Editorial

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Reshaping the Chromatin and Epigenetic Landscapes with Quantitative Mass Spectrometry*

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The solving of the structure of DNA by Watson and Crick is arguably one of the major breakthroughs in all of science during the 20th century (1). Subsequent feverish research led to the discovery of a DNA genetic code, and that this code was utilized by living cells to encode proteins, the workhorse molecules of most cellular functions (2). The fundamental idea of genetic information transfer encoded by a DNA code has revolutionized the biological sciences and has led to the creation of new related fields and industries such as genomics, proteomics and biotechnology. However, not all gene expression patterns and subsequent phenotype changes can be explained by changes in this genetic code (DNA sequence). Epigenetics refers to stable heritable changes in gene expression that are not due to changes in DNA sequence, caused by DNA methylation, RNA interference and histone post-translational modifications (PTMs)[1] (3). These epigenetic changes are responsible for modulating transcriptional states that in part generate different cell types originating from identical genomes. Additionally, even though all genes exist in every cell, only a small number of genes are expressed in any given cell type and these expression patterns can be "memorized." Inheritance of transcription patterns through DNA replication and chromatin remodeling that accompanies each cell division is critical for cell proliferation, but the mechanisms for maintenance of epigenetic memory remain unclear (4). Additionally, many epigenetic processes are involved in several aspects of human physiology such as cellular differentiation and DNA damage repair. Most importantly, growing evidence has linked alterations to epigenetic mechanisms with various human diseases such as autoimmune, neurological disorders and various cancers (5–7). Evidence for epigenetic origins of many human diseases is mounting, and has led to organization of a possible large-scale Human Epigenome Project similar to the Human Genome Project (8).

Developments in technology have played central roles in advancing our understanding in epigenetic studies. Next generation sequencing technology has for example, allowed for the mapping of chromatin proteins and their PTMs to specific genomic loci. Mass spectrometry (MS) has also had a starring role in chromatin biology and epigenetic research over the years (9). There have been many reports detailing MS detection of DNA and histone modifications, starting as early as when these modifications were discovered. However, the advent of electrospray ionization allowed for much more elegant studies to be performed. One of the earlier applications of MS to histone PTM analysis was by Edmonds *et al.*, who used quadrupole and ion trap instruments to perform tandem mass spectrometry experiments to localize histone modification sites with both Top Down and Bottom Up MS approaches (10). Hunt and co-workers demonstrated the ability of MS to identify novel histone modifications, in this case arginine methylation, demonstrating the first site-specific *in vivo* occurrence of this type of PTM (11). The first comprehensive analyses of histone PTMs using a more modern multifaceted approach was accomplished by Al Burlingame's research group (12, 13). They identified nearly all of the major modification sites on histones H3 and H4, sites that now have been extensively functionally annotated by the chromatin community. MS has also contributed much to what is now known as the "Histone Code" hypothesis, (14) where histone PTMs, either alone or in combination, are responsible for specific functional outputs (15). In fact, MS has led to the identification of >500 different modification sites on histones. These sites are composed of a large variety of modification types, thus establishing MS as an indispensable tool for chromatin and epigenetics research (16).

Here in this special MCP issue focused on Chromatin Biology and Epigenetics, we gather MS based manuscripts that continue to drive and reshape the chromatin and epigenetic landscape. van Nuland *et al.* provides a detailed review on arguably the most versatile and abundant histone mark, histone H4 Lys^{20} methylation. Several manuscripts detail new advances in instrumentation or methodology geared toward enhanced measurements of chromatin targets. Kelleher and

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[1] The abbreviations used are: PTM, post-translational modification.

co-workers describe newer mass spectrometry instrumentation for Top Down MS analysis of histone H3, the mostly highly PTM modified histone. Similarly, Anderson *et al.* demonstrates the usefulness of front end electron transfer dissociation for Top Down analysis of all histones on a chromatographic time-scale, detecting several unique proteoforms. Noberini *et al.* describes new procedures for extraction of histones from formalin-fixed paraffin-embedded samples, a significant advance to the pathology field. Han *et al.* demonstrates a chemical proteomics technique for mapping phosphorylated proteins to their target genes on chromatin. Vermuelen and co-workers introduce a streamlined cross-linking MS method for obtaining structural information from minute amounts of immunoprecipitated chromatin complexes such as the cohesion complex. Sengupta *et al.* investigates the role of histone H3 Lys^{27} methylation in a cell line model of melanoma, finding that this mark and the EZH2 methyltransferase repress transcription of important tumor suppressor genes. Imhof and coworkers employ sequential window acquisition of all theoretical mass spectra (SWATH) analyses to quantitatively define the composition and kinetics of *in vitro* assembled chromatin utilizing extracts *Drosophila melanogaster* embryos.

Work on histone variants is presented by Chen *et al.* investigating the modification of histone H1 variants in breast cancer cell models. Additionally, Bailey *et al.* focuses their investigations on characterizing nucleosomes that contain the histone H3 variant CENP-A, finding centromere specific histone modification patterns. Interrogation of other chromatin related proteins and their effect on transcription is also the emphasis of some contributions to this special issue. Nguyen and coworkers describe most comprehensive examination of lysine mono-methylation reported to date, and then specifically identify and validate several novel substrates of the lysine methyltransferase SMYD2. Giguère *et al.* nicely show that the protein deleted in breast cancer 1 (DBC1) interacts with several chromatin-associated proteins to regulate gene expression, unexpectedly during circadian rhythm processes. Faca and coworkers provide a large-scale proteome analysis following overexpression of the transcription factor SNAIL. They find that SNAIL regulates expression of several epigenetic factors including HDAC1, which modulate the epithelial to mesenchymal transition in breast cancer. Bode *et al.* studies the role of two compositionally different Nucleosome Remodeling and Deacetylase (NuRD) complexes in mouse embryonic stem cells, suggesting the importance of these complexes in regulation of gene transcription in the pluripotent stem cell state. MS is also used to characterize modifications on nucleic acids as well. Rose *et al.* utilizes a combination of MS techniques including ion mobility MS to catalogue RNA modifications from *S. cerevisiae* that are differentially expressed during oxidative stress. Wang and coworkers similarly demonstrate that oxidative stress induces alterations to DNA modifications in a rat model of human Wilson's disease. Overall, we are pleased to bring you this collection of manuscripts which continue the longstanding and impactful MS research contributions to the chromatin biology and epigenetics fields.

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REFERENCES

1. Watson, J. D., and Crick, F. H. (1953) Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature* **171,** 737–738
2. Crick, F. H., Barnett, L., Brenner, S., and Watts-Tobin, R. J. (1961) General nature of the genetic code for proteins. *Nature* **192,** 1227–1232
3. Goldberg, A. D., Allis, C. D., and Bernstein, E. (2007) Epigenetics: a landscape takes shape. *Cell* **128,** 635–638
4. Campos, E. I., Stafford, J. M., and Reinberg, D. (2014) Epigenetic inheritance: histone bookmarks across generations. *Trends Cell Biol.* **24,** 664–674
5. Ning, B., Li, W., Zhao, W., and Wang, R. (2016) Targeting epigenetic regulations in cancer. *Acta Biochim. Biophys. Sinica* **48,** 97–109
6. Zhang, Z., and Zhang, R. (2015) Epigenetics in autoimmune diseases: Pathogenesis and prospects for therapy. *Autoimmunity Rev.* **14,** 854–863
7. Landgrave-Gomez, J., Mercado-Gomez, O., and Guevara-Guzman, R. (2015) Epigenetic mechanisms in neurological and neurodegenerative diseases. *Frontiers Cell. Neurosci.* **9,** 58
8. (2008) Moving AHEAD with an international human epigenome project. *Nature* **454,** 711–715
9. Huang, H., Lin, S., Garcia, B. A., and Zhao, Y. (2015) Quantitative proteomic analysis of histone modifications. *Chem. Rev.* **115,** 2376–2418
10. Edmonds, C. G., Loo, J. A., Smith, R. D., Fuciarelli, A. F., Thrall, B. D., Morris, J. E., and Springer, D. L. (1993) Evaluation of histone sequence and modifications by electrospray ionization mass spectrometry and tandem mass spectrometry. *J. Toxicol. Environmental Health* **40,** 159
11. Strahl, B. D. Briggs, S. D. Brame, C. J. Caldwell, J. A. Koh, S. S. Ma, H. Cook, R. G. Shabanowitz, J. Hunt, D. F. Stallcup, M. R. and Allis, C. D. (2001) Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. *Current Biol.* **11,** 996–1000
12. Zhang, K., Williams, K. E., Huang, L., Yau, P., Siino, J. S., Bradbury, E. M., Jones, P. R., Minch, M. J., and Burlingame, A. L. (2002) Histone acetylation and deacetylation: identification of acetylation and methylation sites of HeLa histone H4 by mass spectrometry. *Mol. Cell. Proteomics* **1,** 500–508
13. Zhang, K., Tang, H., Huang, L., Blankenship, J. W., Jones, P. R., Xiang, F., Yau, P. M., and Burlingame, A. L. (2002) Identification of acetylation and methylation sites of histone H3 from chicken erythrocytes by high-accuracy matrix-assisted laser desorption ionization-time-of-flight, matrix-assisted laser desorption ionization-postsource decay, and nanoelectrospray ionization tandem mass spectrometry. *Anal. Biochem.* **306,** 259–269
14. Strahl, B. D., and Allis, C. D., (2000) The language of covalent histone modifications. *Nature* **403,** 41–45
15. Garcia, B. A., Pesavento, J. J., Mizzen, C. A., and Kelleher, N. L. (2007) Pervasive combinatorial modification of histone H3 in human cells. *Nat. Methods* **4,** 487–489
16. Zhao, Y., and Garcia, B. A. (2015) Comprehensive catalog of currently documented histone modifications. *Cold Spring Harbor Perspectives Biol.* **7,** a025064