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Epigenetic regulation of chromatin structure and gene function by biotin:

are biotin requirements being met?

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Abstract

Histones H2A, H3, and H4 are modified by covalent binding of the vitamin biotin to distinct lysine residues. Binding of biotin to histones is mediated by holocarboxylase synthetase (HCS) and perhaps biotinidase. Biotinylation of lysine- 12 in histone H4 (K12BioH4) plays roles in gene repression, stability of repeat regions and transposable elements, and regulation of biotin transporter expression in eukaryotes. Decreased biotinylation of histones in biotin-deficient and HCS-deficient human cells and *Drosophila melanogaster* impairs stress resistance, life span, and biotin homeostasis.

CHROMATIN

Chromatin is comprised of DNA and DNA-binding proteins, i.e., histones and non-histone proteins. Histones play a predominant role in the folding of DNA into chromatin.¹ 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 amino terminus (histone “tail”). DNA and histones form repetitive nucleoprotein units, the nucleosomes.¹ Each nucleosome (“nucleosomal core particle”) consists of 146 base pairs of DNA wrapped around an octamer of core histones (one H3-H3-H4-H4 tetramer and two H2A-H2B dimers).

The amino terminal tail of histones protrudes from the nucleosomal surface; covalent modifications of this tail affect the structure of chromatin and form the basis for gene regulation.¹⁻³ Amino acid residues in histone tails are modified by covalent acetylation, methylation, phosphorylation, ubiquitination, and poly(ADP-ribosylation). The various modifications of histones have distinct functions. For example, trimethylation of K4 in histone H3 is associated with transcriptional activation of surrounding DNA, whereas dimethylation of K9 is associated with transcriptional silencing.^{2,3} Covalent modifications of histones are reversible and may have clinical implications.³

BIOTINYLATION OF HISTONES

Recently, we identified a novel modification of histones by investigating the enzymatic biotinylation of histone-based, synthetic peptides: covalent binding of the vitamin biotin to K9, K13, K125, K127, and K129 in histone H2A;⁴ K4, K9, and K18 in histone H3;⁵ and K8 and

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Declaration of interest. JZ receives royalties from the sales of antibodies to biotinylated histones, based on a licensing agreement with Upstate, Inc. and is named as a co-inventor on U.S. Patent Application No. 20,060,286,611, which covers products and methods relating to antibodies against biotinylated histones and related proteins and assays related thereto. The other authors have no interests to declare.

K12 in histone H4.⁶ Confirmation of biotinylation sites by mass spectrometry (MS) is currently in progress using histone extracts from human lymphoid (Jurkat) cells.⁷ Biotinylated histones were detected in primary human cells, transformed cell lines, and in other eukaryotes such as *Drosophila melanogaster*.⁸⁻¹⁰ Biotinylation of histones is catalyzed by holocarboxylase synthetase, HCS, and perhaps biotinidase, BTB.^{11,12} HCS and BTB knockdown studies in *Drosophila melanogaster* have provided evidence that HCS is more important than BTB for biotinylation of histones.¹⁰ Previous studies have also revealed that HCS is a chromosomal protein¹⁰ and that chromatin can be immunoprecipitated with an antibody to HCS.⁹ Our laboratory is in the process of identifying zinc-finger proteins that direct HCS to specific regions in chromatin and tyrosine kinases that regulate the nuclear translocation of HCS (Hassan YI and Zempleni J, unpublished observation). Biotinylation of histones is a reversible process, but the identity of the histone debiotinylase(s) is uncertain.¹³ It has been proposed that BTB might participate in the hydrolytic debiotinylation of histones,^{13,14} but other mechanisms, such as histone replacement, might also participate in the removal of biotinylated histones.¹⁻³

BIOLOGICAL FUNCTIONS OF HISTONE BIOTINYLATION

Over the past few years substantial progress has been made towards identifying the biological functions of biotinylated species' histone H4. First, it was shown that K12BioH4 and perhaps lysine (K)-8 biotinylated histone H4 (K8BioH4) are integral components of repeat regions in (peri)centromeric chromatin and mediate silencing of transcriptionally competent chromatin.¹⁵ Second, evidence was provided that K12BioH4 is enriched at transposable elements in the eukaryotic genomes (Chew YC, West J, Zempleni J, unpublished observation). These studies also provided evidence that biotin deficiency is associated with decreased abundance of biotinylated histones at transposable elements, increasing the transcriptional activity of endogenous retroviruses and genomic instability. K-9 biotinylated histone H2A (K9BioH2A) and K-13 biotinylated histone H2A (K13BioH2A) also appear to be enriched at transposable elements. Third, a model has been proposed in which biotin homeostasis in human cells is regulated by enrichment of K12BioH4 at promoters driving the expression of biotin transporters.⁹ In this model, biotin deficiency is associated with decreased abundance of BioK12H4 at biotin transporter loci, causing de-repression of transporter expression and increased biotin uptake. Administration of pharmacological doses of biotin is associated with increased abundance of BioK12H4 at biotin transporter loci, causing repression of transporter expression and decreased biotin uptake.

HCS AND BIOTIN DEFICIENCY

Decreased biotinylation of histones caused by long-term biotin deficiency or by HCS knockdown is associated with decreased life span and stress resistance, and aberrant gene-expression patterns in *Drosophila*.¹⁰ These effects were caused by decreased biotinylation of histones rather than decreased biotinylation of carboxylases, another substrate for biotinylation by HCS.¹⁶ In flies, it takes several generations of feeding a biotin-deficient diet before decreased biotinylation of histones in bulk extracts of histones is evident.¹⁷ Short-term biotin deficiency has no apparent effect on histone biotinylation,¹⁸ although potential effects in confined regions of the genome (e.g., transposable elements) have not been formally excluded.

What is the dietary relevance of these studies? The majority of the studies described above were conducted in cultures of human cells, using biotin concentrations representative of those seen in biotin-deficient, biotinnormal, and biotin-supplemented humans. Both biotin deficiency and supplementation are prevalent in the North American diet. For example, moderate biotin deficiency has been observed in up to 50% of pregnant women.^{19,20} About 20% of the US population reports taking biotin supplements,²¹ producing supraphysiological

concentrations of the vitamin in tissues and body fluids.^{22,23} Biotin deficiency and overdose likely have effects on gene regulation and genome stability that go far beyond the classical coenzymic role of biotin in metabolism.

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REFERENCES

1. Wolffe, A. Chromatin. 3th ed.. Academic Press; San Diego, CA: 1998.
2. Fischle W, Wang Y, Allis CD. Histone and chromatin cross-talk. *Curr Opin Cell Biol* 2003;15:172–183. [PubMed: 12648673]
3. Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;293:1074–1080. [PubMed: 11498575]
4. Chew YC, Camporeale G, Kothapalli N, Sarath G, Zempleni J. Lysine residues in N- and C-terminal regions of human histone H2A are targets for biotinylation by biotinidase. *J Nutr Biochem* 2006;17:225–233. [PubMed: 16109483]
5. Kobza K, Camporeale G, Rueckert B, et al. K4, K9, and K18 in human histone H3 are targets for biotinylation by biotinidase. *FEBS J* 2005;272:4249–4259. [PubMed: 16098205]
6. Camporeale G, Shubert EE, Sarath G, Cerny R, Zempleni J. K8 and K12 are biotinylated in human histone H4. *Eur J Biochem* 2004;271:2257–2263. [PubMed: 15153116]
7. Chew YC, Raza AS, Sarath G, Zempleni J. Biotinylation of K8 and K12 co-occurs with acetylation and mono-methylation in human histone H4. *FASEB J* 2006;20:A610.
8. Stanley JS, Griffin JB, Zempleni J. Biotinylation of histones in human cells: effects of cell proliferation. *Eur J Biochem* 2001;268:5424–5429. [PubMed: 11606205]
9. Gralla M, Camporeale G, Zempleni J. Holocarboxylase synthetase regulates expression of biotin transporters by chromatin remodeling events at the SMVT locus. *J Nutr Biochem* 2008;19:400–408. [PubMed: 17904341]
10. Camporeale G, Giordano E, Rendina R, Zempleni J, Eissenberg JC. *Drosophila* holocarboxylase synthetase is a chromosomal protein required for normal histone biotinylation, gene transcription patterns, lifespan and heat tolerance. *J Nutr* 2006;136:2735–2742. [PubMed: 17056793]
11. Narang MA, Dumas R, Ayer LM, Gravel RA. Reduced histone biotinylation in multiple carboxylase deficiency patients: a nuclear role for holocarboxylase synthetase. *Hum Mol Genet* 2004;13:15–23. [PubMed: 14613969]
12. Hymes J, Fleischhauer K, Wolf B. Biotinylation of histones by human serum biotinidase: assessment of biotinyl-transferase activity in sera from normal individuals and children with biotinidase deficiency. *Biochem Mol Med* 1995;56:76–83. [PubMed: 8593541]
13. Chew YC, Sarath G, Zempleni J. An avidin-based assay for quantification of histone debiotinylase activity in nuclear extracts from eukaryotic cells. *J Nutr Biochem* 2007;18:475–481. [PubMed: 17156993]
14. Ballard TD, Wolff J, Griffin JB, Stanley JS, van Calcar S, Zempleni J. Biotinidase catalyzes debiotinylation of histones. *Eur J Nutr* 2002;41:78–84. [PubMed: 12083317]
15. Camporeale G, Oommen AM, Griffin JB, Sarath G, Zempleni J. K12-biotinylated histone H4 marks heterochromatin in human lymphoblastoma cells. *J Nutr Biochem* 2007;18:760–768. [PubMed: 17434721]
16. Camporeale G, Zempleni J, Eissenberg JC. Susceptibility to heat stress and aberrant gene expression patterns in holocarboxylase synthetase-deficient *Drosophila melanogaster* are caused by decreased biotinylation of histones, not of carboxylases. *J. Nutr* 2007;137:885–889. [PubMed: 17374649]
17. Smith EM, Hoi JT, Eissenberg JC, et al. Feeding *Drosophila* a biotin-deficient diet for multiple generations increases stress resistance and lifespan and alters gene expression and histone biotinylation patterns. *J. Nutr* 2007;137:2006–2012. [PubMed: 17709434]

18. Landenberger A, Kabil H, Harshman LG, Zempleni J. Biotin deficiency decreases life span and fertility but increases stress resistance in *Drosophila melanogaster*. *J Nutr Biochem* 2004;15:591–600. [PubMed: 15542350]
19. Mock DM, Stadler DD. Conflicting indicators of biotin status from a cross-sectional study of normal pregnancy. *J Am Coll Nutr* 1997;16:252–257. [PubMed: 9176832]
20. Mock DM, Stadler D, Stratton S, Mock NI. Biotin status assessed longitudinally in pregnant women. *J Nutr* 1997;127:710–716. [PubMed: 9164991]
21. National, RC. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B₆, Folate, Vitamin B₁₂, Pantothenic Acid, Biotin, and Choline. National Academy Press; Washington, DC: 1998.
22. Mock DM, Lankford GL, Mock NI. Biotin accounts for only half of the total avidin-binding substances in human serum. *J Nutr* 1995;125:941–946. [PubMed: 7722698]
23. Zempleni J, Helm RM, Mock DM. In vivo biotin supplementation at a pharmacologic dose decreases proliferation rates of human peripheral blood mononuclear cells and cytokine release. *J Nutr* 2001;131:1479–1484. [PubMed: 11340103]