Repression of Transposable Elements by Histone Biotinylation^{1,2}

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Abstract

Transposable elements constitute >40% of the human genome; transposition of these elements increases genome instability and cancer risk. Epigenetic mechanisms are important for transcriptional repression of retrotransposons, thereby preventing transposition events. Binding of biotin to histones, mediated by holocarboxylase synthetase (HCS), is a novel histone mark that plays a role in gene regulation. Here, we review recent findings that biotinylation of lysine-12 in histone H4 (H4K12bio) is an epigenetic mechanism to repress long terminal repeat (LTR) retrotransposons in human and mouse cell lines, primary cells from human adults, and in *Drosophila melanogaster*. Further, evidence is summarized that supports a causal relationship between the repression of LTR in H4K12bio-depleted cells and increased production of viral particles, increased frequency of retrotransposition events, and increased frequency of chromosomal abnormalities in mammals and *Drosophila*. Although HCS interacts physically with histones H3 and H4, the mechanism responsible for targeting HCS to retrotransposons to mediate histone biotinylation is uncertain. We hypothesize that HCS binds specifically to genomic regions rich in methylated cytosines and catalyzes increased biotinylation of histone H4 at lysine-12. Further, we hypothesize that this biotinylation promotes the subsequent dimethylation of lysine-9 in histone H3, resulting in an overall synergistic effect of 3 diet-dependent covalent modifications of histones in the repression of LTR. J. Nutr. 139: 2389–2392, 2009.

Transposable elements

Type I transposable elements constitute ~42% of the human genome; they predominantly fall into 2 categories, the long terminal repeat (LTR)⁴-containing retrotransposons and the non-LTR long interspersed nucleotide elements (1–3). This study focuses on LTR. Mammalian genomes contain 2 types of LTR elements, intact retrotransposon LTR and solitary LTR. In intact retrotransposons, the viral genes *gag*, *pol*, and *env* are flanked by 2 repeat regions: 5'-LTR and 3'-LTR. The expression of retroviral genes is regulated by promoters in the 5'-LTR (4). Transcription of intact retrotransposons by a reverse transcriptase and subsequent translocation impair genomic stability and

are associated with various disease states such as cancer and autoimmunity (1,5). In solitary LTR, the retroviral genes have been deleted by recombination between the LTR (1,4,6,7). Although solitary LTR cannot produce viral proteins, they may have promoter activity and can cause abnormal patterns of host gene expression (6,7). Most retrotransposons are inactive, but 54 promoter-active retrotransposons have been identified in human testes (4). Repression of intact retrotransposons and solitary LTR is important to prevent abnormal gene activity and to decrease the incidence of retrotranspositions.

Epigenetic repression of retrotransposons

Histones are posttranslationally modified by various epigenetic marks (Fig. 1) (8–15). In addition, methylation of cytosine residues in DNA contributes to the arsenal of known chromatin modifications (16). K9-dimethylation of histone H3 (H3K9me2) and methylation of cytosine residues in DNA are known to repress transposable elements (2,17,18). Evidence suggests that chromatin modifications other than methylation marks are also critical for silencing of retroviral elements and for preserving chromosomal stability and human health (19). One of these modifications appears to be biotinylation of histones. The covalent binding of the vitamin biotin to histones is mediated by holocarboxylase synthetase (HCS) (20,21). The following biotinylation sites have been identified: K9, K13, K125, K127, and K129 in histone H2A (13); K4, K9, K18, and perhaps K23 in histone H3 (11); and K8 and K12 in histone H4 (10).

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⁴ Abbreviations used: H3K9me2, K9-dimethylated histone H3; H4K12bio, K12-biotinylated histone H4; HCS, holocarboxylase synthetase; LTR, long terminal repeats.

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FIGURE 1 Modification marks in histones H2A, H3, and H4 (8–15). Biotinylation sites in histones H1 and H2B have not yet been investigated, and thus, H1 and H2B are not depicted. Abbreviations: Ac, acetate; B, biotin; M, methyl; P, phosphate; U, ubiquitin. Marks labeled with “?” are based on preliminary observations and await confirmation (10,12).

Preliminary evidence suggests that K5 and K16 in histone H4 are also targets for biotinylation (10,12). Importantly, K12-biotinylation of histone H4 (H4K12bio) is a mark for repeat regions and heterochromatin, plays a role in gene repression, and colocalizes with the repression mark H3K9me2 (22).

Chromatin immunoprecipitation assays provided evidence that H4K12bio is greatly enriched at loci coding for both intact and solitary LTR in a human lymphoid cell line. Similar observations were made in human and mouse cell lines that originated in other tissues (23). Importantly, the enrichment of H4K12bio depends on the concentration of biotin in cell culture media at nutritionally relevant levels, consistent with a diet (biotin)-dependent epigenetic mark. Similar observations were made in a supplementation study in apparently healthy human adults, where supplementation with a typical over-the-counter biotin supplement increased the enrichment of H4K12bio at LTR in primary peripheral blood mononuclear cells (23).

These observations relate to a mechanism that likely has substantial biological importance as follows. First, decreased enrichment of H4K12bio at LTR in biotin-deficient or HCS knockdown cells is associated with increased LTR transcript abundance (23). Transcription of LTR is the first and critical step in retrotransposition events (2). Second, murine Mm5MT mammary carcinoma cells are known to produce particles of mouse mammary tumor virus (24). Decreased enrichment of H4K12bio in biotin-deficient Mm5MT cells is associated with increased secretion of viral particles, encoded by the viral genes *gag*, *pol*, and *env*, into culture media (23). Third, studies in *Drosophila melanogaster* are consistent with the hypothesis that decreased histone biotinylation increases the frequency of retrotransposition events. In *Drosophila*, ovoD1 females are sterile because of a point mutation that resulted in the dominant arrest of oogenesis (25). HCS knockdown, ovoD1 females revert spontaneously to fertility as a result of the insertion of the endogenous retrovirus gypsy or the retrotransposon copia (26). If histone biotinylation in ovoD1 females is decreased by knocking down HCS, the frequency of retrotransposition events is ~4 times that in HCS wild-type ovoD1 females (23). Fourth, some of the known histone biotinylation marks are not enriched

at LTR, suggesting specificity for H4K12bio in the repression of retrotransposons (23). Fifth, chromosomal abnormalities are detectable in biotin-deficient human lymphoid cells but not in biotin-normal and biotin-supplemented cells (23).

Repression of LTR (and other gene loci) depends on cross-talk between H4K12bio and methylation marks. If cytosine methylation is decreased by treating cells with 5'-azacytidine, the enrichment of H4K12bio at LTR is reduced by ~50% (23). In contrast, if the abundance of H4K12bio is decreased in biotin-deficient cells, cytosine methylation remains unaltered. However, if H4K12bio is decreased in biotin-deficient cells of HCS knockdown cells, the local enrichment of H3K9me2 at the same locus decreases substantially (22,23,27). The latter is consistent with preliminary observations that HCS physically interacts with a histone H3 K9-methyltransferase (28). Based on these observations we propose a model in which methylcytosine-binding proteins such as MeCP2 direct HCS (and other enzymes) to loci rich in methylated DNA, triggering local biotinylation of histone H4 (Fig. 2). Histone H3 K9-methyltransferases are also part of this multiprotein complex, leading to enrichment of H3K9me2. According to this model, 3 nutrient-dependent repression marks (cytosine methylation, H4K12bio, H3K9me2) synergize in the repression of LTR.

Holocarboxylase synthetase

Attachment of biotin to histones is mediated by holocarboxylase synthetase (HCS, EC 6.3.4.10) (20,21,27). The following 4 domains have been identified and characterized in human HCS: N-terminal domain, central domain, linker domain, and C-terminal domain (29). Both N- and C-termini of HCS participate in substrate recognition (29). The central domain in HCS contains binding sites for both ATP and biotin (30–32). Mutations and knockdown of HCS decrease the abundance of

FIGURE 2 Epigenetic synergies between biotin- and folate-dependent chromatin marks in the repression of LTR.

biotinylated histones (20,21,27), leading to abnormal patterns of gene expression (21,27) and phenotypes such as altered life span and stress resistance (21,33).

HCS does not interact directly with histone H2A; biotinylation of histone H2A is mediated by diffusion of the HCS-generated intermediate biotinyl-5'-AMP to its target site (34). In contrast, HCS appears to interact physically with histones H3 and H4 to mediate biotinylation (B. Baolong, V. Pestinger, Y. I. Hassan, and J. Zemleni, unpublished data). These observations suggest that interactions between HCS and the H3/H3/H4/H4 tetramer (but not H2A/H2B dimers) in nucleosomes might contribute to the binding of HCS to chromatin. This theory is not mutually exclusive with the methyl cytosine-directed targeting of HCS to distinct chromosomal loci (Fig. 2).

Conclusions

This article summarizes recent evidence concerning the repression of transposable elements by histone biotinylation and offers a novel mechanistic hypothesis concerning the diet-dependent epigenetic repression of transposable elements. Biotin-dependent repression is reinforced by newly discovered epigenetic synergies between H4K12bio and folate-dependent marks such as H3K9me2 and methylation of cytosine residues. The phenomena described here are potentially important for human health and provide a mechanistic link between biotin deficiency and chromosomal abnormalities in humans.

Other articles in the supplement include references (35–38).

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