Clinical Chemistry 66:6
852–853 (2020)

Citation Classic

Sequencing of the First Draft of the Human Acetylome

Chunaram Choudhary[a,*] and Matthias Mann[a,b,*]

Featured Article: Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV, Mann M. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 2009;325:834-40.[1]

Biologists have long known that posttranslational modifications (PTMs), such as phosphorylation, play important regulatory roles in eukaryotic signaling. However, the pervasiveness of such regulation was not fully appreciated until the beginning of this millennium, when sequencing of the human genome revealed far fewer protein-coding genes than anticipated. This finding led to the appreciation that PTMs may provide an important layer in diversifying the regulatory landscape of proteomes. A challenge was then to map the landscape of PTM-based regulation. Concurrent with the emergence of the genomic era, mass spectrometry (MS) emerged as a powerful tool for identifying proteins and for focused analysis of PTMs. Initially, however, the sensitivity and the resolution of MS instruments were not sufficiently high for large-scale PTM mapping. The situation changed markedly with the commercial introduction of Orbitrap-based mass spectrometers in 2005 and sample-preparation and bioinformatics advances. By combining these tools with efficient phosphorylated peptide enrichment strategies and SILAC (stable isotope labeling by amino acids in cell culture)-based quantification, our group provided the first time-resolved map of phosphorylation in human cells *(1)*.

Successful decoding of the human phosphoproteome encouraged us to explore lesser known PTMs, in particular, lysine acetylation. Although acetylation was discovered in the early 1960s, just a few years after the discovery of phosphorylation, the fate of these PTMs diverged greatly in subsequent years. Phosphorylation was identified on hundreds of proteins and established as a major regulatory PTM in cell signaling, whereas for decades, acetylation remained confined to just a few proteins, mostly histones and tubulin. The picture started to change in the mid- to late 1990s following a wave of discoveries, including the identification of the first acetyltransferases and deacetylases and the bromodomain as an acetyllysine reader. Furthermore, acetylation was described for transcription factors, most prominently the tumor suppressor p53. This led to speculation that the regulatory scope of acetylation could rival that of phosphorylation *(2)*. However, without a proper toolbox for identification and quantification, it was difficult to confirm this prediction and to identify which proteins and which of their lysine residues were modified by acetylation. Armed with the latest MS technology, and with antiacetyllysine antibodies sufficiently specific for enrichment, we started to explore the "acetylome" in depth. We were elated to find that even with a few trial MS runs, we were able to identify more acetylation sites than reported in all previous studies in the literature combined. Nevertheless, our depth was still limited to about 1000 acetylation sites. To increase the depth of acetylated peptide sequencing, we used the so-called OFFGEL-based peptide fractionation approach to separate peptides following the acetylated peptide–enrichment step. This enabled us to isolate and sequence low abundant peptides, greatly enhancing the depth of measured acetylome. Finally, to enable site-specific relative quantification of acetylation, we combined these approaches with the SILAC method. As reported in our 2009 article discussed here, This powerful combination identified approximately 3600 acetylation sites on 1750 proteins, dramatically expanding the known universe of lysine acetylation. For the first time, these data allowed systematic assessment of the global properties of this PTM. Contrary to common beliefs, it turned out that acetylation was not just confined to the nucleus but rather occurred across diverse subcellular compartments, even in mitochondria, where protein phosphorylation is underrepresented. Notably, we detected acetylation on multiple components of large protein complexes, suggesting their coregulation. Furthermore, our quantitative analyses of lysine deacetylase inhibitor–regulated

[a] Proteomics Program, the Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[b] Proteomics and Signal Transduction, Max Planck Institute for Biochemistry, Martinsried, Germany.
*Address correspondence to: C.C. at Proteomics Program, the Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3B, DK2200, Copenhagen, Denmark; E-mail chuna.choudhary@cpr.ku.dk. M.M. at Proteomics and Signal Transduction, Max Planck Institute for Biochemistry, 82152 Martinsried, Germany; E-mail mmann@biochem.mpg.de.
[1] This article has been cited more than 2400 times since publication.
Received March 25, 2020; accepted March 27, 2020.
DOI: 10.1093/clinchem/hvaa087

acetylation revealed that inhibition of classical zinc-dependent deacetylases affected a much smaller subset of the acetylome than previously thought. Together, these results demonstrated that our approach enabled unbiased, systems-wide quantification of perturbation-induced acetylation changes.

In addition to providing a high-confidence set of acetylation sites and revealing global properties of acetylation, our work raised many new questions about the occurrence of acetylation in evolutionarily diverse organisms, the mechanism causing acetylation (enzymatic vs nonenzymatic), and the dynamics and stoichiometry of this PTM. In the ensuing years, we combined quantitative MS with chemical and genetic tools to reveal the stoichiometry and dynamics of acetylation. Perhaps surprisingly, these analyses clearly demonstrated that most acetylation sites have very low stoichiometry (<1%) and that a vast majority of acetylation events in bacteria, and a large fraction of mitochondrial acetylation, occur through nonenzymatic reactions *(3–5)*. Although much remains to be learned about the biological functions, kinetics, and enzymatic regulation of acetylation, the pioneering work discussed here firmly put acetylation on the global map of reversible modifications. We anticipate that in the next decade, these discoveries will be translated into further functional understanding of how acetylation and related regulatory enzymes control diverse biological processes and how dysregulation affects disease states.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: The Novo Nordisk Foundation Center for Protein Research is financially supported by the Novo Nordisk Foundation (NNF14CC0001).
Expert Testimony: None declared.
Patents: None declared.

Acknowledgments: We thank our collaborators for contributing to the discussed work, and the members of our laboratories at the Max Planck Institute of Biochemistry and the Center for Protein Research in Copenhagen for their helpful discussions.

References

1. Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell 2006;127:635–48.
2. Kouzarides T. Acetylation: a regulatory modification to rival phosphorylation? EMBO J 2000;19:1176–9.
3. Weinert BT, Iesmantavicius V, Moustafa T, Schölz C, Wagner SA, Magnes C, et al. Acetylation dynamics and stoichiometry in *Saccharomyces cerevisiae*. Mol Syst Biol 2014;10:716.
4. Weinert BT, Iesmantavicius V, Wagner SA, Schölz C, Gummesson B, Beli P, et al. Acetyl-phosphate is a critical determinant of lysine acetylation in *E. coli*. Mol Cell 2013;51:265–72.
5. Weinert BT, Moustafa T, Iesmantavicius V, Zechner R, Choudhary C. Analysis of acetylation stoichiometry suggests that SIRT3 repairs nonenzymatic acetylation lesions. EMBO J 2015;34:2620–32.