

The role of holocarboxylase synthetase in genome stability is mediated partly by epigenomic synergies between methylation and biotinylation events

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Holocarboxylase synthetase (HLCS) catalyzes the covalent binding of biotin to histones. Biotinylated histones are gene repression marks and are particularly enriched in long terminal repeats, telomeres and other repeat regions. The effects of HLCS in gene regulation are mediated by its physical interactions with chromatin proteins such as histone H3, DNMT1, MeCP2 and EHMT-1. It appears that histone biotinylation depends on prior methylation of cytosines. De-repression of long terminal repeats in biotin- or HLCS-deficient cell cultures and organisms is associated with genome instability.

Background

The human *holocarboxylase synthetase* (HLCS) gene was characterized in 1994.¹ Originally it was believed that the role of HLCS is limited to that as a biotin:carboxylase ligase, catalyzing the covalent binding of the vitamin biotin to 3-methylcrotonyl-CoA carboxylase, propionyl-CoA carboxylase, pyruvate carboxylase and acetyl-CoA carboxylases 1 and 2 in humans.^{2,3} Subsequently, it was demonstrated that HLCS and its microbial ortholog BirA can also biotinylate histones.^{4,5} To date, at least five biotinylation sites have been identified in histones H3 [lysine (K)-4, K9, K18 and probably K23] and H4 (K8, K12 and probably K16).^{4,6,7} K9 and K13 in histone H2A might also be biotinylated,⁸ but the abundance of these two marks appears to be very low.⁹ Studies with synthetic HLCS substrates provide unambiguous evidence that biotinylation

of histones by HLCS is a substrate-specific process,¹⁰ contrary to claims that histone biotinylation is a random event.¹¹

Biotinylation of Histones is a Real Epigenetic Mark

The existence of biotinylated histones was recently questioned by Healy et al.¹² but three independent laboratories, in addition to ours, confirmed that biotinylation is a natural histone modification.¹³⁻¹⁵ These studies included analysis of histone biotinylation by mass spectrometry and suggested that, at least in *Candida albicans*, up to 50% of histones might be biotinylated.¹⁴ In contrast, histone biotinylation is a comparably rare event in humans (<0.1% of histones are biotinylated),¹⁵ but the abundance of an epigenetic mark is no marker for its importance. For example, serine-14 phosphorylation in histone H2B and histone poly(ADP-ribosylation) are detectable only after induction of apoptosis and major DNA damage, respectively, but the role of these epigenetic marks in cell death is unambiguous.^{16,17} The abundance of histone biotinylation marks is much greater in confined genomic loci compared with bulk histones. For example, about one out of three molecules of histone H4 is biotinylated at K12 in telomeric chromatin.¹⁸

Cellular Distribution of HLCS and Regulation of Expression

Consistent with its role as carboxylase and histone biotinyl ligase, HLCS can be found in both extranuclear and nuclear

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Abbreviations: DNMT1, DNA methyltransferase 1; EHMT-1, euchromatic histone methyltransferase; H3K9me2, K9-dimethylated histone H3; H3K9me3, H4K12bio, K12-biotinylated histone H4; HLCS, holocarboxylase synthetase; K, lysine; LTR, long terminal repeat; MeCP2, methyl-CpG-binding domain protein 2

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compartments.^{8,19,20} Nuclear HLCS is associated with chromatin and the nuclear lamina.^{19,20} The binding of HLCS to chromatin is mediated by physical interactions with histones H3 and H4;⁵ the recruitment of HLCS to particular loci is probably mediated by interactions between HLCS and other chromatin proteins (see below). HLCS binding sites in chromatin have been mapped by using both DNA adenosyl methyltransferase technology and chromatin immunoprecipitation assays.²¹⁻²³

Our knowledge of HLCS regulation is limited to the following observations: (1) Both the abundance of HLCS mRNA and the nuclear translocation of HLCS depend on biotin.²² (2) The human HLCS promoter has been tentatively identified²⁴ but not yet characterized in great detail. (3) The expression of HLCS is repressed by miR-539.²⁵

Phenotypes of HLCS Deficiency

HLCS deficiency causes severe phenotypes, consistent with the key roles that HLCS plays in intermediary metabolism and gene regulation. No living HLCS null individual has ever been reported, suggesting embryonic lethality. HLCS knockdown studies (~30% residual activity) produced phenotypes such as decreased life span and heat resistance in *Drosophila melanogaster*,²⁰ and aberrant gene regulation in human cell lines.²² Low levels of biotinylated proteins have been linked to de-repression of retrotransposons and chromosomal abnormalities as described below in reference 21.

Numerous mutations in the human *HLCS* gene have been identified and characterized at both the enzymatic and clinical level; these mutations cause a substantial decrease in HLCS activity.^{26,27} Unless diagnosed and treated early, HLCS deficiency appears to be uniformly fatal.²⁸ Three independent cancer and patent databases correlate HLCS loss or mutation with human tumors.²⁹⁻³¹

Histone Biotinylation is a Repression Mark

All known species of biotinylated histones are gene repression marks.^{21,22,32,33}

Atomic force microscopy studies suggest that nucleosomal condensation increases in response to biotinylation of K12 and possibly other residues, in histone H4.³⁴ Biotinylation marks such as K-12-biotinylated histone H4 (H4K12bio) colocalize with repression marks such as methylated cytosines and K9-dimethylated histone H3 (H3K9me2).^{21,22,32,33}

Debiotinylation of Histones

The binding of biotin to histones is a reversible process, but the identity of the histone debiotinylase is uncertain. Circumstantial evidence has been provided that biotinidase has histone debiotinidase activity.^{35,36} This notion is consistent with the classical role of biotinidase as a biotin- ϵ -lysine hydrolase.³⁷ The hydrolysis of the biotin- ϵ -lysine bond by biotinidase is a reversible reaction and the equilibrium can be shifted toward the binding of biotin to histones by providing high concentrations of biotin- ϵ -lysine in vitro.^{6,38}

Epigenomic Synergies between Biotinylation and Methylation Marks

Depletion of histone biotinylation can be achieved by biotin depletion or HLCS knockdown and causes deregulation of genes.^{7,22,32,33} Consistent with this notion, depletion of histones biotinylation deregulates long-terminal repeats (LTRs). The production of viral particles, the frequency of retrotransposition events, and the number of chromosomal abnormalities increase when LTRs are de-repressed by biotin depletion or HLCS knockdown in cell cultures, humans and *Drosophila melanogaster*.²¹ Retrotransposition events may cause cancer,³⁹⁻⁴⁵ and de-repression of retroelements by biotin depletion and HLCS deficiency unambiguously links biotin status with cancer risk.

Nutrients may have synergistic effects in gene regulation by epigenomic mechanisms. Evidence suggests cross-talk between biotinylation and methylation marks in maintaining genome stability.^{21,22,32,33} For example, histone biotinylation is substantially impaired when cytosine methylation marks are erased

by treating cells with 5-aza-2'-deoxycytidine;²¹ in contrast, depletion of biotinylated histones does not affect cytosine methylation, suggesting that methylation is the primary loading factor. The local enrichment of another repression mark, H3K9me2, depends on prior enrichment of H4K12bio.^{21,22,32,33} Importantly, preliminary evidence suggests that HLCS physically interacts with the maintenance DNA methyltransferase DNMT1, the methyl-CpG-binding domain protein 2 (MeCP2) and the H3K9 methyltransferase EHMT-1 (Xue J, et al. unpublished; Li Y, et al. unpublished).

Causal links between histone biotinylation and the teratogenic effects of biotin deficiency remain to be demonstrated.^{46,47} Note that the human biotin requirement is unknown and that recommendations for biotin intake are based solely on the typical intake of biotin in the general, apparently healthy, population.⁴⁸ This approach is flawed in the case of biotin where dietary intake data are only crude estimates. Currently, no studies are available that quantified biotin in foods by using chemically specific assays,⁴⁹ and it is not clear whether intake estimates exceed or underestimate the true biotin intake. Also, the "normal state" is defined by using biotin-dependent carboxylases or urinary metabolites as markers, while ignoring the apparently subtle changes occurring at the chromatin level.

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