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RESEARCH HIGHLIGHT

Histone lysine de-β-hydroxybutyrylation by SIRT3

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Recent studies have demonstrated the addition and removal of a smorgasbord of site-specific acylation modifications on lysine residues of histone tails. The study by Zhang et al. now shows how the SIRT3 histone deacylase exhibits class specificity, acting on only a subset of β-hydroxybutyrylated lysines.

Modification of histones by acetylation was discovered more than half a century ago. Studies in the intervening decades have uncovered the histone acetyltransferases (HATs) responsible for this modification and the protein complexes in which these enzymes are embedded (reviewed in[1]). Acetylation primarily occurs on lysine residues in the unstructured amino-terminal tails of all four histone proteins. Modifications in the tails of H3 and H4, in particular, have been shown to play two fundamental roles. First, acetylation neutralizes or masks the positive charge on lysine residues, thereby reducing the strength of the interaction between the acidic patch in the histone tails of H2A/B and the negatively charged DNA in chromatin. These changes are believed to lead to decondensed chromatin that is transcriptionally more permissive. Second, acetylated lysine residues in histone tails also act as signals and interact with bromodomains in gene regulatory proteins. Both of these roles are primarily pro-transcriptional functions, with the HATs commonly referred to as writers and interacting proteins as readers.

In the past decade, studies have shown that acetylation is only one of the possible acylations that occurs on histone proteins and, like acetylation, is tied to transcriptional activity (reviewed in[2,3]). In fact, ten different histone acylations have now been identified, including acetylation, crotonylation, butyrylation, hydroxybutyrylation (hib), 2 hydroxyisobutyrylation (bhb), succinylation, glutarylation, malonylation, benzyoylation, and propionylation (reviewed in[4]).

In contrast to the pro-transcriptional role of histone acyltransferase "writers" and bromodomain-containing "readers", the enzymes (erasers) that remove these acyl marks generally contribute to gene silencing. The human genome encodes 18 different histone deacylases (HDACs) that fall into 4 categories. Class I includes Rpd3-like HDACs 1, 2, 3 and 8, while Class II include HDACs 4, 5, 6, 7, 9, 10. Class III is composed of the sirtuin proteins SIRT1, 2, 3, 4, 5, 6, 7. Lastly, Class IV includes HDAC11. These enzymes also fall into two distinct functional types: Class I and II are zinc dependent with conserved residues in the active site; by contrast, deacylation by the Class III sirtuin enzymes is NAD^+ dependent (reviewed in[5]). Within these subgroups, however, our understanding of the substrate specificity of each enzyme is far from comprehensive. Substrate specificity includes two different components. The first is the action of each enzyme on specific acyl modifications (so-called site specificity) while the second is the enzyme's action on particular lysine residues within the histone tails (class specificity). Importantly, Zhang et al.[6] have now provided new insights into the poorly understood class specificity of these deacylases, revealing an additional complexity to gene regulation by histone deacylation.

Prior to the study by Zhang et al., our understanding of substrate specificity in removal of acyl modifications resided solely in the modification itself (reviewed in[2,4,7]). In general, the Class III sirtuins SIRTs 1, 2 and 3 are active in removing hydrophobic modifications, which include propionylation, butyrylation and crotonylation. SIRT2 is also active on benzoylated residues. Of the remaining Class III sirtuins, SIRT5 acts on acidic modifications that include malonylation and glutyrylation while both SIRT5 and SIRT7 remove succinyl modifications. Classes I and II HDACs are efficient deacetylases. Class I HDACs have limited activity on histone KCr and KGlu. However, efficient decrotonylation requires SIRT3 or SIRT5 (see below). Class I HDACs are also active on 2 hydroxyisobutyrylated lysines. Some sirtuins, such as SIRT5, actually prefer other acylations over acetyllysine. Thermodynamic binding studies with *Thermotoga maritima* SIR2 (SIR2Tm) confirmed that interactions with the acyl modification are important for binding; unacylated versions of substrate peptides bind to SIR2Tm with at least 1,000-fold lower affinity. SIR2Tm can accommodate both acetyl and propionyl lysines in its active site, but depropionylation activity is slightly less efficient than deacetylation activity. SIRT5 deacetylase activity is relatively weak compared to that of SIRT1 and SIRT2 but has strong activity in removing succinyl and malonyl modified lysines. Lastly, Goudarzi et al.[8] have shown that competition between these modified residues can act as a regulatory mechanism.

SIRT3, the focus of the work by Zhang et al., has previously been shown to prefer crotonylated residues over acetylated residues. HDAC3, SIRT1 and SIRT2 are able to decrotonylate histone peptides in vitro. However, SIRT3 appears to be the major decrotonylase in vivo and loss of SIRT3 results in higher levels of KCr than loss of either SIRT1 or SIRT2[4,9] (reviewed in[4]). Zhang et al. now show strong activity for SIRT3 on β-hydroxylbutyryl modifications (Kbhb). However, in contrast to its broad classspecificity for the KCr modification, SIRT3 activity on Kbhb depends on the site of modification. Notably, they show that SIRT3 can remove bhb from H3K4, K9, K18, K23, K27 and H4K16 but has no activity on β–hydroxybutyrylated H4K5, K8 or K12 (Fig. 1). This class specificity is due to an entropically unfavorable barrier: H4K5, K8 and K12 lie in a non-beta sequence motif in which glycine residues directly flank these bhb-modified lysines. These glycines have a high degree of rotational freedom that is detrimental to particular interactions of SIRT3 with the βbackbone of the modified residue. By comparison, substrate recognition by the

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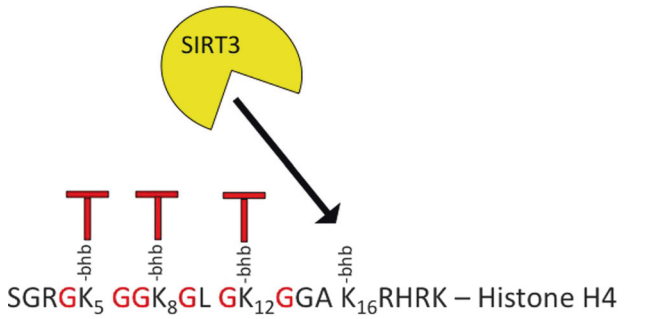

Fig. 1 SIRT3 is only able to bind and remove bhb from lysine 16 of histone H4. Removal of bhb from lysines 4, 8 and 12 is blocked by flanking glycine residues. The rotational freedom of these glycines is detrimental to interaction of SIRT3 with the backbone of these modified lysines

Zinc-dependent Class I HDACs is dependent on a βbackbone but not on its particular conformation. Thus, while HDAC3 also possesses histone de-β-hydroxybutyrylation activity, it can remove Kbhb regardless of adjacent glycines.

Zhang et al. also demonstrate that SIRT3 specificity for Kbhb is sensitive to the R/S enantiomer form of bhb. S-form bhb is stabilized by hydrogen bonding and hydrophobic contacts, but the hydrogen bonding interactions of R-form Kbhb are imperfect and SIRT3 binding is therefore weaker. R-form β-hydroxybutyrate is a component of ketone bodies, which increase in the blood during fasting, starvation, or prolonged intense exercise. Fed conditions favor formation of S-form β-hydroxybutyrate through short-chain fatty acid metabolism. As previous studies have also shown, the levels and types of histone acylation are sensitive to the cellular concentrations of the associated metabolites (reviewed in[10]). Thus, these features provide mechanisms that link histone Kbhb and gene regulation to metabolic state.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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