. H. (1992) *Mol. Endocrinol.* **6**, 647–655
- 25 Howard, P., Day, K. H., Kim, K. E., Richardson, J., Thomas, J., Abraham, I., Fleischmann, R. D., Gottesman, M. M. and Maurer, R. A. (1991) *J. Biol. Chem.* **266**, 10189–10195
- 26 Gil-Gómez, G., Ayté, J. and Hegardt, F. G. (1993) *Eur. J. Biochem.* **213**, 773–779
- 27 Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1987) *Current Protocol in Molecular Biology*, Green Publishing Associates/Wiley-Interscience, New York
- 28 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- 29 Dignam, J. D., Lebovitz, R. M. and Roeder, R. G. (1983) *Nucleic Acids Res.* **11**, 1475–1489
- 30 Kneipel, W., Jepeal, L. and Habener, J. F. (1990) *J. Biol. Chem.* **265**, 8725–8735
- 31 Comb, M., Mermoud, N., Hyman, S. E., Pearlberg, J., Ross, M. E. and Goodman, H. M. (1988) *EMBO J.* **7**, 3739–3805
- 32 Quinn, P. G., Wong, T. W., Magnusson, M. A., Shabb, J. B. and Granner, D. K. (1988) *Mol. Cell. Biol.* **8**, 3467–3475
- 33 Montminy, M. R. and Bilezikjian, L. M. (1987) *Nature (London)* **328**, 175–178
- 34 Yamada, K., Duong, D. T., Scott, D. K., Wang, J.-C. H. and Granner, D. K. (1999) *J. Biol. Chem.* **274**, 5880–5887
- 35 Deleage, A. M., Ferland, L. H. and Mellon, P. L. (1987) *Mol. Cell. Biol.* **7**, 3994–4002
- 36 Dean, D. C., Blakely, M. S., Newby, R. F., Ghazal, P., Henninghausen, L. and Bourgeois, S. (1989) *Mol. Cell. Biol.* **9**, 1498–1506
- 37 von der Ahe, D., Pearson, D. and Nagamine, N. (1990) *Nucleic Acids Res.* **18**, 1991–1999