The Nuclear Abundance of Transcription Factors Sp1 and Sp3 Depends on Biotin in Jurkat Cells

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ABSTRACT Biotin affects gene expression in mammals; however, the signaling pathways leading to biotin-dependent transcriptional activation and inactivation of genes are largely unknown. Members of the Sp/Krüppel-like factor family of transcription factors (e.g., the ubiquitous Sp1 and Sp3) play important roles in the expression of numerous mammalian genes. We tested the hypothesis that the nuclear abundance of Sp1 and Sp3 depends on biotin in human T cells (Jurkat cells) mediating biotin-dependent gene expression. Jurkat cells were cultured in biotin-deficient (0.025 nmol/L) and biotin-supplemented (10 nmol/L) media for 5 wk prior to transcription factor analysis. The association of Sp1 and Sp3 with DNA-binding sites (GC box and CACCC box) was 76–149% greater in nuclear extracts from biotin-supplemented cells compared with biotin-deficient cells, as determined by electrophoretic mobility shift assays. The increased DNA-binding activity observed in biotin-supplemented cells was caused by increased transcription of genes encoding Sp1 and Sp3, as shown by mRNA levels and reporter-gene activities; increased transcription of Sp1 and Sp3 genes was associated with the increased abundance of Sp1 and Sp3 protein in nuclei. Notwithstanding the important role for phosphorylation of Sp1 and Sp3 in regulating DNA-binding activity, the present study suggests that the effects of biotin on phosphorylation of Sp1 and Sp3 are minor. The increased nuclear abundance of Sp1 and Sp3 in biotin-supplemented cells was associated with increased transcriptional activity of 5′-flanking regions in Sp1/Sp3-dependent genes in reporter-gene assays. This study provides evidence that some effects of biotin on gene expression might be mediated by the nuclear abundance of Sp1 and Sp3.

KEY WORDS: • biotin • human • Jurkat cells • Sp1 • Sp3

In mammals, biotin serves as a covalently bound coenzyme for the following carboxylases: acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase and 3-methylcrotonyl-CoA carboxylase (1). These enzymes catalyze essential steps in the metabolism of glucose, amino acids and fatty acids (1). Consistent with the classical roles for biotin in carboxylase-dependent metabolic pathways, biotin deficiency may cause decreased rates of cell proliferation (2,3), impaired immune function (4–6) and abnormal fetal development (7–9).

Evidence shows that biotin might also play a role in the expression of various genes in mammals. For example, the expression of genes encoding glucokinase (10–14), holocarboxylase synthetase (15,16), 3-methylcrotonyl-CoA carboxylase (17), biotin transporters (3,18), interleukin-2 receptor (17), interleukin-2 (19), interleukin-1β (17) and interferon-γ (17) correlates with the biotin supply in humans, human cell lines and rats. In contrast, biotin supplementation causes decreased expression of genes encoding phosphoenolpyruvate carboxykinase in the liver from diabetic rats (20) and interleukin-4 in peripheral blood mononuclear cells from healthy adults (17). DNA microarray studies have suggested that biotin supplementation affects the expression of ~300 genes in peripheral blood mononuclear cells from healthy adults (Rodríguez-Melendez, R. and Zempleni, J., unpublished results). Theoretically, abnormal gene expression might account for some of the adverse effects of biotin deficiency in humans and animals. The signaling pathways leading to biotin-dependent transcriptional activation and inactivation of genes are largely unknown.

Sp1 and Sp3 belong to the Sp/Krüppel-like factor (KLF)3 family of transcription factors (3). The Sp/KLF family comprises at least 20 identified transcription factors in mammals, including Sp1, Sp3, ubiquitous and basic KLF; many of these proteins exhibit a widespread or ubiquitous tissue distribution (21). Sp/KLF transcription factors bind to GC-rich sequences (e.g., GC boxes and CACCC boxes) located in the regulatory regions of numerous genes; these sequences are collectively referred to as Sp1 sites. Members of the Sp/KLF family have overlapping DNA-binding specificities; thus, competition among family members for binding to regulatory sequences in

1 This work was supported by NIH Grant DK 60447. This paper is a contribution of the University of Nebraska Agricultural Research Division, Lincoln, NE 68583 (Journal Series No. 14189).
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3 Abbreviations used: EMSA, electrophoretic mobility shift assay; KLF, Krüppel-like factor; PHA, phytohemagglutinin; PMA, phorbol-12-myristate-13-acetate; SERCA3, sarco/endoplasmic reticulum Ca2+/ATPase 3; SV40, simian virus 40.
DNA plays an important role in the regulation of gene expression (21). Sp/KLF transcription factors differ in their transcriptional activity with some being activating and some being repressive, establishing a very complex network of regulation. For example, Sp3 may repress Sp1-dependent activation of genes encoding dihydrofolate reductase and α1(I) procollagen by competing with Sp1 for binding to Sp1 sites (22,23). In the present study we tested the hypothesis that the concentration of biotin in culture media affects the DNA-binding activity of transcription factors Sp1 and Sp3 in a human T cell line (Jurkat cells), mediating some of the effects of biotin on gene expression.

MATERIALS AND METHODS

Cell culture. Jurkat cells (clone E6–1) were purchased from American Type Culture Collection (Manassas, VA). Cells were cultured (5% CO2 at 37°C in a humidified atmosphere) for at least 5 wk prior to sample collection in a biotin-defined media, i.e., 0.025 nmol/L biotin (deficient) or 10 nmol/L biotin (supplemented); culture medium was replaced every 48 h. The media were prepared using biotin-depleted fetal bovine serum as described previously (3); biotin concentrations in media were confirmed by avidin-binding assay (24) with modifications (3). Biotin concentrations were chosen based on the reasoning that 0.025 nmol/L of biotin is >2 SD below the mean physiologic concentration in normal plasma (25); thus, 0.025 nmol/L equals a deficient concentration of biotin. Ingestion of a typical biotin supplement providing 25 times the adequate intake of biotin with 2 μg of antibody to either Sp1 or Sp3 for 60 min at room temperature prior to adding oligonucleotide probes. The incubation of nuclear proteins with antibodies i) may prevent formation of transcription factor-oligonucleotide complexes (if antibodies block oligonucleotide-binding sites in transcription factors); or ii) may decrease the electrophoretic mobility of the transcription factor-oligonucleotide complex (if antibodies bind to transcription factors without blocking their oligonucleotide-binding sites).

Gene expression. The expression of genes encoding Sp1, Sp3 and histone H3 (control) was quantified by RT-PCR as previously described (28) with 45°C being used as the annealing temperature for PCR amplifications of Sp1 and Sp3. The following customized primers were used for PCR (Integrated DNA Technologies, Coralville, IA): i) 5'-CTACCCCTACCTCAAGAGG-3' and 5'-CTCTCTCTCTTCTTGCTGCG-3' for human Sp1 (29); ii) 5'-TCAAGTGTTATTGCTCTCT-3' and 5'-TGAAGTGTGCTTTAAGAAT-3' for human Sp3 (29); and iii) 5'-ATGGCGCTACTAAGCCAGGC-3' and 5'-TACGCCCTCTCCCACGAGTGGC-3' for human histone H3; histone H3 was chosen as control because the 5’-flanking of this gene does not contain Sp1 sites (30). cDNA was quantified by gel densitometry using the Kodak EDAS 290 Documentation and Analysis System (Rochester, NY); only values from within the exponential phase of PCR amplification were considered for data analysis. The abundance of cDNA encoding Sp1 and Sp3 was normalized by the abundance of cDNA encoding histone H3.

Western blot analysis of Sp1 and Sp3. Sp1 and Sp3 were extracted from Jurkat cells using detergents and protease inhibitors (31). Equal amounts of protein were electrophoresed using 3–8% Tris acetate gels (Invitrogen) as previously described (31). Sp1 and Sp3 were probed using goat and rabbit polyclonal IgG anti-human antibodies (Santa Cruz Biotechnology) as previously described (31). Histone H3 (control) was extracted from cell nuclei using hydrochloric acid as previously described (32). Equal amounts of histones (as judged by gel densitometry after staining with coomassie blue) were electrophoresed using 18% Tris glycine gels (Invitrogen); histones were used for PCR (Integrated DNA Technologies, Coralville, IA).

Electrophoretic mobility shift assays (EMSA). Approximately 9 × 10⁶ cells (in 10 mL of medium) were cultured with 50 μg/L of phorbol-12-myristate-13-acetate (PMA) and 2 mg/L of phytohemagglutinin (PHA) for up to 6 h to stimulate the nuclear translocation of transcription factors. Nuclear extracts were prepared using a Nuclear Extract Kit (Active Motif, Carlsbad, CA) according to the manufacturer’s instructions. Protein concentrations in extracts were determined using the bicinchoninic acid method (Pierce, Rockford, IL). Double-stranded oligonucleotides (Integrated DNA Technologies, Coralville, IA) containing Sp1 sites (GC box and CACC box; Table 1) were radiolabeled using [³²P]dCTP (specific radioactivity = 111 TBq/mmol) and the Megaprime DNA labeling system (Amersham Biosciences, Piscataway, NJ). Nuclear protein (5 μg) was incubated with 2 pmol/L of [³²P]-labeled oligonucleotides for 20 min on ice using the following buffer in a total volume of 10 μL: 1 mmol/L MgCl₂, 0.5 mmol/L EDTA, 0.5 mmol/L dithiothreitol, 50 mmol/L sodium chloride, 10 mmol/L Tris hydrochloride (pH 7.5), 0.05 g/L poly(dl-dC), 0.04 L glycerol/L buffer [a concentrate of this buffer (Gel Shift Binding 5x Buffer) was purchased from Promega, Madison, WI]. The following controls were used in EMSA (Table 1): ³²P-labeled oligonucleotides containing a mutated Sp1 site (GCmut); ³²P-labeled oligonucleotides containing a consensus sequence for the transcription factor Oct-1 (which is not a member of the Sp/KLF family); unlabeled oligonucleotides (Sp1 sites) at concentrations 100 times greater than the corresponding ³²P-labeled probe; and samples with no nuclear extract. Samples were electrophoresed using 6% DNA retardation gels (Invitrogen, Carlsbad, CA). Gels were dried under vacuum at 70°C for 30 min; autoradiography film was exposed to the dried gels and was developed using a Konica SRX101 developer (Wayne, NJ).

In some cases, transcription factor-oligonucleotide complexes were supershifted using goat and rabbit polyclonal IgG anti-human antibodies to Sp1 and Sp3 (Santa Cruz Biotechnology, Santa Cruz, CA). These samples were prepared by incubating 5 μg of nuclear protein with 2 μg of antibody to either Sp1 or Sp3 for 60 min at room temperature prior to adding oligonucleotide probes. The incubation of nuclear proteins with antibodies i) may prevent formation of transcription factor-oligonucleotide complexes (if antibodies block oligonucleotide-binding sites in transcription factors); or ii) may decrease the electrophoretic mobility of the transcription factor-oligonucleotide complex (if antibodies bind to transcription factors without blocking their oligonucleotide-binding sites).

Table 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Consensus binding sequence</th>
<th>Nucleotide sequence†</th>
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<tbody>
<tr>
<td>GC box</td>
<td>Sp1, Sp3</td>
<td>5'-ATTCCATGCAAGGCAGGGCAGG-3'</td>
</tr>
<tr>
<td>GCmut</td>
<td>None</td>
<td>5'-GGTCTCGGGGCCAGAAG-3'</td>
</tr>
<tr>
<td>CACC box</td>
<td>Various members of the Sp/KLF family</td>
<td>5'-ACAGATTGGCCAGAGCCCTGCGTAAAGCGCGTC-3'</td>
</tr>
<tr>
<td>Oct-1</td>
<td>Oct-1</td>
<td>5'-TTCTAGGATTCCATGGCA-3'</td>
</tr>
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† The minimal essential binding sequences for transcription factors are underlined. Lower case letters indicate the mutated sequences in mutant oligonucleotides.
tome H3 was probed as described above using goat polyclonal IgG antihuman histone H3 (Santa Cruz Biotechnology).

**Reporter-gene constructs.** The following reporter-gene constructs were used to quantify the effects of biotin on the transcriptional activity of the gene encoding Sp1: (i) a construct of the luciferase reporter gene driven by the 5′-flanking region of the Sp1 gene (basepairs −1612 to +20 from the ATG start codon) was provided by Carlos J. Ciudad (University of Barcelona, Spain); this plasmid was denoted pGL3FOR5 (33); (ii) a promoter-free plasmid containing the luciferase gene (pGL3-Basic) was purchased from Promega; and (iii) a construct of the RSV promoter linked to the β-galactosidase reporter gene (RSV βgal) was provided by Brett R. White (University of Nebraska-Lincoln).

Jurkat cells were transfected with reporter-gene constructs using SuperFect (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Forty-eight hours after transfection, cells were stimulated with 50 μg/L PMA and 2 mg/L PHA for 3 h. Luciferase and β-galactosidase activities were quantified in lysed cells as previously described (19). Luciferase activities were normalized for transfection efficiency using β-galactosidase activities. Data are expressed as ratios of luciferase activities in cells transfected with pGL3FOR5 to activities in cells transfected with pGL3-Basic.

The regulatory regions of the following genes contain Sp1 sites (GC boxes or CACCC boxes) and were used as models to investigate the effects of biotin on the transcriptional activity of Sp-dependent genes: the 5′-flanking region of the human sarco/endoplasmic reticulum Ca2+ -ATPase 3 (SERCA3; containing 14 GC boxes and 11 CACCC boxes) and the simian virus 40 (SV40) early promoter (6 GC boxes) in the T antigen gene (34-35). The following reporter-gene constructs were used to investigate the effects of biotin on the transcriptional activity of these genes: (i) The region −135 to +55 (transcription start site +1) of human SERCA3 was ligated into the pGL3-Basic vector; this plasmid was denoted PstI-del and was generously provided by Frank Wuytack (Katholieke Universiteit Leuven, Belgium) (34). This region of human SERCA3 contains 5 GC boxes, 1 CACCC box, and 1 Sp1-like site. (ii) The SV40 early promoter and SV40 enhancer A were ligated into the pGL3-Basic vector; this plasmid was denoted pGL3-Control (Promega). Transcriptional activities were quantified as described above with the following modifications: (i) pGL3FOR5 was substituted with PstI-del or pGL3-Control; and (ii) cells were stimulated with PMA and PHA for 6 h.

**Dephosphorylation assays.** The DNA-binding activity of Sp1 is regulated by phosphorylation of this transcription factor (36-39). We determined whether biotin concentrations in culture media affect the phosphorylation of Sp1. Nuclear extracts were prepared as described above. Proteins were dephosphorylated using calf intestinal alkaline phosphatase as previously described (40); controls were incubated in the absence of phosphatase. DNA-binding activity was quantified by EMSA as described above.

**Statistical analysis.** A paired t or Wilcoxon test was used to determine significance of differences (41). StatView 5.0.1 (SAS Institute, Cary, NC) was used to perform all calculations. Differences were considered significant at P < 0.05. Data are expressed as means ± SD.

**RESULTS**

**DNA-binding activity of transcription factors.** Nuclear extracts of Jurkat cells were collected at timed intervals after stimulation with PHA and PMA. Three bands (a, b and c) were observed in EMSA if transcription factors in nuclear extracts were probed with the oligonucleotide GC box that contains an Sp1/Sp3 consensus sequence (Fig. 1A). The binding of transcription factors to probe GC box reached maximal levels 2–4 h after the stimulation of cells with PHA and PMA (Fig. 1A). Six hours after stimulation with PHA and PMA the DNA-binding activity in nuclear extracts returned to prestimulation levels. In subsequent experiments cells were stimulated with PHA and PMA for 3 h prior to isolation of the nuclear extracts.

The binding of transcription factors to the probe GC box was greater in biotin-supplemented cells compared with biotin-deficient cells for bands c and b (Fig. 1A). This finding was confirmed by gel densitometry in nuclear extracts collected 3 h after stimulation with PHA and PMA. The DNA-binding activities associated with bands c and b were 76 and 149% greater, respectively, in biotin-supplemented cells compared with biotin-deficient cells (Fig. 1B). The DNA-binding activity associated with band a was not affected by biotin.

If transcription factors were probed using a different Sp1 consensus site (CACCC box), the binding pattern was similar to that observed for probe GC box: CACCC-binding activity was
greater in biotin-supplemented cells compared with biotin-deficient cells for proteins associated with bands b and c (Fig. 1C).

These data suggest that the binding of transcription factors to GC and CACCC boxes depends on the concentration of biotin in culture medium. Theoretically, increased binding activity in biotin-supplemented cells could have been caused by a greater abundance of transcription factors or by posttranslational modifications of transcription factors. These two possibilities are not mutually exclusive and were addressed in the experiments described below.

**Identification of transcription factors.** We determined whether the binding of probes to Sp1 and Sp3 caused the shifts observed for the electrophoretic mobility of oligonucleotide GC box and CACCC box. Supershift assays were conducted using antibodies to human Sp1 and Sp3. The incubation of nuclear extracts with antibody to Sp1 severely reduced formation of the bands previously denoted b and c (Fig. 2). Similarly, incubation of nuclear extracts with antibody to Sp3 prevented the formation of band b and severely reduced the formation of band c. These data suggest that Sp1 and Sp3 participated in the formation of the complexes associated with bands b and c. Thus, bands b and c were redenoted Sp1/Sp3 I and Sp1/Sp3 II, respectively (Fig. 2). Band a (Fig. 1A) was not affected by incubation with antibodies to either Sp1 or Sp3 (Fig. 2), suggesting that band a was caused by members of the Sp/KLF family other than Sp1 and Sp3.

**Specificity.** Does the biotin concentration in culture media specifically affect the DNA-binding activities of Sp1 and Sp3 or does the biotin concentration globally affect the abundance of various transcription factors? Do transcription factors in nuclear extracts specifically bind to probe GC box as opposed to unspecific binding of various proteins to this probe? The following negative controls caused no gel shift in EMSA (Fig. 3): (i) incubation of nuclear extract with radiolabeled GC box in the presence of a molar excess of unlabeled GC box; (ii) incubation of nuclear extract with GCmut (mutated Sp1 site); and (iii) incubation of GC box without nuclear extract. Moreover, the abundance of proteins binding to an Oct-1 consensus sequence was not affected by biotin concentrations in culture media (Fig. 3). These observations are consistent with the hypothesis that the concentration of biotin in culture media specifically affects Sp1 and Sp3.

**Expression of genes encoding Sp1 and Sp3.** Transcriptional activity of the gene encoding Sp1 was greater in biotin-supplemented cells compared with biotin-deficient cells as judged by reporter-gene activity. If biotin-supplemented cells were transfected with pGL3FOR5, luciferase activity was 357 ± 17 times the luciferase activity in cells transfected with pGL3-Basic; if biotin-deficient cells were transfected with pGL3FOR5, luciferase activity was only 250 ± 6.2 times the luciferase activity in cells transfected with pGL3-Basic (P < 0.01; n = 3 independent experiments).

The abundance of mRNA encoding Sp1 and Sp3 was quantified in nontransfected cells. The abundance of mRNA encoding Sp3 was greater in biotin-supplemented cells compared with biotin-deficient cells, as judged by gel densitometric analysis of RT-PCR; data were normalized by the abundance of mRNA encoding histone H3 (control). The abundance of mRNA encoding Sp3 in biotin-supplemented cells was 7.8 ± 7.7 times the abundance in biotin-deficient cells (P < 0.05; n = 5; Fig. 4A). The analysis of mRNA encoding Sp1 by RT-PCR produced a weak signal and was not considered for quantitative analysis (data not shown).

The abundance of Sp1 and Sp3 protein in cell nuclei was quantified by Western blot analysis. The abundance of Sp1 was greater in biotin-supplemented cells compared with biotin-deficient cells (Fig. 4B). For Sp1, one single band (~100 kDa) was detected, suggesting that the two known isoforms of Sp1 (95 and 106 kDa) comigrated on the gel, or that one of the two isoforms was below detection limits. The abundance of Sp3 was moderately greater in biotin-supplemented cells compared with biotin-deficient cells. Both of the two known isoforms of Sp3 were detected (60 and 100 kDa). The abundance of a control protein (histone H3) was not affected by biotin. Collectively, these experiments are consistent with the
hypothesis that the expression of genes encoding Sp1 and Sp3 depends on the concentration of biotin in culture media.

**Phosphorylation status of Sp1 and Sp3.** When Sp1 and Sp3 in nuclear extracts were dephosphorylated using calf intestinal phosphatase, binding to probe GC box increased moderately (Fig. 5). The DNA-binding activity increased by 67% in extracts from biotin-deficient cells and by 22% in extracts from biotin-supplemented cells, as judged by gel densitometric analysis of complex Sp1/Sp3 II (n = 3 independent samples). This finding suggests that dephosphorylation of Sp1 and Sp3 was associated with increased DNA-binding activity in Jurkat cells. The DNA-binding activity in dephosphorylated extracts from biotin-deficient cells remained lower than the DNA-binding activity in dephosphorylated extracts from biotin-supplemented cells (Fig. 5, lanes D and H+). This finding suggests that effects on Sp1/Sp3 by the biotin concentration in media were not primarily mediated by phosphorylation of these transcription factors.

**Transcriptional activity of Sp1/Sp3-dependent genes.** The increased abundance of Sp1 and Sp3 in biotin-supplemented cells was associated with increased transcriptional activity of Sp1/Sp3-dependent genes. Reporter-gene constructs were tested for the gene promoters SERCA3 and SV40 early promoter. The transcriptional activity (luciferase activity) of the SERCA3 promoter (basepairs −135 to +55) was 39% greater in biotin-supplemented cells compared with biotin-deficient cells (Table 2). Preliminary studies in our laboratory suggested that regulatory sequences upstream from basepair −135 in SERCA3 may contain recognition sites for biotin-dependent repressors of transcription (see Discussion). The transcriptional activity of the SV40 early promoter was also greater in biotin-supplemented compared with biotin-deficient cells, but the difference (24%) was smaller than that observed for SERCA3 (Table 2).

**DISCUSSION**

The present study provides evidence that the expression of genes encoding Sp1 and Sp3 depends on the concentration of biotin in culture media. The increased nuclear abundance of Sp1 and Sp3 in biotin-supplemented cells compared with biotin-deficient cells was associated with increased transcrip-

**TABLE 2**

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Biotin in culture medium, nmol/L</th>
<th>0.025</th>
<th>10</th>
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<tr>
<td>SERCA3</td>
<td>50 ± 25°</td>
<td>74 ± 49</td>
<td></td>
</tr>
<tr>
<td>SV40 early</td>
<td>53 ± 7.5°</td>
<td>64 ± 5.8</td>
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1 Values are ratios of luciferase activities in cells transfected with Sp1/Sp3-dependent luciferase constructs to activities in cells transfected with promoter-less luciferase constructs. *Significantly different compared with cells cultured in medium containing 10 nmol/L biotin (P < 0.05; n = 6 independent experiments).
tional activity of 5′-flanking regions in Sp1/Sp3-dependent reporter genes. These findings are consistent with the hypothesis that some of the effects of biotin on gene expression are mediated by the nuclear abundance of Sp1 and Sp3.

The effects of biotin on the expression of members of the Sp/KLF family of transcription factors are physiologically important. Sp1 and Sp3 are ubiquitous transcription factors that play important roles in the regulation of numerous genes, including TATA-less housekeeping genes. Examples for Sp1/Sp3-dependent genes include SERCA3 (containing 14 GC boxes and 11 CACCC boxes), dihydrofolate reductase (4 GC boxes), telomerase reverse transcriptase (5 GC boxes) and poly(ADP-ribose) polymerase (5 GC boxes) (34,42–44). These genes play essential roles in processes such as intracellular calcium flux, folate metabolism, synthesis of telomeric DNA (and thus cell proliferation) and DNA repair. The findings in the present study are consistent with the hypothesis that biotin affects the expression of Sp1/Sp3-dependent genes; the transcriptional activities of the 5′-flanking region of SERCA3 and the SV40 (translimian vector) early promoter were greater in biotin-supplemented cells compared with biotin-deficient cells.

Previous studies have provided evidence that Sp1 acts as a transcriptional activator whereas Sp3 may act as a transcriptional repressor (21–23). The present study provides evidence that the transcriptional activity of GC box-containing genes is upregulated in biotin-supplemented cells despite the increased abundance of the transcriptional repressor Sp3. The following explains this apparent paradox.

i) In the present study, the abundance of both Sp1 and Sp3 increased in response to biotin supplementation. Thus, the ratio of Sp1 to Sp3, which compete for binding to the same recognition sites in regulatory elements of genes, might not have changed.

ii) Previous studies have provided evidence that repression of promoters by Sp3 depends on the context and the number of functional Sp1/Sp3-binding sites in the regulatory sequences of genes. For example, the dihydrofolate reductase promoter (containing 4 functional GC boxes) displays Sp3 repression of Sp1 activation, whereas the single GC boxes in histone H4 and thymidine kinase promoters are not responsive to repression by Sp3 (22). Preliminary studies in our laboratory also suggest that the regulation of transcriptional activity by Sp1 and Sp3 is context dependent, i.e., when Jurkat cells were transfected with a reporter-gene construct spanning the region −1312 to +55 of human SERCA3, luciferase activity was substantially smaller in biotin-supplemented cells compared with biotin-deficient cells (data not shown). In contrast, when Jurkat cells were transfected with a reporter-gene construct spanning the region −135 to +55 of human SERCA3, luciferase activity was substantially greater in biotin-supplemented cells compared with biotin-deficient cells (data not shown). This region between basepairs −1312 and −135 contains an additional 3 GC boxes and 7 CACCC boxes compared with plasmid PstI-del (34); some of these sites might bind Sp3 preferably over Sp1, leading to biotin-dependent repression of the full-length 5′-flanking region in SERCA3. Studies of the effects of biotin on the gene encoding SERCA3 are currently underway in our laboratory.

iii) Other transcription factors might cooperate with Sp1 and Sp3 in the regulation of transcriptional activity. For example, an NF-κB/Sp1 region is essential for chromatin remodeling and transcription of a human granulocyte-macrophage colony-stimulating factor transgene in T cells (45). Likewise, Sp1 interacts with the transcription factors E2F (46), c-Myc (42) and GATA-1 (47). Evidence has shown that the nuclear translocation of NF-κB increases in response to biotin deficiency (Rodriguez-Melendez, R., Schwab, L. D. and Zempleni, J., unpublished results), whereas expression of the gene encoding c-Myc decreases in response to biotin deficiency (48). Interactions among Sp1, Sp3 and other transcription factors might account for the distinct responses of the 5′-flanking region of SERCA3 and the SV40 early promoter to biotin observed in the present study. The 5′-flanking region of SERCA3 contains binding sites for Sp1/Sp3, Ets-1, Oct-1, c-Myc, MyoD and AP-2, whereas the SV40 early promoter contains only Sp1 sites and an A/T-rich TATA-like element.

Posttranslational modifications affect the transactivation activity of members of the Sp/KLF family. For example, transcriptional activity of Sp3 decreases in response to acetylation (49) and after conjugation to a small ubiquitin-like modifier (50); Sp3 is a phosphoprotein but the roles for phosphorylation are uncertain (21). Depending on cell type and stimuli, phosphorylation of Sp1 can increase (36), decrease (37–39) or have no effect on Sp1 binding activity (51). O-linkage of N-acetylglucosamine to the Sp1 activation domain increases (52,53) or decreases (54) Sp1 transactivation activity, probably by targeting Sp1 for proteasome-dependent degradation (53). Finally, noncovalent binding of Sp1 to the nuclear protein, p74, might inhibit Sp1-mediated transcription (54). Notwithstanding the important role for posttranslational modifications (and in particular phosphorylation) of Sp1 and Sp3 in regulating DNA-binding activity, the present study provides evidence that the effects of biotin on phosphorylation of Sp1 and Sp3 are quantitatively moderate. These findings are consistent with the hypothesis that the effects of biotin on transcriptional activation by Sp1 and Sp3 are mediated by increased abundance of these transcription factors rather than by posttranslational modifications.

The mechanism by which biotin causes increased transcription of the genes encoding Sp1 and Sp3 is unknown. The proximal region of the Sp1 promoter contains putative binding sites for p53, E2F, Sp1, C/EBP (CCAAT-enhanced binding protein), NF-Y (nuclear factor-Y), CREB, AP-1 and AP-2 (33). Theoretically, biotin supplementation might increase the abundance of any of these transcription factors, leading to increased expression of the Sp1 gene. The sequence of the human Sp3 promoter has been submitted to GenBank (accession #AY251018) but the publication of the promoter analysis is still pending (Monasterio, P., Nicolas, M., Noe, V. and Ciudad, C. J., unpublished results). Future studies will be necessary to identify those factors that mediate increased expression of genes encoding Sp1 and Sp3 in response to biotin.

Multimerization of Sp1 and Sp3 might play a role in the formation of complexes Sp1/Sp3 I and Sp1/Sp3 II, given that the formation of complexes Sp1/Sp3 I and Sp1/Sp3 II was almost completely prevented by using antibodies to either Sp1 or Sp3 (supershift experiments). Previous studies are consistent with this hypothesis; these studies have provided evidence that the activation of the human insulin-like growth factor-binding protein 3 promoter involves an Sp1/Sp3 multiprotein complex that may bind the histone acetyl transferase p300 and histone deacetylase-1 (55,56).

ACKNOWLEDGMENTS

We thank Carlos J. Ciudad (University of Barcelona, Spain), Brett White (University of Nebraska-Lincoln), and Frank Wuytack (Katholieke Universiteit Leuven, Belgium) for generously providing plasmids.

LITERATURE CITED


