Published in final edited form as:

Trends Biochem Sci. 2017 July ; 42(7): 501–503. doi:10.1016/j.tibs.2017.05.006.

Cycling Around Lysine Modifications

Surinder Kumar¹ and David B. Lombard^{1,2,*}

¹Department of Pathology, University of Michigan, Ann Arbor, MI 48109, USA

²Institute of Gerontology, University of Michigan, Ann Arbor, MI 48109, USA

Abstract

Recent studies have revealed the existence of a plethora of previously unknown protein acyl-lysine modifications, affecting the functions of targets involved in diverse cellular processes. A recent study from the Hirschey laboratory has provided new chemical insights into the mechanisms of protein acylation.

Keywords

non-enzymatic acylation; HMGylation; MGylation; MGcylation

Acetylation of proteins on lysine residues was first identified in the 1960s in the context of nuclear histones, and has subsequently been described on many non-histone proteins, including mitochondrial proteins. In recent years, high-resolution proteomics studies have revealed the existence of a large number of novel protein lysine acyl modifications: crotonylation, succinylation, glutarylation, malonylation, beta-hydroxybutyrylation, long chain fatty acid acylation, and many others [1]. These modifications affect target proteins involved in many biological processes, notably chromatin functions and metabolic pathways such as glycolysis and mitochondrial metabolism. Indeed, many of these modifications are particularly prominent on mitochondrial proteins. How these modifications are conjugated to target proteins in the first place has remained an important outstanding question; the putative acyltransferases responsible for catalyzing most of these modifications are largely unknown.

Evidence has accumulated strongly supporting the notion that many of these lysine acyl modifications occur via a spontaneous, non-enzymatic mechanism involving reaction of acyl-CoA species with lysine residues [2]. The presence of an intrinsic thioester functional group renders acyl-CoAs highly reactive biomolecules. Moreover, the mitochondrial matrix provides a particularly conducive chemical environment for such non-enzymatic modifications. The notion of non-enzymatic acylation is further bolstered by data suggesting that much protein acetylation occurs at very low stoichiometry (<1%) in wild-type cells under basal conditions [3, 4]. In this regard, a recent study described the development of a

*correspondence: davidlom@umich.edu (D.B. Lombard).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

novel chemoproteomic method to identify targets of intrinsic non-enzymatic acylation, focusing on a role for malonyl-CoA mediated protein modification in inhibiting cytosolic glycolytic enzyme activity [5]. In mitochondria, new work implicates the malonyl-CoA synthetase enzyme ACSF3 in generating malonyl-CoA to drive lysine malonylation in this compartment [6].

Now, an elegant new study from the Hirschev laboratory has contributed important insights into chemical mechanisms of non-enzymatic protein acylation. Wagner *et al.* uncover a mechanism for lysine acylation by negatively charged dicarboxyl CoA thioesters with four- and five-carbon acyl backbones, such as succinyl-CoA and glutaryl-CoA [7]. They show that these acyl-CoAs are much more chemically reactive than uncharged acyl-CoAs, such as acetyl-CoA and butyryl-CoA, or than malonyl-CoA, a negatively charged, three-carbon acyl-CoA. Wagner *et al.* show these species undergo an intramolecular reaction to form high-energy cyclic anhydride intermediates, which in turn robustly react with free lysine ϵ -amino groups (Fig. 1). Consistently, succinyl- and glutaryl-CoAs, which undergo self-hydrolysis at a rate much higher than malonyl-CoA, acylate protein lysine residues much more efficiently [7].

Based on this mechanism, Wagner and colleagues predict and validate a novel protein lysine modification, 3-hydroxy-3-methylglutaryl (HMG)-lysine. HMG-CoA, an important intermediate metabolite in leucine catabolism, ketogenesis, and cholesterol biosynthesis, possesses a structure similar to glutaryl-CoA. Like glutaryl- and succinyl-CoAs, HMG-CoA can undergo intramolecular hydrolysis to form a high-energy cyclic anhydride intermediate, inducing protein lysine HMGylation over a wide pH range. Additionally, the authors provide evidence for the existence of two related protein modifications; 3-methylglutaryl-lysine (MGylation) and 3-methylglutaconyl-lysine (MGylation), derived from 3-methylglutaryl-CoA and 3-methylglutaconyl-CoA, respectively [7]. In a complementary study, Anderson, Huynh *et al.*, show that SIRT4 catalyzes removal of these newly described modifications from lysine residues of target proteins [8].

Wagner and colleagues use quantitative proteomics to identify proteins subject to HMGylation and glutarylation in wild-type liver mitochondria as well as mitochondria from mutant mice with elevated levels of HMG-CoA or glutaryl-CoA (HMGCL and GCDH KO mice, respectively). Since HMG-CoA is an intermediate metabolite in leucine metabolism and ketogenesis, whereas glutaryl-CoA is generated in lysine metabolism, the authors focused on a comparison of HMGylation and glutarylation levels on proteins in these pathways. Interestingly, in absence of HMGCL, HMG-CoA heavily HMGylates proteins involved in ketogenesis, whereas in GCDH KO mice, proteins in lysine metabolism are heavily glutarylated [7]. These findings indicate that proteins from specific metabolic pathways are more susceptible to non-enzymatic acylation by acyl-CoAs generated in that pathway, presumably due to high local levels of the cognate CoA species. Interestingly, proteins in leucine metabolism and the TCA cycle display increases in both glutaryl and HMG modifications, suggesting there are proteins in specific pathways that can be modified promiscuously [7]. Although previous studies had demonstrated that inborn errors of metabolism can provoke altered cellular protein acylation [9, 10], the studies of Wagner *et al.* provide new insights into the target specificity of these modifications.

This new work bolsters the emerging case that non-enzymatic lysine modifications can occur extremely efficiently for certain chemical classes of modifications, and provides important new mechanistic insights into this process. Moreover, these results may have important clinical implications. In particular, the statins, the major class of cholesterol-lowering drugs taken by millions of individuals worldwide to mitigate cardiovascular disease risk, inhibit conversion of HMG-CoA to mevalonate. These new results would suggest that increased HMG-CoA levels occurring in the setting of statin therapy might promote increased protein HMGylation, perhaps contributing to the therapeutic effects of these agents, and/or to their well-known off-target effects, such as muscle cramps.

Acknowledgments

We thank Dr. Lauren Rodenbarger for useful suggestions. Support: Melanoma Research Alliance, NIH (R01GM101171, 2R01HL114858, and R21AG053561), DoD (OC140123), the Glenn Foundation for Medical Research, Love Your Melon/St. Baldrick's Foundation, the Harrington Discovery Institute, and the University of Michigan Cancer Research Committee.

References

1. Sabari BR, et al. Metabolic regulation of gene expression through histone acylations. *Nat Rev Mol Cell Biol.* 2017; 18:90–101. [PubMed: 27924077]
2. Wagner GR, Hirschey MD. Nonenzymatic protein acylation as a carbon stress regulated by sirtuin deacylases. *Mol Cell.* 2014; 54:5–16. [PubMed: 24725594]
3. Lombard DB, et al. Acetyl-ed question in mitochondrial biology? *EMBO J.* 2015; 34:2597–2600. [PubMed: 26369717]
4. Weinert BT, et al. Analysis of acetylation stoichiometry suggests that SIRT3 repairs nonenzymatic acetylation lesions. *EMBO J.* 2015; 34:2620–2632. [PubMed: 26358839]
5. Kulkarni RA, et al. Discovering Targets of Non-enzymatic Acylation by Thioester Reactivity Profiling. *Cell Chem Biol.* 2017; 24:231–242. [PubMed: 28163016]
6. Bowman CE, et al. The Mammalian Malonyl-CoA Synthetase ACSF3 Is Required for Mitochondrial Protein Malonylation and Metabolic Efficiency. *Cell Chem Biol.* 2017
7. Wagner GR, et al. A Class of Reactive Acyl-CoA Species Reveals the Non-enzymatic Origins of Protein Acylation. *Cell Metab.* 2017; 25:823–837. e828. [PubMed: 28380375]
8. Anderson KA, et al. SIRT4 Is a Lysine Deacylase that Controls Leucine Metabolism and Insulin Secretion. *Cell Metab.* 2017; 25:838–855. e815. [PubMed: 28380376]
9. Pougovkina O, et al. Aberrant protein acylation is a common observation in inborn errors of acyl-CoA metabolism. *J Inher Metab Dis.* 2014; 37:709–714. [PubMed: 24531926]
10. Tan M, et al. Lysine glutarylation is a protein posttranslational modification regulated by SIRT5. *Cell Metab.* 2014; 19:605–617. [PubMed: 24703693]

Figure 1. A mechanism for non-enzymatic protein lysine acylation

The terminal carboxylic acid in four- and five-carbon backbone acyl-CoAs, such as succinyl-CoA, glutaryl-CoA, HMG-CoA, and 3-methylglutaryl-CoA, undergo intramolecular general base catalysis to form highly reactive cyclic anhydride intermediates, which react with free lysine ϵ -amino groups to generate protein lysine-acyl modifications.