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The expression of genes encoding ribosomal subunits and eukaryotic translation initiation factor 5A depends on biotin and bisnorbiotin in HepG2 cells☆

Rocio Rodriguez-Melendez[a], Jacob B. Griffin[a], Janos Zempleni[a,b,c,*]

[a]Department of Nutrition and Health Sciences, University of Nebraska at Lincoln, Lincoln, NE 68583-0806, USA
[b]Department of Biochemistry, University of Nebraska at Lincoln, Lincoln, NE 68583-0806, USA
[c]Department of Animal Science, University of Nebraska at Lincoln, Lincoln, NE 68583-0806, USA

Abstract

Biotin affects gene expression at both the transcriptional and the posttranscriptional level; biotin metabolites might have biotin-like activities with regard to gene expression. Here, human hepatocarcinoma (HepG2) cells were used (i) to identify clusters of biotin-dependent genes, (ii) to determine whether the naturally occurring metabolite bisnorbiotin affects gene expression and (iii) to determine whether biotin and bisnorbiotin affect the expression of genes coding for ribosomal subunits and translation initiation factors. HepG2 cells were cultured in media containing deficient (0.025 nmol/L), physiological (0.25 nmol/L, control) and pharmacological (10 nmol/L) concentrations of biotin; a fourth treatment group consisted of cells cultured in biotin-deficient medium (0.025 nmol/L) supplemented with bisnorbiotin (0.225 nmol/L). Gene expression was quantified by using DNA microarrays and reverse transcriptase polymerase chain reaction. The expression of 1803 genes depended on biotin concentrations in culture media; the expression of 618 genes depended on bisnorbiotin. Biotin deficiency was associated with increased expression of a gene cluster encoding ribosomal subunits and eukaryotic translation initiation factor 5A; this effect was reversed by supplementation with biotin and bisnorbiotin. Additional prominent clusters of (bisnor)biotin-dependent genes included DNA-, RNA-, and nucleotide-binding proteins, consistent with a role for biotin in cell signaling and gene expression. Collectively, these data suggest that bisnorbiotin has biotin-like activities regarding gene expression, and that clusters of (bisnor)biotin-dependent genes include genes that play roles in translational activity.

Keywords: Biotin; Gene expression; HepG2 cells; Human; Translation

1. Introduction

Biotin has the following biological functions in mammals: First, biotin serves as a covalently bound coenzyme for acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase (PCC) and 3-methylcrotonyl-CoA carboxylase [1]. These enzymes catalyze steps in the metabolism of glucose, amino acids and fatty acids [1]. Second, histones (DNA-binding proteins) contain covalently bound biotin [2]. Evidence has been provided that biotinylation of histones might play a role in gene silencing [3], cell proliferation [2,4], and DNA repair or apoptosis [3]. Third, biotin affects gene expression at both the transcriptional [5–7] and the posttranscriptional level [6,8,9].

Recently, evidence emerged that the following signaling mechanisms mediates effects of biotin on gene expression; these mechanisms are not mutually exclusive. (i) Biotinyl-AMP (an intermediate in holocarboxylase synthesis) activates soluble guanylate cyclase, increasing the generation of cyclic guanosine monophosphate [10]. Subsequently, cyclic guanosine monophosphate-dependent protein kinase phosphorylates and activates proteins that enhance transcriptional activity of genes. (ii) Biotinylation of histones may affect the transcriptional activities of genes [11]. (iii) Biotin affects the nuclear abundance of transcription factors. For example, biotin deficiency is associated with increased nu-

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* Corresponding author. Department of Nutrition and Health Sciences, University of Nebraska at Lincoln, Lincoln, NE 68583-0806, USA. Tel.: +1 402 472 3270; fax: +1 402 472 1587.
E-mail address: jzempleni2@unl.edu (J. Zempleni).

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clear translocation of nuclear factor κB (NF-κB), mediating activation of NF-κB-dependent genes [12]. Moreover, biotin supplementation is associated with increased nuclear abundance of Sp1 and Sp3 [13]; these transcription factors may act as transcriptional activators or repressors, depending on the context [14,15].

Synthetic biotin analogs such as diaminobiotin and desthiobiotin have biotin-like activities with regard to gene expression [16]. These analogs can substitute for biotin in the transcriptional activation of genes encoding interleukin-2 and interleukin-2 receptor γ. Currently, it is unknown as to whether any of the naturally occurring biotin metabolites also affect gene expression.

Biotin metabolites are quantitatively important in mammalian tissues and body fluids; biotin metabolites account for 50–70 mol% of total biotinyl compounds (biotin+metabolites) [17–19]. Two pathways of biotin catabolism have been identified: (i) β-oxidation of the valeric acid side chain [1,17,19–21], leading to the formation of bisnorbiotin, tetranorbiotin and related metabolites that are known to result from β-oxidation of fatty acids, and (ii) sulfur oxidation in the heterocyclic ring, leading to the formation of biotin-*l*-sulfoxide, biotin-*d*-sulfoxide and biotin sulfone [20,21]. Bisnorbiotin is the quantitatively most important biotin metabolite in human body fluids [18,19,22]. Thus, in the studies described below, we used bisnorbiotin to model the biological activity of biotin metabolites.

Effects of biotin on gene expression are not limited to transcriptional events but extend to the posttranscriptional level. Specifically, evidence has been provided that the synthesis of asialoglycoprotein receptor and PCC depends on biotin [8,9]. The mechanisms that mediate the effects of biotin on gene expression at the posttranscriptional level are unknown. The following proteins play critical roles in posttranscriptional (translational) events, given their essential role in the assembly of the 80S translation initiation complex [23]; it is not unreasonable to assume that effects of biotin at the translational level might be mediated by the expression of genes coding for these proteins. (i) Ribosomal subunits: greater than 80 subunits form the backbone of mammalian ribosomes, and (ii) eukaryotic translation initiation factors (eIFs): eIFs play roles at various steps during the initiation of translation. For example, the first step in the initiation of translation requires the formation of a ternary complex containing eIF2, GTP and initiator tRNA [23]. In the studies described below, we will repeatedly refer to eIF5A. Evidence has been provided that eIF5A plays a role in the translation of proteins that promote cell proliferation and inhibit apoptosis [24,25].

In the present study, we used human hepatocarcinoma (HepG2) cells (i) to identify clusters of biotin-dependent genes, (ii) to determine whether the naturally occurring metabolite bisnorbiotin affects gene expression and (iii) to determine whether biotin and bisnorbiotin affect the expression of genes coding for ribosomal subunits and translation initiation factors. As a secondary aim, we determined whether bisnorbiotin could substitute for biotin as a coenzyme for carboxylases.

HepG2 cells were selected as a cell model for the following reasons. First, biotin-dependent carboxylases are abundant in HepG2 cells, generating a large demand for biotin; biotin deficiency greatly decreases proliferation and viability in HepG2 cells [10]. Second, biotin-dependent signaling cascades have been characterized in HepG2 cells [10]. Third, hepatic cells are capable of catabolizing biotin by all known pathways of biotin degradation, including generation of bisnorbiotin [17].

2. Materials and methods

2.1. Cell culture

HepG2 cells were purchased from American Type Culture Collection (Manassas, VA). Cells were cultured (5% CO_2 at 37°C in humidified atmosphere) in the following biotin-defined media for 2 weeks prior to sample collection: 0.025 nmol/L of biotin (denoted "deficient"), 0.25 nmol/L of biotin ("physiological"), 10 nmol/L of biotin ("pharmacological") or 0.025 nmol/L of biotin plus 0.225 nmol/L of bisnorbiotin (denoted "bisnorbiotin-supplemented"). Culture medium was replaced with fresh medium every 48 h. Media were prepared by using biotin-depleted fetal bovine serum as described previously [26]; biotin concentrations in media were confirmed by avidin-binding assay [27] with modifications [26]. Cultures were trypsinized and split when cells reached confluence (typically, two times per week). For the assays described below, cell pellets were collected at 60% to 70% confluence.

Biotin concentrations in media were chosen based on the following lines of reasoning: (i) 0.25 nmol/L of biotin represents the physiological concentration of biotin in plasma from healthy adults [22]; (ii) 0.025 nmol/L of biotin is greater than two standard deviations below the mean physiological concentration in normal plasma [22]; thus, 0.025 nmol/L equals a deficient concentration of biotin; (iii) ingestion of a typical biotin supplement providing 25 times the adequate intake of biotin for adults [28] is associated with plasma concentrations of approximately 10 nmol/L of biotin in healthy adults [29]; thus, this concentration represents a pharmacological concentration of biotin in plasma.

2.2. Biotin-dependent carboxylases

Biotin-dependent carboxylases are reliable markers for cellular biotin [1]. Biotinylated carboxylases in cell extracts were resolved by polyacrylamide gel electrophoresis and were probed using streptavidin peroxidase [26]. In addition, activities of PCC in cell lysates were quantified as described previously [26].

2.3. DNA microarrays

Total RNA was extracted from HepG2 by using TRIzol reagent according to the manufacturer's instructions (Invi-

trogen, Carlsbad, CA); RNA from seven culture flasks was combined for each treatment group. Absorbances at 260 and 280 nm were measured for each RNA sample; the absorbance ratio (260:280 nm) was 1.8±0.2, consistent with the absence of significant contamination with protein. To confirm integrity of RNA, 22 μg of RNA was electrophoresed using a formaldehyde–agarose gel [30]; RNA was stained with ethidium bromide and visualized using a Kodak EDAS 290 Documentation and Analysis System (Rochester, NY). Two major ribosomal bands (28S and 18S rRNA) but no degraded RNA were detected (data not shown). DNA microarrays were conducted as described previously, using the Human Genome U133A Array (Affymetrix, Santa Clara, CA) for hybridizations [31].

2.4. Microarray data normalization and analysis

The Microarray Suite 5.0, Micro DB and Data Mining Tool 3.0 software (Affymetrix) were used for normalization and analysis of microarray data. The signal value of the experimental array was multiplied by a normalization factor to make its mean intensity equivalent to the mean intensity of the control array, using Microarray Suite software according to the manufacturer's protocol. The absolute call (present, marginal, absent) and average difference of about 14,000 gene expressions in a sample and the absolute call difference, percent change and average difference of gene expressions between samples were identified using the abovementioned software. The genes showing altered expression were categorized on the basis of their suggested molecular functions, using Onto-Express [32]. Genes that were not annotated or not easily classified were excluded from the functional clustering analysis.

2.5. Reverse transcriptase polymerase chain reaction (RT-PCR)

The relative abundance of mRNA encoding selected genes was quantified by RT-PCR to confirm DNA microarray data. For analysis by RT-PCR, we selected genes that were up-regulated or down-regulated in treatment groups compared to physiological controls (as judged by DNA microarray). Abundance of mRNA encoding these genes was assayed at least three times by RT-PCR [33], using the customized primers (Integrated DNA Technologies, Coralville, IA) listed in Table 1. The expression of β-actin was not affected by biotin and was used as control. Polymerase chain reaction products were collected at timed intervals for up to 32 PCR cycles, and were quantified by gel densitometry as described [5]; only values from within the exponential phase of PCR amplification (typically up to 28 PCR cycles) were considered for data analysis.

2.6. Statistical analysis

Homogeneity of variances among groups was tested using Bartlett's test [34]. Variances were homogeneous, that is, data were not log-transformed before further statistical analysis. Significance of differences among groups was tested by one-way ANOVA. Fisher's protected least significant difference procedure was used for posthoc testing [34]. StatView 5.0.1 (SAS Institute, Cary, NC) was used to perform all calculations. Differences were considered significant if $P<.05$. Data are expressed as mean±S.D.

Table 1
Oligonucleotide primers used for RT-PCR[a]

Gene	Primer
ACADM	5′-TTT AGT TTT GAG TTC ACC GA-3′
	5′-TCC TCT AGT ATC TGA ACA TCG-3′
β-actin	5′-ATC TGG CAC CAC ACC TTC TAC AAT GAG CTG CG-3′
	5′-CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC-3′
eIF5A	5′-ACT TGG ACT TCG AGA CAG-3′
	5′-GAT CTC TTC TCC ACA GTC G-3′
IMPA1	5′-GGG AAA AAA GTA TCT TAA CCG-3′
	5′-TAA TTA ATC TTC GTC GTC TC-3′
L27	5′-AAC TTC GAC AAA TAC CAC-3′
	5′-CTC TTA ATC TTC TCC TCA-3′
L38	5′-GTT GCT GCT TGC TGT GAG-3′
	5′-ATA TAA TAC AGT TCC AAT CA-3′
LP2	5′-GTC GCC TCC TAC CTG CT-3′
	5′-GGG GAG CAG GAA TTT AAT-3′
RANBP9	5′-ATC TAC AGA CCA GAC CGT-3′
	5′-ACT AGG AAG CAA TGT AAA GCG-3′
RBP1	5′-TCT CTA CCT CCC CTA CAA-3′
	5′-TGC TCC TGA AGA GTC CGG-3′
S11	5′-AGC CCC TGC GTA ATC GA-3′
	5′-GTT GTA CTT GCG GAT G-3′
S19	5′-ACT GGT TCT ACA CGC GA-3′
	5′-TCT AAT GCT TCT TGT TGG-3′

[a] Abbreviations (GenBank accession numbers): ACADM=acyl-coenzyme A dehydrogenase (NM_000016); eIF5A=eukaryotic translation initiation factor 5A (AA393940); IMPA1=inositol (myo)-1 (or 4)-monophosphatase 1 (NM_005536); L27=ribosomal protein 27a (BE737027); L38=ribosomal protein 38 (NM_000999); LP2=ribosomal protein large P2 (NM_001004); RANBP9=RAN binding protein 9 (NM_005493); RBP1=ribosome binding protein 1 (NM_004587.1); S11=ribosomal protein S11 (BF680255); S19=ribosomal protein S19 (NM_001022.1).

3. Results

3.1. Biotin-dependent carboxylases

Biotinylation of carboxylases in HepG2 cells paralleled biotin concentrations in culture media. If cells were cultured in biotin-deficient medium, biotinylated acetyl-CoA carboxylase, pyruvate carboxylase, PCC and 3-methylcrotonyl-CoA carboxylase were barely detectable in cell extracts, using streptavidin peroxidase as a probe (Fig. 1A). In contrast, holocarboxylases were easily detectable in extracts from cells cultured in medium containing a physiological concentration of biotin; holocarboxylases were even more abundant in cells cultured in medium containing a pharmacological concentration of biotin. If biotin-deficient cells were supplemented with bisnorbiotin, the abundance of holocarboxylases was greater than in biotin-deficient cells but smaller than in physiological controls. Note that the

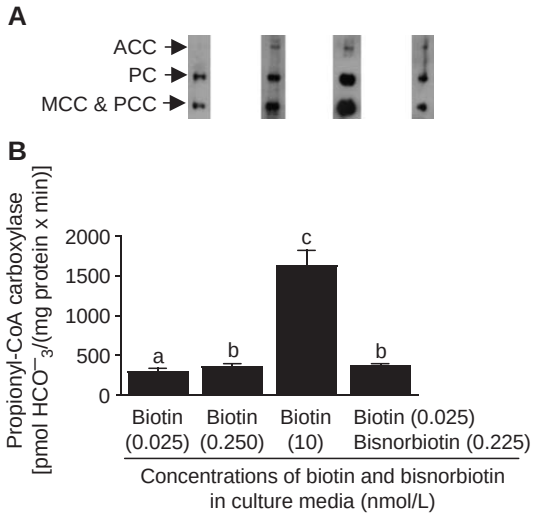

Fig. 1. Concentrations of biotin and bisnorbiotin in culture media affect the abundance of holocarboxylases in HepG2 cells. Cells were cultured in media containing deficient (0.025 nmol/L), physiological (0.25 nmol/L) or pharmacological (10 nmol/L) concentrations of biotin for 14 days; a fourth treatment group consisted of cells cultured in biotin-deficient medium (0.025 nmol/L) supplemented with bisnorbiotin (0.225 nmol/L). Panel A, Representative Western blot depicting acetyl-CoA carboxylase, pyruvate carboxylase, α-chain of PCC and α-chain of 3-methylcrotonyl-CoA carboxylase. Panel B, PCC activity. Values are means±S.D. ($n=3$). Columns not sharing the same superscript (a, b and c) are significantly different ($P<.05$).

biotin-containing α-chains of PCC (molecular mass=80 kDa) and 3-methylcrotonyl-CoA carboxylase (molecular mass=83 kDa) migrate as one single band on the polyacrylamide gels used here.

Propionyl-CoA carboxylase activities paralleled the concentrations of biotinyl compounds in culture media: biotin deficient<physiological=bisnorbiotin<pharmacological (Fig. 1B). Collectively, these findings suggest (i) that biotin concentrations in culture media affect intracellular biotin concentrations and (ii) that bisnorbiotin might serve as a coenzyme for biotin-dependent carboxylases.

3.2. Gene expression analysis

The concentration of (bisnor)biotin in culture media affected the abundance of mRNA encoding 2421 genes, as judged by DNA microarray analysis. Genes were considered (bisnor)biotin responsive if the mRNA abundance in a given treatment group (deficient, pharmacological or bisnorbiotin supplemented) changed by at least 30% compared to physiological controls. If cells were cultured in biotin-deficient medium, the expression of 102 and 597 genes increased and decreased, respectively, compared to physiological controls. If cells were cultured in medium containing a pharmacological concentration of biotin, the expression of 243 and 861 genes increased and decreased, respectively, compared to physiological controls. If cells were cultured in bisnorbiotin-supplemented medium, the expression of 259 and 359 genes increased and decreased, respectively, compared to physiological controls. Note that the total concentration of biotinyl compounds was the same in bisnorbiotin-supplemented medium and physiological control medium, suggesting that bisnorbiotin has unique effects on gene expression. Notwithstanding this observation, bisnorbiotin can substitute for biotin regarding the expression of some genes (see the following discussions).

Table 2 provides a list of those genes that were affected most dramatically by the concentrations of biotin and bisnorbiotin in culture media, as judged by DNA microarray. The relative abundance of mRNA encoding these highly biotin-responsive genes is expressed in units of percent of controls (cells cultured in physiological medium). The relative abundance of mRNA encoding highly biotin-activated genes was 200–2111% of controls; the relative abundance of mRNA highly biotin-repressed genes was 6.3–62% of controls.

Table 2
Biotin-responsive and bisnorbiotin-responsive genes in HepG2 cells

Treatment group	Gene expression compared to physiological control	
	Increase	Decrease
Gene (relative abundance of mRNA)[a]		
Deficient	eIF5A (283%)[b]	AND-1 (6.3%)
	APOE (264%)	MSF-FN70 (23%)
	S19 (230%)	FLJ20060 (31%)
	L27 (214%)	HQ0131 (33%)
	S11 (200%)	FLJ12102 (37%)
Bisnorbiotin	ENC1 (2111%)	UFE4B (37%)
	KIAA0630 (857%)	RBP1 (52%)
	eIF5A (606%)	SBNE (55%)
	IGF1R (566%)	HNRH1 (55%)
	DGK (459%)	HPFLJ13033 (62%)
Pharmacological	FLJ11267 (606%)	HZFH (12%)
	ULBP1 (348%)	SH3P5 (49%)
	PKLD (324%)	HPFLJ20060 (55%)
	PLCE (303%)	KIAA1109 (55%)
	cPLA2 (263%)	SPLCB2 (61%)

[a] Cells cultured in physiological medium (0.25 nmol/L of biotin) were used as a reference. mRNA abundance is shown in parentheses, using cells in physiological medium as control (=100%).

[b] Abbreviations (GenBank accession numbers): AND-1=AND-1 protein (NM_007086.1); APOE=apolipoprotein E (AI358867); cPLA2=phosphatidylcholine 2-acyl hydrolase (M68874.1); DG=diacylglycerol kinase (NM_004717.1); eIF5A=eukaryotic translation initiation factor 5A (AA393940); ENC1=ectodermal–neural cortex with BTB-like domain (NM_003633.1); FLJ11267=hypothetical protein FLJ11267 (NM_019607); FLJ12102=cDNA FLJ12102 (AU147194); FLJ20060=hypothetical protein FLJ20060 (NM_017645.1); HNRH1=heterogenous nuclear ribonucleoprotein H1 (BF983406); HPFLJ13033=hypothetical protein FLJ13033 (NM_025191.1); HPFLJ20060=hypothetical protein FLJ20060 (NM_017645.1); HQ0131=clone HQ0131 (AF090896.1); HZFH=human zinc finger homeodomain protein (U12170.1); IGF1R=insulin-like growth factor (NM_000875.2); KIAA0630=KIAA0630 protein (AI393355); KIAA1109=KIAA1109 protein (AB029032.1); L27=ribosomal protein 27a (BE737027); MSF-FM70=migration stimulating factor FN70 (AJ276395.1); PKLD=protein kinase lysine deficient (NM_018979.1); PLCE=phospholipase C, epsilon (NM_006226.1); RBP1=ribosome binding protein 1 (NM_004587.1); SBNE=spectrin, beta, nonerythrocytic 1 (NM_003128.1); SH3P5=SH3-domain protein 5, ponsin (NM_015385.1); SPLCB2=serine palmitoyl transferase LCB2 (U15555.1); S11=ribosomal protein S11 (BF680255); S19=ribosomal protein S19 (NM_001022.1); UFE4B=ubiquitination factor e4B (NM_006048); ULBP1=UL16-binding protein 1 (NM_025218.1).

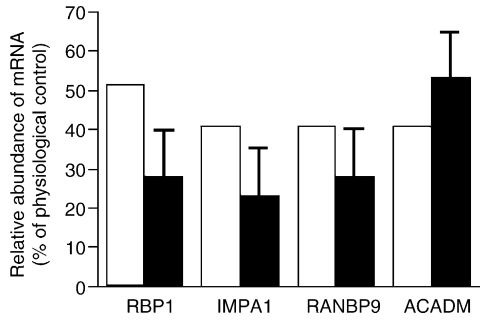

Fig. 2. Comparative analysis of gene expression by DNA microarrays and RT-PCR. Abundances of mRNA encoding the following four genes were quantified: RBP1, ribosome-binding protein 1 (GenBank accession number: NM_004587); IMPA1, inositol (myo)-1 (or 4)-monophosphatase 1 (NM_005536); RANBP9, RAN-binding protein 9 (NM_005493); ACADM, acyl-CoA dehydrogenase (NM_000016). Open bars = DNA microarray; black bars = RT-PCR ($n = 3–4$ for RT-PCR).

The relative abundances of mRNA as determined by DNA microarray were independently confirmed by RT-PCR as follows. First, we selected the following four biotin-responsive genes from our DNA microarray experiments; the expression of these genes decreased if cells were cultured in media containing bisnorbiotin or a pharmacological concentration of biotin compared to physiological controls: ribosome-binding protein 1 (decreased expression in response to bisnorbiotin supplementation), inositol (myo)-1 (or 4) monophosphatase 1 (decreased expression in response to pharmacological biotin), RAN-binding protein 9 (decreased expression in response to pharmacological biotin) and acyl-coenzyme A dehydrogenase (decreased expression in response to pharmacological biotin). Next, the abundance of mRNA encoding these four genes was quantified by RT-PCR. Comparative analysis of mRNA abundance by DNA microarrays and RT-PCR produced the same trends for all four genes (Fig. 2). For example, both DNA microarrays and RT-PCR identified RPBP1 as a gene that is down-regulated in response to pharmacological concentrations of biotin. Moreover, the magnitude of effects was similar if quantified by DNA microarray and RT-PCR. The abundance of mRNA encoding β-actin (control) was not affected by biotin (data not shown). Collectively, these data suggest that the data collected by using DNA microarrays are both accurate and precise.

3.3. Gene clusters

Biotin-dependent genes were not randomly distributed in the HepG2 cell genome but could be assigned to gene clusters. Five predominant clusters of biotin-dependent genes were identified if classification was based on the molecular function of gene products. These clusters comprised DNA-binding proteins, RNA-binding proteins, genes that play roles in translational activity, nucleotide-binding proteins and proteins with transferase activity; numerous minor clusters accounted for the balance (Table 3). Clusters of biotin-dependent genes were further broken down into subclusters. For example, the following subclusters were identified for nucleotide-binding proteins: ATP-binding proteins and GTP-binding proteins (Table 3). In previous studies, we provided evidence that biotin-dependent genes can also be clustered according to cellular localization of gene products, chromosomal localization of genes or biological functions of gene products in human lymphocytes [31]. Similar clusters

Table 3
Clusters of biotin-dependent and bisnorbiotin-dependent genes in HepG2 cells

Gene cluster	Transcriptional response to culture conditions, number of genes (n)					
	Increased transcription[a]			Decreased transcription		
	Def.[b]	Pharm.	BNB	Def.	Pharm.	BNB
DNA binding						
(a) Transcription factor	1	9	8	13	22	16
(b) Others	2	6	8	34	33	20
RNA binding						
(a) mRNA binding	2	3	5	5	17	4
(b) Double-stranded RNA binding	0	0	3	1	4	2
(c) Other	11	10	7	16	12	8
Translational activity						
(a) Ribosomal structure	14	10	0	4	11	3
(b) Translation initiation factor	1	1	4	1	5	2
Nucleotide binding						
(a) ATP binding	1	9	7	50	51	20
(b) GTP binding	1	3	6	13	17	4
Transferase activity	3	18	5	20	38	16

[a] Compared to cells cultured in physiological control medium (0.25 nmol/L of biotin).
[b] Abbreviations: Def. = biotin-deficient medium (0.025 nmol/L of biotin); pharm. = pharmacological concentration of biotin (10 nmol/L of biotin); BNB = medium supplemented with bisnorbiotin (0.025 nmol/L of biotin plus 0.225 nmol/L of bisnorbiotin).

were identified in the present study in hepatocarcinoma cells (data not shown).

In the present study, we hypothesized that biotin affects the transcription of genes encoding ribosomal subunits and eIF, mediating effects on gene expression at the posttranscriptional level. Indeed, we identified a cluster of biotin-dependent genes that play roles in translational activity: biotin deficiency was associated with transcriptional activation of genes encoding a translation initiation factor and proteins that play a role in ribosomal structure (Table 2). Specifically, biotin deficiency was associated with increased expression of ribosomal subunits LP2, L27a, L38, S11 and S19, and translation initiation factor eIF5A. This effect was reversed by supplementation of cells with biotin and bisnorbiotin. The only exception to this pattern was eIF5A expression, which was activated in both biotin-deficient and bisnorbiotin-supplemented cells (Table 2). Note that supplementation of cells with pharmacological concentrations of biotin caused transcriptional activation of genes coding for some ribosomal subunits: L37a, S5 and S21.

Expression profiles of ribosomal subunits and eIF5A as per DNA microarray were confirmed by RT-PCR. First, we selected the following six biotin-responsive genes from our DNA microarray experiments: ribosomal subunits LP2, L27a, L38, S11, S19 and eIF5A. Next, we quantified the abundance of mRNA encoding these six genes by RT-PCR, using samples from biotin-deficient cells and physiological controls. Comparative analysis of mRNA abundance by DNA microarrays and RT-PCR produced the same trends for all six genes: mRNA abundance was greater in biotin-deficient cells compared to physiological controls (Fig. 3). Abundance of mRNA encoding β-actin (control) was not affected by biotin (data not shown). Collectively, these data suggest that biotin affects the synthesis of proteins that play roles in translational activity, and that bisnorbiotin has biotin-like activities regarding expression of genes that play roles in translation.

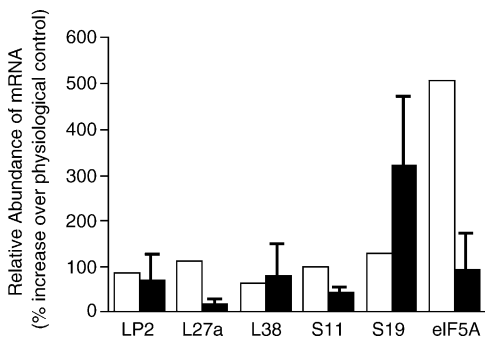

Fig. 3. Expression of genes coding for ribosomal subunits and eIF5A increases in response to biotin-deficiency in HepG2 cells. The abundance of mRNA coding for ribosomal subunits LP2, L27a, L38, S11 and S19, and eIF5A was quantified by DNA microarray (open bars) and RT-PCR (black bars). The figure depicts the percent increase in mRNA abundance in biotin-deficient cells compared to physiological controls ($n = 3–4$ for RT-PCR).

4. Discussion

The present study provides evidence (i) that biotin-dependent genes cluster around the following molecular functions: DNA-binding proteins, RNA-binding proteins, proteins that play roles in translational activity, nucleotide-binding proteins and proteins with transferase activity; (ii) that the naturally occurring metabolite bisnorbiotin has biotin-like effects regarding gene expression; and (iii) that biotin affects the transcription of genes encoding ribosomal subunits and eIF, mediating effects on gene expression at the posttranscriptional level.

These findings are physiologically important for the following reasons: (i) The present study points to novel pathways by which biotin might exert its well-known effects on gene expression [5–7]. For example, this study provides evidence that biotin affects the expression of transcription factors, mRNA-binding proteins, GTP-binding proteins and proteins that play roles in translational activity (see the following discussions). Identification of these biotin-dependent signaling molecules will aid in further deciphering the complex network by which biotin orchestrates its effects on gene expression.

(ii) The present study suggests that biotin metabolites are not metabolic waste but may have biotin-like activities. This is consistent with a previous study using synthetic biotin analogs [16]. These findings have the following important implications. First, the content of biotin metabolites in foods needs to be considered when estimating dietary biotin intake. For example, bisnorbiotin may account for close to 50% of the combined total of all biotinyl compounds in human breast milk [35]. Second, biotin metabolites should be included in the assessment of an individual's biotin status if based on the concentrations of biotinyl compounds in plasma or urine. Bisnorbiotin is the single most important biotin metabolite in human body fluids [18,22].

(iii) To our knowledge, this study for the first time provides evidence that biotin affects the transcriptional activity of genes coding for ribosomal subunits and eIF. Likely, the abundance of ribosomal subunits and eIF modifies the translational activity in cells, mediating effects at the posttranscriptional level. Currently, the net effect of biotin status on translational activity is uncertain, based on the following lines of reasoning. The transcription of some genes encoding ribosomal subunits and eIF was greater in biotin-deficient cells compared to controls, whereas the transcription of some other genes that play roles in translational activity was greater in biotin-supplemented cells compared to controls. Currently, studies are underway in our laboratory to investigate effects of biotin on the translational activity in human cells.

We speculate that the increased expression of eIF5A observed in biotin-deficient cells helps to maintain normal

rates of cell proliferation and to prevent apoptosis. This is consistent with the following observations from previous studies. First, eIF5A is known to play a role in promoting cell proliferation and preventing apoptosis [24,25]. Second, we have provided evidence that human cells maintain normal rates of cell proliferation and apoptosis if cultured in biotin-deficient medium for several weeks [26,36]. Note, however, that other factors also contribute to preventing apoptosis in biotin-deficient cells. For example, evidence has been provided that human cells respond to biotin deficiency with increased nuclear translocation of NF-κB, enhancing the expression of antiapoptotic genes [12].

In the present study, biotinylated and bisnorbiotinylated carboxylases were less abundant in bisnorbiotin-supplemented cells compared to physiological controls, as judged by probing with streptavidin. This was despite the fact that the concentration of total biotinyl compounds was the same in both bisnorbiotin-supplemented medium and physiological control medium. We offer the following explanations for this observation; these explanations are not mutually exclusive. First, binding of (bisnor)biotin to carboxylases is mediated by holocarboxylase synthetase [1]; this enzyme may have a lower affinity for bisnorbiotin compared to biotin. Second, in the present study, we used streptavidin to probe carboxylase-bound bisnorbiotin. Previous studies provided evidence that avidin and streptavidin have a lower affinity for biotin analogs compared to biotin [27,37]. Thus, probing bisnorbiotinylated carboxylases with streptavidin may moderately underestimate the true abundance of bisnorbiotin in holocarboxylases. Note that carboxylases from bisnorbiotin-supplemented cells contained more streptavidin-reactive material than carboxylases from biotin-deficient cells, suggesting that binding of bisnorbiotin to carboxylases took place. Consistent with this hypothesis, PCC activities were similar in bisnorbiotin-supplemented cells and physiological controls in the present study; PCC activities were significantly lower in biotin-deficient cells. Collectively, our data are consistent with the hypothesis that bisnorbiotin is incorporated into carboxylases, creating bioactive enzymes.

We cannot formally exclude the possibility that bisnorbiotin is converted to biotin by elongation of its propionate side chain. Also, we cannot formally exclude the possibility that bisnorbiotin has a biotin-sparing effect in HepG2 cells, for example, by reducing the rate of biotin breakdown. We address these uncertainties as follows: (i) Synthetic biotin analogs (diaminobiotin, desthiobiotin) that cannot be converted to biotin have biotin-like effects regarding gene expression [16]. These observations are consistent with the hypothesis that biotin metabolites do not need to be converted to biotin in order to affect gene expression. (ii) Previous studies have suggested that biotin is metabolized to bisnorbiotin in rat liver homogenates; no conversion of bisnorbiotin to biotin was apparent [17]. (iii) Some naturally occurring biotin metabolites (e.g., bisnorbiotin methyl ketone) cannot be converted to biotin [19]. In future studies, we will determine whether these metabolites affect gene expression. (iv) We are currently pursuing preparation of recombinant holocarboxylase synthetase and apo-PCC. We intend to use these reagents to investigate the binding of biotin metabolites to carboxylases in a cell-free system, eliminating confounders such as biotin-sparing effects and conversion of metabolites to biotin.

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