Protein Modifications

DOI: 10.1002/anie.201200316

Expansion of the Lysine Acylation Landscape**

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In memory of Jerzy W. Jaroszewski

acetylation · crotonylation · epigenetics · protein modifications · succinylation

The eukaryotic genome is densely packed into chromatin fibers consisting of nucleosomes, which are made up of histone octamer complexes around which the DNA is wrapped (Figure 1).[1] In order for the transcription machinery to gain access to the DNA a variety of enzymes serve to modify the side chains of the histone tails, which in turn regulate how tightly packed the nucleosome particles appear.[2] These posttranslational modifications (PTMs) give rise to a so-called "histone code" that is believed to control the recruitment of transcription and/or repression factors, thereby regulating the degree of transcription of the genome. This "code" is highly dynamic, and the modified histones should perhaps be considered as nodes in a dynamic network. The combinations of PTMs are constantly altered by enzymes such as histone acetyltransferases (HATs), lysine deacetylases (HDACs and sirtuins), serine/threonine kinases, and lysine/arginine methylases and demethylases, as well as ubiquitin ligases.[3] Although, these processes are not yet well understood, the effects of lysine acetylation (structure **1**) on chromatin structure and function have been studied extensively, and two drugs targeting HDAC enzymes have been marketed for the treatment of cutaneous T-cell lymphoma.[4] Lysine acetylation in proteins, however, was recently shown to be a general posttranslational modification with cellular regulatory functions beyond epigenetic processes related to histone acetylation,[5] and more specifically, protein acetylation has been shown to have implications in metabolism.[6]

Now, a series of additional acyl groups—crotonyl, malonyl, and succinyl groups (see structures **2**–**4** in Figure 1)—have been identified as posttranslational modifications of lysines in histones as well as in other proteins.[7] Here these new developments are summarized, and selected state-of-the-art methods suitable for further investigation of these novel PTMs are discussed in brief.

In a recently reported mass spectrometry (MS) based proteomics study, the number of known histone modification marks was increased by approximately 70%. On 28 of the modified lysine residues from core histone peptides, the identity of the modification was shown to be the not previously reported *ε*-*N*-crotonyllysine (Kcr) group.[7a] Compelling evidence for this finding was provided by several methods. First, the authors compared in vivo derived peptides with chemically synthesized reference peptides by high-resolution MS/MS and co-elution experiments using high-performance liquid chromatography (HPLC). Furthermore, a specific anti-Kcr antibody was generated and applied in corroborating Western blot and immunostaining experiments, and finally isotopic labeling with [D_4]crotonyl confirmed the existence of 19 of the 28 Kcr marks in HeLa cells in culture. Interestingly, some of the modified histone lysine residues appeared to be prone to crotonylation but not acetylation, and lysine acetylation (Kac) and Kcr marked different sets of genes.[7a] The study now leads to several interesting questions, including: Which proteins will emerge as the "writers, erasers, and/or readers" of this novel histone PTM, and what are the effects of Kcr on chromatin structure and function?

Lysine succinylation was initially identified and verified as another novel PTM in *E. coli*,[7b] and both malonylation and succinylation of lysine residues were very recently demonstrated in mammalian cells.[7c] The latter study was inspired by an X-ray crystal structure of sirtuin 5, which is a nicotinamide adenine dinucleotide (NAD)-dependent hydrolase of the silent information regulator 2 (Sir2) protein family. In the co-crystal with an *ε*-*N*-thioacetyllysine-containing peptide, the authors noticed a buffer molecule containing a sulfonate group bound in the active site. Based on this finding, together with the observation that sirtuin 5 is a very weak deacetylase, a series of alternative substrates were designed including versions containing malonylated and succinylated lysines. The catalytic efficiency of the enzyme proved to be up to 1000 times higher against certain succinylated substrates than against acetylated counterparts, and a second co-crystal structure of sirtuin 5 with an *ε*-*N*-succinyllysine-containing substrate confirmed the predicted enzyme–substrate interactions.[7c] By capitalizing on these binding affinities, FLAG-tagged sirtuin 5 was used to affinity purify several malonyl- and succinyl-containing proteins (the FLAG tag has the following peptide sequence, N→C: DYKDDDDK). Furthermore, the degree of lysine succinylation observed for sirtuin 5 knock-out mice was significantly less than for wild-type mice.[7c] The identified proteins were all metabolic enzymes, indicating that these newly discovered lysine acylation marks likely play important roles in the regulation of metabolism alongside the *N*-acetyl functionalization.[6,7c] Future investi-

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[**] Financial support from the Lundbeck Foundation (Young Group Leader Fellowship) and the Danish Independent Research Council – Natural Sciences (Steno Grant No. 10-080907) is gratefully acknowledged.

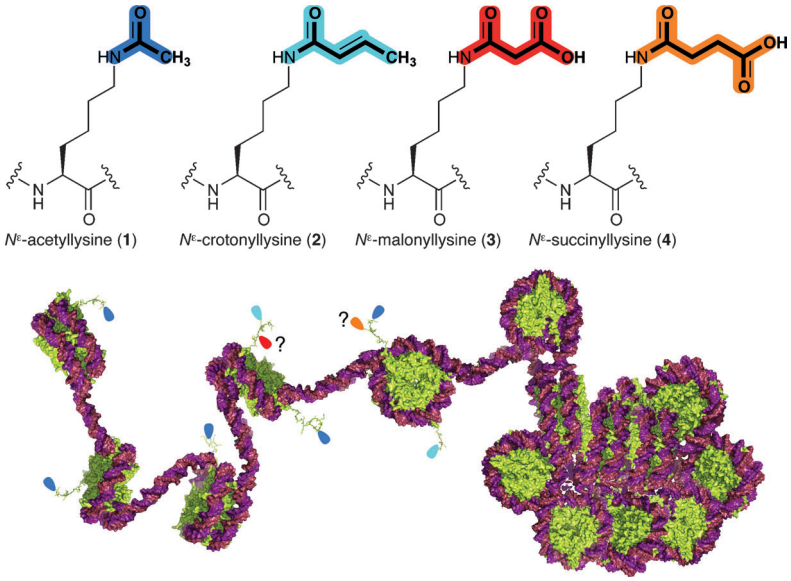

Figure 1. Top: Chemical structures of the discussed ε-*N*-acyllysine residues. Bottom: Nucleosome particles (pdb 1ZBB)[1b] with color-coded marks symbolizing posttranslational acylation of lysines. Acetyl and crotonyl marks have been identified in histone tails, while malonyl and succinyl marks have been identified on non-histone proteins thus far.

gations of the possible biochemical consequences of these lysine acylation marks, however, will be highly important.

Sirtuin 5 was the only human sirtuin shown to harbor demalonylase and desuccinylase activity in this new study, and since the enzyme localizes in mitochondria, it is not surprising that the peptides shown to contain malonyl and succinyl marks were all from mitochondrial proteins. It remains to be seen whether the purpose of lysine malonylation and succinylation is to augment lysine acetylation in metabolic regulation, or if these new marks play a role in the epigenetic regulation of chromatin structure and function as well. Lysine crotonylation, on the other hand, was found on histone lysine residues, and appears to supplement lysine acetylation in its role as epigenetic regulator. Whether this PTM has an influence on chromatin remodeling and/or transcription needs further investigation.

The important studies highlighted here raise a plethora of interesting questions. To address some of these issues, the ribosomal[8] and chemical synthesis[9] of peptides containing well-defined combinations of the various PTM marks should yield valuable information. Furthermore, advances in chemical protein synthesis have proven instrumental in investigations of chromatin compaction.[10] Also, in addition to MS-based proteomics methods, which have proved highly rewarding in the investigation of these systems,[11] the use of nuclear magnetic resonance (NMR) techniques may be beneficial in providing insights regarding these processes in situ.[12]

Received: January 12, 2012
Published online: February 28, 2012

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