Transcriptional regulation of lipid biosynthetic genes by FBI-1, SREBP-1a, and Sp1

Hyejin Park

Department of Medical Science

The Graduates School, Yonsei University

Transcriptional regulation of lipid biosynthetic genes by FBI-1, SREBP-1a, and Sp1

Hyejin Park

Department of Medical Science

The Graduates School, Yonsei University

Transcriptional regulation of lipid biosynthetic genes by FBI-1, SREBP-1a, and Sp1

Directed by professor Man-Wook Hur

A master's thesis submitted to the Department of Medical Science, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science

Hyejin Park

December, 2005

This certifies that the master's thesis of

Hyejin Park is approved

Thesis Supervisor : Man-Wook Hur

Kyung-Sup Kim

Soon-Jung Park

The Graduate School

Yonsei University

December, 2005

ACKNOWLEDGEMENT

I would like to express my gratitude to my supervisor Prof. Man-Wook Hur for his excellent guidance and valuable advice throughout my Master's degree course. During this course, his generous personality and encouragement gave me support to finish my research successfully. I wish to express my appreciation to the members of my dissertation committee, Dr. Kyung-Sup Kim and Dr. Soon-Jung Park for their criticism and thoughtful suggestion. I would like to thank to Dr. Yong-Ho Ahn, Dr. Kun-Hong Kim, Dr. Ho-Geun Yoon, and Dr. Ha-Il Kim who provided me best research environment and useful knowledge. I would like to thank all members in the department of Biochemistry and Molecular biology. I especially wish to thank Hee-Eun, Myung-Hwa, Min-Nyung, Jung-Yoon, Bu-Nam, Won-Il, and Mi-young. It was a great pleasure to work with them and many inspiring discussions with them were encouraging and helpful to my research.

Most of all, I thank my family for their role for my education. I thank my parents for teaching me a value of education, instilling me a higher education and supporting me during this course. I am deeply grateful to my mother-in-law and father-in-law who supported me and took care of my family instead of me during this course. I cannot thank enough my beloved husband Jung-il for his consistent support, encouragement and love throughout the past two years. And my son, Junhyung also give me great pleasure during my Master's degree course. No research today can be accomplished without supporting my family and fellows.

TABLE OF CONTENTS

LIST OF FIGURES

Abstract

Transcriptional regulation of lipid biosynthetic genes by FBI-1, SREBP-1a, and Sp1

Hyejin Park

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor **Man-Wook Hur**)

SREBP-1a and Sp1 are major transcription regulators of many genes related with cholesterol and fatty acid biosynthesis. SREBP-1a and Sp1 activate transcription synergistically by binding to SRE (or E-box) and GC box, respectively. The protein interacting either with SREBP-1a or Sp1 can be important in the transcriptional regulation of the genes involved in lipid biosynthesis. We investigated whether FBI-1 could regulate transcription of the lipid biosynthetic genes such as, fatty acid synthase (FAS), ATP-citrate lyase (ACL), and LDL receptor (LDLR). We observed FBI-1 regulated transcription

by Sp1 and SREBP-1a in lipogenic genes via transient transfection assay in SL2 cells and CV-1 cells. FBI-1 repressed transcription of both ACL promoter and artificial promoter with SRE and GC box. However, on FAS and LDLR promoter, FBI-1 activated transcription. GST fusion protein pulldown assay show that the POZ domain of FBI-1 directly interacts with Sp1ZFDBD and SREBP-1a. Electrophoretic mobility shift assays showed that the POZ domain of FBI-1 and FBI-1ZFDBD inhibit binding of Sp1 to the GC boxes of lipid biosynthetic genes. Taken together, we found that molecular interaction among cis-regulatory elements, SREBP-1a, Sp1, and FBI-1 is important in transcriptional regulation of major genes of lipid metabolism. FBI-1 acts as transcription activator or repressor depending on the promoter context and cellular context.

Key words: transcriptional regulation, fatty acid synthase, ATP-citrate lyase, LDL receptor, FBI-1, SREBP-1a, Sp1

Transcriptional regulation of lipid biosynthetic genes by FBI-1, SREBP-1a, and Sp1

Hyejin Park

Department of Medical Science

The Graduate School, Yonsei University

(Directed by Professor Man-Wook Hur)

I. Introduction

FBI-1 (factor that binds to the inducer of short transcripts of human immunodeficiency virus-1; also called Pokemon) is a BTB/POZ (broad complex, Tramtrack, and bric-a-brac/poxvirus and zinc finger) domain protein that has been shown to modulate HIV-1 Tat trans-activation and to repress transcription of some cellular genes.[1-3] Molecular cloning revealed that FBI-1 is a 61.5-kDa protein with a POZ domain at the N terminus and Krüppel-type four C2H2 zinc fingers at the C terminus and Western blotting showed that FBI-1 is a 75 kDa protein, suggesting possible posttranslational modification.

The POZ domain is an evolutionarily conserved protein-protein interaction motif found in many transcription factors, oncogenic proteins, ion channel proteins, and some actin-associated proteins.[4] The POZ domain genes, which were first identified in a set of Drosophila and poxvirus, have since been found in organisms ranging from yeast to humans. The POZ domain proteins are strongly involved in many critical cellular processes such as development, oncogenesis, apoptosis, ion channel activity, and transcription.[5-9] The domain has been shown to form homomeric and heteromeric associations with other POZ domains. Given the large number of POZ domain proteins in the higher eukaryotes, this domain is likely to regulate many more critical biological processes by a combinatorial diversity of complexes.

The most striking and common property of the POZ domain transcription factors is their ability to repress transcription via their POZ domains, as reported for PLZF (promyelocytic leukemia zinc finger transcription factor), Bcl-6 (B cell lymphoma transcription factor-6), FBI-1, MIZF, and Kaiso.[10-14] The ability of the domain to interact with other key regulatory proteins such as corepressor proteins and other transcription factors such as GATA 1, 2[15-16] (PLZF), Jun proteins[17] (Bcl-6), Sp1[12] (FBI-1), CTCF[18] (kaiso) appears to be important for repression. In particular, the POZ domains of human PLZF and Bcl-6 were shown to interact with SMRT/N-CoR, mSin3A, B-CoR, and histone deacetylase.[19-21] By referring to the molecular mechanisms proposed for the

nuclear receptors, chromatin compaction by histone deacetylase complex recruited by the POZ domain was suggested to repress transcription.[22-24]

FBI-1 has been shown to repress transcription of some extracellular matrix genes[25] (these authors refer to FBI-1 as hcKroxβ) and of the *ADH5/FDH* promoter. In the latter case, the proposed mechanism of repression is the interference of Sp1 binding to the GC-box by FBI-1 bound to upstream FBI-1 binding site located adjacent [12] The mouse counterpart of FBI-1, LRF (leukemia/lymphoma-related factor), is co-immunoprecipitated and co-localized with Bcl-6.[43] The rat homolog of FBI-1, OCZF (osteotclast-derived zinc finger), is a transcription repressor and is involved in osteoclastogenesis.[44] FBI-1 represses the transcription of the tumor suppressor gene $p19^{ARF}$ through direct binding[26] (these authors refer to FBI-1 as pokemon). $p19^{ARF}$ is aberrantly expressed in knock out mice of coding gene of FBI-1, *Zbtb7* null MEFs. Conversely, FBI-1 overexpression leads to overt oncogenic transformation. SAGE (serial analysis of gene expression) analysis shows that the expression of FBI-1 is increased in cancer tissues (available at www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Hs&CID=465623). These data suggest that FBI-1 or pokemon may be proto oncogene. Recently, FBI-1 was identified as a possible active participant in the adipocyte differentiation process in both murine and human and suggested that FBI-1 may be promoting growth arrest and terminal differentiation during adipocyte

differentiation.[27] Most recently, the POZ domain of FBI-1 was identified to interact with the subunit of the p65 subunit of NF-κB and IκB.[45]

SREBPs (sterol-regulatory-element-binding proteins) are important regulators of mammalian lipid metabolism.[28] The SREBP-1 gene encodes two almost identical proteins, SREBP-1a and SREBP-1c, which are expressed from overlapping mRNAs.[29] A separate gene encodes a single mRNA and protein for SREBP-2 (Fig. 1A). The two SREBP-1 proteins are identical except at the extreme N-termini. SREBP-1a and 1c have different N-termini but are identical from the common second exon onwards. SREBP-1a has 28 unique amino acids from its first exon and SREBP-1c has only four (in addition to the initiator methionine residue). The activation domain of the SREBP-1 proteins is located in this N-terminal region. SREBP-1a has a potent activation domain, which interacts efficiently with co-activators such as p300/CREB-binding protein (where CREB stands for cAMP-response-element-binding protein) and the mammalian mediator complex.[30] The shorter N-terminus of SREBP-1c is a weak activation domain and interacts weakly with the same transcriptional co-activators. SREBPs directly activate the expression of more than 30 genes dedicated to the synthesis and uptake of cholesterol, fatty acids, triglycerides, and phospholipids, as well as the NADPH cofactor required to synthesize these molecules. SREBP-1a is a potent activator of all SREBP-responsive genes, including those that mediate the synthesis of cholesterol, fatty acids, and

triglycerides. SREBP-1c preferentially enhances transcription of genes required for fatty acid synthesis but not cholesterol synthesis. Like SREBP-1a, SREBP-2 has preferentially activates cholesterol synthesis.

SREBPs are unique membrane-bound transcription factors important in cholesterol and lipid metabolism. SREBPs are structurally composed of four domains with two membrane-spanning regions.[31-32] Both amino- and carboxyl-terminal portions of the proteins project into the cytoplasm. The N-terminal domain of approximately 480 amino acids is a basic-helix-loop-helix leucine zipper (bHLHZip), and it is a functionally active portion as a transcription factor. C-terminal portion, regulatory domain, forms a complex with the Trp-Asp (WD) repeat of SREBP cleavage activating protein (SCAP). When cells are cultured in medium containing sufficient cholesterol the SREBPs are sequestered in the ER by two membrane spanning domains. When cellular sterol levels drop, the amino-terminal portions of the SREBPs are released from the ER by two ordered proteolytic events. The liberated mature SREBPs then enter the nucleus where they activate transcription of various genes in the fatty acid and cholesterol metabolic pathways such as LDL receptor, ATP-citrate lyase, Fatty acid synthase (Fig. 1B).[33-35]

Sp1 (specificity protein 1) is a sequence-specific transcription factor that binds GC box and activates a wide range of viral and cellular genes.[36-37] Sp1 belongs to the Krüppel-like C2 H2-type zinc finger superfamily. The protein is

composed of several modules, N terminus inhibitory domain (aa 1-82), serine/threonine-rich domains (aa 87-143; aa 243-350), glutamine-rich domains (aa 138-232; aa 51-500) important in transcription activation, zinc finger DNA binding domain (aa 622-720), and C terminus D domain (aa 721-788). Because almost all genes involved in cholesterol and fat metabolism contain Sp1-binding GC box in their promoters and Sp1 often plays a critical role in the transcription activation of many genes, there must be a mechanism regulating the activity of Sp1 and thus the expression of cholesterol and lipid metabolism.

In SREBP target gene promoters, SRE, NF-Y or Sp1 binding site is frequently found and NF-Y and Sp1 directly bind to SREBP.[28, 38] Through the binding, NF-Y and Sp1 seem involved in recruitment of basic transcription machinery. SREBP-1 and Sp1 activate transcription synergistically by binding to SRE (or E-box) and GC box, respectively.[39-40] The protein interacting either with SREBP-1 or Sp1 can be important in the transcriptional regulation of the genes involved in lipid biosynthesis.

Fatty acid synthase (FAS), one of the main lipogenic enzymes, converts dietary calories into a storage form of energy. The transcription factors, specificity proteins 1 and 3 (Sp1 and Sp3), nuclear factor Y (NF-Y), upstream stimulatory factor (USF), and sterol regulatory element binding protein-1 (SREBP-1) have cognate binding sites on the promoter of the FAS gene. It is demonstrated that SREBP and Sp1 synergistically activate the FAS promoter in

Drosophila SL2 cells, which lack endogenous Sp1. It is demonstrated that the SREBP and Sp1 proteins interact together in the absence of DNA through a small region in Sp1 called the buttonhead domain (btd) and the DNA binding/dimerization domain of SREBP.[46]

ATP-citrate lyase (EC 4.1.3.8, ACL) is a cytosolic enzyme which catalyzes the formation of acetyl CoA and oxaloacetate from citrate and CoA. ACL, an enzyme catalyzing the first step in biosynthesis of fatty acids, is induced during the lipogenesis and cholesterologenesis. It was reported that the change in the expression of Sp1 family proteins play an important role in activation of the ACL promoter by glucose.[35] ACL plays an important role in fatty acid and cholesterol biosynthesis and is highly controlled by diet at the transcription level.

Cellular sterol balance is efficiently maintained by a feedback mechanism. When cellular sterol levels are low genes involved in cholesterol synthesis and uptake are activated. When sufficient cholesterol is amassed they are efficiently shut off. The protein involved in cholesterol uptake is a cell surface receptor that rapidly internalizes cholesterol-rich low density lipoprotein (LDL) particles from outside the cell. Transcription from the LDL receptor gene is efficiently regulated by cholesterol, and its simple promoter is composed of three related sequence elements referred to as repeats 1-3 located upstream of a TATA box-like element. Repeats 1 and 3 bind the universal transcription factor Sp1.

However, repeat 2 binds SREBPs. The SRE-1 functions as a conditionally positive element activating expression only when sterol levels are low. It cannot function efficiently by itself even when present in multiple copies. In the native LDL receptor promoter the adjacent binding site for transcription factor Sp1 located in repeat 3 is also required.[47]

Because FBI-1 is the gene most abundantly expressed in the process of terminal differentiation of pre-adipocytes,[27] and FBI-1 and Sp1 interact with each other and repress the transcription activation by Sp1[12], we investigated and found that SREBP and Sp1 are involved in protein-protein interactions with FBI-1. We also determined that these interactions are important in the transcriptional regulation of lipid biosynthetic genes. This may have significant implication in understanding how FBI-1 can play a part in lipid biosynthesis.

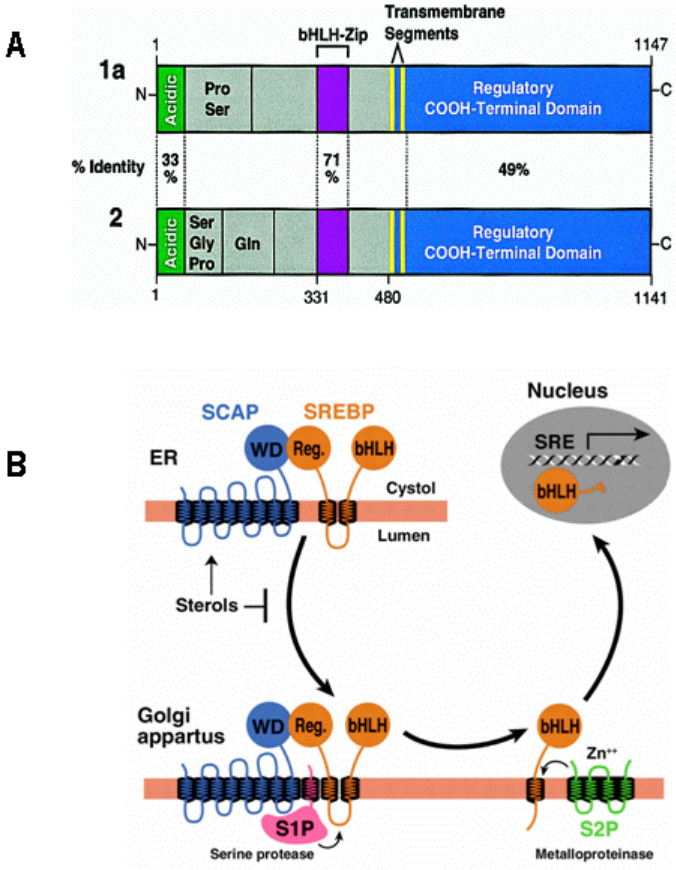

Figure 1. Domain structures of human SREBP-1a and SREBP-2 and model for the sterol-mediated proteolytic release of SREBPs from membranes. (A) The sequence of SREBP-1c (not shown) is identical to that of SREBP-1a except for a shortened NH_2-terminal acidic domain (24 amino acids in SREBP-1c versus 42 amino acids in SREBP-1a).[31] (B) Model for the sterol-mediated proteolytic release of SREBPs from membranes. SCAP is a sensor of sterols and an escort of SREBPs. When cells are depleted of sterols, SCAP transports SREBPs from the ER to the Golgi apparatus, where two proteases, Site-1 protease (S1P) and Site-2 protease (S2P), act sequentially to cut and release the NH_2-terminal bHLH-Zip domain from the membrane. The bHLH-Zip domain enters the nucleus and binds to a sterol response element (SRE) in the enhancer/promoter region of target genes, activating their transcription.[47]

II. Materials and Methods

1. Plasmids

Mammalian and insect cell expression vector for human Sp1, pCMV-Sp1 and pPacSp1 were kind gifts from Dr. Robert Tjian. pcDNA3-FBI-1 and pPac-FBI-1 plasmids were reported by elsewhere.[41] pG5-1x (SRE+GC)-Luc and pG5-2x (SRE+GC)-Luc plasmids were prepared by cloning one or two copies of annealed oligonucleotides composed of SRE and GC box (Top strand, 5'-aattcAAAATCACCCCACTGCAATTCGATCGGGGCGGGGCGAG-3'; antisense strand, 5'-aattcTCGCCCCGCCCCGATCGAATTGCAGTGGGGT-GATTTTG-3') into pG5 luciferase/*EcoR*1 (Promega, Madison, WI, USA).

Fatty acid synthase promoter-luciferase reporter gene, FAS 1 (FAS-150/+65), FAS 2 (FAS-135/+65), FAS 3 (FAS-150/-73 TATA), LDLR-tk-Luc and pPac-SREBP-1a insect cell expression vector were kindly provided by Dr. Timothy F. Osborne of University of California, Irvine. ATP-citrate lyase promoter-luciferase reporter gene, pCMV-SREBP-1a, pQE30-SREBP-1a generous gifts from Dr. Kyung-Sup Kim. pGEX4T3-POZ_{FBI-1} and pQE30-POZ_{FBI-1} plasmids were reported elsewhere.[12] To make recombinant Sp1 zinc finger protein, cDNA fragment encoding ZF DNA binding region from amino acids 624 to 718 of Sp1 was cloned into pTrcHisB expression vector/Kpn1-EcoR1 (Invitrogen, Carlsbad, CA, USA). Two oligonucleotides (5' PCR primer:

#484, Sp1-622-Kpn1: 5'-ACGTGGTACCAAACAGCATATTTGCCAC-3' 3' PCR primer: #563, Sp1ZF3': 5'-GATCTCTAGAGAATTCCTAACTCAGAG-CTACACCTGGGCCTC-3') was used to PCR the ZFDBD region out of Sp1 cDNA using following amplification condition: 94℃ denaturation 5 min, 30 cycles of amplification , 94 ℃ 30 sec, 55 ℃ 30 sec, 72 ℃, 30 sec, extension 72℃ 10 min.). PCR product was digested with Kpn1-EcoR1 and cloned into pTrcHisB.

2. RT-PCR of FBI-1 mRNA in C57Bl/6J mice and *ob/ob* C57Bl/6J mice

Total RNA was isolated from abdominal adipose tissues using TRIzol reagent (Invitrogen). cDNAs were synthesized using 1 mg total RNA, random hexamer (10 pmol), and superscript reverse transcriptase II (200 units) in 20 ㎕ using reverse transcription kit (Invitrogen). PCR were performed by using following amplification condition: 94 ℃ denaturation 5 min, 35 cycles of amplification reaction, 94 ℃ 1 min., 60 ℃ 45 sec, 72 ℃ 1.5 min, final extension reaction at 72 ℃ 10 min.

3. Cell culture and Transient transfection

CV-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) supplemented with 10% fetal bovine serum (FBS) (Invitrogen). CV-1 cells were seeded onto 6-well plates and grown for 24 hrs before

transfection. The cells were transfected with 1.2 μg of plasmid DNA mixture by using Lipofectamine Plus reagent (Invitrogen) according to the manufacturer's protocols. Briefly, the plasmid DNA and 3 $\mu\ell$ of PLUS reagent were mixed in 100 $\mu\ell$ Opti-MEM (Gibco BRL) and then added 100 $\mu\ell$ of containing 3 $\mu\ell$ of Lipofectamine reagent were added. The cells were washed with PBS and supplied with 800 $\mu\ell$ of serum free Opti-MEM. After 15 min of incubation, Lipofectamine-DNA mixtures were added into wells. The cells were transfected for 3 hrs with the plasmid, then grown in DMEM supplemented with 10% FBS. The cells were harvested 48 hrs after transfection and lysed in 100 $\mu\ell$ of reporter lysis buffer (Promega), and cell debris was removed by centrifugation and the supernatant was collected. Luciferase assays were conducted with 5 $\mu\ell$ of cell extracts and 50 $\mu\ell$ of luciferase assay reagent (Promega). Luciferase activity was normalized by β-galactosidase activity.

Drosophila SL2 cells were cultured in Schneider’s Drosophila medium (GIBCO BRL) supplemented with 10% fetal bovine serum. SL2 cells were seeded onto 6-well plates and grown for 12 hrs before transfection in Schneider’s Drosophila medium without FBS. The cells were transfected with 2.4 μg of plasmid DNA by using Cellfectin reagent (Invitrogen) according to the manufacturer's protocols. Briefly, the plasmid DNA and 3 $\mu\ell$ of Cellfectin reagent were mixed in 100 $\mu\ell$ Schneider’s Drosophila medium with FBS. After 45 min of incubation, Lipofectamine-DNA mixtures were added into

wells. The cells were transfected for 24 hrs with the plasmid, then grown in Schneider's Drosophila medium supplemented with 10% FBS. The cells were harvested 48 hrs after transfection and lysed in 100 ㎕ of reporter lysis buffer (Promega). Luciferase activities were measured using the Luciferase Assay reagent. Luciferase activity was normalized by β-galactosidase activity.

4. Preparation of recombinant His-Sp1ZF, His-SREBP-1a, His-POZ_{FBI-1}, GST-POZ_{FBI-1}, GST-FBI-1ZFDBD proteins in *E. coli*

To prepare bacterially overexpressed recombinant fusion proteins, pGEX4T3-POZ_{FBI-1}, pGEX4T3-FBI-1ZFDBD, pQE30-POZ_{FBI-1}, pQE30-SREBP-1a, pTrcHisB-Sp1ZFDBD plasmids were transformed into *E. coli* (BL21-DE3). To purify proteins from bacteria transformed by pGEX4T series expression plasmid vectors, bacteria were grown in 200 ml of LB broth to A_{600}=0.7 at 37 ℃, and induced with 0.1 mM of IPTG for overnight at 23 ℃. Bacteria were collected by centrifugation and lysed with cold lysis buffer (1x PBS, 2 mM EDTA, 1 mM PMSF, 0.2 mg/ml lysozyme). Bacteria were disrupted by sonication (amplitude 50 %, cycle 0.5, 30 sec.; Fisher Scientific, USA). Disrupted mixture was centrifuged at 12,000 rpm at 4 ℃ and soluble fractions of lysate were incubated with 2 ml of GSH-agarose resin (Peptron, Taejeon, Korea) for 2 hrs at 4 ℃. After washing, bound proteins were eluted

with 2 ml of elution buffer (15 mM GSH, 50 mM Tris-HCl, final pH 8.0), and dialyzed with 1000 volume of cold PBS for overnight at 4 ℃.

To purify proteins from bacteria transformed by pQE30 and pTrcHisB derived expression plasmids, bacteria were grown in 200 ml of LB broth to A_{600}=0.7 at 37 ℃, and induced with 0.1 mM of IPTG for 6 hrs at 23 ℃. To over express His-SREBP-1a, expression was induced by 0.2 mM of IPTG for O/N at 23 ℃. To over express His-Sp1ZFDBD, expression was induced by 0.2 mM of IPTG for O/N at 18 ℃. To over express His-POZ_{FBI-1}, induction was made with 1 mM of IPTG for 6 hrs at 30 ℃. Bacteria were collected by centrifugation and lysed with cold lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM Imidazole, 1 mM PMSF, pH 8.0). Bacteria were disrupted by sonication (amplitude 50 %, cycle 0.5, 30 sec). Disrupted mixture was centrifuged at 12,000 rpm at 4 ℃ and soluble fractions of lysate were incubated with 2 ml of Ni^+-NTA resin (Qiagen, Hilden, Germany) for 2 hrs at 4 ℃. After washing the resin with washing buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM Imidazole, 1 mM PMSF, pH 8.0), bound proteins were step-gradiently eluted with 2 ml of elution buffer (50 mM Na_2HPO_4, pH 8.0, 300 mM NaCl, 50, 75, 100, 150, 250 mM Imidazole), and dialyzed with 1000 volume of cold PBS for overnight at 4 ℃.

5. In vitro protein-protein interaction

To investigate interaction between the SREBP-1a and the POZ domain of FBI-1, purified GST protein (5 μg) and GST-POZ protein (5 μg) were bound to GSH-agarose (50 ㎕ slurry) (Peptron, Taejeon, Korea) for 1 hr in 500 ㎕ TNE buffer (50 mM Tris HCl, pH 8.0, 1 % Nonidet P-40, 2 mM EDTA, and one tablet/50 ml protease inhibitor mixture (Roche, Mannheim, Germany) at 4 ℃. Agarose-bound GST or GST-POZ protein was collected by centrifugation at 3000x *g* for 1 min at 4 ℃ and washed four times with cold TNE buffer. Agarose-bound GST or GST-POZ protein was incubated with 5 ㎍ of 6x His-SREBP-1 in binding buffer (25 mM Tris HCl, pH7.5, 75 mM NaCl, 0.25 % Nonidet P-40, 15 mM β-mercaptoethanol) for 6 hrs at 4 ℃. After the agarose-protein complex was washed three times with cold TNE buffer, the bound proteins were resolved by 15 % SDS-PAGE and subjected to Western Blot analysis using anti-SREBP-1 antibody (1:1000 dilution for mouse primary monoclonal antibody against SREBP-1a and 1:2000 dilution for rabbit secondary anti mouse antibody) (Santa Cruz Biotechnology, CA, USA).

6. Electrophoretin Mobility Shift Assay (EMSA)

The oligonucleotide probes (500 picomoles each in 83.3 mM Tris-HCl , pH=8.0, 16.7 mM $MgCl_2$, 166.7 mM NaCl) were annealed by heating at 93 ℃ for 5 min and cooling down slowly to room temperature. After diluting the

solution containing the annealed oligonucleotides with water to 50 mM Tris-HCl , pH 8.0, 10 mM $MgCl_2$, 100 mM NaCl, 100 picomoles of annealed oligonucleotides for EMSAs were labeled with [α-^{32}P] ATP and Klenow enzyme (Roche, Mannheim, Germany) by incubating 30 min. at 37 ℃. α-^{32}P labeled, double-stranded oligonucleotides were purified with PROBER™ spin column (iNtRON biotechnology, Seoul, Korea). Sequences of various oligonucleotides used in EMSA are as follows (only the top strand is shown):

LDLR Sp1: 5'-GATCGCAACTCCTCCCCCTGCTA-3';

FAS Sp1: 5'-GATCTGGCCGGGCGGCGCAGCCA-3';

ACL Sp1: 5'-GATCCGATGGGGGCGGGGAAAAG-3'.

Each binding reaction was carried out in 20 ㎕ of binding buffer containing 10 mM HEPES, pH 7.6, 75 mM KCl, 1 mM EDTA, 10 % Glycerol, 500 ng poly(dI-dC), 2 mM DTT, and purified recombinant Xpress tagged Sp1 ZFDBD (280 ng), GST-FBI-1 ZFDBD (280 ng), and the POZ domain of FBI-1 polypeptides (160 ng - 640 ng). 1.2 ㎍ of Anti-Xpress antibody against Sp1ZFDBD (Invitrogen, CA, USA) was added to the binding mixture in some EMSA reactions for super-shift assays. The protein-DNA complexes were resolved from free probe by 6 % non-denaturing polyacrylamide gel electrophoresis at room temperature in 0.5x TBE (89 mM Tris-Borate, 2 mM EDTA, pH 8.3) buffer. The dried gels were exposed to X-ray film at -70 ℃ with a Kodak intensifying screen (Kodak, NY, USA).

III. Results

1. FBI-1 is expressed more in *ob/ob* C57BL/6J mice compared to normal C57BL/6J mice

Recently, FBI-1 was identified as a possible active participant in the adipocyte differentiation process in both mice and human, and suggested that FBI-1 may be promoting growth arrest and terminal differentiation during adipocyte differentiation.[27]

First, we tested expression level of FBI-1 in mouse adipose tissue (Fig. 2). Total RNA was extracted from normal mouse adipose tissue and obese *ob/ob* mouse adipose tissues. RT-PCR was performed to measure the mRNA expression of mouse FBI-1 (also called LRF) in normal mouse and *ob/ob* mouse adipose tissues. We observed higher FBI-1 mRNA level in obese *ob/ob* mouse.

Based on this initial observation, we suspected that FBI-1 may play a role in lipid metabolism and there might be correlation between FBI-1 expression and fat mass.

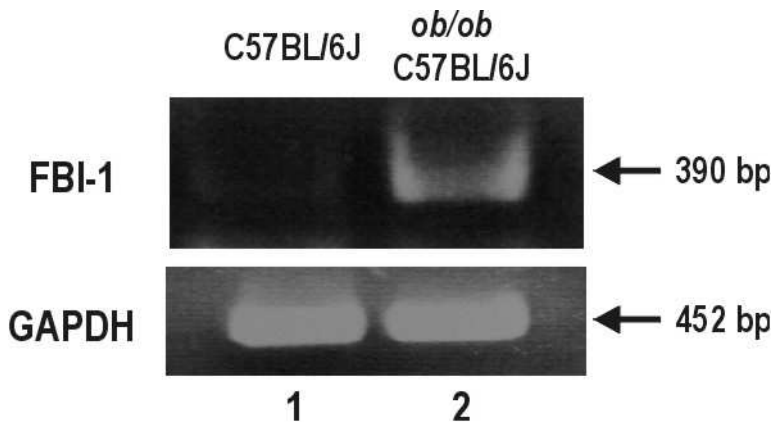

Figure 2. The expression of FBI-1 mRNA in *ob/ob* C57BL/6J mice and normal C57BL/6J mice. RT-PCR analysis of mRNA of FBI-1 and control GAPDH. Total RNA was isolated from the abdominal adipose tissues of normal C57BL/6J and *ob/ob* C57BL/6J mouse with TRIzol reagent. Two oligonucleotides were used to amplify the POZ-domain of FBI-1 (390 bp). Amplification of mouse GAPDH mRNA with the oligonucleotides designed to anneal to conserved regions of mouse and human GAPDH cDNA resulted in 452 bp PCR product. *ob/ob* C57BL/6J mouse shows higher level of FBI-1 mRNA than control C57BL/6J mouse. Control, mRNA of GAPDH does not show difference.

2. FBI-1 represses transcriptional activation by SREBP-1a and Sp1, and also represses synergistic activation by SREBP-1a and Sp1 on artificial promoters in SL2 cells and CV-1 cells

Sp1 is an important transcription activator of lipogenic genes by collaborating with SREBP. We reported previously that Sp1ZFDBD interacts with the POZ domain of FBI-1.[12] Accordingly, the molecular interaction of Sp1 and FBI-1 suggest a possibility that FBI-1 may play a role in transcription regulation of lipid biosynthetic genes by affecting either Sp1 or SREBP, or the synergistic interaction between Sp1 and SREBP.

To investigate whether FBI-1 affects the transcription of the lipogenic genes, we tested whether ectopic expression of FBI-1 affects the SREBP-1ą and Sp1-mediated transcription of the artificial promoters with the SRE and GC box in the proximal promoters (Fig. 3A). The proximal promoters of lipogenic genes such as FAS, ACL, and LDLR have SRE or E-box, and GC-boxes in various configurations. We introduced artificial reporter promoter with SRE and GC-box (pG5 1x(SRE+GC box) Luc:1x(SRE+GC); pG5 2x(SRE+GC box) Luc: 2x(SRE+GC)), SREBP-1a, Sp1, and FBI-1 expression plasmids in various combination into Drosophila SL2 cells lacking mammalian transcription factors to test the possible molecular interaction among three regulatory factors (total plasmid 2.4 μg: reporter plasmid reporter 0.8 μg, each transcription factor expression plasmids, 0.4 μg, pCMV-β-Gal, 0.4 μg). After 48 hrs of

cotransfection with calcium phosphate or Cellfectin (Invitrogen, CA, USA), cells were harvested, and analyzed for luciferase gene expression.

With 1x(SRE+GC) and 2x(SRE+GC) in Drosophila SL2 cells, Sp1 activated transcription by more than 4-7 fold, but FBI-1 did not activate or repress transcription significantly (Fig. 3B, 3C). Interestingly, SREBP-1a did not activates transcription in 1x(SRE+GC) construct and rather appeared to repress transcription by 80% and FBI-1 activated transcription by SREBP-1a by 4 fold, up to the control level. With 2x(SRE+GC) constructs, SREBP-1a activates transcription by 2.4 fold and FBI-1 repressed transcription by SREBP-1a by 20 %. Interestingly, FBI-1 repressed Sp1 dependent transcription only by 20-10 % both in 1x(SRE+GC) and 2x(SRE+GC) construct. Sp1 and SREBP showed synergistic transcription activation (7.7-82 fold), which is potently repressed by FBI-1 as much as 43-54% (Fig. 3B, 3C). Although action of FBI-1 by SREBP-1a or Sp1 is inconsistent, we observed FBI-1 represses synergistic activation by Sp1 and SREBP-1a.

To investigate whether FBI-1 has any effect on transcription regulation by SREBP-1a and Sp1 on the artificial promoter with SRE and GC-box in mammalian cells, we carried out transfection assays in African green monkey kidney CV-1 cells. The cells were cotransfected with FBI-1, SREBP-1a, Sp1 expression vectors, and artificial promoter-luciferase reporter gene fusion plasmids with 1x(SRE+GC) and 2x(SRE+GC) (total plasmid 1.2 μg: reporter

plasmid reporter 0.4 μg, each transcription factor expression plasmids, 0.2 μg, pCMV-β-Gal, 0.2 μg). After 48 hrs of cotransfection of plasmids with lipofectamine plus reagent (Invitrogen), cells were harvested, and analyzed for luciferase gene expression.

In artificial promoter-luciferase reporter gene constructs, 1x (SRE+GC) and 2x (SRE+GC), ectopic Sp1 weakly transactivated transcription by 1.6-1.3 fold, probably because of already enough endogenous Sp1 in CV-1 cells. FBI-1 rather potently repressed Sp1-dependent transcription activation by 50-72%. Constitutive active form of SREBP-1a (a.a.1-490) potently activated transcription by 12-31 fold and cotranscfection of Sp1 and SREBP further activated transcription 17-41 fold. Strong transcription activation by SREBP-1a or cotrasfected SREBP-1a and Sp1 was potently repressed by cotransfected FBI-1 by 80-62 % and 88-70 %, respectively (Fig. 3D, 3E). Although FBI-1 appeared to repress transcription activation by either Sp1 or SREBP-1a, it is unclear whether FBI-1 really repress SREBP-1a activity because endogenous Sp1 can be critical in transcriptional activation of SREBP-1a (Fig. 3D, 3E). One possible scenario is that FBI-1 represses Sp1 and thus abolishes synergistic transcription activation by Sp1 and SREBP-1a on the 1x(SRE+GC) and 2x(SRE+GC) construct, as we observed with in Drosophila SL2 cells (Fig. 3D, 3E).

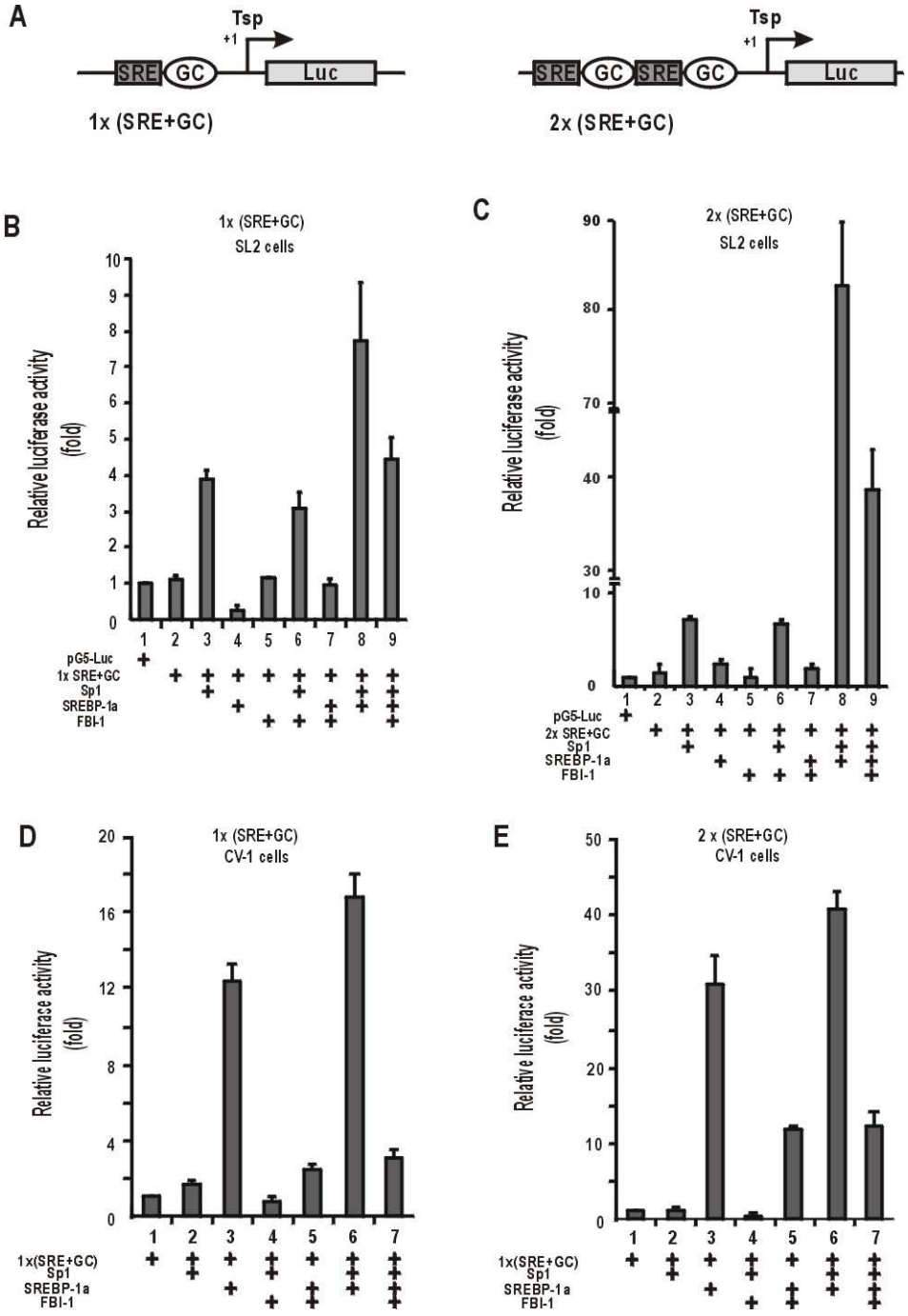
A
Tsp
+1
SRE
GC
Luc
1x (SRE+GC)
SRE
GC
SRE
GC
Luc
2x (SRE+GC)
B
1x (SRE+GC)
SL2 cells
Relative luciferase activity (fold)
pG5-Luc
1x SRE+GC
Sp1
SREBP-1a
FBI-1
C
2x (SRE+GC)
SL2 cells
Relative luciferase activity (fold)
pG5-Luc
2x SRE+GC
Sp1
SREBP-1a
FBI-1
D
1x (SRE+GC)
CV-1 cells
Relative luciferase activity (fold)
1x(SRE+GC)
Sp1
SREBP-1a
FBI-1
E
2 x (SRE+GC)
CV-1 cells
Relative luciferase activity (fold)
1x(SRE+GC)
Sp1
SREBP-1a
FBI-1

Figure 3. The transcriptional regulatory roles of FBI-1, SREBP-1a and Sp1 on the artificial promoters in SL2 cells and CV-1 cells. FBI-1 potently repressed synergistic transcription activation by cotransfected Sp1 and SREBP-1a. (A) The structures of artificial promoter reporter gene fusion plasmid constructs with either 1x (SRE+GC-box) or 2x (SRE+GC-box). SRE was adapted from well characterized SREBP binding element of LDLR gene promoter. GC-box was from the consensus sequence of SV40 promoter. SRE is recognized by SREBP and GC-box is bound by Sp1. Tsp, transcription start point, Luc, luciferase. (B, C) Transient transfection assays in the promoters with proximal 1x (SRE+GC) and 2x (SRE+GC) by Sp1, SREBP-1a, and FBI-1 in SL2 cells. Sp1 is a more potent activator than SREBP-1a, and when Sp1 and SREBP-1a are cotransfected, strong transcription activation is observed. FBI-1 had weak or no effect on transcription activation by Sp1 and SREBP-1a. However, FBI-1 potently repressed synergistic transcription activation by cotransfected Sp1 and SREBP-1a. (D, E) Transient transfection assay of the promoters with proximal 1x (SRE+GC) and 2x (SRE+GC) by Sp1, SREBP-1a, and FBI-1 in monkey kidney CV-1 cells. Ectopic Sp1 weakly activated transcription and SREBP-1a potently activated transcription. When Sp1 and SREBP-1a are cotransfected, addictive transcription activation is observed. FBI-1 potently repressed transcription either by Sp1 or SREBP-1a, and also inhibited addictive transcription activation by cotransfected Sp1 and SREBP-1a.

3. FBI-1 represses transcriptional activation of lipid biosynthetic genes (FAS) by SREBP-1a in SL2 cells

In proximal promoter of rat FAS gene, SRE, GC-box, and E-box (recognized by bHLH type transcription factors) are present. Because of additional E-box that can be recognized by SREBP-1a as reported by others.[42] we divided the promoter into two constructs shown in Figure 4A in order to study the function of E-box and SRE in term of transcriptional regulation by SREBP-1a, Sp1 and FBI-1.

In all three FAS1, 2, 3 constructs tested, Sp1 activated transcription by 89, 88 fold (FAS 1, 2), 3.4 fold (FAS 3). SREBP-1a activates transcription potently by 76, 38 fold (FAS1, 2), 1.2 fold (FAS3) depending on the types of promoters (Fig. 4B-D). We could observe lower relatives transcription activity in FAS3. It appears that having intact GC-box-E-box elements is critical in transcription activation by Sp1 or SREBP-1a. In all constructs tested, FBI-1 alone did not affect the transcription of reporter gene (Fig. 4B-D). In FAS 1, 2, 3, FBI-1 did not repress transcription by Sp1 significantly. With respect to SREBP-1a action, FBI-1 repressed transcription by SREBP-1a by 44 % in FAS1, 49 % in FAS2 construct. However, in FAS3, FBI-1 did not affect transcription by SREBP-1a. It appears that FBI-1 represses transcription by acting on SREBP-1a and depends on the presence of E-box rather than SRE on FAS promoter (Fig. 4B-D).

Once Sp1 and SREBP-1a are cotransfected, transcription was increased by two fold compared to Sp1 or SREBP-1a dependent transcription activation in FAS1, possibly suggesting an additional effects of two transcription factors. In FAS2 or FAS3 promoters, cotransfection of two factors did not result in transcription activation over either Sp1 or SREBP-1a dependent transcription and rather decreased transcription by half in FAS2. However, in FAS2, FBI-1 activated transcription by Sp1 and SREBP-1a by 2.3 fold. FBI-1 did not affect transcription activation by SREBP and Sp1 in FAS1 and FAS3 (Fig. 4B-D).

These data suggest that in FAS1 and FAS2, FBI-1 apparently repressed transcription activation by SREBP-1a and no significant effect on Sp1 dependent transcription, suggesting that the E-box is important in the transcription repression by FBI-1 (Fig 4B-D).

4. FBI-1 represses transcription activation by Sp1. However FBI-1 further activates transcription activation of lipid biosynthetic genes (FAS) by SREBP-1a and cotransfected Sp1 and SREBP-1a in CV-1 cells

In mammalian CV-1 cells, Sp1 did not particularly activate transcription above the control pGL2 basic or pSYNt-Luc (TATA box of HMG-CoA synthetase cloned in pGL2 basic in case of FAS3) in FAS1, 2, and 3 constructs tested, probably because of endogenous Sp1 (Fig. 4E-G). SREBP-1a potently activated transcription by 11, 21.7, 6.3 fold depending on the types of promoters. Although FBI-I repressed transcription activation by Sp1 by 60, 36, 20%, interestingly, FBI-1 activated transcription by SREBP-1a by 1.6 fold in FAS1 and 3.8 fold in FAS2 construct where E-box juxtaposed to Sp1 binding GC-box. However, in FAS 3 lacking E-box, FBI-1 repressed transcription by 47 %. Overall transcription level of FAS2 construct is at least 2-4 fold higher compared to FAS1. Presence of additional SRE upstream of GC-box has lowered overall transcription level compared to FAS1, of which the reason is obscure.

FBI-1 further activated transcription contributed by SREBP-1a and Sp1 in FAS1 by 2.5 fold, FAS2 by 3 fold, once FBI-1, Sp1 and SREBP-1a were cotransfected. This is rather unexpected because FBI-1 was known as transcription repressor in general due to the presence of POZ-domain that can interact with corepressors molecules.[19-21] In FAS3 lacking E-box, FBI-1

repressed transcription by 64 % as we have seen in artificial promoters with SRE and GC-box (Fig. 4E-G). These data suggest that in FAS promoter, FBI-1 can act as transcription activator and the E-box are particularly important in the transcription activation by SREBP-1a and FBI-1.

In FAS promoter, relative configuration of GC-box and E-box, potential hydrophobic interaction between HLH of SREBP-1a and POZ domain of FBI-1 may result in transcription repression by FBI-1 in SL2 cells and transcription activation by FBI-1 in CV-1 cells.

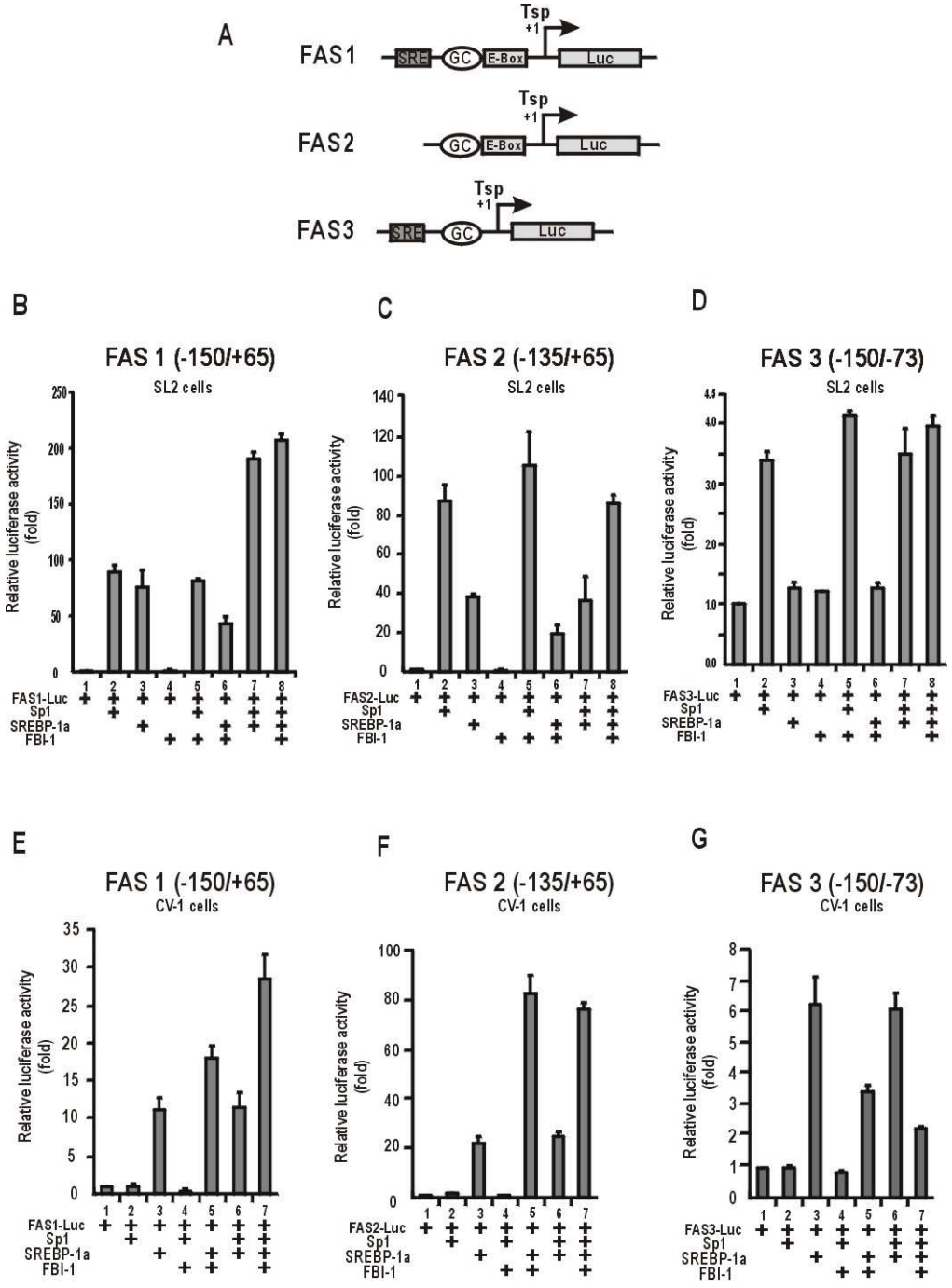
A
FAS1
FAS2
FAS3
Tsp
+1
SRE
GC
E-Box
Luc
B
FAS 1 (-150/+65)
SL2 cells
C
FAS 2 (-135/+65)
SL2 cells
D
FAS 3 (-150/-73)
SL2 cells
Relative luciferase activity (fold)
FAS1-Luc
FAS2-Luc
FAS3-Luc
Sp1
SREBP-1a
FBI-1
E
FAS 1 (-150/+65)
CV-1 cells
F
FAS 2 (-135/+65)
CV-1 cells
G
FAS 3 (-150/-73)
CV-1 cells

Figure 4. The transcriptional regulatory roles of FBI-1, SREBP-1a and Sp1 on the FAS promoter in SL2 cells and CV-1 cells. FBI-1 activated or repressed transcription activation by Sp1 and SREBP-1a depending on the FAS promoter constructs tested. (A) The structures of three FAS promoter reporter gene fusion plasmid constructs with SRE, GC-box, and E-box. SRE and E-box are recognized by SREBP and GC-box is bound by Sp1. (B, C, D) Transient transfection assays of the promoters with FAS1, FAS2, FAS3 by Sp1, SREBP-1a, and FBI-1 in SL2 cells. Both Sp1 and SREBP-1a are potent transcription activators. However, when Sp1 and SREBP-1a were cotransfected, strong transcription activation was not observed. FBI-1 repressed transcription activation by SREBP-1a by 40-50% and required E-box located immediately downstream GC-box. But FBI-1 had little effect on transcription activation by Sp1. (E, F, G) Transient transfection assay of the promoters with FAS1, FAS2, FAS3 by Sp1, SREBP-1a, and FBI-1 in CV-1 cells In FAS1, FAS2 reporter construct, FBI-1 repressed transcription activation by Sp1. However, FBI-1 activated transcription activation by SREBP-1a, and further activated transcription activation by cotransfected Sp1 and SREBP-1a. But, in FAS 3 reporter construct, FBI-1 potently represses transcription activation either by Sp1 or SREBP-1a.

5. FBI-1 represses transcriptional activation of lipid biosynthetic genes (ACL) by SREBP-1a and Sp1, and also represses synergistic activation by SREBP and Sp1 in SL2 cells and CV-1 cells

In proximal promoter of rat ACL gene (bp -114 to +67), two SRE flanking a Y box, and a GC-box are present (Fig. 5A). It was shown previously by others that Y-box can also be recognized by SREBP and activates transcription.[35] With pACL114-Luc construct cotransfected into Drosophila SL2 cells, Sp1 activated transcription by 1.5 fold, but neither SREBP-1a nor FBI-1 activate or repress transcription significantly (Fig. 5B). And FBI-1 did not affect Sp1 or SREBP-1a dependent transcription in this promoter. However, once Sp1 and SREBP-1a expression plasmids were cotransfected, transcription was synergistically activated (5.4 fold), which is repressed by FBI-1 by 40% (Fig. 5B).

To investigate whether FBI-1 has any effect on transcriptional regulation by SREBP-1 and Sp1 on ACL promoter in mammalian CV-1 cells, we carried out similar transfection assays. In ACL promoter luciferase construct, ectopic Sp1 did not significantly activated transcription, probably because of enough endogenous Sp1 in CV-1 cells. FBI-1 rather potently repressed Sp1-dependent transcription activation by 56%. However, SREBP-1a potently activated transcription by 19.8 fold and cotranscfection of Sp1 and SREBP-1a further activated transcription 26.7 fold. Strong transcription activation by SREBP-1a

or cotrasfected SREBP-1a and Sp1 was potently repressed by cotransfected FBI-1 by 47% and 38%, respectively (Fig. 5C).

Our data suggest that SREBP-1a and Sp1 are major transcription regulators of ACL genes both in SL2 and CV-1 cells, while FBI-1 represses transcription activation by both SREBP-1a and Sp1.

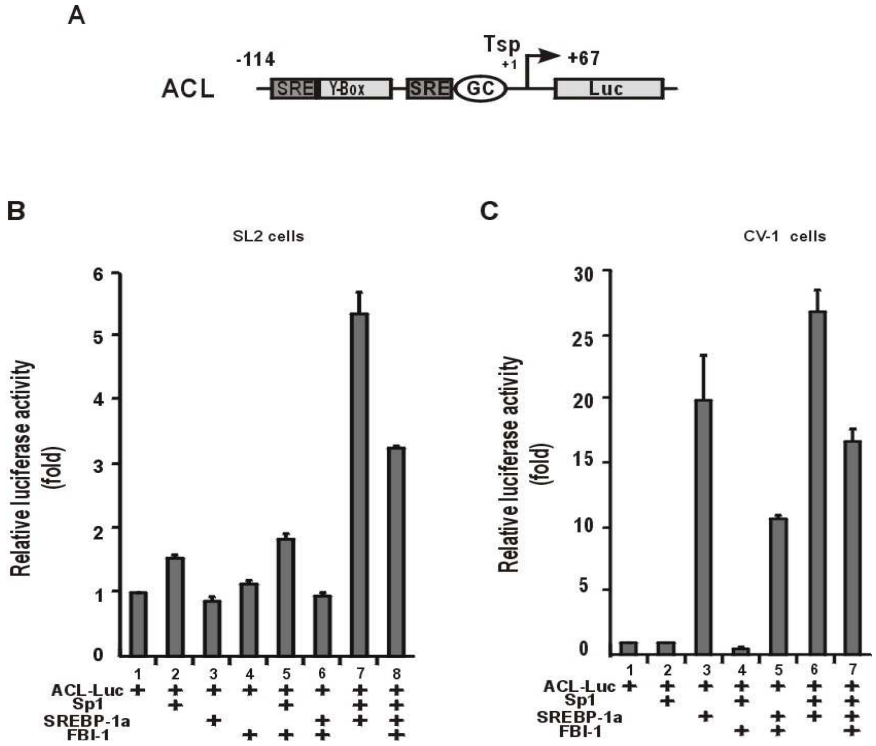

Figure 5. The transcriptional regulatory roles of FBI-1, SREBP-1a and Sp1 on the ACL promoter in SL2 cells and CV-1 cells. (A) The structures of proximal ACL promoter reporter gene fusion plasmid construct with two SRE, Y-box, and GC-box. (B) Transient transfection assay of ACL promoter by Sp1, SREBP-1a, and FBI-1 in SL2 cells. Sp1 is a more potent activator than SREBP-1a, and when Sp1 and SREBP-1a are cotransfected, strong transcription activation is observed. FBI-1 had weak or no effect on transcription activation either by Sp1 or SREBP-1a. However, FBI-1 potently repressed synergistic transcription activation by cotransfected Sp1 and SREBP-1a. (C) Transient transfection assay of ACL promoter by Sp1, SREBP-1a, and FBI-1 in CV-1 cells. Ectopic Sp1 weakly activated transcription and SREBP-1a potently activated transcription. When Sp1 and SREBP-1a are cotransfected, addictive transcription activation is observed. FBI-1 potently repressed transcription either by Sp1 or SREBP-1a, and also inhibited addictive transcription activation by cotransfected Sp1 and SREBP-1a.

6. FBI-1 modulates transcriptional activation of lipid biosynthetic genes (LDLR) by SREBP-1a or Sp1 in SL2 cells and CV-1 cells

In proximal promoter (bp -123 to -24) of human LDL receptor gene, one SRE and a GC-box are present (Fig. 6A). With LDLR-tk-Luc construct in Drosophila SL2 cells, Sp1 and SREBP-1a activated transcription by 12.7 fold and 2.0 fold respectively, but and FBI-1 alone did not affect transcription significantly (Fig. 5B). Interestingly, FBI-1 activated transcription by SREBP-1a by 1.9 fold. Sp1 and SREBP-1a showed synergistic transcription activation (96.9 fold), which is weakly further activated by FBI-1 by 1.4 fold (Fig. 6B). Intriguingly, FBI-1 activated transcription by Sp1 by 3.8 fold in this promoter.

To investigate whether FBI-1 has any effect on transcription regulation by SREBP-1a and Sp1 on LDLR promoter in mammalian cells, we also carried out transfection assays in CV-1 cells. In LDLR promoter luciferase construct, ectopic Sp1 transactivated transcription by 1.5 fold and FBI-1 potently repressed Sp1-dependent transcription activation by 89%. SREBP-1a activated transcription by 4.4 fold and interestingly, cotranscfection of Sp1 and SREBP-1a further activated transcription 10.4 fold. Transcription activation by SREBP-1a or cotransfected SREBP-1a and Sp1 was potently activated by cotransfected FBI-1 by 2.8 fold and 1.5 fold, respectively as FAS 1 promoter construct (Fig. 6C).

In SL2 cells, transcription was potently activated by cotransfection of Sp1 and FBI-1 and rather weakly activated by cotransfection of SREBP-1a and FBI-1. However, in CV-1 cells, the strong transcription activation was observed by the cotransfection of SREBP-1a and FBI-1 although FBI-1 repressed transcription activation by Sp1. (Fig. 6C).

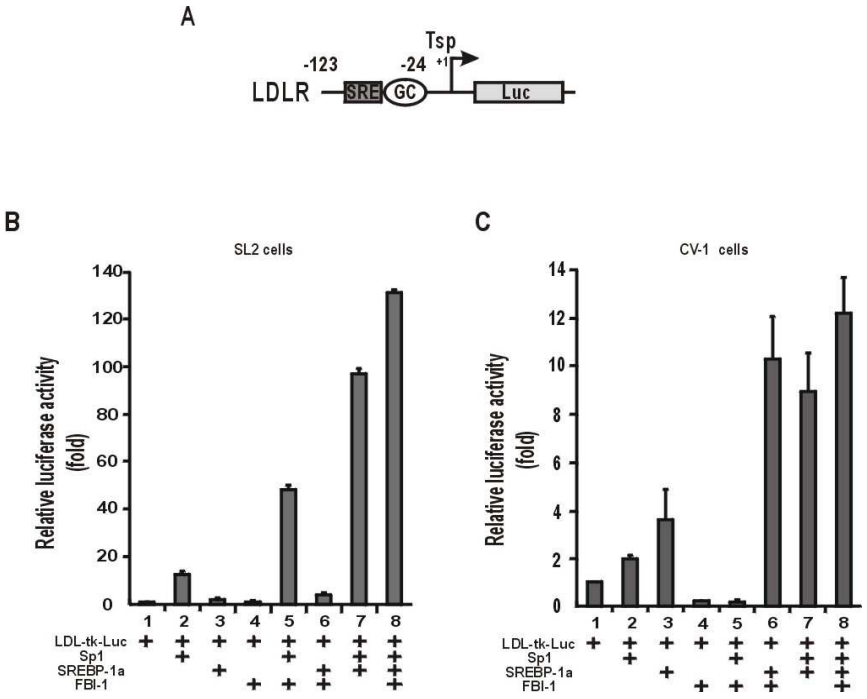

Figure 6. The transcriptional regulatory roles of FBI-1, SREBP-1a and Sp1 on the LDLR promoter in SL2 cells and CV-1 cells. FBI-1 activated transcription activation by SREBP-1a. (A) The structures of proximal LDLR promoter reporter gene fusion plasmid constructs with a SRE and a GC-box. (B) Transcriptional regulation of the LDLR promoters by Sp1, SREBP-1a, and FBI-1 in SL2 cells. Sp1 is a more potent activator than SREBP-1a, and when Sp1 and SREBP-1a are cotransfected, strong transcription activation is observed. FBI-1 strongly activated transcription by Sp1 and also activated transcription by SREBP-1a by 2 fold. And FBI-1 further activated synergistic transcription activation by cotransfected Sp1 and SREBP-1a. (C) Transcriptional regulation of the LDLR promoters by Sp1, SREBP-1a, and FBI-1 in CV-1 cells. Ectopic Sp1 weakly activated transcription and SREBP-1a potently activated transcription. When Sp1 and SREBP-1a were cotransfected, addictive transcription activation was observed. FBI-1 potently repressed transcription by Sp1. However, FBI-1 activated transcription by SREBP-1a, and also weakly activated addictive transcription activation by cotransfected Sp1 and SREBP-1a.

7. The POZ domain of FBI-1 interacts with SREBP-1a and Sp1ZFDBD

Based on transcription data in this study, the POZ domain of FBI-1 represses or activates transcription probably interacting either with Sp1 or SREBP-1a. We were interested in how FBI-1 regulates transcription of promoters with SRE and Sp1 binding sites. We reported previously that the Sp1 zinc finger interacts with the POZ domain of FBI-1.[12] Sp1 transcription factor was shown to play an important role in the transcription of lipogenic genes activated by SREBP.[40] Accordingly, the molecular interaction between Sp1 and FBI-1 suggests that FBI-1 may play a certain role in transcription regulation of lipogenic genes probably by interacting with Sp1 or SREBP-1a on the lipogenic gene promoters. Initially, we suspected that the POZ domain of FBI-1 might also interact with SREBP-1a, and investigated whether FBI-1 interacts with SREBP and Sp1 by carrying out GST fusion protein pulldown assay (Fig. 7). Recombinant GST or GST-POZ was allowed to interact with recombinant 6x His-Xpress-Sp1ZFDBD and 6x His-SREBP-1a, pulled down, and analyzed by western blotting using anti Xpress-tag antibody or anti-SREBP-1 antibody. The POZ domain of FBI-1 interacted not only with the Sp1ZFDBD (Fig. 7A) but also with SREBP-1a directly (Fig. 7B).

Although we confirmed direct interaction between FBI-1 and SREBP-1a through GST fusion protein pulldown assay, we could not observe apparent change of transcription level by cotransfection of SREBP-1a and FBI-1 in SL2

cells (Fig. 4-6). To investigate whether FBI-1 has any effect on transcription by SREBP-1a in SL2 cells, we introduced 6x SRE-tk-Luc construct. This construct has six SRE sand a thymidine kinase (tk) promoter which has two potential Sp1 binding GC-boxes (Fig. 7C). FBI-1 repressed transcription by SREBP-1a by 50% and strong synergistic effect by Sp1 and SREBP-1a by 38%. As well as above transient transcription, FBI-1 did not affect transcription activation by Sp1 (Fig. 7C). The data suggests that FBI-1 may repress transcription through the direct interaction with Sp1 or SREBP-1a.

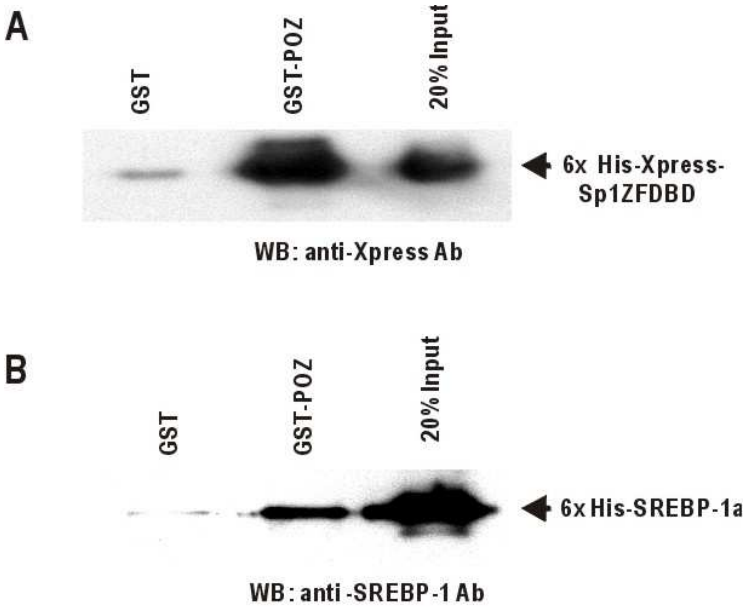
A
GST
GST-POZ
20% Input
6x His-Xpress-Sp1ZFDBD
WB: anti-Xpress Ab
B
GST
GST-POZ
20% Input
6x His-SREBP-1a
WB: anti -SREBP-1 Ab
C
6x SRE-tk-Luc
Tsp
+1
GC
GC
Luc
6x SRE
Tk promoter
Relative luciferase activity (fold)
200
150
100
50
0
1
2
3
4
5
6
7
8
6x SRE-tk-Luc
Sp1
SREBP-1a
FBI-1

Figure 7. GST fusion protein pulldown assays showed that the POZ domain of FBI-1 interact with Sp1 and SREBP-1a. (A) recombinant GST or GST-POZ protein bound onto GSH-agarose bead was incubated with Xpress tagged Sp1ZFDBD protein (5 μg) and carried out Western blot analysis using anti Xpress-tag antibody. (B) GST or GST-POZ protein bound onto GSH-agarose bead was incubated with purified full length SREBP-1a protein (5 μg) and carried out Western blot analysis using anti-SREBP-1 antibody. (C) The structure of 6xSRE-tk-Luc construct and transient transfection assay using SREBP-1a, Sp1, and FBI-1 on 6x SRE-tk-Luc reporter plasmid. FBI-1 represses transcription by SREBP-1a and Sp1 on 6x SRE-tk-Luc reporter plasmid.

8. The POZ domain of FBI-1 reduces binding affinity of Sp1 to the GC-boxes of FAS, ACL, and LDLR genes

Sp1 binds to regulatory elements on the promoters of FAS, ACL, and LDLR genes, and acts as a major activator of transcription in the genes with SRE and GC-box. Previously, Sp1ZFDBD was shown to interact with the POZ domain of FBI-1, and we have shown that transcription of the genes activated by Sp1 was repressed by FBI-1 in ACL, FAS, and LDLR promoters. Accordingly, we suspected that FBI-1 affects on transcription of lipogenic genes through the interaction with Sp1ZFDBD. Therefore we investigated whether the POZ domain of FBI-1 has any effect on binding of Sp1ZFDBD to the target GC boxes of lipogenic genes using EMSA. The sequences of the probes used in EMSA were shown in Fig. 8A. EMSA showed that the purified recombinant 6x His-Xpress-Sp1ZFDBD (280 ng) binds to the GC box probes of three genes above mentioned but with variety affinity (Fig. 8B-D, lanes 2,3). Adding recombinant 6x His tagged POZ_{FBI-1} polypeptides (160 ng, 320 ng, 640 ng) reduced binding by Sp1ZFDBD significantly, suggested that FBI-1 could repress transcription by inhibiting Sp1 binding to the key regulatory GC-boxes of the genes by its POZ domain and the inhibition of Sp1 binding could be important in transcription repression by Sp1 (Fig. 8B-D, lanes 4, 5, 6).

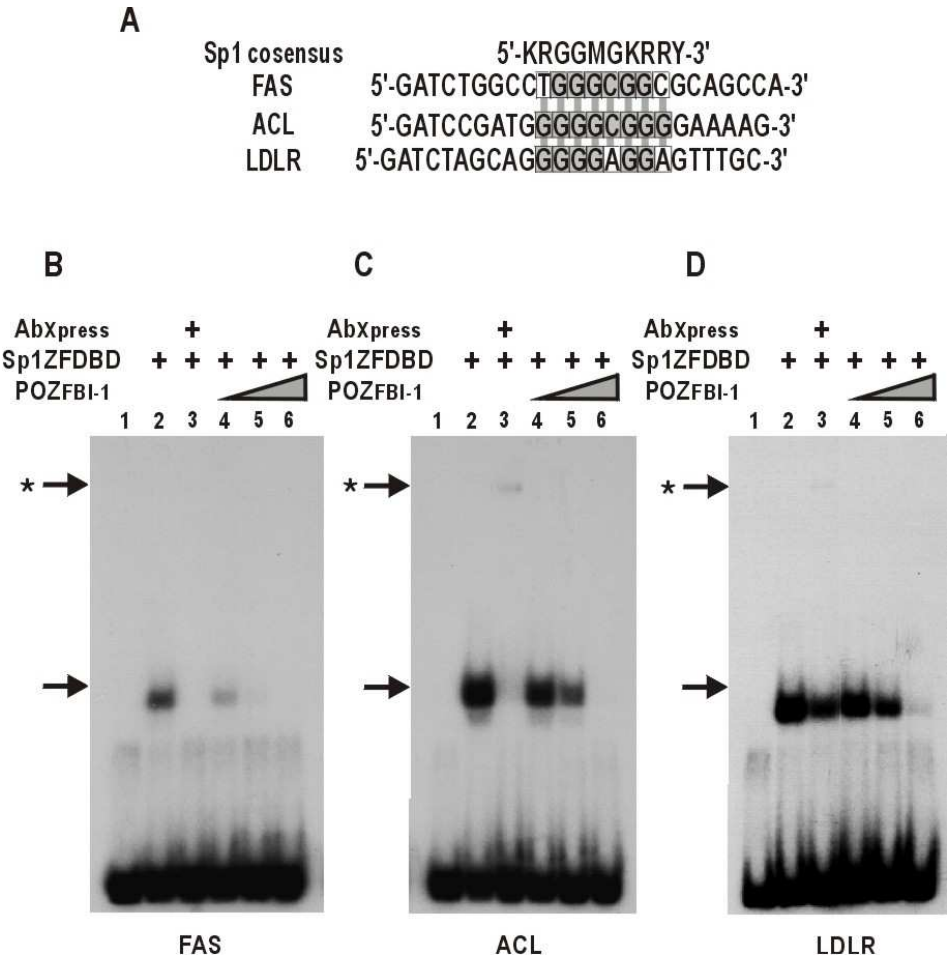

Figure 8. Direct interaction between the POZ domain of FBI-1 and Sp1ZFDBD reduces DNA binding of Sp1ZFDBD to the GC-box probes of FAS, ACL and LDLR genes. (A) Sequences comparisons of Sp1 binding to the GC-box probes used in EMSA. (B) Sp1ZFDBD binds GC-box of FAS promoter (lanes 2, 3). POZ domain decreases DNA binding of Sp1ZFDBD to the GC-box probe (lanes 4-6). To confirm Sp1 protein binding to the probe, we use anti Xpress-tag antibody to supershift Sp1-probe complex. (C) The POZ domain of FBI-1 decreases DNA binding affinity of Sp1ZFDBD to the GC-box of ACL promoter (lanes 4-6). (D) The POZ domain of FBI-1 decreases DNA binding of Sp1ZFDBD to the GC-box of LDLR promoter (lanes 4-6). Arrow, Sp1ZFDBD-probe complex; Arrow with asterisk, band supershifted by antibody.

9. FBI-1ZFDBD can bind to the GC-boxes of FAS, ACL, and LDLR genes

We recently found that FBI-1 binds to some of the GC rich sequences through CAST assay (unpublished data). To investigate whether FBI-1 binds to the GC boxes of ACL, FAS, and LDLR promoters, we performed EMSA using the probes listed in Fig. 9A. The purified recombinant 6x His-Xpress-Sp1ZFDBD (280 ng) binds to the GC box probes of FAS, ACL, and LDLR genes as expected (Fig. 9B-E, lanes 2, 3 of each panel). We also tested whether FBI-1ZFDBD (280 ng) binds to the some GC-box probes of FAS, ACL, and LDLR promoters that were shown to bind to Sp1ZFDBD. We used the same quantity of labeled probes and performed EMSA under the same assay condition. Interestingly, FBI-1 binds strongly to the GC-box of ACL gene and rather weakly to GC-box of FAS gene and LRLR genes (Fig. 9B-E, lanes 4, 5). We could also confirm that FBI-1ZFDBD binds to the probe of FAS when the x-ray film was exposed for long time (Fig. 9B, lanes 4, 5).

The fact that FBI-1 repressed or activated transcription by SREBP-1a according to promoters can be explained by difference of binding affinity observed Fig. 9. FBI-1ZFDBD strongly bound to GC-box of ACL promoter. And in transient transfection assay, FBI-1 repressed transcription by SREBP-1a in ACL promoter construct. In contrast to ACL, in FAS and LDLR promoter construct where FBI-1 may bind weakly, FBI-1 further activated transcription by SREBP-1a. If FBI-1 binds to the GC-boxes strongly, transcription activity

can be repressed as in ACL promoter. In contrast, when FBI-1 cannot bind to any of the promoter element, FBI-1 only interacts with SREBP-1a and Sp1 bound to regulatory elements of promoter and transcription activity is potently activation by unknown mechanism as in FAS and LDLR.

The result suggests that competitive binding of Sp1 and FBI-1 to the GC-boxes may be important in transcription activation or repression by FBI-1 depending on the promoter configuration of SRE, FRE, E-Box, and GC-boxes of the lipid biosynthetic genes shown in Figs. 3-6.

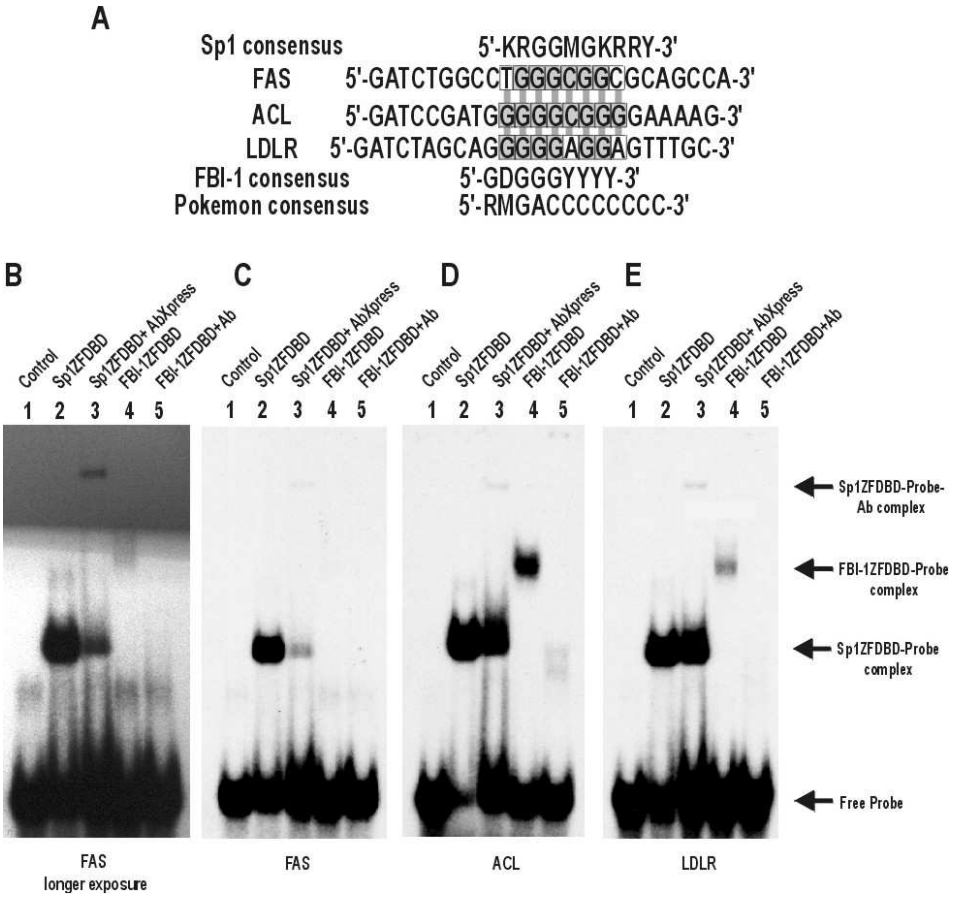

Figure 9. FBI-1ZFDBD can bind to the Sp1 binding GC-boxes of FAS, ACL, LDLR genes. FBI-1 may compete with Sp1. (A) Sequences comparisons of Sp1 binding GC-boxes and FBI-1 binding consensus sequence. (B, C) Sp1ZFDBD weakly binds GC-box of FAS promoter compare to either ACL or LDLR (lanes 2, 3). Also, FBI-1ZFDBD very weakly binds GC rich region (lanes 4, 5). To confirm FBI-1ZFDBD protein binding to the probe, we used anti GST-POZ_{FBI-1} antibody. (D) The Sp1ZFDBD strongly binds the GC-box probe of FAS promoter compare to ACL (lanes 2, 3). The FBI-1ZFDBD also strongly binds GC rich region (lanes 4, 5) but weakly compared to Sp1ZFDBD. (E) The Sp1ZFDBD binds the GC-box probe of FAS promoter (lanes 2, 3). FBI-1ZFDBD weakly binds the GC-box probe (lanes 4, 5).

IV. Discussion

More than 300 known human proteins are estimated to contain the POZ domain (smart.embl-heidelberg.de/smart/). We found that about 150 candidate proteins might interact with the POZ domain of FBI-1 using proteom and genome assay methods. Of the candidates, genes or proteins related to lipid metabolism such as RXR, SREBP, PPARγ appear to interact with the POZ domain of FBI-1 (data not shown).

We initially suspected that FBI-1 might participate in lipid metabolism. The facts that FBI-1 interacts with Sp1, a co-regulatory factor of SREBPs and is a participant of adipocyte differentiation supported our suspicion. We found that FBI-1 mRNA expression level was increased in *ob/ob* C57BL/6J mice compare to normal C57BL/6J mice (Fig. 2). To investigate whether FBI-1 participates in lipid metabolism, we tested transcriptional regulation of major genes regulating lipid metabolism such as FAS, ACL, and LDLR genes through transient transfection assay of FBI-1, SREBP-1a, and Sp1 in mammalian and insect cell lines. Using artificial promoters with SRE and GC-box (Fig. 3), we observed FBI-1 acts as transcriptional repressor. And also, we showed that FBI-1 represses transcription by SREBP-1a and Sp1 of the lipogenic gene, ACL (Fig. 5). However, surprisingly, in CV-1 cells, we also found that FBI-1 can activate transcription by collaborating with SREBP-1a in certain promoter contexts such

as FAS1, FAS2 and LDLR (Figs. 4, 6). It appears that the presence of E-box is essential in transcription activation by FBI-1 in case of FAS1 and 2. In FAS 3 lacking E-box, we observed the same result as artificial promoter. It may suggest that FBI-1 regulates transcription oppositely either as activator or repressor depending on configuration of proximal regulatory elements and cellular context.

In SL2 cells, Sp1 was a stronger activator than SREBP-1a in all the promoters tested such as FAS, ACL, and LDLR. We observed apparent synergistic activation by cotransfected Sp1 and SREBP-1a. In artificial and ACL promoters, strangely, FBI-1 did not affect transcription by Sp1 and SREBP-1a separately. This may be caused by relatives low expression of FBI-1 compared to the level of Sp1 or SREBP-1a. We may need to evaluate the expression level individual transcription factor in the future investigation. However, synergistic transcription activation was clearly repressed by FBI-1. Because FBI-1 repressed transcription activation by Sp1 and SREBP-1a separately in CV-1 cells, we expected to observe similar repression in SL2 cells. We guess that difference in co-regulatory factors that interact with Sp1 or SREBP-1a and in cellular context between insect cells and mammalian cells might also give different results in transcriptional regulation of artificial and ACL promoters by FBI-1. To dissect the regulatory roles of FBI-1 on transcriptional regulation by Sp1 and SREBP-1a, it may be more reasonable to

knock down the mRNA of Sp1, SREBP-1 or FBI-1 by RNA interference in mammalian cells.

In FAS promoter in SL2 cells, FBI-1 also did not affect transcription by Sp1 as in artificial or ACL promoter. However, FBI-1 efficiently repressed transcription activation by SREBP-1a except FAS3 promoter with deleted E-box. These data suggest that, in FAS promoter, FBI-1 can act as transcription repressor and the E-box are particularly important in the transcription repression by SEEBP-1a and FBI-1. In LDLR promoter in SL2 cells, surprisingly, FBI-1 also activated transcription by both Sp1 and SREBP-1a. Although, the results between two promoters, FAS and LDLR promoters were different, we observed that FBI-1 could modulate transcription by SREBP-1a either as activator or repressor. FBI-1 possibly acted via different mechanisms refracting difference in promoter contexts and cellular context, which remain to be characterized.

In CV-1 cells, SREBP-1a was a stronger activator than Sp1 in all the promoters tested such as FAS, ACL, and LDLR. It appeared that ectopic Sp1 could not do much on transcription because of enough endogenous Sp1 present in CV-1 cells. FBI-1 repressed transcription activation by Sp1 in all cases. However, FBI-1 had different action either as activation or repression on transcription by SREBP-1a depending on the promoters tested. FBI-1 activated transcription by SREBP-1a or both Sp1 and SREBP-1a in FAS1, FAS2, and LDLR. We analyzed transcription data and EMSA data together. The EMSA

data showed that the POZ domain of FBI-1 inhibited to bind Sp1 to GC-box and FBI-1ZFDBD could compete to the same GC-box recognized by Sp1ZFDBD (Figs. 8, 9). In the genes like ACL in which transcription is repressed by FBI-1, there is reasonable possibility that FBI-1 may bind to the GC-box of the proximal promoter. Once FBI-1 was targeted and bound to the GC-box, it could recruit co-repressors via its POZ domain and resulting chromatin compaction could repress transcription. In other genes such as FAS, LDLR, there is little possibility that FBI-1 may actually bind to the GC-box. Sp1 preferentially bind to the GC-box. In this case, FBI-1 can also be localized to the proximal promoter by interaction with SREBP-1a and Sp1 that were already bound on promoter. FBI-1, SREBP-1a, and Sp1 complex can recruit the co-regulatory factors that are quite different from the one recruited by promoter bound FBI-1 and the complex activates transcription (Fig. 10). It is also possible to promote binding SRE of SREBP-1a by FBI-1 through direct interaction with SREBP-1a and FBI-1 and transcription is activated. Taken together, we found that molecular interaction among cis-regulatory element, SREBP-1a, Sp1 and FBI-1 is important in transcriptional regulation of major genes of lipid metabolism.

Once we compared transient transfection data obtained from SL2 cells with CV-1 cells, we could find some contradicting results. In FAS promoters, FBI-1 repressed transcription activation by SREBP-1a in SL2 cells, but FBI-1 further activated transcription activation by SREBP-1a in CV-1 cells. And also, in

LDLR promoter, FBI-1 repressed transcription activation by Sp1 in SL2 cells but, activated in CV-1 cells. Although the reason is unclear, we guess that different co-regulatory factors, hydrophobic interaction between bHLH of SREBP-1a and the POZ domain of FBI-1 and weak binding of FBI-1 to GC-box may result in transcription repression by FBI-1 in SL2 cells and transcription activation by FBI-1 in CV-1 cells. Because co-regulatory factors involved in the transcriptional regulation of FAS and LDLR genes in insect cells could be different from the co-regulatory factors in mammalian cell, it may be reasonable to get contradicting results in two different cell line. To understand transcriptional regulation by FBI-1, we may have to carry out some additional experiments such as co-immunoprecipitation assay, chromatin immunoprecipitation assay, RNA interference, transfection assay in preadipocyte 3T3-L1 and hepatocytes stably overexpressing FBI-1, and the dynamics of promoter occupancy by FBI-1, SREBP-1a, and Sp1 under various conditions.

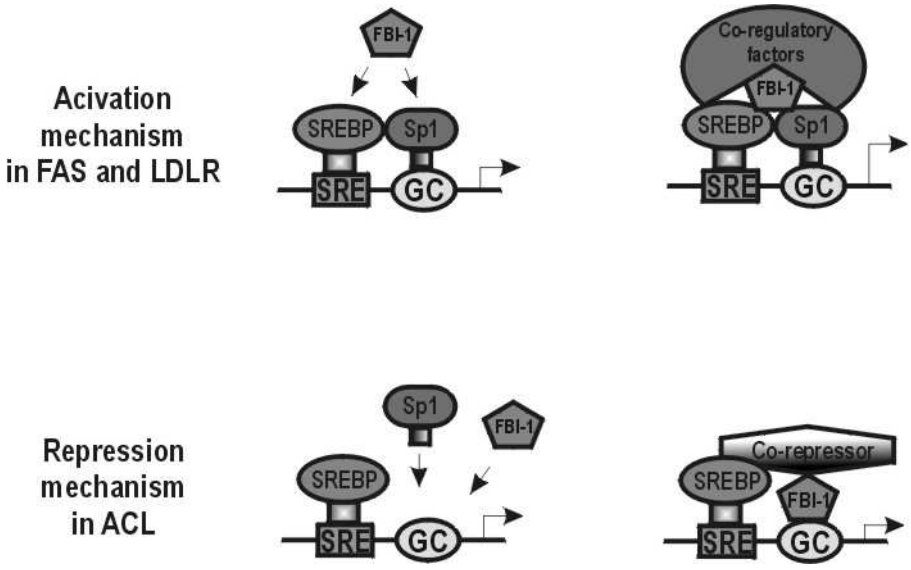

Figure 10. Hypothetical mechanism of transcriptional regulation by FBI-1. Activation mechanism: FBI-1 interacts with Sp1 or SREBP-1a that were already bound onto promoter and the interaction recruit the co-regulatory factors. FBI-1, Sp1, and SREBP-1a complex activates transcription. Repression mechanism: FBI-1 recognizes GC boxes and inhibits promoter binding by Sp1. After FBI-1 binding, chromatin compaction by co-repressors recruited by the POZ domain of FBI-1 may repress transcription.

V. Conclusion

1. FBI-1 mRNA levels increase in adipose tissue of *ob/ob* mouse. It implies that FBI-1 may play an important role in lipid metabolism.

2. FBI-1 may has different action depending on promoter context of lipogenic genes and cellular context. In three FAS constructs, FBI-1 activates or represses transcription depending on configuration of response elements. In ACL construct, FBI-1 represses transcription by Sp1 or SREBP-1a. In LDLR construct, FBI-1 activates transcription by SREBP-1a.

3. The POZ domain of FBI-1 interacts with SREBP-1a and Sp1ZFDBD. FBI-1 regulates transcription by Sp1 or SREBP-1a through the direct interaction.

4. The POZ domain of FBI-1 reduces binding affinity of Sp1 to the GC-boxes of FAS, ACL, and LDLR genes.

5. FBI-1ZFDBD can bind to the GC-boxes of FAS, ACL, and LDLR genes. FBI-1 may compete with Sp1.

6. FBI-1 plays an important role in lipid metabolism via interaction with SREBP-1a or Sp1.

References

1. Morrison DJ, Pendergrast PS, Stavropoulos P, Colmenares SU, Kobayashi R, Hernandez N. FBI-1, a factor that binds to the HIV-1 inducer of short transcripts (IST), is a POZ domain protein. Nucleic Acids Res. 1999; 27: 1251-1262.

2. Pessler F, Pendergrast PS, Hernandez N. Purification and characterization of FBI-1, a cellular factor that binds to the human immunodeficiency virus type 1 inducer of short transcripts. Mol Cell Biol. 1997; 17: 3786-3798.

3. Pendergrast PS, Wang C, Hernandez N, Huang S. FBI-1 can stimulate HIV-1 Tat activity and is targeted to a novel subnuclear domain that includes the Tat-P-TEFb-containing nuclear speckles. Mol Biol Cell. 2002; 13: 915-929.

4. Bardwell VJ and Treisman R. The POZ domain: a conserved protein-protein interaction motif. Genes Dev. 1994; 8: 1664-1677.

5. Farkas G, Gausz J, Galloni M, Reuter G, Gyurkovics H, Karch F. The Trithorax-like gene encodes the Drosophila GAGA factor. Nature. 1994; 371(6500): 806-808.

6. Chen Z, Brand NJ, Chen A, Chen SJ, Tong JH, Wang ZY, et al. Fusion between a novel Kruppel-like zinc finger gene and the retinoic acid receptor-alpha locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. The EMBO Journal 1993; 12: 1161-1167.

7. Yamochi T, Kaneita Y, Akiyama T, Mori S, Moriyama M. Adenovirus-

mediated high expression of BCL-6 in CV-1 cells induces apoptotic cell death accompanied by down-regulation of BCL-2 and BCL-X(L). Oncogene. 1999; 18(2): 487-494.

8. Aravind L, Koonin EV. Fold prediction and evolutionary analysis of the POZ domain: structural and evolutionary relationship with the potassium channel tetramerization domain. J Mol Biol. 1999; 285(4): 1353-1361.

9. Dong S, Zhu J, Reid A, Strutt P, Guidez F, Zhong HJ, et al. Amino-terminal protein-protein interaction motif (POZ-domain) is responsible for activities of the promyelocytic leukemia zinc finger-retinoic acid receptor-α fusion protein. Proc. Natl. Acad. Sci. U. S. A. 1996; 93: 3624-3629.

10. Li JY, English MA, Ball HJ, Yeyati PL, Waxman S, Licht JD. Sequence-specific DNA binding and transcriptional regulation by the promyelocytic leukemia zinc finger protein. J Biol Chem. 1997; 272(36): 22447-22455.

11. Chang CC, Ye BH, Chaganti RS, Dalla-Favera R. BCL-6, a POZ/zinc-finger protein, is a sequence-specific transcriptional repressor. Proc Natl Acad Sci U S A. 1996; 93(14): 6947-6952.

12. Lee DK, Suh D, Edenberg HJ, Hur MW. POZ domain transcription factor, FBI-1, represses transcription of ADH5/FDH by interacting with the zinc finger and interfering with DNA binding activity of Sp1. J. Biol. Chem. 2002; 277: 26761-26768.

13. Sekimata M, Homma Y. Sequence-specific transcriptional repression by an

MBD2-interacting zinc finger protein MIZF. Nucleic Acids Res. 2004; 32(2): 590-597.

14. Yoon HG, Chan DW, Reynolds AB, Qin J, Wong J. N-CoR mediates DNA methylation-dependent repression through a methyl CpG binding protein Kaiso. Mol Cell. 2003; 12(3): 723-734.

15. Labbaye C, Quaranta MT, Pagliuca A, Militi S, Licht JD, Testa U, et al. PLZF induces megakaryocytic development, activates Tpo receptor expression and interacts with GATA1 protein. Oncogene. 2002; 21(43):6669-6679.

16. Tsuzuki S, Enver T. Interactions of GATA-2 with the promyelocytic leukemia zinc finger (PLZF) protein, its homologue FAZF, and the t(11;17)-generated PLZF-retinoic acid receptor alpha oncoprotein. Blood. 2002; 99(9): 3404-3410.

17. Vasanwala FH, Kusam S, Toney LM, Dent AL. Repression of AP-1 function: a mechanism for the regulation of Blimp-1 expression and B lymphocyte differentiation by the B cell lymphoma-6 protooncogene. J Immunol. 2002; 169(4): 1922-1929.

18. Defossez PA, Kelly KF, Filion G, Perez-Torrado R, Magdinier F, Menoni H, et al. The human enhancer-blocker CTCF interacts with the transcription factor kaiso. in press.

19. Huynh KD, Bardwell VJ. The BCL-6 POZ domain and other POZ domains interact with the co-repressors N-CoR and SMRT. Oncogene. 1998; 17(19):

2473-2484.

20. Melnick A, Carlile G, Ahmad KF, Kiang CL, Corcoran C, Bardwell V, et al. Critical residues within the BTB domain of PLZF and Bcl-6 modulate interaction with corepressors. Mol Cell Biol. 2002; 22(6): 1804-1818.

21. Dhordain P, Albagli O, Lin RJ, Ansieau S, Quief S, Leutz A, et al. Corepressor SMRT binds the BTB/POZ repressing domain of the LAZ3/BCL6 oncoprotein. Proc. Natl. Acad. Sci. U. S. A. 1997; 94: 10762-10767.

22. Heinzel T, Lavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, et al. A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature. 1997; 387(6628): 43-48.

23. Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, et al. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell. 1997; 89(3): 373-380.

24. Lin RJ, Nagy L, Inoue S, Shao W, Miller WH Jr, Evans RM. Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature. 1998; 391(6669): 811-814.

25. Widom RL, Lee JY, Joseph C, Gordon-Froome I, Korn JH. The hcKrox gene family regulates multiple extracellular matrix genes. Matrx Biology 2001; 20: 451-462.

26. Maeda T, Hobbs RM, Merghoub T, Guernah I, Zelent A, Cordon-Cardo C, et al. Role of the proto-oncogene Pokemon in cellular transformation and ARF

repression. Nature. 2005; 433(7023): 278-285.

27. Laudes M, Christodoulides C, Sewter C, Rochford JJ, Considine RV, Sethi JK, et al. Role of the POZ zinc finger transcription factor FBI-1 in human and murine adipogenesis. J. Biol. Chem. 2004; 279: 11711-11718.

28. Sanchez HB, Yieh L, Osborne TF. Cooperation by sterol regulatory element-binding protein and Sp1 in sterol regulation of low density lipoprotein receptor gene. J. Biol. Chem. 1995; 270: 1161-1169.

29. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J Clin Invest. 1997; 99(5): 838-845.

30. Oliner JD, Andresen JM, Hansen SK, Zhou S, Tjian R. SREBP transcriptional activity is mediated through an interaction with the CREB-binding protein. Genes Dev. 1996; 10(22): 2903-2911.

31. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997; 89: 331-340.

32. Wang X, Sato R, Brown MS, Hua X, Goldstein JL. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. Cell. 1994; 77, 53-62.

33. Yokoyama C, Wang X, Briggs MR, Admon A, Wu J, Hua X, et al. SREBP-1,

a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell. 1993; 75: 187-97.

34. Bennett MK, Lopez JM, Sanchez HB, Osborne TF. Sterol regulation of fatty acid synthase promoter. Coordinate feedback regulation of two major lipid pathways. J Biol Chem. 1995; 270(43): 25578-25583.

35. Moon YA, Lee JJ, Park SW, Ahn YH, Kim KS. The roles of sterol regulatory element-binding proteins in the transactivation of the rat ATP citrate-lyase promoter. J. Biol. Chem. 2000; 275(39): 30280-30286.

36. Suske G. The Sp-family of transcription factors. Gene. 1999; 238(2):291-300.

37. Courey AJ, Tjian R. (1992) in Transcriptional Regulation. McKnight SL, Yamamoto KR, editors, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. p. 743-769.

38. Dooley KA, Millinder S, Osborne TF. Sterol regulation of 3-hydroxy-3-methylglutaryl-coenzyme A synthase gene through a direct interaction between sterol regulatory element binding protein and the trimeric CCAAT-binding factor/nuclear factor Y. J Biol Chem. 1998; 273(3):1349-1356.

39. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin. Invest. 2002; 109: 1125-1131.

40. Yieh L, Sanchez HB, Osborne TF. Domains of transcription factor Sp1

required for synergistic activation with sterol regulatory element binding protein 1 of low density lipoprotein receptor promoter. Proc Natl Acad Sci USA. 1995; 92: 6102-6165.

41. Kwon HS, Kim MS, Edenberg HJ, Hur MW. Sp3 and Sp4 Can Repress Transcription by Competing with Sp1 for the Core cis-Elements on the Human ADH5/FDH Minimal Promoter J. Biol. Chem. 1999; 274: 20-28.

42. Magana MM, Osborne TF. Two tandem binding sites for sterol regulatory element binding proteins are required for sterol regulation of fatty-acid synthase promoter. J. Biol. Chem. 1996; 271: 32689-32694.

43. Davies JM, Hawe N, Kabarowski J, Huang QH, Zhu J, Brand NJ, et al. Novel BTB/POZ domain zinc-finger protein, LRF, is a potential target of the LAZ-3/BCL-6 oncogene. Oncogene. 1999; 18(2): 365-375.

44. Kukita A, Kukita T, Ouchida M, Maeda H, Yatsuki H, Kohashi O. Osteoclast-Derived Zinc Finger (OCZF) protein with POZ domain, a possible transcriptional repressor, is involved in osteoclastogenesis. Blood 1999; 94 (6); 1987-1997.

45. Lee DK, Kang JE, Park HJ, Kim MH, Yim TH, Kim JM, et al. FBI-1activates transcription of NF-kB responsive E-selectin gene by nuclear localization of p65 subunit of NF-B. J. Biol. Chem. 2005; 280(30): 27783-27791.

46. Bennett MK, Ngo TT, Athanikar JN, Rosenfeld JM, Osborne TF. Co-

stimulation of Promoter for Low Density Lipoprotein Receptor Gene by Sterol Regulatory Element-binding Protein and Sp1 Is Specifically Disrupted by the Yin Yang 1 Protein J Biol Chem, 1999; 274(19): 13025-13032.

47. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002; 109(9): 1125-1131.

Abstract (in Korean)

FBI-1, SREBP-1a, Sp1 전사인자에 의한 지질대사 관련 유전자 전사조절

지도교수 허만욱

연세대학교 대학원 의과학과

박혜진

FBI-1 은 단백질-단백질 상호작용에 관여하는 BTB/POZ domain 과 Krüppel-like zinc finger DNA binding domain 으로 구성된 전사조절 기능을 가진 단백질이다. FBI-1 은 일반적으로 BTB/POZ domain 을 통해 전사를 억제하고, 지방세포 분화에 관여한다고 밝혀져 있으나 지질대사에 있어 FBI-1 의 역할에 대한 명확한 연구결과는 없는 상태이다. 본 연구에서는 지질대사에 관여하는 SREBP-1a 나 Sp1 전사인자와 함께 FBI-1 이 지질대사 관련 유전자의 전사에 어떤 영향을 미치는지 조사하였다. *ob/ob* C57BL/6J 생쥐의 지방조직에서 FBI-1 mRNA 가 증가되어 있음을 관찰하였다. FAS, ACL, LDLR promoter 를 이용한 transient transfection assay 를 통해 FBI-1 이 SREBP-

1a 나 Sp1 에 의한 전사활성을 유전자의 promoter context 나 cellular context 에 따라 억제하거나 활성화할 수 있다는 사실을 발견하였다. GST 융합단백질 pulldown assay 를 통해 FBI-1 은 Sp1 또는 SREBP-1a 와 직접적으로 결합한다는 사실을 보였다. 또한 EMSA 결과를 통해 FBI-1 BTB/POZ domain 이 FAS, ACL, LDLR ptomoter 에 존재하는 Sp1 결합 부위인 GC-box 에 Sp1 이 결합하는 것을 저해한다는 사실을 밝혔다. 더불어 FBI-1ZFDBD 역시 FAS, ACL, LDLR 에 존재하는 GC-box 에 결합할 수 있는 능력이 있다는 것을 알아냈다. FBI-1 은 Sp1 과 경쟁적으로 GC-box 에 결합하여 지질대사 관련 유전자의 전사를 활성화하거나 억제하는 것으로 여겨진다. 이러한 결과들을 통해 FBI-1 은 FAS, ACL, LDLR 등의 지질대사 관련 유전자의 전사를 조절하여 지질대사에 직접적으로 관여하리라 사료된다.

핵심되는 말: 전사 조절, 지질대사 관련 유전자, FBI-1, SREBP-1a, Sp1