Biochemical and Biophysical Research Communications 404 (2011) 376–381

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Role of FOXA and Sp1 in mitochondrial acylcarnitine carrier gene expression in different cell lines

Paolo Convertini [a], Vittoria Infantino [a,b], Faustino Bisaccia [b], Ferdinando Palmieri [a,*], Vito Iacobazzi [a]

[a] Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari, Bari, Italy
[b] Department of Chemistry, University of Basilicata, Potenza, Italy

ARTICLE INFO

Article history:
Received 23 November 2010
Available online 3 December 2010

Keywords:
Carnitine/acylcarnitine carrier
FOXA
Gene expression
Mitochondria
Promoter
Sp1
Transcription
Transporter

ABSTRACT

This study investigates the transcriptional role of the human mitochondrial carnitine/acylcarnitine carrier (CAC) proximal promoter. Through deletion analysis, an activation domain (−334/−80 bp) was identified which contains FOXA and Sp1 active sites. The wild-type (but not mutated) −334/−80 bp region of the CAC gene conferred 74% LUC transgene activity in HepG2 cells, 17% in HEK293 cells and 14% in SK-N-SH cells as compared to that observed with the entire −1503/+3 bp proximal promoter. Overexpression and silencing of FOXA2 or Sp1 in HepG2 cells enhanced and diminished, respectively, LUC activity, CAC transcript and CAC protein. In HEK293 and SK-N-SH cells, which do not contain FOXA1-3, LUC activity was increased by FOXA2 overexpression to a greater extent than in HepG2 cells. Both FOXA2 and Sp1 in HepG2, and only Sp1 in HEK293 and SK-N-SH cells, were found to be bound to the CAC proximal promoter. These results show that FOXA and Sp1 sites in HepG2 cells and only the Sp1 site in HEK293 and SK-N-SH cells have a critical role in the transcriptional regulation of the CAC proximal promoter.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

The carnitine/acylcarnitine carrier (CAC), encoded by the SLC25A20 gene, is an inner mitochondrial membrane protein which belongs to the mitochondrial carrier protein family [1,2]. Its function, conserved in all eukaryotes, is to transport acylcarnitines across the inner mitochondrial membrane in exchange for free carnitine. By catalyzing this exchange, CAC allows the import of fatty acyl moieties into the mitochondria where they are oxidized by the β-oxidation enzymes. Fatty acid β-oxidation is the major source of energy for heart and skeletal muscles during fasting and physical exercise. CAC has been purified and reconstituted into liposomes, kinetically characterized, cloned and expressed in *Escherichia coli* and yeast [3–8]. The human gene spans about 42 kb of DNA, contains 9 coding exons and maps to chromosome 3p21.31 [9,10]. Mutations in SLC25A20 are responsible for a disease named CAC deficiency (OMIM 212138) which is characterized by life-threatening episodes of coma upon fasting due to hypoglycemia, cardiomyopathy, muscle weakness and abnormal liver function [2]. Management of patients affected by CAC deficiency consists of fasting prevention with frequent meals, a diet rich in carbohydrates, low in lipids and supplemented with essential polyunsaturated fatty acids [2]. Recently, we proposed a pharmacological treatment of patients affected with mild phenotype on the basis of the finding that statins and fibrates up-regulate the transcription of the human CAC gene via the PPRE site of its promoter [11]. Apart from this information, the mechanisms of transcriptional regulation of the CAC gene are yet to be understood.

To shed more light on the transcriptional regulatory role of the CAC gene promoter, herein we have functionally analyzed the proximal promoter of this gene. Evidence is provided for the presence of an activation domain between −334 and −80 bp which contains active FOXA and Sp1 sites. Moreover, it is shown that both these sites in hepatic cells and only the Sp1 site in HEK293 and SK-N-SH cells act as enhancers in the transcriptional regulation of CAC gene expression.

2. Materials and methods

2.1. Construction of plasmids

Progressive deletion fragments of the region from −1503 to +3 bp of the CAC gene promoter were amplified by PCR and cloned

Abbreviations: CAC, carnitine/acylcarnitine carrier; FOXA, forkhead box A; Sp1, stimulating protein 1; PPRE, peroxisome proliferator-activated receptor element; ChIP, chromatin immunoprecipitation; LUC, luciferase; siRNA, small interfering RNA; wtFOXA, wtSp1a and wtSp1b, wild-type FOXA, Sp1a and Sp1b sites, respectively; mutFOXA, mutSp1a and mutSp1b, mutated FOXA, Sp1a and Sp1b sites, respectively; *mut*FOXA/Sp1, double mutated FOXA and Sp1 sites.

* Corresponding author. Address: Department of Pharmaco-Biology, University of Bari, Via Orabona 4, 70125 Bari, Italy. Fax: +39 (0)805442770 (F. Palmieri).

E-mail address: fpalm@farmbiol.uniba.it (F. Palmieri).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.11.126

into the pGL3 basic-LUC vector (Promega) upstream of the LUC gene-coding sequence. In order to generate *mut*FOXA, *mut*Sp1a, *mut*Sp1b and *mut*FOXA/Sp1a in the C5 DNA fragment, wtDNA was mutagenized using the QuikChange® Site-Directed Mutagenesis Kit (Stratagene) and the following complementary forward and reverse primer sets: 5′-CTCAGAGGACCCATGGAAGT-3′ (mutFOXA), 5′-CCAAACCCCGAACATGCGGC-3′ (mutSp1a) or 5′-CTCGTGCCCCG-AACACCCAA-3′ (mutSp1b). For heterologous promoter expression, a 3-fold repeat wtFOXA, mutFOXA, wtSp1a, mutSp1a, wtSp1b or mutSp1b site was cloned into the pGL3 promoter-LUC vector (Promega) upstream of the SV40 basal promoter. The sequences of all constructs were verified by DNA sequencing. The FOXA2 and Sp1 expression vectors (pcDNA3-FOXA2 and pcDNA3-Sp1, respectively) were obtained by cloning the human FOXA2 (Accession No. NM_021784) or Sp1 (Accession No. NM_138473) cDNA into the pcDNA3 vector (Invitrogen).

2.2. Cell culture, RNA interference and transient transfection

HepG2 and HEK293 cells (Sigma) were maintained in high glucose DMEM (Dulbecco's modified Eagle's medium) containing 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 U penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO_2; C9 rat hepatocytes (Sigma) in Ham's F12 containing 10% (v/v) heat-inactivated fetal calf serum and 2 mM L-glutamine at 37 °C in 5% CO_2; and SK-N-SH cells (ICLC, Interlab Cell Line Collection) in RPMI 1640 medium (Roswell Park Memorial Institute). Transient transfection was performed as described [12] using 0.5 μg of each construct reported above, and 10 ng of pRL-CMV (Promega) to normalize the extent of transfection [13]. In RNA interference experiments, the specific pre-designed siRNA targeting human FOXA2 (s6691, Ambion) or human Sp1 (s13319, Ambion) was transfected in HepG2 cells or C9 rat hepatocytes using siPORT™ NeoFX™ Transfection Agent (Ambion). A siRNA (Catalog No. C6A-0126, Ambion) with no significant similarity to human, mouse, or rat gene sequences was used as negative control. For overexpression, HepG2 cells or C9 rat hepatocytes were transfected with pcDNA3-FOXA2 or pcDNA3-Sp1 vector, and HEK293 and SK-N-SH cells with pcDNA3-FOXA2 vector. Forty-eight hours after transfection, cells were assayed for LUC activity using the Dual-Luciferase® Reporter Assay System (Promega).

2.3. Chromatin immunoprecipitation

ChIP experiments were performed as previously reported [14]. Briefly, 2×10^7 of HepG2, HEK293 and SK-N-SH cells were fixed by 1% formaldehyde at 37 °C for 10 min; afterwards, the cells were lysed and sheared by sonication in a 1% SDS lysis buffer to generate cellular chromatin fragments of 400–500 bp. The chromatin was immunoprecipitated for 14–16 h at 4 °C using specific antibodies to FOXA2 (Santa Cruz Biotechnology, Catalog No. sc-6554X) and to Sp1 (Santa Cruz Biotechnology, Catalog No. sc-59X). After reverse cross-linking, chromatin immunoprecipitates were purified, then 2 μl of each sample were analyzed by PCR (35 cycles) using a forward primer (5′-GGACAGGGACCTGTGTGTTGTAC-3′) and a reverse primer (5′-AGTTGGCACGGTAGGGCTTC-3′) suitable to amplify the −410/−141 bp region of the CAC gene promoter.

2.4. Other methods

Electrophoretic mobility shift assays (EMSA) were performed as described [15]. The double-stranded oligonucleotide probes were 5′-end labeled using T4 polynucleotide kinase and [γ^{32}P]-ATP at 37 °C for 30 min. The gels were dried and images acquired by phosphoimager (Bio-Rad). Total RNA was extracted from 1×10^6 cells, and reverse transcription was performed as reported [16]. Real-time PCR was carried out as described previously [13]. Assay-on-demand for human CAC (Catalog No. Hs01088810_g1), Sp1 (Catalog No. Hs00412720_m1), FOXA1 (Catalog No. Hs00293689_s1), FOXA2 (Catalog No. Hs00232764_m1), FOXA3 (Catalog No. Hs00270130_m1), and actin (Catalog No. Hs00357333_g1) were purchased from Applied Biosystems. All transcript levels were normalized against the β-actin expression levels. For Western blot analysis, proteins were electroblotted onto nitrocellulose membranes (Bio-Rad) and subsequently treated with anti-CAC (specific for total rat mitochondrial CAC) [17], the β-actin (BioLegend) antibodies. The immunoreaction was detected by the Immobilon Western ECL system (Millipore).

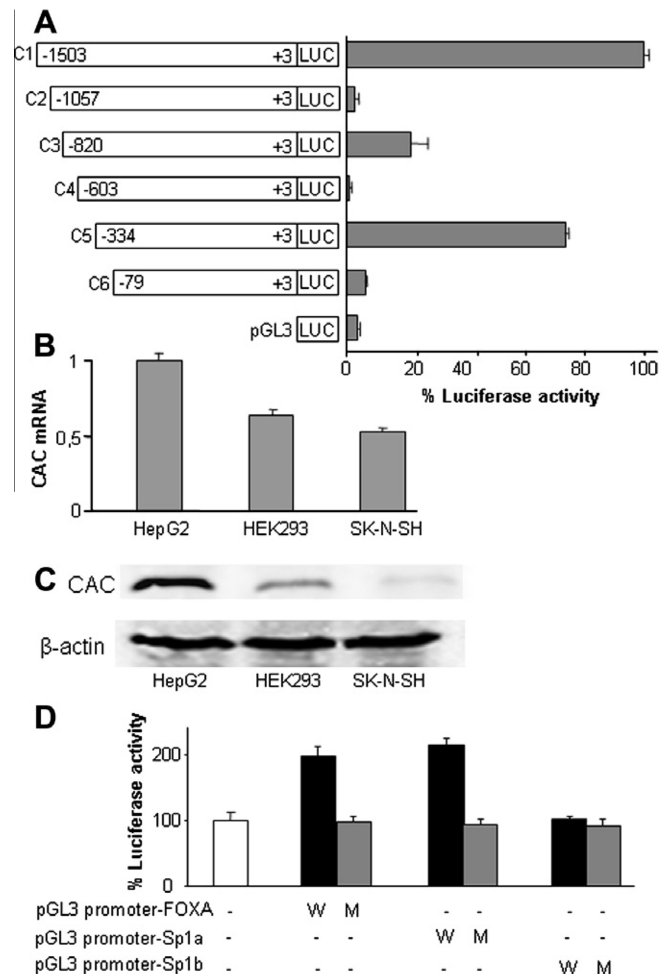

Fig. 1. Identification of the activation domain in the CAC proximal promoter containing active FOXA and Sp1a sites, and CAC expression in different cell lines. (A) Deletion analysis of the 5′-flanking region of the human CAC gene. The deletion fragments named C1–C6 were cloned into the pGL3 basic-LUC vector and tested for expression activity in transfected HepG2 cells. pGL3 indicates the pGL3 basic-LUC vector alone. Numbering indicates the extent of fragments, while gray bars indicate LUC activity. The values were set relative to construct C1. Means ± SD of six duplicate independent experiments are shown. (B and C) CAC expression in different cell lines. In (B) total RNA from HepG2, HEK293 and SK-N-SH cells was used to quantify CAC mRNA by real-time PCR. Means ± SD of three duplicate independent experiments are shown. In (C) CAC and β-actin of each cell line were immunodecorated with specific antibodies. (D) FOXA- or Sp1a-driven gene reporter activity. HepG2 cells, transfected with pGL3 promoter-LUC vector containing 3-fold repeated wtFOXA, mutFOXA, wtSp1a, mutSp1a, wtSp1b, mutSp1b or none were assayed for LUC expression activity. Means ± SD of three duplicate independent experiments are shown. Only the differences between sample of wtFOXA or wtSp1a (black bars) and control (white bar) were significant ($P < 0.05$, one-way ANOVA).

3. Results

3.1. Identification of an activation domain within the proximal promoter of the CAC gene

To investigate CAC promoter activity, deletion mutants of CAC promoter-driven LUC reporter gene constructs and control pGL3 basic-LUC vector were transfected into HepG2 cells, and the relative LUC expression activity of each reporter gene construct was measured (Fig. 1A). Our serial promoter deletions from −1503 to +3 bp led to the identification of an activation domain between −334 and −80 bp (construct C5 in Fig. 1A) in the proximal promoter of the human CAC gene. Thus in HepG2 cells construct C5 conferred 74% LUC transgene activity of that measured with the entire −1503/+3 bp region. Notably, when HEK293 or SK-N-SH cells were transfected with construct C5 a much lower LUC activity was obtained, i.e. 17% in HEK293 and 14% in SK-N-SH cells (data not shown). This finding parallels the observation that HepG2 cells exhibited a higher level of CAC gene expression in terms of both CAC transcript (Fig. 1B) and CAC protein (Fig. 1C) than HEK293 and SK-N-SH cells.

3.2. The activation domain of the CAC gene proximal promoter contains cis-elements for FOXA and Sp1

A computer search of a vertebrate transfactor database (http://www.cbrc.jp/research/db/TFSEARCH.html) with the −5000/+3 bp region of the CAC gene revealed the presence in the proximal promoter of cis-elements for FOXA at −300/−290 and Sp1 at −237/−227 bp and at −115/−105 bp, named Sp1a and Sp1b, respectively. The first of these elements shares 87% identical nucleotides with the canonical FOXA-binding site [18] and the other two 94% and 91% identical nucleotides with the canonical Sp1-binding site, respectively [19]. The protein-binding activities of the CAC FOXA and Sp1 sites were investigated by EMSA experiments using nuclear extracts of HepG2 cells and a labeled DNA probe from −305 to −285 bp (FOXA), from −242 to −222 bp (Sp1a) and from −120 to −100 bp (Sp1b). No shift was obtained with the Sp1b labeled probe. On the contrary, a band shift was observed with either the FOXA or the Sp1a probe (data not shown). These bands disappeared by competition with unlabeled probe (wtFOXA or wtSp1a), whereas they were unaffected by the presence of mutated probes (mutFOXA or mutSp1a). Moreover, both bands were also no longer present in super-shifts experiments carried out by pre-incubating HepG2 nuclear extracts with an antibody directed against the transcription factor (FOXA or Sp1) under investigation. These results indicate that the FOXA and Sp1a elements present in the CAC proximal promoter are functional, whereas the Sp1b site is inactive.

3.3. FOXA2 and Sp1 are involved in the transcriptional regulation of the CAC gene in HepG2 cells

To investigate the role of FOXA and Sp1 sites in the regulation of CAC gene expression *in vivo*, we measured the LUC gene reporter activity in HepG2 cells transfected with the pGL3 promoter-LUC vector containing a 3-fold wtFOXA, wtSp1a or wtSp1b site. LUC activity was enhanced in cells transfected with the pGL3 promoter-LUC vector harboring the wtFOXA or the wtSp1a site by 97% and 115%, respectively, as compared to cells transfected with the empty vector (Fig. 1D). By contrast, no increase was observed in cells transfected with the same vector containing a 3-fold mutFOXA, mutSp1a, wtSp1b or mutSp1b site (Fig. 1D).

Moreover, we measured the LUC activity in HepG2 cells transfected with the pcDNA3 vector containing FOXA2, Sp1 or no insertion in the presence of the pGL3 basic-LUC vector harboring the C5 fragment of the CAC gene promoter. LUC activity was increased about 2-fold in cells overexpressing FOXA2 (Fig. 2A) or Sp1 (Fig. 2D) as compared to controls. In agreement with these results, FOXA2 and Sp1 overexpression also induced a parallel increase of both CAC transcript and protein levels (Fig. 2B, C and E, F, respectively). In another set of experiments, HepG2 cells were co-transfected with siRNA targeting human FOXA2 or Sp1, or control siRNA, and with the pGL3 basic-LUC vector containing the C5 fragment, and measured for the gene reporter activity (Fig. 3A and D). LUC activity was considerably diminished by FOXA2 or Sp1 silencing as compared to controls (Fig. 3A and D, respectively). Consistently, both CAC transcript and protein levels were significantly decreased by FOXA2 or Sp1 silencing as compared to those in control cells (Fig. 3B, C and E, F, respectively). It is worth mentioning that similar effects of overexpression and silencing of FOXA2 or Sp1 on LUC transgene activity were observed using rat hepatocytes instead of HepG2 cells, i.e. an about 2-fold increase and a 60–75% decrease, respectively (data not shown). All together, the above-reported results provide evidence for direct involvement of FOXA2 and Sp1 in the regulation of CAC gene expression in hepatic cells.

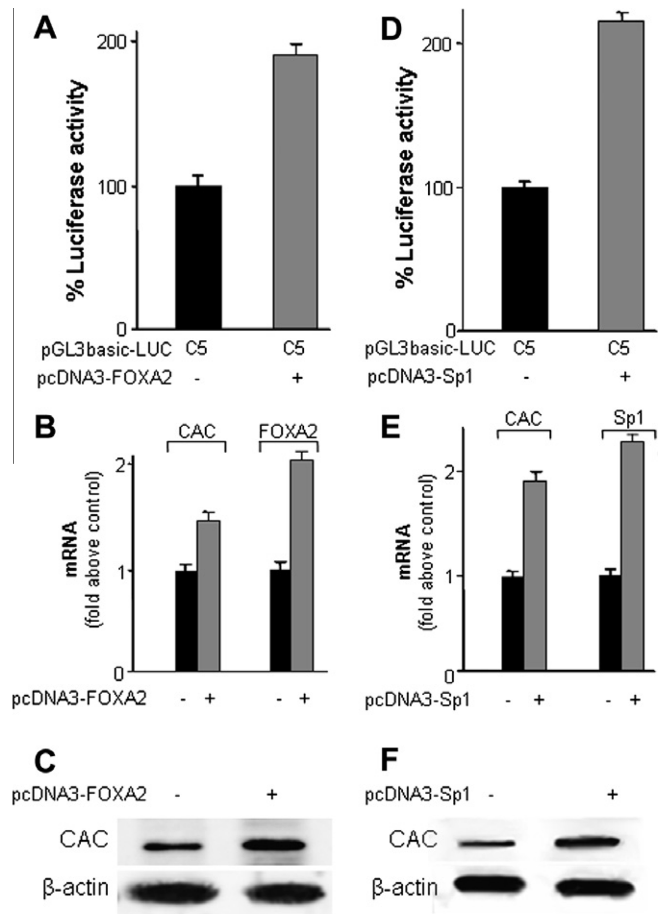

Fig. 2. Effect of FOXA2 and Sp1 overexpression on CAC gene expression. (A and D) HepG2 cells, co-transfected with the pcDNA3-FOXA2 (+) and the pcDNA3-Sp1 (+), respectively, or empty vector (−) and with pGL3 basic-LUC vector containing the C5 fragment of CAC gene promoter, were assayed for LUC activity. (B and E) Total RNA extracted from HepG2 cells transfected with the pcDNA3-FOXA2 (+) and the pcDNA3-Sp1 (+), respectively, or empty vector (−), was used to quantify FOXA2, Sp1 and CAC mRNAs. (C and F) CAC and β-actin of HepG2 cells transfected as reported in B and E, respectively, were immunodecorated with specific antibodies. Means ± SD of three duplicate independent experiments are shown; differences between samples and relative controls (set at 100%) were significant ($P < 0.05$, one-way ANOVA).

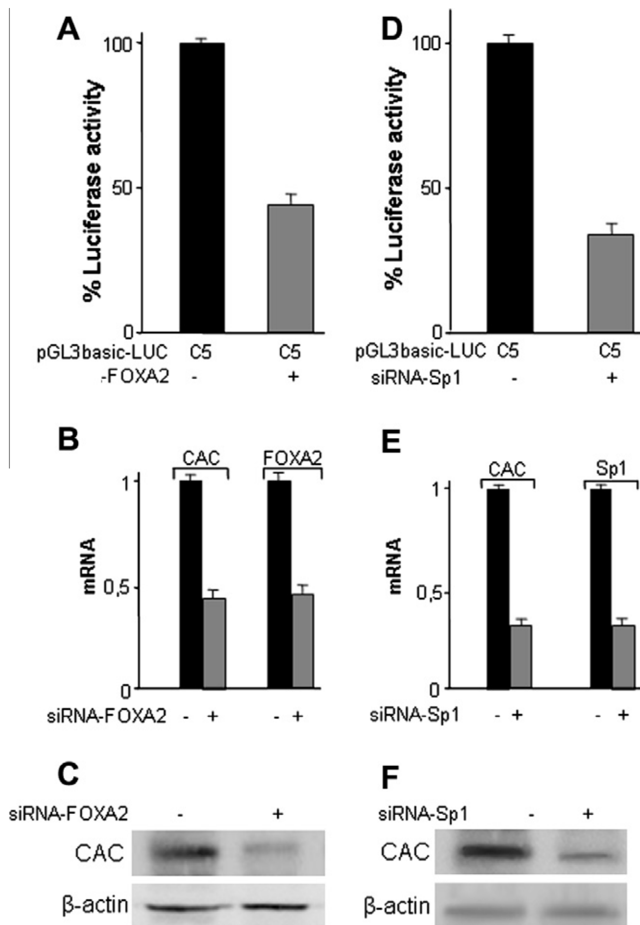

Fig. 3. Effect of FOXA2 and Sp1 silencing on CAC gene expression. (A and D) HepG2 cells, co-transfected with siRNA targeting human FOXA2 (+) and Sp1 (+), respectively, or control siRNA (−) and with pGL3 basic-LUC vector containing the C5 fragment of CAC gene promoter, were assayed for LUC activity. (B and E) Total RNA extracted from HepG2 cells, transfected with siRNA targeting human FOXA2 (+) and Sp1 (+), respectively, or control siRNA (−), was used to quantify FOXA2, Sp1 and CAC mRNAs. (C and F) CAC and β-actin of HepG2 cells transfected as reported in B and E, respectively, were immunodecorated with specific antibodies. Means ± SD of three duplicate independent experiments are shown; differences between samples and relative controls (set at 100%) were significant ($P < 0.05$, one-way ANOVA).

Given that CAC gene expression was lower in HEK293 and SK-N-SH cells than in HepG2 cells at transcript and protein levels (see Fig. 1B and C) and upon transfection with construct C5 (see above), we measured the transcript levels of FOXA1, FOXA2 and FOXA3 in HEK293 and SK-N-SH cells by real-time PCR and investigated whether the overexpression of FOXA2 in these cells enhanced CAC gene expression. It was found that FOXA1, FOXA2 and FOXA3 mRNAs were virtually absent in HEK293 and SK-N-SH cells, and the LUC gene reporter activity of these cells transfected as in Fig. 2A was increased 4.2-fold in HEK293 cells and 5.3-fold in SK-N-SH cells as compared to controls (data not shown), i.e. much more than in HepG2 cells. Therefore, in HEK293 and SK-N-SH cells the CAC gene expression is limited by the absence of FOXA transcription factors.

3.4. Effect of FOXA and Sp1 site mutations on CAC gene transcription

To further assess the role of FOXA- and Sp1-binding sites in the basal expression of CAC gene, the FOXA and each of the two Sp1 sites of the CAC proximal promoter were mutated individually or in combination within the DNA sequence of the C5 fragment. The pGL3 basic-LUC vectors containing wild-type or mutated C5 fragments were transfected into HepG2, HEK293 or SK-N-SH cells and luciferase assays were performed (Fig. 4A). Disruption of FOXA site, Sp1a site or both (but not that of Sp1b) diminished the gene reporter activity almost completely in HepG2 cells. In HEK293 and SK-N-SH cells LUC activity was also strongly decreased by mutations of the Sp1a site, whereas it was unaffected by FOXA and Sp1b mutations. These results show that both the FOXA and Sp1a sites in HepG2 cells and only the Sp1a site in HEK293 and SK-N-SH cells are important in the regulation of the CAC gene basal expression.

3.5. FOXA2 and Sp1 bind to the CAC proximal promoter

To demonstrate that FOXA2 and Sp1 bind to the CAC gene proximal promoter in intact cells, ChIP analysis was performed. FOXA2- and Sp1-specific antibodies immunoprecipitated the −410/−141 bp region of the CAC gene proximal promoter from HepG2 cells (Fig. 4B, lane FOXA2 and Sp1, respectively). The same DNA fragment was immunoprecipitated by anti-Sp1 antibodies, but not by anti-FOXA2 antibodies, from HEK293 and SK-N-SH cells (Fig. 4B), in agreement with the fact that Sp1 is present and FOXA2 is absent in these cells. No PCR product was observed without addition of antibodies (Fig. 4B, lanes No Ab). In addition, a product of the expected molecular weight was obtained when amplification was performed using total chromatin before immunoprecipitation (Fig. 4B, lanes I). These data clearly indicate that both FOXA2 and Sp1 in HepG2, and only Sp1 in HEK293 and SK-N-SH cells, are bound to the CAC proximal promoter.

4. Discussion

In the present study we have functionally analyzed the proximal promoter of the CAC gene. Preliminary *in silico* analysis of the core promoter revealed the absence of TATA box and the presence of the BRE element (cGGCGCC) at −81/−75 bp and the DPE element (AGTGAC) at −18/−13 bp. DPE allows binding of the general transcription factor, TFIID, as found in most TATA-less promoters [20,21], and BRE of TFIIB, another general transcription factor [22,23]. Furthermore, through deletion analysis of the CAC gene 5′-flanking region, we have identified an activation domain between −334 and −80 bp, which contains an active FOXA site at −300/−290 bp and an active Sp1 site at −237/−227 bp besides the previously characterized [11] PPRE site. FOXA and Sp1 sites are also present in the promoter of other mitochondrial CAC genes sequenced such as those of *Rattus norvegicus*, *Bos taurus*, *Danio rerio* and *Gallus gallus*.

The newly identified FOXA and Sp1 cis-elements of the CAC promoter act as strong enhancers in the regulation of CAC gene expression in liver. This conclusion is demonstrated by the observations that in hepatic cells (a) *in vitro* mutagenesis of FOXA or Sp1 sites almost abolishes LUC activity; (b) overexpression and silencing of either FOXA2 or Sp1 cause increase and decrease, respectively, of LUC activity and CAC mRNA and protein levels; and (c) both FOXA2 and Sp1 are bound to the CAC proximal promoter sequence. These findings demonstrate that in hepatic cells CAC gene expression is up-regulated by the interactions of the FOXA and Sp1 sites with their cognate transcriptional factors FOXA2 and Sp1. Both FOXA2 and Sp1 enable chromatin access to other transcriptional factors [24,25]. However, Sp1 is an ubiquitously expressed transcription factor, whereas FOXA2 is a liver and pancreas specific transcriptional factor [26] essential for glucose and lipid homeostasis [27]. In this respect it is interesting to note that, besides SLC25A20 encoding CAC, other genes encoding hepatic enzymes involved in metabolism during fasting and energy deprivation contain FOXA-binding sites [27–29]. Among these are

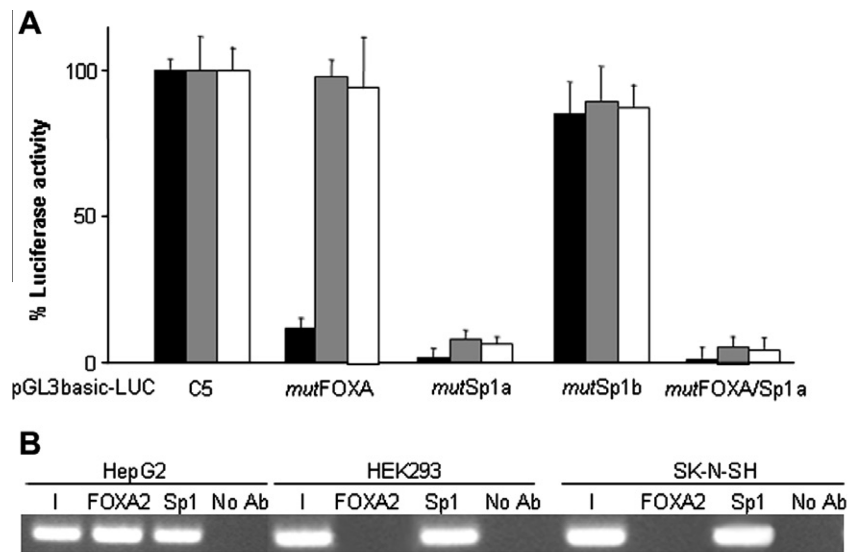

Fig. 4. Effect of mutations of FOXA, Sp1a and Sp1b CAC promoter sites on gene reporter activity, and ChIP analysis. (A) HepG2 (black bars), HEK293 (gray bars) and SK-N-SH (white bars) cells, transfected with the pGL3 basic-LUC vector containing the wild-type C5 fragment or the *mut*FOXA, *mut*Sp1a, *mut*Sp1b or *mut*FOXA/Sp1a C5 fragment of CAC gene promoter, were assayed for LUC activity. Means ± SD of three duplicate independent experiments are shown. Differences between samples and controls (set at 100%) were significant in HepG2 cells for *mut*FOXA, *mut*Sp1a and *mut*FOXA/Sp1a, and in HEK293 and SK-N-SH cells for *mut*Sp1a and *mut*FOXA/Sp1a ($P < 0.05$, one-way ANOVA). (B) Chromatin of HepG2, HEK293 and SK-N-SH cells was immunoprecipitated by anti-FOXA2 (lanes FOXA2) or by anti-Sp1 (lanes Sp1) antibodies. PCR was performed using forward and reverse primers encompassing the CAC gene promoter from −410 to −141 bp. Lanes No Ab, PCR of the precipitates without antibody; lanes I, PCR of input DNA dilutions (1/10).

enzymes of lipid catabolism, such as carnitine palmitoyltransferase 1, hydroxyacyl-CoA dehydrogenase, lipoprotein lipase, of ketogenesis, such as 3-hydroxy-3-methylglutaryl-CoA synthase 1, and of gluconeogenesis, such as phosphoenopyruvate carboxykinase and glucose-6-phosphatase. It is likely that in liver Sp1 alone is not enough to activate the transcription of the CAC gene to a level sufficient to match the requirement of this tissue with respect to fatty acid metabolism and FOXA2 is required for maximal activation of the CAC gene transcription. Our results further demonstrate that the FOXA site present in the CAC gene proximal promoter does not contribute to this gene expression in non-hepatic cells such as HEK293 or SK-N-SH cells. This conclusion is substantiated by the experimental observations reported in this study and in particular by the lack of FOXA transcription factors in HEK293 and SK-N-SH cells and by the very high increase in gene reporter activity caused by FOXA2 transfection in these cells. Therefore, this study explains at the molecular level, at least in part, the differences in CAC levels between liver and other tissues [30].

Acknowledgments

This work was supported by grants from MIUR, The Center of Excellence in Genomics, Apulia Region, the Fondazione Cassa di Risparmio di Puglia, and The Italian Human ProteomeNet No. RBRN07BMCT_009.

References

[1] F. Palmieri, The mitochondrial transporter family (SLC25): physiological and pathological implications, Pflugers Arch. 447 (2004) 689–709.
[2] F. Palmieri, Diseases caused by defects of mitochondrial carriers: a review, Biochim. Biophys. Acta 1777 (2008) 564–578.
[3] C. Indiveri, A. Tonazzi, F. Palmieri, Identification and purification of the carnitine carrier from rat liver mitochondria, Biochim. Biophys. Acta 1020 (1990) 81–86.
[4] C. Indiveri, A. Tonazzi, G. Prezioso, F. Palmieri, Kinetic characterization of the reconstituted carnitine carrier from rat liver mitochondria, Biochim. Biophys. Acta 1065 (1991) 231–238.
[5] C. Indiveri, A. Tonazzi, F. Palmieri, Characterization of the unidirectional transport of carnitine catalyzed by the reconstituted carnitine carrier from rat liver mitochondria, Biochim. Biophys. Acta 1069 (1991) 110–116.
[6] C. Indiveri, V. Iacobazzi, N. Giangregorio, F. Palmieri, The mitochondrial carnitine carrier protein: cDNA cloning, primary structure and comparison with other mitochondrial transport proteins, Biochem. J. 321 (1997) 713–719.
[7] C. Indiveri, V. Iacobazzi, N. Giangregorio, F. Palmieri, Bacterial overexpression, purification, and reconstitution of the carnitine/acylcarnitine carrier from rat liver mitochondria, Biochem. Biophys. Res. Commun. 249 (1998) 589–594.
[8] L. Palmieri, F. Lasorsa, V. Iacobazzi, M. Runswick, F. Palmieri, J. Walker, Identification of the mitochondrial carnitine carrier in *Saccharomyces cerevisiae*, FEBS Lett. 462 (1999) 472–476.
[9] V. Iacobazzi, M. Naglieri, C. Stanley, R. Wanders, F. Palmieri, The structure and organization of the human carnitine/acylcarnitine translocase (CACT1) gene, Biochem. Biophys. Res. Commun. 252 (1998) 770–774.
[10] L. Viggiano, V. Iacobazzi, R. Marzella, C. Cassano, M. Rocchi, F. Palmieri, Assignment of the carnitine/acylcarnitine translocase gene (CACT) to human chromosome band 3p21.31 by in situ hybridization, Cytogenet. Cell Genet. 79 (1997) 62–63.
[11] V. Iacobazzi, P. Convertini, V. Infantino, P. Scarcia, S. Todisco, F. Palmieri, Statins, fibrates and retinoic acid upregulate mitochondrial acylcarnitine carrier gene expression, Biochem. Biophys. Res. Commun. 388 (2009) 643–647.
[12] V. Iacobazzi, V. Infantino, P. Costanzo, P. Izzo, F. Palmieri, Functional analysis of the promoter of the mitochondrial phosphate carrier human gene: identification of activator and repressor elements and their transcription factors, Biochem. J. 391 (2005) 613–621.
[13] V. Iacobazzi, V. Infantino, F. Palmieri, Epigenetic mechanisms and Sp1 regulate mitochondrial citrate carrier gene expression, Biochem. Biophys. Res. Commun. 376 (2008) 15–20.
[14] T. Pollicino, L. Belloni, G. Raffa, N. Pediconi, G. Squadrito, G. Raimondo, M. Levrero, Hepatitis B virus replication is regulated by the acetylation status of hepatitis B virus cccDNA-bound H3 and H4 histones, Gastroenterology 130 (2006) 823–837.
[15] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbour Laboratory, New York, 1989.
[16] V. Infantino, V. Iacobazzi, F. De Santis, M. Mastrapasqua, F. Palmieri, Transcription of the mitochondrial citrate carrier gene: role of SREBP-1, upregulation by insulin and downregulation by PUFA, Biochem. Biophys. Res. Commun. 356 (2007) 249–254.
[17] M. Di Noia, S. Van Driesche, F. Palmieri, L. Yang, S. Quan, A. Goodman, N. Abraham, Heme oxygenase-1 enhances renal mitochondrial transport carriers and cytochrome *C* oxidase activity in experimental diabetes, J. Biol. Chem. 281 (2006) 15687–15693.
[18] D. Overdier, A. Porcella, R. Costa, The DNA-binding specificity of the hepatocyte nuclear factor 3/forkhead domain is influenced by amino-acid residues adjacent to the recognition helix, Mol. Cell. Biol. 14 (1994) 2755–2766.

[19] M. Briggs, J. Kadonaga, S. Bell, R. Tjian, Purification and biochemical characterization of the promoter-specific transcription factor, Sp1, Science 234 (1986) 47–52.
[20] Q. Yan, W. Liu, J. Qin, J. Liu, H. Chen, X. Huang, L. Chen, S. Sun, M. Deng, L. Gong, Y. Li, L. Zhang, Y. Liu, H. Feng, Y. Xiao, D. Li, Protein phosphatase-1 modulates the function of Pax-6, a transcription factor controlling brain and eye development, J. Biol. Chem. 282 (2007) 13954–13965.
[21] T. Burke, J. Kadonaga, Drosophila TFIID binds to a conserved downstream basal promoter element that is present in many TATA-box-deficient promoters, Genes Dev. 10 (1996) 711–724.
[22] T. Lagrange, A. Kapanidis, H. Tang, D. Reinberg, R. Ebright, New core promoter element in RNA polymerase II-dependent transcription: sequence-specific DNA binding by transcription factor IIB, Genes Dev. 12 (1998) 34–44.
[23] F. Tsai, P. Sigler, Structural basis of preinitiation complex assembly on human pol II promoters, EMBO J. 19 (2000) 25–36.
[24] J. Friedman, K. Kaestner, The Foxa family of transcription factors in development and metabolism, Cell. Mol. Life Sci. 63 (2006) 2317–2328.
[25] L. Li, J. Davie, The role of Sp1 and Sp3 in normal and cancer cell biology, Ann. Anat. 192 (2010) 275–283.
[26] K. Kaestner, H. Hiemisch, B. Luckow, G. Schütz, The HNF-3 gene family of transcription factors in mice: gene structure, cDNA sequence, and mRNA distribution, Genomics 20 (1994) 377–385.
[27] C. Wolfrum, E. Asilmaz, E. Luca, J. Friedman, M. Stoffel, Foxa2 regulates lipid metabolism and ketogenesis in the liver during fasting and in diabetes, Nature 432 (2004) 1027–1032.
[28] J. Le Lay, K. Kaestner, The Fox genes in the liver: from organogenesis to functional integration, Physiol. Rev. 90 (2010) 1–22.
[29] C. Wolfrum, D. Shih, S. Kuwajima, A. Norris, C. Kahn, M. Stoffel, Role of Foxa-2 in adipocyte metabolism and differentiation, J. Clin. Invest. 112 (2003) 345–356.
[30] M. Huizing, W. Ruitenbeek, L.P. van den Heuvel, V. Dolce, V. Iacobazzi, J. Smeitink, F. Palmieri, F.J.M. Trijbels, Human mitochondrial transmembrane metabolite carriers: tissue distribution and its implication for mitochondrial disorders, J. Bioenerg. Biomembr. 30 (1998) 277–284.