Effects of insulin, glucose and polyunsaturated fatty acid on transcriptional regulation of leptin gene

Hitomi Fukuda, Nobuko Iritani

Abstract

The DNA regulatory sequences required for stimulation and suppression of leptin gene expression were investigated in primary cultured hepatocytes and adipocytes of rats. When two copies of the sequences spanning -101 to -83 of leptin promoter were linked to a reporter gene and were used for transfection, the reporter activity significantly increased in the presence of insulin/glucose in comparison with glucose alone in hepetocytes and adipocytes of rats. This increase was inhibited by addition of polyunsaturted fatty acid (PUFA). Cotransfection studies in rat hepatocytes and adipocytes, with the Sp1 expression vector and lepin (-101/-83) constructs, showed the inactivation of the leptin promoter by Sp1. Gel mobility shift assays using an end-labeled leptin (-101 to -83) construct as a probe revealed that nuclear factor (s) from rat liver or adipose tissue specifically formed complexs with the sequence. The region from -101 to -83 of leptin gene competed for the formation of DNA-protein complexes to the region from -101 to -83 in the gel shift assay. By antibody supershift assays, the transcriptional factor Sp1 was found to bind the GC-rich region located within -101 to -83 of the leptin gene. Mutational analysis of this region showed that the sequence was essential for the binding of Sp1 and Sp3. It has been demonstrated that the regions from -101 to -83 of the leptin gene was responsible for regulation due to insulin/glucose and PUFA, and Sp1 and Sp3 may be involved in the regulation. These results were similar to those for the region from -57 to -35 of fatty acid synthase (FAS) and -64 to -41 of ATP citrate-lyase (ACL) genes.

Thus, we postulated that the leptin, ACL and FAS genes contain common DNA sequences responsible for the insulin/glucose stimulation, suggesting that these gene are similarly regulated.

Introduction

The products of the *ob* gene is leptin, a 16-kDa peptide hormone produced by adipose cells that acts in the hypothalamus and plays a central role in regulation of energy expenditure, food

intake and adiposity (1,2). Leptin expression is regulated by glucocorticoids (3-5), insulin (6,7), β-aderenergic agonist (3,8), thiozolidinediones (9-11), adiposity (12,13) and fasting (14-16). Sp1 has been shown to be a transcription factor of leptin gene expression, binding to nucleotides -101 to -83, and mutation in this region abolishes transcription factor binding and reduces promoter activity (17). We previously mapped the sequences responsible to insulin/glucose-stimulation in the proximal promoter region from -57 to -35 of fatty acid synthase (FAS) [EC 2.3.1.85] and -64 to -41 of ATP citrate-lyase (ACL) [EC 4.1.3.8.] of rat liver (18, 19). When two copies of a synthetic nucleotide probe of region -57 to -35 of FAS gene were linked to a reporter gene and the resultant construct was used for transfection, the reporter gene activity was significantly increased in response to insulin/glucose treatment and the insulin stimulation was suppressed by polyunsaturated fatty acid (PUFA), Sp1 and leptin in hepatocytes (20). The same results were observed with the -64 to -41 region of the ACL gene (19). Nucleotides -101 to -83 of the leptin gene, -57 to -35 of the FAS gene and -64 to -41 of the ACL gene contain overlapping binding sites for Sp1 proteins (17, 20, 21).

It is not clear whether regulation of the leptin promoter is controlled by the relative expression levels of these transcription factors or by changes in their functional activity. In the present study, we have investigated the effect of insulin/glucose and PUFA on the transcription of leptin gene, using a -101 to -83 of leptin linked to a reporter gene in cultured hepatocytes and adipocytes of rats.

Materials and methods

Materials Restriction endonuclease and other enzymes were purchased from Takara Shuzo. The PGL3 promoter vector and dual luciferase assay system were purchased from Promega. The sequence kit and luciferase assay kit were from Takara Syuzo and Toyo Ink, respectively. William's medium E was purchased from Flow Laboratories and other culture media were obtained from Nissui Seiyaku. [^{14}C]Chloramphenicol (2.22 GBq/mmol) and [γ-^{32}P]ATP (110 GBq/mmol) was from ICN. Antibody against Sp1 and Sp3 were from Santa Cruz Biotec, lipofectin reagent was from Life Technologies and lecombinant mouse leptin was obtained from R & D Systems Inc.

Plasmid constructs Plasmid pactL, a luciferase vector containing β-actin enhancer and promoter, and PRL-SV40 were used as an internal control to normalize for variations in CAT and luciferase transfection efficiency (22). Plasmid PL1cat, which contains fragment -94 to +37 of L-type pyruvate kinase (LPK) gene, was produced from LPKcat (23). Plasmid pRSVSp1 (24) were

generously gifted by Prof. Y. Fujii-Kuriyama (Touhoku University). Plasmid pRCMVSp3 (25) was a generous gift from Prof. G. Suske (Philipps Universitat Marburg, Germany). The following single-stranded oligonucleotides were synthesized by GIBCO Ltd. Leptin (-101/-83): 5'-AGCTGGGGCGGGAGTTGGCGCTCG-3', Leptin (-101/-83)-M: 5'-AGCTGAAGCTTGAGTTGGCGCTCG-3', FAS (-57/-35): 5'-GATCGTGGCCGCGCGGGGATGGCCGCG-3', ACL (-64/-41): 5'-GATCTGATGGGGGGCGGGGAGGAGCCCG-3', Sp1: 5'-ATTCGATCGGGGCGGGGCGAGC-3', Sp1-M: 5'-ATTCGATCGGTTCGGGGCGAGC-3'. Underlined sequences are the mutations from the substituted bases. Double-stranded oligonucleotides of leptin (-101/-83) or Lep (-101/-83) M werre inserted into the PGL3 promoter . Double-stranded oligonucleotides of FAS (-57/-35) was inserted into the BamHI sites of PL1cat (20). Double-stranded oligonucleotides of ACL (-64/-41) was inserted into ACLcat20 (21). The sequence of these inserts was verified by dideoxy sequencing, using a sequence kit (26). Two copies of these sequences spanning were linked to reporter gene.

Primary cell culture and transfection Male Wistar rats (200-250 g) maintained on a standard diet (Oriental Koubo, MF, Tokyo) were fasted for 16 h before experiments. The rat hepatocytes and adipocytes were isolated by collagenase digestion, and the DNA reporter constructs were transfected into the cells, as described previously (27). Subsequently, the cells were cultured for 48 h in experimental medium (with 100 μg/ml streptomycin and 100 units/ml penicillin) containing 20 mM glucose with or without 0.1 μM insulin. To some cultures, 0.1 mM arachidonic acid or 0.3 μM recombinant mouse leptin was added. All transfections were performed at least three times in duplicate.

Reporter gene assay The cells were incubated for 48 h after transfection, harvested and lysed by sonication. The extracts were heated at 60℃ for 10 min to inactivate endogenous acetylase (28, 29). Prior to the heating step, aliquots were removed for luciferase assays (30). The heated extracts were centrifuged and the supernatants were assayed for CAT activities. Amounts of the cell extracts normalized by the luciferase activity were used for CAT assays, and the acetylated and nonacetylated forms of [^{14}C]chloramphenicol were determined using a scintillation counter. The percentages of the acetylated forms were calculated. The luciferase reporter activity was assayed by the dual luciferase assay system from Promega according to manufacturer's instructions and measured with a Turner Design TD-20/20 luminometer (31). The luciferase activity driven by the -101 to -83 region of the leptin promoter was expressed as a percentage of the SV40 promoter-driven luciferase activity, which served as a positive control in every transfection experiment.

Gel mobility shift assay Nuclear extracts from rat liver and adipocyte were prepared as

described by Gorski *et al* (32). The end-labeled double-stranded oligonucleotide corresponding to leptin nucleotides -101/-83 was incubated with the indicated amount of nuclear proteins as described previously (20). The reaction mixture was incubated for 1 h at room temperature and then loaded onto a 4 % non-denaturing polyacrylamide gel and separated by electrophoresis at 200V for 1 h (23) In competition studies, the indicated amounts of double-stranded oligonucleotide were added to the reaction mixtures. The nucleotide sequences of FAS (-57/-35) (20), ACL (-64/-41) (21) and Sp1 (33) are shown above. For antibody supershift assays, Sp1 or Sp3 antibody was added to the binding reaction mixture and incubated for 1 h at room temperature prior to adding the labeled probes. The gel was then fixed in 10% methanol, 10% acetic acid, dried, and auto radio graphed.

Results and Discussion

Effects of insulin/glucose, PUFA, Sp1 family and leptin on leptin transcription To explore possible regulatory elements in the 5'-flanking region of leptin gene, double strand oligonucleotides -101 to -83 of the leptin gene was synthesized and linked into PGL3 promoter. The plasmid DNA was trasfected to rat hepatocytes and adipocytes. The cells were cultured in the presence of 20 mM glucose with or without 0.1 μM insulin for 48 h, and then used to prepare extracts which were assayed for luciferase activity, as a reporter activity for leptin transcription. As shown in Fig.1, in the presence of insulin/glucose, the reporter activity of leptin gene transcription was markedly increased (by 1.5-fold) above that in the presence of glucose alone in rat hepatocytes. The stimulation by insulin/glucose was reduced by 50% in arachidonic acid treated hepatocytes. In adipocytes, the reporter activity was also stimulated by insulin/glucose and suppressed by PUFA or leptin. To determined directly the binding of Sp1 to these sequence, rat hepatocytes and adipocytes were cotransfected with leptin (-101/-83) linked to reporter gene (PGL3 promoter) and the Sp1 expression vector. The leptin gene reporter activity was reduced in the presence of the Sp1 expression vector. It is suggested that the Sp1 consensus sequence located between -101 to -83 of the leptin gene can bind to Sp1 and that bound Sp1 inactivated transcription. To further define the sequence involved in the insulin/glucose responsiveness of the leptin promoter, mutagenesis was performed in the region from -100 to -95 of the leptin gene. The mutation resulted in a loss of responsiveness to insulin/ glucose (data not shown). The leptin gene reporter activity was not significantly changed in the presence of the Sp3 expression vector.

As a positive control, the transcriptional activities of FAS (-57/-35) linked to PL1cat or ACL

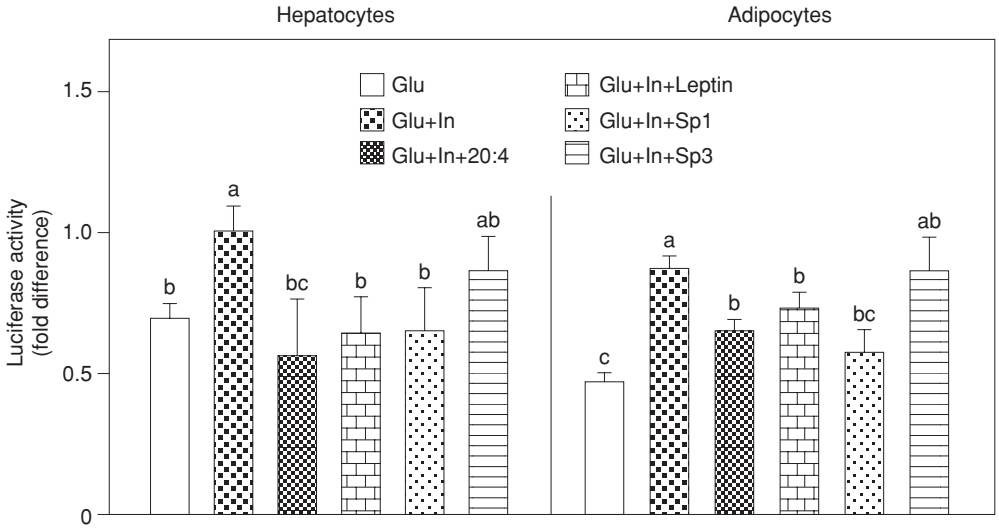

Fig. 1 Effect of insulin/glucose, PUFA, leptin and Sp1 family on leptin gene expression in hepatocytes and adipocytes of rat. The plasmide constuct containing leptin (-101/-83) linked PGL3 promoter was introduced into primary hepatocytes or adipocytes. The cells were cultured for 48 h to measure the luciferase activities in William's medium E containing 20 mM glucose (Glu) with or without 0.1 μM insulin (In). When included, 0.1 mM arachidonic acid (20:4) or 0.3 μM leptin was added. To some cultures, leptin (-101/-83) linked to PGL3 promoter was cotransfected into cells with the Sp1 expression vector (Sp1) or the Sp3 expression vector (Sp3). The relative luciferase activities, the construct of leptin (-101/-83) linked to PGL3 promoter were introduce into cells, are normalized to Glu+In addition. Means with different superscript letters in each column are signifacantly different at $P<0.05$. Mean ± SD (n=4).

(-64/-41) linked to ACLcat20 were measured in the same hepatocytes and adipocytes used for leptin transcription studies. The transcriptional activities of the FAS and ACL genes were stimulated by insulin/glucose, and the stimulation was suppressed by PUFA or leptin in a manner similar to the leptin gene. The results for FAS transcription are shown in Fig. 2, and the results for the ACL gene were very similar (data not shown). These results confirmed those of previous studies (18, 19). The transcriptional activities of the FAS and ACL genes stimulated by insulin/glucose were suppressed in the presence of Sp1 expression vector (20, 21). Leptin (-101/-83), FAS (-57/-35) and ACL (-64/-41) contain similar GC-rich sequences in which an Sp1 consensus sequence is found.

Gel mobility shift assays. To identify and characterize potential protein binding activity associated with the leptin (-101/-83), an electrophoresis mobility shift assay was used. End-labeled oligonucleotide leptin (-101/-83) was incubated with nuclear extract of rat liver and subjected to a non-denaturing polyacrylamide gel electrophoresis. Three bands of DNA-protein complexes were observed, the complexes were competed away by unlabeled oligonucleotide of

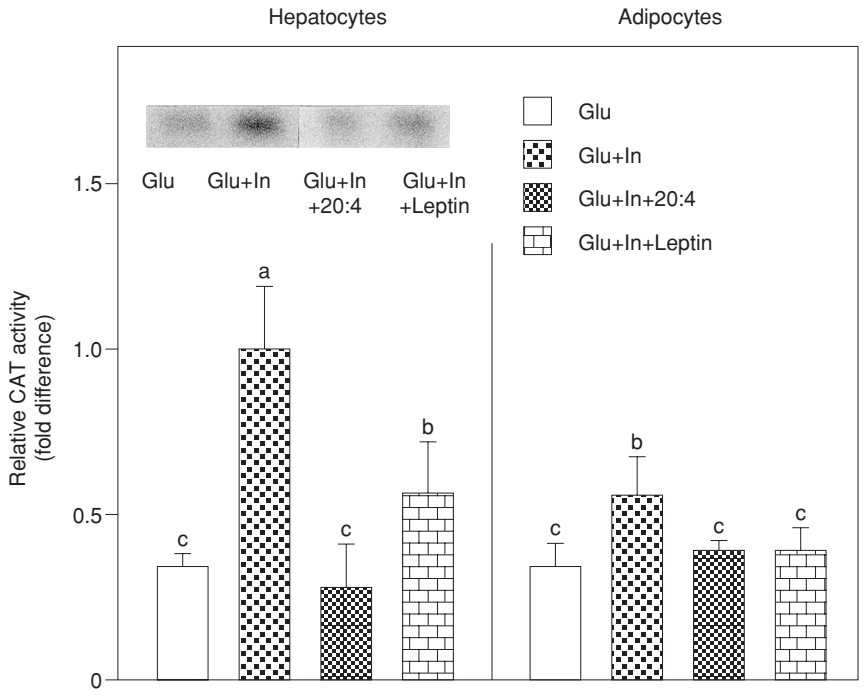

Fig.2. The CAT activities (transcriptional activities) of the construct of FAS (-57/-35). The construct of FAS (-57/-35) linked to PL1cat was introduced into rat hepatocytes or adipocytes. The cells were incubated with or without 0.1 μM insulin (In) for 48h in the presence of 20 mM glucose (Glu). When included, 0.1 mM arachidonic acid (20:4) or 0.3 μM leptin was added. A typical result is shown. The percentages of acetylated forms are normalized to the value for insulin/glucose treated cell (In + Glu). Mean ± SD (n=4).

leptin (-101/-83) (Fig. 3). The result demonstrated specificity of the DNA-protein complex formation. In the presence of excess unlabeled leptin (-101/-83)-M (mutated oligonucleotides), no competition was observed. This suggests that sequences between -101 to -83 of leptin essentially for the binding of nuclear factor (s) to this putative inslin/glucose response sequence in the leptin gene.

On the other hand, we showed previously that the ACL gene promoter segment -64 to -41, the FAS gene promoter segment -57 to -35 are the insulin/glucose-stimulation and PUFA-suppression response elements (20, 21). Since the similarity between nucleotides -54 to -43 of the FAS gene, -60 to -49 of the ACL gene and -101 to -83 of leptin gene was 9 out of 12, we tested whether these FAS and ACL sequence competes for the nuclear factors binding to leptin (-101/-83). As shown in Fig. 3, FAS (-57/-35) and ACL (-64/-41) oligonucleotide as well as leptin (-101/-83) itself, could effectively compete for the formation of the three bands of DNA-protein complexes at 20- molar excess (in ratio) to labeled leptin (-101/-83) probe in the liver nuclear

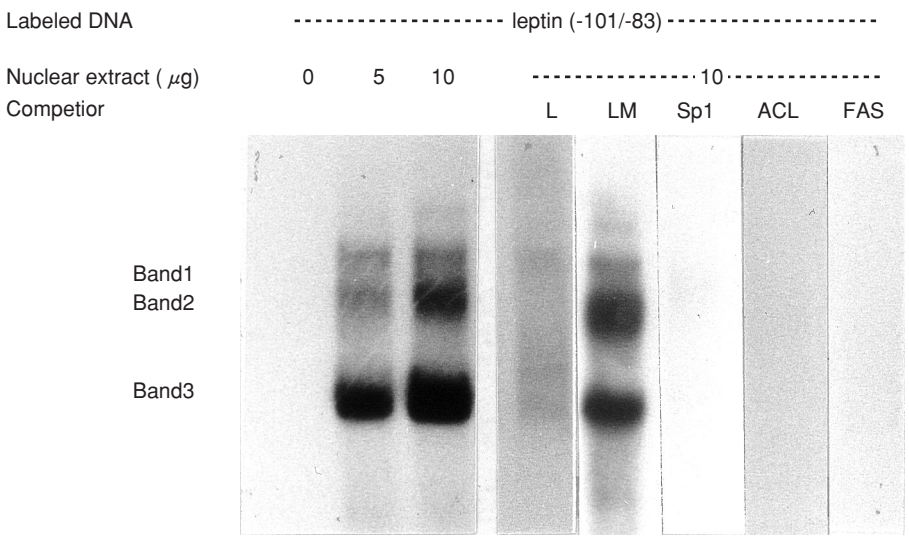

Fig. 3. Gel mobility shift assays using liver nuclear extracts with end-labeled leptin (-101/-83). Increasing amounts of rat liver nuclear extracts were incubated in the presence of end-labeled oligonucleotide leptin (-101/-83). Competitor DNA was added in 20-fold molar excess relative to labeled DNA. The species of competitor DNAs are indicated at the top. L: leptin (-101/-83), LM: mutant leptin (-101/-83), ACL: ACL (-64/-41), FAS: FAS (-57/-35).

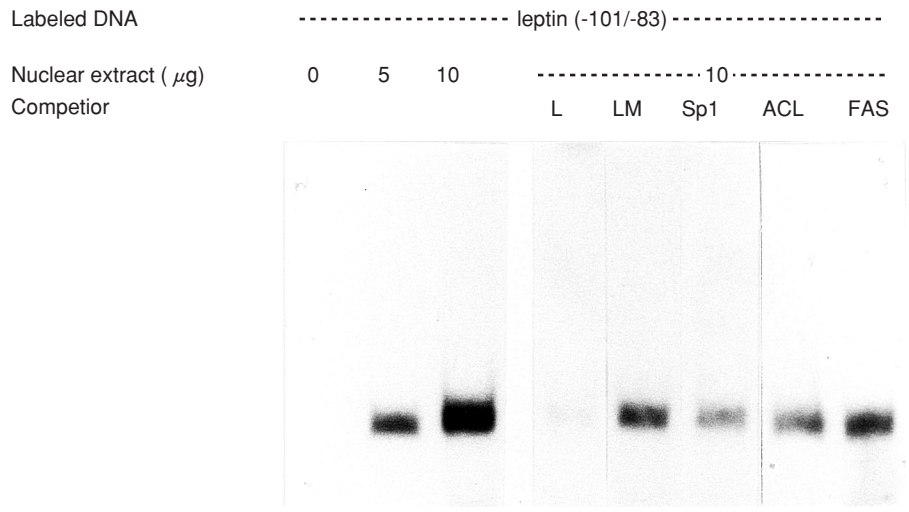

Fig.4. Gel mobility shift assays using adipose tissue nuclear extracts with end-labeled leptin (-101/-83). Increasing amounts of rat adipose tissue nuclear extracts were incubated in the presence of end-labeled oligonucleotide leptin (-101/-83). Competitor DNA was added in 10-fold molar excess relative to labeled DNA. The species of competitor DNAs are indicated at the top. L: leptin (-101/-83), LM: mutant leptin (-101/-83), ACL: ACL (-64/-41), FAS: FAS (-57/-35).

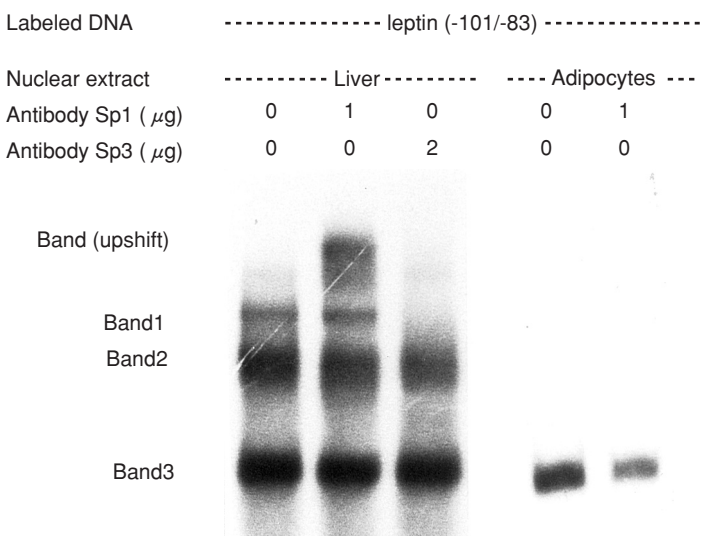

Fig. 5. DNA mobility supershift assay. Mobility shift assays were performed with or without Sp1 and Sp3 antibody. Labeled leptin (-101/-83) was used as probes in the binding assay.

extracts. Addition of the same amount of Sp1 probe to the gel mobility shift reactions had effectively compete to the labeled leptin (-101/-83) probe. These results suggested that Sp1 can bind to the insulin/glucose response elements of leptin (-101/-83) as well as to those of FAS (-57/-35) and ACL (-64/-41).

The results for nuclear extracts of adipose tissue are shown in Fig. 4. Only one band of DNA-protein complex (band 3) was seen in the fluorogram. Addition of Sp1 probe to the gel mobility shift reactions competed against the labeled leptin probe, although the competition was not so strong as in the liver nuclear extracts. After incubation of nuclear extracts of adipose tissue with the leptin probe in the presence of Sp1 antibody, the supershift band disappeared (Fig. 5). Therefore, any other binding protein than Sp1 may exist for DNA-protein complex formation in adipose tissue. The physiological singnificance of these findings awaits further investigation.

Since the Sp1 consensus sequence is known to bind Sp1 and other related protein, particularly Sp3, were attempted to further demonstrate that Sp1 binding to the leptin gene, Sp1- or Sp3-specific antibody was added to the gel mobility shift assay. After incubation of liver nuclear extract with leptin (-101/-83) probe in the presence of Sp1 antibodies, band 2 resulted in supershift, as shown in Fig. 5. The formation complex (band 1) was efficiently inhibited in the presence of Sp3 antibody. The result shows that band 1 and band 2 represents an leptin (-101/-83) specific protein-DNA complex, which contains Sp3 and Sp1, respectivly. It was

reported that the glucose activation of acetyl-CoA carboxylase gene promoter II was mediated by a member of the Sp1 family of transcription factor (34), as members of a gene family , Sp1, Sp3 and Sp4 show similar structural characteristics and identical DNA binding specificity (35, 36).

Daniel and Kim (34) reported that the promoter II of acetyl-CoA carboxylase gene (-340 to -249) was activated by high concentrations of glucose and the effect of glucose was mediated by Sp1. On the other hand, Rolland et al. (37) reported that FAS promoter activity mainly depended on a region from -200 to-126 and this sequence exerted a strong negative effect on FAS promoter in adipocytes from lean rats but not in those from obese rats. They demonstrated that Sp1 or Sp1-like proteins were bound to this DNA subregion. It appears that while Sp1 can directly suppress gene transcription by DNA binding (38-40), Sp3, by competing for Sp1 binding or by interacting with other nuclear factor, leads to repression of gene transcription (41, 42).

The present studies demonstrated that the sequences located between -101 and -83 of the leptin gene can bind to Sp1 and Sp3, and that the bound Sp1 inactivated the leptin transcription. Similarly, the nucleotide -57 to -35 of FAS gene and the nucleotide -64 to -41 of ACL gene bound to Sp1 and Sp3, and the bound Sp1 inactivated its own transcription and the bound Sp3 stimulated its own transcription, respectively (43). On the other hand, these three nucleotides have been demonstrated to be the response elements of the insulin/glucose activation and the PUFA inactivation of the leptin gene. Thus, we postulated that Sp1 and Sp3 are somehow involved in the insulin/glucose activation and the PUFA inactivation of the leptin gene, the FAS gene and ACL gene.

References

(1) Zahng, Y., Proenca, R., Maffei, M., Borone, M., Leopold, L., and Freidman, J. M. (1994) Nature, 372, 425-4432.

(2) Flier, J. S. (1997) Proc. Natl. Acad. Sci. USA, 94, 4242-4245.

(3) Murakami, T., Iida, M. and Shima, K. (1995) Biochem. Biophys. Acta, 214, 1260-1267.

(4) De Vos, P., Saladin, R., Auwerx, J. and Staels, B. (1995) J. Biol. Chem. 270, 15958-15961.

(5) Slieker, L. J., Sloop, K. W., Surface, P. L., Kriauciunas, A., LaQuier, F. Manetta, J,m Bue-Vallesky, J. and Stephens, T. W. (1996) J. Biol. Chem. 271, 5301-5304.

(6) Saladin, R., De Vos, P., Guerre-Millo, M., Leturque, A., Girard. J., Staels, B., and Auwerx, J. (1995) Nature, 377, 527-529.

(7) Cusin, I., Sainsbury, A., Doyle, P., Roher-Jeanrenaud, F. and Jeanrenaud, B. (1995) Diabetes, 44, 1467-1470.

(8) Mantzoros, C. S., Qu, D., Frederich, R. C., Susulic, V. S., Lowell, B. B., Maratos,-Filer, E. and Flier, J. S. (1996) Diabetes, 45, 909-9014.

(9) De Vos, P., Lefebvre, A. M., Miller, S. G., Guerre-Milo, M., Wong, K., Saladin, R., Hamann, L. G., Staels,

B., Briggs, M. R. and Auwerx, J. (1996) J. Clin. Invest, 98, 1004-1009.

(10) Zahng, G., Graziano, M. P., Doebber, T. W., Leibowitz, M. D., White-Carringtomn, S., Szalkowski, D. M., Hey, P. J., Wu, M., Cullinan, C. A., Bailey, P., Lollmann, B., Frederich, R., Flier, J. S., Strader, C. D. and Smith, R. G. (1996) J. Biol. Chem. 271, 9455-9459.

(11) Nolan, J. J., Olefsky, J. M., Nyce, M. R., Considine, R. V. and Caro, J. F. (1996) Diabetes, 45, 1276-1278.

(12) Lonnqvist, M. K., Arner, P., Nordfors, L. and Schalling, M. (1995) Nature Medi. 1, 950-953.

(13) Sinha, M. K., Ohannesian, J. P., Heiman, M. L., Kriauciunass, A., Stephens, T. W., Magosin, S., Marco, C. and Caro, J. F. (1996) J. Clin. Invest. 97, 1344-1347.

(14) Ahima, R. S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E. and Flier, J. S. (1996) Nature, 382, 250-252.

(15) Kolaczynski, J. W., Considine, R. V., Ohannesian, J., Marco, C., Opentanova, I., Nyce, M. R., Myint, M. and Caro, J. F. (1996) Diabetes, 45, 1511-1515.

(16) Boden, G., Chen, X., Mozzoli, M. and Ryan, I. (1996) J. Clin. Endocrinol. Metab. 81, 3419-3423.

(17) Mason, M. M., HE, Y., Chen, H., Quon, M. J. and Reitman, M. (1998) Endocrinol. 139, 1013-1022.

(18) Fukuda, H., Iritani, N., Katsurada, A. and Noguchi, T. (1996) Biochem. Mol. Biol. Int. 38, 987-996.

(19) Fukuda, H., Katsurada, A., Iritani, N. and Noguchi, T. (1996) FEBS Letters, 380, 204-207.

(20) Fukuda, H., Iritani, N. and Noguchi, T. (1997) FEBS Letters, 406, 243-248.

(21) Fukuda, H., Iritani, N. and Noguchi, T. (1997) Eur. J. Biochem. 247,497-502.

(22) Wang, Z., Takenaka, M. Imai, E., Yamada, K., Tanaka, T. and Noguchi, T. (1994) Eur. J. Biochem. 220, 301-307.

(23) Yamada, K., Noguchi, T., Matsuda, T., Takenaka, M., Monaci,P., Nicosia, A. and Tanaka, T. (1990) J. Biol. Chem. 265, 19885-19891.

(24) Imataka, H., Sogawa, K., Yasumoto, K., Kikuchi, Y., Sasano, K.,Kobayashi, A., Hayami, M. and Fujii-Kuriyama, Y. (1992) EMBO J., 11, 3663-3671.

(25) Hagen, G., Muller, S., Beato, M. and Suske, G. (1994) EMBO J. 73, 3843-3851.

(26) Sanger, F., Nicklen, S. and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.

(27) Fukuda, H., Iritani, N., Sugimoto, T. and Ikeda, H. (1999) Eur. J. Biochem. 260, 505-511.

(28) Crabb, B. W. and Dixon, J. E. (1987) Anal. Biochem. 163, 88-92.

(29) Gorman, C. M., Moffat, L. F. and Howard, B. H. (1982) Mol. Cell Biol. 2, 1044-1051.

(30) Williams, T. M., Burlein, J. E., Ogden, S., Kricka, L. J. and Kant, J. A. (1989) Anal. Biochem. 176, 28-32.

(35) Gorski, K., Carniero, M. and Schibler, U. (1986) Cell 47, 767-776. (36) Kadonaga, J. T., Carner, K. R. , Masiarz, F. R. and Tjian, (1987) Cell, 51, 1079-1090.

(31) Menon, R. K., Stephan, D> A., Singh, M., Morris, S. M. Jr., and Zhou, L. (1995) J. Biol. Chem. 270, 8851-8859.

(32) Gorski, K., Carniero, M. and Schibler, U. (1986) Cell 47, 767-776.

(33) Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 163, 156-159.

(34) Daniel, S. and Kim, K.-H. (1996) J.Biol. Chem. 271, 1385-1392.

(35) Kingsley, C. and Winto, A. (1992) Mol. Cell Biol. 12, 4251-4261.

(36) Hagen, G., Muller, S., Beato, M. and Suske, G. (1992) Nucle. Acid Res. 20, 5519-5525.

(37) Rolland, V., Liepvre, X. L., Jump, D. B., Lavau, M. and Dugail, I. (1996) J. Biol. Chem. 271, 21297-21302.

(38) Li, R., Hodny, Z., Luciakova, K., Barath, P. and Nelson, B. D. (1996) J. Biol. Chem. 271, 18925-18930.

(39) Sengupta, P. K., Lavelle, D. and DeSimone, J. (1994) Am. J. Hematology 46, 169-172.

(40) Madsen, C. S., Hershey, J. C., Hautmann, M. B., White, S. L. and Owens, G. K. (1997) J. Biol. Chem. 272, 6332-6340.

(41) Denning, J., Beato, M. and Suske, G. (1996) EMBO J. 15, 5659-5667.

(42) Gao, J. and Tseng, L. (1996) Mol. Endocrinol. 10, 613-621.

(43) Fukuda, H., Noguchi, T. and Iritani, N. (1999) FEBS Letters, 464, 113-117.