Regulation of gene expression by biotin☆ (Review)

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Abstract

In mammals, biotin serves as coenzyme for four carboxylases, which play essential roles in the metabolism of glucose, amino acids, and fatty acids. Biotin deficiency causes decreased rates of cell proliferation, impaired immune function, and abnormal fetal development. Evidence is accumulating that biotin also plays an important role in regulating gene expression, mediating some of the effects of biotin in cell biology and fetal development. DNA microarray studies and other gene expression studies have suggested that biotin affects transcription of genes encoding cytokines and their receptors, oncogenes, genes involved in glucose metabolism, and genes that play a role in cellular biotin homeostasis. In addition, evidence has been provided that biotin affects expression of the asialoglycoprotein receptor and propionyl-CoA carboxylase at the post-transcriptional level. Various pathways have been identified by which biotin might affect gene expression: activation of soluble guanylate cyclase by biotinyl-AMP, nuclear translocation of NF-κB (in response to biotin deficiency), and remodeling of chromatin by biotinylation of histones. Some biotin metabolites that cannot serve as coenzymes for carboxylases can mimic biotin with regard to its effects on gene expression. This observation suggests that biotin metabolites that have been considered "metabolic waste" in previous studies might have biotin-like activities. These new insights into biotin-dependent gene expression are likely to lead to a better understanding of roles for biotin in cell biology and fetal development. © 2003 Elsevier Inc. All rights reserved.

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1. Introduction

In mammals, biotin serves as a covalently bound coenzyme for four carboxylases: acetyl-CoA carboxylase (isoforms α and β; E.C. 6.4.1.2); pyruvate carboxylase (E.C. 6.4.1.1); propionyl-CoA carboxylase (E.C. 6.4.1.3); and 3-methylcrotonyl-CoA carboxylase (E.C. 6.4.1.4) [1]. The attachment of biotin to the ϵ-amino group of a specific lysine moiety in carboxylases is catalyzed by holocarboxylase synthetase (E.C. 6.3.4.10) in an ATP-dependent reaction [2]. Biotin-dependent carboxylases catalyze essential steps in the metabolism of glucose, amino acids, and fatty acids [1]. Proteolytic degradation of holocarboxylases leads to the formation of biotinyl peptides. These peptides are further degraded by biotinidase (E.C. 3.5.1.12) to release biotin, which can then be used for the synthesis of new holocarboxylases [3].

Consistent with the classical roles for biotin in carboxylase-dependent metabolic pathways, biotin deficiency may cause decreased rates of cell proliferation [4, 5], impaired immune function [6–8], and abnormal fetal development [9–11]. The arrival of molecular biology techniques has added a new dimension to our understanding of roles for biotin in mammalian metabolism that go beyond the classical roles for biotin in carboxylations. Pioneering studies by Dakshinamurti and coworkers revealed that biotin may affect gene expression [12–14]; these studies are consistent with the hypothesis that some of the effects of biotin on cell biology and fetal development might be mediated by alterations in gene expression in response to biotin supply. Recently, significant progress has been made (*i*) in the identification of genes that are affected by biotin at the transcriptional level and the post-transcriptional level; (*ii*) in the elucidation of mechanisms that mediate effects of biotin on gene expression; and (*iii*) in the identification of roles for biotin metabolites in gene expression. These recent developments in the field of biotin research are reviewed here.

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2. Genes affected by biotin at the transcriptional level

2.1. Glucose metabolism

More than thirty years ago, initial evidence has been provided that biotin might affect the expression of the gene encoding glucokinase, a key enzyme in glycolysis. These pioneering studies revealed that biotin deficiency in rats causes a 40% to 45% reduction of liver glucokinase activity, and that enzyme activity can be restored to normal by biotin administration [12]. Also it was demonstrated that biotin administration to biotin-deficient rats increases the synthesis rates of protein, rRNA, and mRNA [13]. Subsequently, it was shown that administration of biotin increases the amount of mRNA coding for glucokinase in rat liver [15, 16] and in cultured beta cells [17].

Glucokinase is not the sole enzyme in glucose metabolism that is affected by biotin; administration of biotin to diabetic rats represses hepatic (but not kidney) phosphoenolpyruvate carboxykinase mRNA to 15% of control levels [2]. Phosphoenolpyruvate carboxykinase is a key enzyme in gluconeogenesis.

2.2. Biotin-dependent carboxylases and holocarboxylase synthetase

Numerous studies have provided evidence that expression of genes encoding biotin-dependent carboxylases and holocarboxylase synthetase depends on biotin. For example, the abundance of mRNA encoding holocarboxylase synthetase decreased significantly in liver, kidney, muscle, and brain from biotin-deficient rats compared to normal controls; the abundance of mRNA increased to near-normal levels if biotin-deficient rats were supplemented with biotin for 24 h [18]. Similar findings were made in a human liver cell line [19]. Decreased expression of the holocarboxylase synthetase gene was associated with decreased biotinylation of pyruvate carboxylase and propionyl-CoA carboxylase in rat tissues [18].

Likewise, expression of genes encoding biotin-dependent carboxylases depends on biotin. For example, in human-derived liver cells the expression of genes encoding acetyl-CoA carboxylase and propionyl-CoA carboxylase decreased to approximately 20% of control values if cells were cultured in biotin-free medium for 15 days [19]. Moreover, supplementation of healthy adults with 8.8 μmol biotin/day for 21 days caused increased expression of the gene encoding 3-methylcrotonyl-CoA carboxylase in peripheral blood mononuclear cells (PBMC) [20]. On the basis of these findings, Solorzano-Vargas et al. have proposed a holocarboxylase-synthetase-dependent signaling pathway that regulates the expression of genes encoding biotin-dependent carboxylases (see below) [19].

2.3. Biotin transporters

Biotin uptake into mammalian cells is mediated by the sodium-dependent multivitamin transporter (SMVT) [21–23] and other unidentified transporters [24–27]. Evidence has been provided that human-derived cell lines respond to biotin deficiency with increased transport rates of biotin, consistent with increased expression of genes encoding biotin transporters [5, 28]. In human choriocarcinoma cells, biotin concentrations in culture media correlated negatively with expression of the biotin transporter SMVT, as judged by cellular transport rates of biotin, abundance of SMVT protein, and transcriptional activity of SMVT reporter-gene constructs [28]. In contrast, the abundance of SMVT protein did not increase in response to biotin deficiency in a human T cell line, despite increased rates of biotin transport in these cells [5]. These studies are consistent with previous observations that immune cells might use proteins other than SMVT to mediate transport of biotin [24–26].

2.4. Cytokines

Immune cells such as T and B cells, monocytes, and macrophages secrete cytokines in response to stimulation of the immune system. Cytokines are small proteins which are grouped in the following families [29]: (*i*) hemopoietins; (*ii*) interferons; (*iii*) interleukin-1 (IL-1) family; (*iv*) tumor necrosis factor family; (*v*) transforming growth factor family; (*vi*) chemokines; and (*vii*) others.

Extracellular cytokines bind to receptors located on the surface of target cells such as T cells and natural killer cells [29]. Receptor binding triggers intracellular signaling cascades, leading to cell growth, proliferation, and differentiation, i.e., processes that are essential for a normal immune response [29]. Some cells that are not immune cells may also secrete or bind cytokines, e.g., fibroblasts, hepatocytes, and smooth muscle cells [29]. Ultimately, cytokine/receptor complexes are endocytosed and degraded [30–36] to avoid excessive stimulation of the immune system by cytokines.

Numerous studies have provided evidence for an essential role of biotin in immune function [6–8, 37–39]. Surprisingly, supplementation of healthy adults with biotin decreases the secretion of IL-2 and IL-1β by PBMC [40]. Likewise, secretion IL-2 by a human T cell line (Jurkat cells) correlated negatively with biotin concentrations in culture media [5], despite decreased expression of the gene encoding IL-2 in biotin-deficient cells compared to biotin-supplemented cells [41].

In subsequent studies the following model was developed to explain the increased (net) secretion of IL-2 despite decreased expression of the IL-2 gene in biotin-deficient Jurkat cells [41]. Jurkat cells express genes encoding IL-2, and the IL-2 receptors α and γ [30, 42]. Expression of genes encoding both IL-2 and IL-2 receptor γ depends on biotin in Jurkat cells [41]. Decreased expression of the gene encoding IL-2 receptor γ causes decreased rates of IL-2 endocytosis
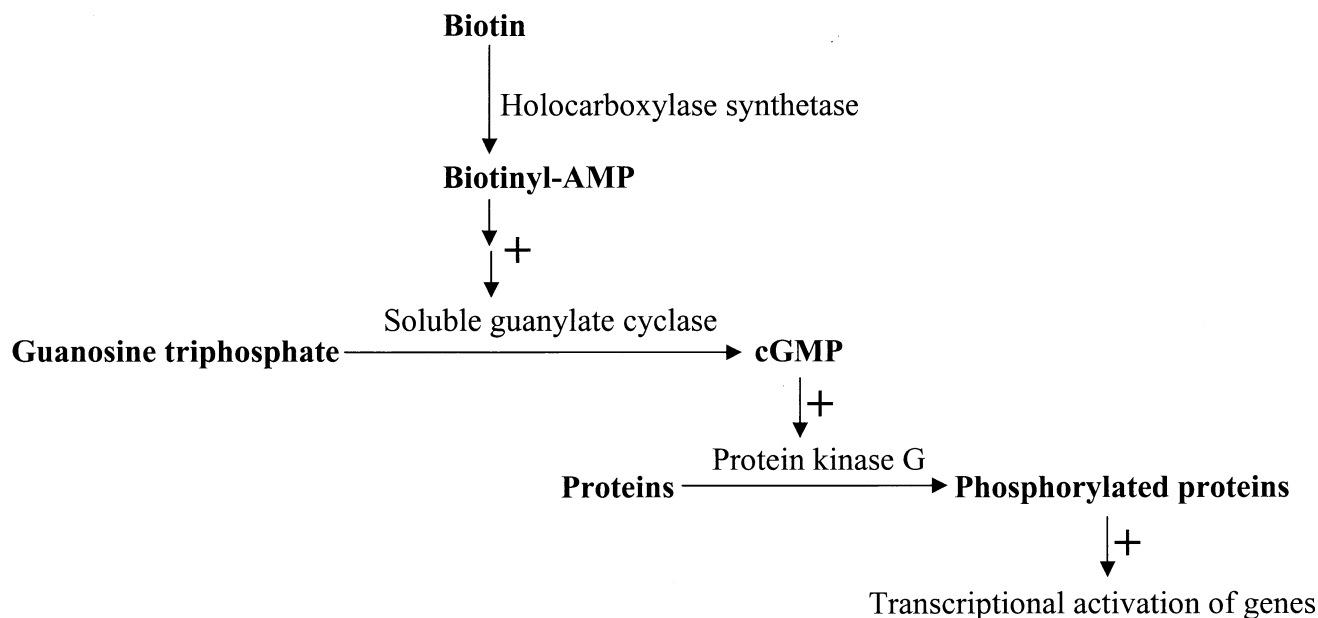
Biotin

| Holocarboxylase synthetase

Biotinyl-AMP

+

Soluble guanylate cyclase

Guanosine triphosphate ⟶ **cGMP**

+

Protein kinase G

Proteins ⟶ **Phosphorylated proteins**

+

Transcriptional activation of genes

Fig. 1. Regulation of gene expression by biotinyl-AMP. Activation of metabolic pathways is denoted by "+".

in biotin-deficient cells. Decreased endocytosis of IL-2 leads to accumulation of IL-2 in biotin-deficient medium despite decreased expression of the IL-2 gene.

Expression of genes encoding cytokines other than IL-2 also depends on biotin in human PBMC [20]. When healthy adults were supplemented with 8.8 μmol biotin/day for 21 days, expression of the gene encoding IL-4 decreased compared to before biotin supplementation, whereas expression of the genes encoding IL-1β and interferon-γ increased in response to biotin supplementation. These cytokines play the following important roles in immune function. (*i*) IL-4 mediates B-cell activation and class switch in B lymphocytes, i.e., the change from the expression of one immunoglobulin class to another [29]. (*ii*) IL-1β causes activation of T_H lymphocytes, maturation and clonal expansion of B lymphocytes, activation of natural killer cells, and attraction of macrophages and neutrophils to sites of inflammatory response [29]. In addition, IL-1β induces synthesis of acute-phase proteins by hepatocytes, osteoblast proliferation, and prostaglandin production in osteoblasts [43]. (*iii*) Interferon-γ induces expression of genes that play important roles in antiviral defense, e.g., 2′,5′-oligoadenylate synthase [29].

2.5. Oncogenes

Evidence has been provided that expression of the following oncogenes depends on biotin in small cell lung cancer cells: N-myc, c-myb, N-ras and raf [44]. Oncogene expression (abundance of mRNA) increased by up to 20% in cells cultured in medium containing 10,000 pM biotin compared to medium containing 250 pM biotin; oncogene expression decreased by up to 47% in cells cultured in medium containing 25 pM biotin compared to medium

containing 250 pM biotin. Biotin-dependent expression of oncogenes is physiologically meaningful, given the important roles of the gene products in signal transduction, gene expression, and cell proliferation and differentiation [45–51].

2.6. Other genes

Biotin deficiency decreases ornithine transcarbamoylase activity and mRNA in rat liver [52]. Ornithine transcarbamoylase plays an essential role in the metabolism of arginine and in the urea cycle. DNA microarray studies that are currently underway in our laboratory have provided preliminary evidence that biotin supplementation affects the expression of greater than 200 genes (out of 12,000 tested) in PBMC from healthy adults. Future studies will attempt to verify these preliminary findings by using techniques such as Northern blot analysis. In-depth analysis of these data is likely to lead to the identification of clusters of biotin-dependent genes and biotin-dependent signaling pathways.

3. Genes affected by biotin at the post-transcriptional level

Biotin might affect the expression of some genes at the post-transcriptional level. If HepG2 (liver) cells are grown in biotin-deficient medium, expression of the asialoglycoprotein receptor is reduced under conditions where protein synthesis, total cellular protein content, and mRNA coding for asialoglycoprotein receptor are comparable to those of control cells; addition of biotin or biocytin restores receptor expression [53]. These findings are consistent with the hy-
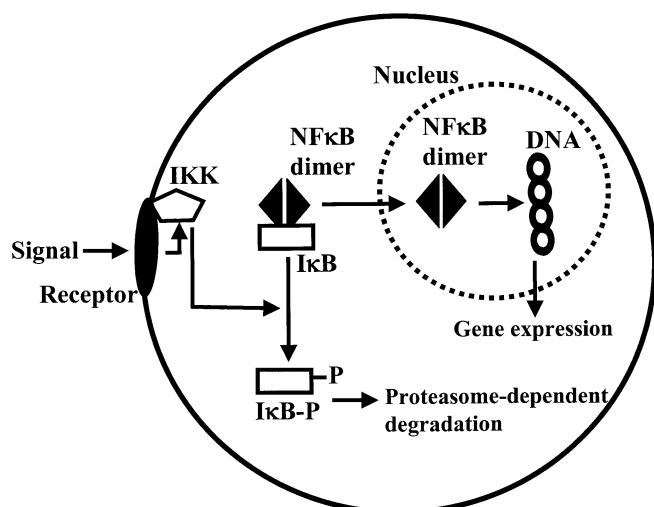
Fig. 2. NF-κB-dependent signaling cascade. ABREVIATIONS. IKK = IκB kinase; IκB = inhibitor of NF-κB; IκB-P = phosphorylated IκB.

pothesis that a biotin-dependent post-transcriptional event permits the ultimate expression of asialoglycoprotein receptor by HepG2 cells. Analogous studies of propionyl-CoA carboxylase in rat hepatocytes suggested that biotin might also affect the expression of this carboxylase at a post-transcriptional step [54].

4. Mechanisms that mediate effects of biotin on gene expression

Currently, three mechanisms have been identified by which biotin might affect gene expression: (*i*) activation of soluble guanylate cyclase by biotinyl-AMP; (*ii*) nuclear translocation of NF-κB (in response to biotin deficiency); and (*iii*) remodeling of chromatin by biotinylation of histones. These mechanisms are not mutually exclusive but might co-exist in human cells; the three mechanisms are reviewed in the following sections.

4.1. Biotinyl adenosine monophosphate (biotinyl-AMP)

Solorzano-Vargas et al. have proposed that biotinyl-AMP plays an essential role in regulating gene expression [19]. Biotinyl-AMP is an intermediate in the synthesis of holocarboxylases; synthesis of biotinyl-AMP is catalyzed by holocarboxylase synthetase (Fig. 1) [1]. Solorzano-Vargas et al. have proposed that biotinyl-AMP activates soluble guanylate cyclase by a yet unknown mechanism; activation of guanylate cyclase leads to increased production of cyclic guanosine monophosphate, cGMP [19]. cGMP stimulates protein kinase G, leading to phosphorylation and activation of proteins that enhance transcriptional activity of genes encoding holocarboxylase synthetase, acetyl-CoA carboxylase 1 (but not acetyl-CoA carboxylase 2), and propionyl-CoA carboxylase. Transcriptional activation of these genes

is impaired by both biotin deficiency and decreased activities of holocarboxylase synthetase, the latter being observed in an inborn error of metabolism known as "multiple carboxylase deficiency" [55, 56]. It remains to be determined whether biotinyl-AMP also plays a role in regulating the expression of genes other than holocarboxylase synthetase, acetyl-CoA carboxylase 1, and propionyl-CoA carboxylase.

In *E. coli* and other enteric bacteria, biotinyl-AMP [complexed with BirA (biotin-protein ligase), the microbial homolog of holocarboxylase synthetase] plays a well-established role in gene expression. The biotinyl-AMP/BirA complex binds to promoter regions in the biotin operon, a cluster of genes mediating biotin biosynthesis; binding of biotinyl-AMP/BirA causes transcriptional repression of these genes [57]. This feedback loop prevents excessive biosynthesis of biotin.

4.2. Nuclear factor kappa B (NF-κB) and other transcription factors

The transcription factor NF-κB plays important roles in regulating processes such as prevention of cell death and embryonic development [58]. Five members of the mammalian NF-κB/Rel family of transcription factors have been cloned and sequenced: c-Rel, NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), and RelB [59]. Briefly, the following mechanistic sequence leads to transcriptional activation of genes by NF-κB (Fig. 2). In unstimulated cells, proteins of the NF-κB family exist as heterodimers or homodimers in the cytoplasm [59]. These inactive dimers are associated with the following monomers of inhibitors of NF-κB: IκBα or IκBβ [59]. Binding to IκB retains NF-κB in the cytoplasm by masking the nuclear localization sequences [59]. Stimulation of IκB kinases by bacteria, cytokines, mitogens, oxidative stress, growth factors, and hormones triggers phosphorylation of IκBs, which are then degraded in proteasome-dependent pathways [58]. The liberated NF-κB dimers translocate to the cell nucleus, where they bind to response elements in regulatory regions of genes, triggering gene expression.

Studies in our laboratory have provided evidence that biotin affects NF-κB-dependent cell signaling (R. Rodriguez-Melendez and J. Zempleni, submitted for publication). In these studies, Jurkat cells were cultured in biotin-defined media (25 and 10,000 pM) for 5 weeks in analogy to our previous studies [5, 41]; cells were stimulated with phytohemagglutinin and phorbol 12-myristate 13-acetate, and variables of NF-κB-dependent signaling were measured. The following observations were made that are consistent with the hypothesis that biotin supply affects NF-κB signaling: (*i*) nuclear translocation of proteins that bind to the NF-κB consensus sequence (AGTTGAGGGGACTTTC-CCAGGC) was greater in biotin-deficient cells compared to biotin-supplemented cells, as judged by electrophoretic mobility shift assay; (*ii*) nuclear abundance of p50 and p65 was greater in biotin-deficient cells compared to biotin-supple-
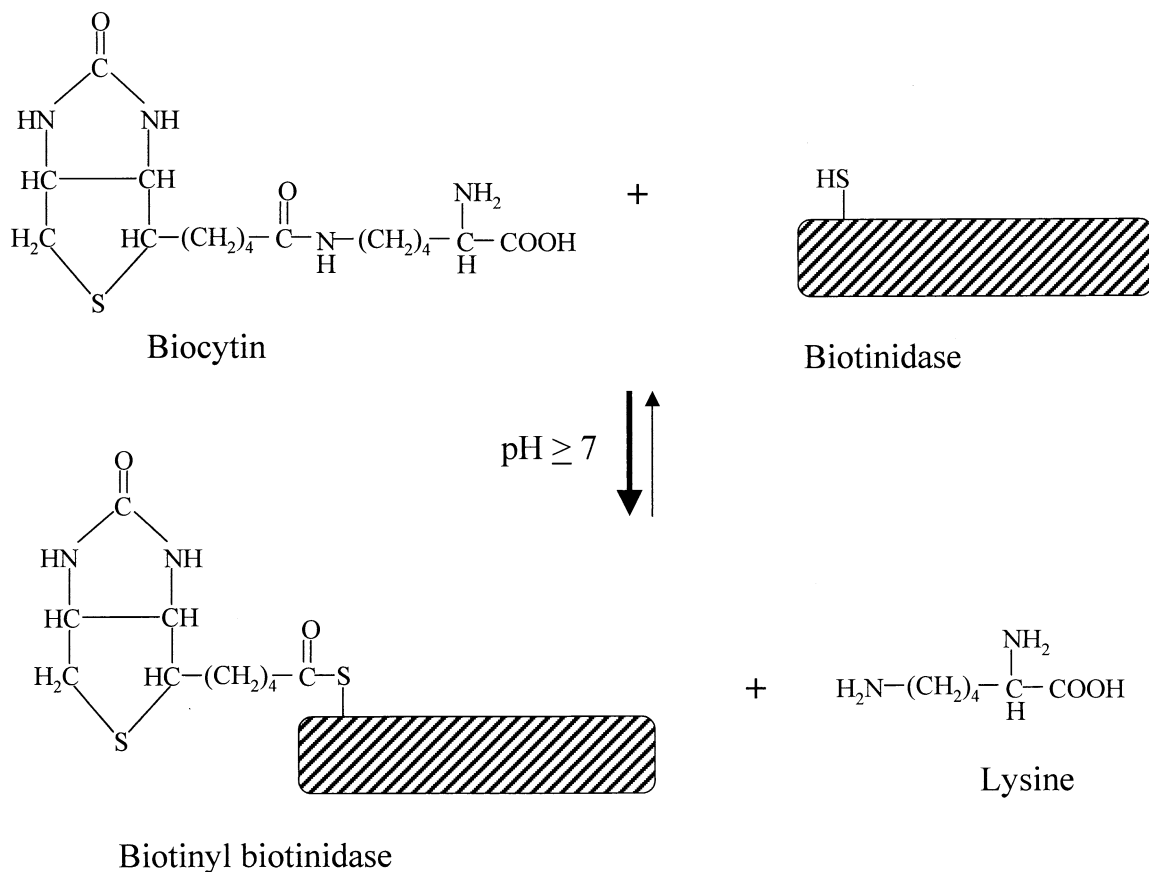
Fig. 3. Cleavage of biocytin (biotin-ϵ-lysine) by biotinidase leads to the formation of a biotinyl-thioester intermediate (cysteine-bound biotin; biotinyl biotinidase) at or near the active site of biotinidase [74].

mented cells, as judged by Western blot analysis and enzyme-linked immunosorbent assay; (*iii*) transcriptional activity of NF-κB-dependent reporter genes was greater in biotin-deficient cells compared to biotin-supplemented cells; and (*iv*) activity of IκBα kinases was greater in biotin-deficient cells compared to biotin-supplemented cells, consistent with increased rates of IκBα degradation and nuclear translocation of NF-κB.

Preliminary evidence has been provided that biotin might also affect signaling pathways involving members of the SP1 and Krüppel-like factor (KLF) family of transcription factors (unpublished observations). This family contains at least 20 identified transcription factors in mammals [60]. Some members of this family are widespread or ubiquitously expressed, whereas other members are highly restricted in their tissue distribution [60]. Regulation of transcription by members of the SP1 and KLF family of transcription factors is highly complex. For example, SP1-binding sites in gene promoters respond differently in cells from distinct tissues [60]. It has been proposed that this high degree of context dependence reflects the differential binding of SP1/KLF factors to different SP1 sites, coupled with variations in the network of SP1/KLF factors that result from differences in the activity and expression of individual family members [60].

4.3. Biotinylation of histones

Components of chromatin include (*i*) DNA; (*ii*) a group of proteins named histones; and (*iii*) various non-histone proteins. Folding of DNA into chromatin is mediated primarily by histones [61]. Five major classes of histones have been identified in mammals: H1, H2A, H2B, H3, and H4. Histones consist of a globular domain and a more flexible and charged amino terminus (histone "tail"). Histones are very basic proteins due to their large content of lysine and arginine residues, which account for a total of more than 20% of the amino acids in histones [61].

DNA and histones in chromatin form repetitive nucleoprotein units, the nucleosomes [61]. Each nucleosome ("nucleosome core particle") consists of 146 basepairs of DNA wrapped around an octamer of core histones (one H3-H3-H4-H4 tetramer and two H2A-H2B dimers). The binding of DNA to histones is of electrostatic nature, and is mediated by the association of negatively charged phosphate groups of DNA with positively charged ϵ-amino groups (lysine moieties) and guanidino groups (arginine moieties) of histones. The amino terminal tail of histones protrudes from the nucleosomes; covalent modifications of this tail affect the structure of chromatin and form the basis for gene regulation, replication, and DNA repair as described below.
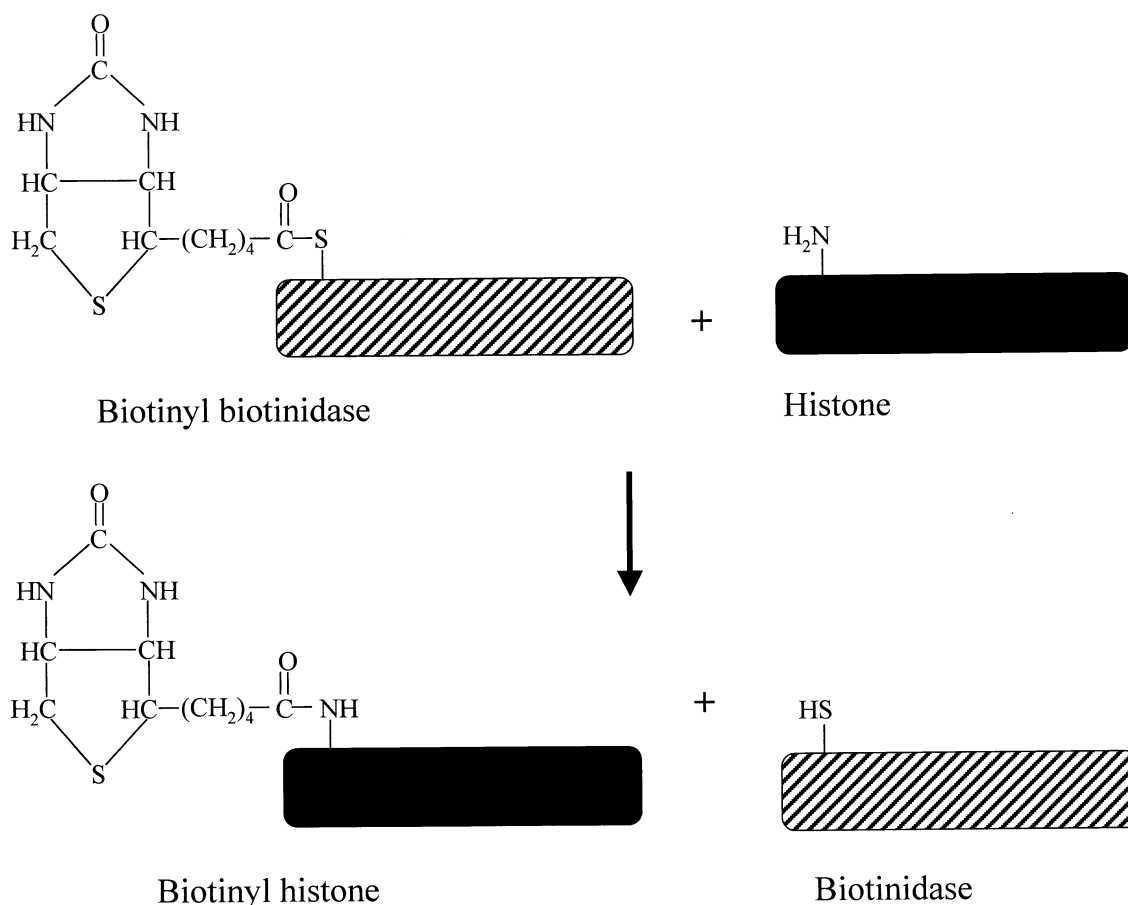
Fig. 4. The biotinyl moiety from the biotinyl biotinidase intermediate is transferred onto the ϵ-amino group of lysine (or other amino groups) in histones [74].

The DNA located between nucleosome core particles is called linker DNA and is associated with linker histone H1.

Histone tails are modified by covalent acetylation [62–64], methylation [61], phosphorylation [61], ubiquitination [61], and poly (ADP-ribosylation) [65–67] of ϵ-amino groups (lysine), guanidino groups (arginine), and hydroxyl groups (serine). These modifications of histone tails ("histone code") may considerably extend the information potential of the DNA code and gene regulation, given that the enzymes catalyzing these modifications are specific for amino acid residues [68–70]. Multiple signaling pathways converge on histones to mediate covalent modifications of histones [71]. For example, binding of nuclear receptors to DNA recruits histone acetyl transferases to chromatin, leading to acetylation of histone tails [72]. Modifications of histone tails may affect binding of chromatin-associated proteins, triggering cascades of downstream histone modifications.

Covalent modifications of histones are reversible, i.e., acetate moieties can be removed by histone deacetylases, and phosphates can be removed by phosphatases. The mechanism leading to removal of methyl groups is uncertain. It has been proposed that methylation marks are removed by proteolytic processing of histones, i.e., the methylated histone tail is clipped off [70]. It is uncertain whether ubiquitination of histones plays a role in this process.

Recently, evidence has been provided for another post-translational modification of histones: biotinylation of lysine residues. Hymes et al. have proposed the following enzymatic mechanism by which biotinidase mediates covalent binding of biotin to histones [73]: cleavage of biocytin (biotin-ϵ-lysine) by biotinidase leads to the formation of a biotinyl-thioester intermediate (cysteine-bound biotin) at or near the active site of biotinidase (Fig. 3) [73, 74]. In a next step, the biotinyl moiety is transferred from the thioester to the ϵ-amino group of lysine in histones (Fig. 4). Biotinidase is ubiquitous in mammalian cells and 26% of the cellular biotinidase activity is located in the nuclear fraction [75].

Studies conducted in our laboratory provided evidence that human cells contain biotinylated histones. Histones were isolated from nuclei of human PBMC by acid extraction [76]. This procedure yielded a pure preparation of histones H1, H2A, H2B, H3, and H4, as judged by comigration with commercially available histones on polyacrylamide gels and by the absence of quantitatively important non-histone bands after coomassie blue staining (Fig. 5, lanes 1 and 2). Biotin in these histones was probed with streptavidin-conjugated peroxidase, using Western blot
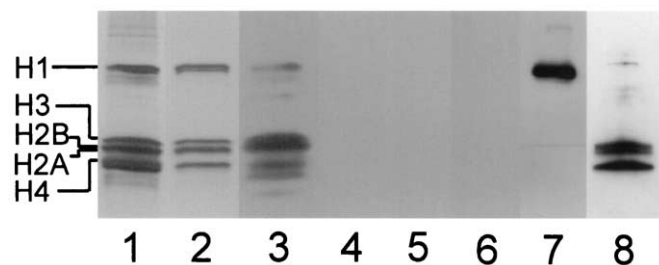
Fig. 5. Nuclei from human PBMC contain biotinylated histones. Histones were extracted from PBMC nuclei and chromatographed using SDS/gel electrophoresis. Non-histone proteins and synthetic polypeptides (poly-L-arginine and poly-L-lysine) were chromatographed as described for histones. Samples were either stained with coomassie blue (lanes 1 and 2), probed with streptavidin-conjugated peroxidase (lanes 3 to 7), or probed with a monoclonal antibody against biotin (lane 8). Lane 1 = histones from human PBMC nuclei; lane 2 = commercially available histones from calf thymus; lane 3 = histones from human PBMC nuclei; lane 4 = mixture of aprotinin, α-lactalbumin, β-lactoglobulin, trypsin inhibitor, and trypsinogen; lane 5 = poly-L-arginine; lane 6 = poly-L-lysine; lane 7 = chemically biotinylated histone H1; lane 8 = histones from human PBMC nuclei. Equal amounts of histones, poly-L-lysine, poly-L-arginine, and non-histone proteins were used except for the chemically biotinylated histone H1 which was diluted approximately 20,000 fold [76].

analysis. Histones H1, H3, and H4 contained streptavidin-binding substances, suggesting that these proteins are biotinylated (Fig. 5, lane 3). Histones H2A and H2B electrophoresed as one single band. Thus, it remains uncertain whether streptavidin bound to histone H2A, H2B, or both.

The following data suggest that streptavidin specifically bound to biotinylated histones rather than unspecifically to non-biotinylated proteins: The (non-biotinylated) proteins aprotinin, α-lactalbumin, β-lactoglobulin, trypsin inhibitor, and trypsinogen have molecular weights similar to histones; no visible bands were observed when these proteins were probed with streptavidin-conjugated peroxidase (Fig. 5, lane 4). The synthetic polypeptides poly-L-lysine and poly-L-arginine mimic lysine- and arginine-rich histones; no visible bands were observed when these polypeptides were probed with streptavidin-conjugated peroxidase (Fig. 5, lanes 5 and 6). Histone H1 was biotinylated chemically [77] and was used as a positive control (Fig. 5, lane 7) [76]. Finally, extracts from PBMC nuclei were probed with a monoclonal antibody against biotin (Fig. 5, lane 8). The antibody bound to histones, providing additional evidence that histones contain biotin. Biotinylated histones were also detected in a human T cell line [5], human small cell lung cancer cells [44], human choriocarcinoma cells [28], and chicken erythrocytes [78].

A tight regulation of gene expression is essential to ensure normal cell proliferation. Consistent with an essential role for biotinylation of histones in cell proliferation, biotinylation of histones increases in response to proliferation of human PBMC [76]; biotinylation of histones increases early in the cell cycle and remains increased during later phases of the cell cycle [76]. It remains uncertain whether genes associated with biotinylated histones are ac-

tivated or silenced, or whether biotinylation of histones is associated with DNA repair processes (e.g., DNA strand breaks occurring during replication of DNA). Recent studies provided evidence that biotinylation of histones increases in response to UV-induced DNA damage and that biotinylated histones are enriched in transcriptionally inactive chromatin in chicken erythrocytes [78].

Recent studies are consistent with the hypothesis that biotinidase may catalyze both, biotinylation [73] and debiotinylation of histones [79]. How do cells regulate biotinylation status of histones despite the fact that both biotinylation and debiotinylation of histones are catalyzed by the same enzyme? The following explanations have been offered [76, 78, 79]: (*i*) Enzymes other than biotinidase might also catalyze biotinylation or debiotinylation of histones. For example, holocarboxylase synthetase catalyzes covalent binding of biotin to lysine residues in mammalian carboxylases [1]. The possibility that holocarboxylase synthetase also catalyzes binding of biotin to histones is an untested hypothesis.

(*ii*) Covalent modification of biotinidase might be a mechanism to favor either biotinylation or debiotinylation of histones. Currently, glycosylation is the only post-translational modification of biotinidase that has been identified [80].

(*iii*) The presence of cofactors might favor either biotinylation or debiotinylation of histones by biotinidase. For example, high concentrations of the substrate biocytin may increase the rate of histone biotinylation. Similarly, biotinylated peptides might inhibit debiotinylation of histones by competing for binding to biotinidase. The pH optimum is similar (pH 8) for both the biotinylating activity [73] and the debiotinylating activity of biotinidase [79]. Thus, changes of the pH in the microenvironment of histones are unlikely to affect biotinylation status.

(*iv*) Binding of accessory proteins to biotinidase or chromatin might regulate enzymatic activity of biotinidase to favor either the forward or the reverse reaction. Accessory proteins have not yet been identified.

5. Effects of biotin metabolites on gene expression

Two pathways of biotin catabolism have been identified in mammals and microorganisms in a series of classical studies by McCormick and coworkers (Fig. 6): (*i*) β-oxidation of the valeric acid side chain [1,81 to 84], leading to the formation of bisnorbiotin, tetranorbiotin, and related metabolites that are known to result from β-oxidation of fatty acids (i.e., α,β-dehydro-, β-hydroxy-, and β-keto-intermediates). (*ii*) Sulfur oxidation in the heterocyclic ring, leading to the formation of biotin-*l*-sulfoxide, biotin-*d*-sulfoxide, and biotin sulfone [81, 82]. Biotin metabolites are quantitatively important in mammalian tissues and body fluids; biotin metabolites account for approximately 50 to 70 mole percent of the total biotinyl compounds [83–85].
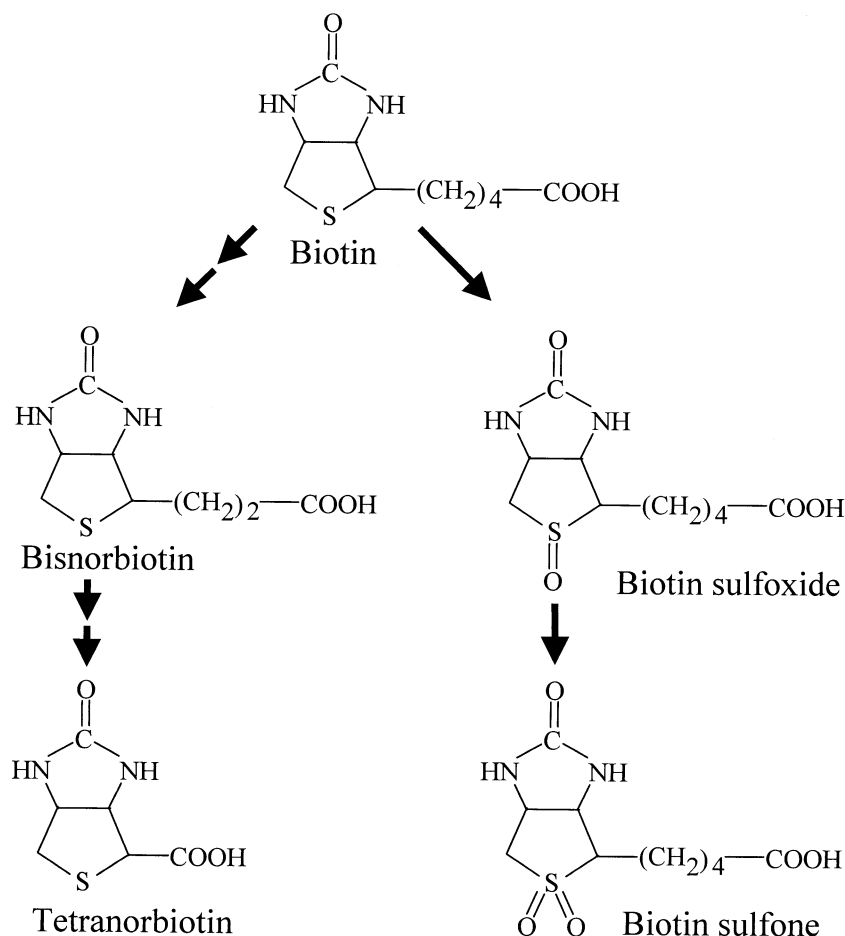
Fig. 6. Pathways of biotin catabolism.

In classical nutrition studies, most biotin metabolites are considered metabolic waste with no known function. For example, metabolites with modifications in the ureido portion of the heterocyclic ring (e.g., the synthetic diaminobiotin) cannot participate in carboxylase reactions because the ureido portion plays an essential role in the carboxylation of organic acids [86]. Similarly, some modifications in the valeric acid side chain of the biotin molecule (e.g., bisnorbiotin methyl ketone and tetranorbiotin methyl ketone) [83, 84, 87] renders these metabolites inactive as they cannot undergo activation by binding of AMP, a prerequisite for binding to carboxylases [86]. In contrast, amino acid or peptide conjugates of biotin (e.g., biotinyl-ε-lysine) have an intact valeric acid side chain and heterocyclic ring; free, bioactive biotin can be released from these metabolites [56].

Recent studies have provided evidence that biotin catabolites might have biotin-like activities with regard to gene expression [88]. In these studies, human lymphoid cells (Jurkat cells) were depleted of biotin, leading to decreased transcriptional activity of the genes encoding IL-2 and IL-2 receptor γ. Supplementation of biotin-depleted cells with the synthetic biotin analogs diaminobiotin (modified ureido portion in the biotin molecule) and desthiobiotin

(modified thiophane portion) caused a significant increase in the transcriptional activity of the genes encoding IL-2 and IL-2 receptor γ. Supplementation with diaminobiotin and desthiobiotin did not affect activities of propionyl-CoA carboxylase and abundance of holocarboxylases. This is consistent with the hypothesis that biotin catabolites have biotin-like activities that are not mediated by binding to carboxylases. Future studies will have to determine whether the observed effects are specific for the synthetic compounds diaminobiotin and desthiobiotin, or whether naturally occurring biotin metabolites also have biotin-like activities.

6. Conclusions and outlook

Clearly, substantial progress has been made in the field of biotin-dependent gene expression since Dakshinamurti and coworkers first described effects of biotin on the expression of the glucokinase gene more than 30 years ago [12, 13]. Since then numerous genes have been identified that are affected by biotin at the transcriptional or post-transcriptional level, and the list continues to grow. Some of

the pathways that mediate effects of biotin on transcriptional activity of genes are likely to have a broad impact on gene expression profiles, e.g., remodeling of chromatin by biotinylation of histones, and nuclear translocation of NF-κB in response to biotin deficiency. Given the importance of covalent modifications of histones and nuclear translocation of NF-κB in processes such as transcriptional activation and silencing of genes, replication and repair of DNA, and response to cell stress [58, 59, 61], future studies of biotin metabolism are likely to generate knowledge in fundamental processes such as fetal development, apoptosis and cancer, and function of the immune system.

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