Histone modifications and a choice of variant: a language that helps the genome express itself

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Abstract

Covalent post-translational modifications on histones impact chromatin structure and function. Their misfunction, along with perturbations or mutations in genes that regulate their dynamic status, has been observed in several diseases. Thus, targeting histone modifications represents attractive opportunities for therapeutic intervention and biomarker discovery. The best approach to address this challenge is to paint a comprehensive picture integrating the growing number of modifications on individual residues and their combinatorial association, the corresponding modifying enzymes, and effector proteins that bind modifications. Furthermore, how they are imposed in a distinct manner during cell cycle and on specific histone variants are important dimensions to consider. Firstly, this report highlights innovative technologies used to characterize histone modifications, and the corresponding enzymes and effector proteins. Secondly, we examine the recent progress made in understanding the dynamics and maintenance of histone modifications on distinct variants. We also discuss their roles as potential carriers of epigenetic information. Finally, we provide examples of initiatives to exploit histone modifications in cancer management, with the potential for new therapeutic opportunities.

Histone marks: an evolving language

The discovery nearly fifty years ago that gene expression correlates with hyperacetylated histones [1] hinted at the importance of factors beyond the DNA sequence and how transcription factors control genome function. In the following years, the identification of histone methylation and phosphorylation [2-4] expanded the repertoire of modifications. Then, work in yeast made the first connection between mutations in histone tails and transcription [5,6], setting the stage for a functional link between histone modifications and gene expression. The connection between acetylation and chromatin function was further supported in Drosophila, using antibodies recognizing acetylated lysines on histone H4 [7]. The characterization of the first histone-modifying enzymes harboring acetyltransferase, deacetylase, methyltransferase, and demethylase activity [8-13] represented major breakthroughs, by providing a first handle on how to control the modifications. The principle of a dynamic system that responds to cellular stimuli and environmental cues to regulate chromatin structure and function emerged [14]. The presence of these modifications (or “marks”) was viewed as acting in two ways: (a) directly, by altering the electrostatic potential and/or the structure of the local chromatin environment to open or restrict access to DNA or (b) indirectly, through the recruitment of effector proteins that carry out a biological event. The latter aspect led to the proposal of the histone code hypothesis that states that histone post-translational modifications (PTMs) act sequentially or in combination to signal downstream events [15,16]. Interestingly, the possibility that these marks could be stably propagated to contribute to the maintenance of cellular identity across many cell
generations placed them as candidate vehicles of epigenetic information [17]. Together, these simple concepts with writers, readers, and erasers initiated a burst of interest spanning a range of scientific disciplines. Yet, we are still debating the existence of a code, defined as one or a combination of marks that signal a particular event. Furthermore, the repertoire of players continues to expand when one considers all histone modifications [18], the enzymes [19], effector proteins [20], and the different histone variants [21,22], and this sophisticated language is evolving with implications in diverse biological activities [23,24]. In this short report, to highlight recent advances in the role of histone marks (modifications and variants), we review novel characterization techniques and provide new insight into their potential inheritance and dynamics in order to discuss emerging approaches in cancer management.

**An interdisciplinary effort to deconvolve a complex language**

Antibodies that recognize site-specific histone modifications remain crucial tools used to study histone marks [7,25], and are routinely exploited in chromatin immuno-precipitation (ChIP) approaches. Combining ChIP with other techniques, such as with next-generation sequencing (ChIP-seq), enables the mapping of histone marks genome-wide [26-28], whereas combining it with bisulfite sequencing correlates histone marks with DNA methylation [29] and can extend to its other modified forms. One limitation is that these approaches require prior knowledge of the associated protein and/or modification, and the availability of a suitable antibody. Importantly, DNA capture techniques, combined with mass spectrometry, provide opportunities to identify unknown modifications and associated proteins. In one approach, work in yeast introduced a unique DNA sequence that is recognized by a DNA-binding protein that acts as a handle, enabling the purification of a single locus [30]. Alternatively, DNA capture using nucleotide derivatives enables the investigation of histone marks and associated proteins at sites of DNA synthesis [31,32], shedding light onto the maintenance of histone marks following replication. Finally, the recent development of gene editing tools, including transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPRs) [33-35], provides opportunities to analyze the impact of the loss of factors involved in the dynamics of histone marks.

Importantly, the binding of proteins, including antibodies, to histone marks can depend on neighboring modifications [36], potentially altering their specificity, and giving rise to artifacts. Here, chemical approaches, including advances in peptide synthesis protocols [37-40], have made significant contributions to efforts to better characterize antibody and protein binding using peptide arrays *in vitro* [41,42]. A useful systematic analysis of nearly 200 antibodies using a standardized method employed by the ENCODE consortium is available online [43] (see http://compbio.med.harvard.edu/antibodies/). Peptide arrays have also helped in elucidating the substrate specificity for the modifying enzymes [44-47]. Remarkable advances in chemical biology include the use of label-free assays, which minimize artifacts associated with labels [48,49], and high-throughput platforms, used to identify small molecule modulators of histone modifying enzymes [50,51]. Finally, mass spectrometry (MS) has proven to be a powerful tool, particularly in its quantitative format [52,53], used to identify novel modifications [54,55], crosstalk [56], and to explore their dynamics [57]. This use of mass spectrometry has been extensively reviewed [58-60].

Notably, many assays including ChIP provide only a snapshot of the histone modification landscape, and generally represent an average, over a population of cells. This hinders studies of dynamics, and by averaging over heterogeneous cell populations, key information can be lost or diluted. This has motivated efforts towards single-cell analyses. An early example of single-cell analysis exploited imaging techniques with the use of antibody Fab fragments coupled to fluorophores, a technique termed FabLEM [61], to visualize the dynamics of endogenous modifications in individual living cells. Another study labeled different antibodies with distinct fluorophores to simultaneously visualize multiple marks in single cells isolated using microfluidic devices [62]. Of note, these single-cell approaches tend to assess histone modifications globally across the genome. An alternative approach aims to investigate individual genomic loci in single cells [63] by combining *in situ* hybridization and the proximity ligation assay (PLA) [64]. This assay is compatible with histological sections [63] and could therefore extend to the clinic. Optimization of these techniques and others, such as the development of ChIP-seq at single-cell levels, will open avenues for assays with applications at the bench and in the clinic.

**Maintenance of histone marks: should I stay or should I go?**

Histone modifications are present not only on nucleosomal histones but also on histones in transit following their synthesis. Exploiting epitope-tagged histone variants in order to purify soluble histone complexes [65] revealed that different pools of non-nucleosomal
histones feature distinct marks [66,67], and some are more prevalent on particular variants [68]. Thus, the issue of the histone variants, whose expression is cell cycle regulated, is a critical component of the picture [21,22].

When present in chromatin, histone modifications are commonly referred to as epigenetic marks, yet often function simply as a signaling module in the short-term and their longevity is still under exploration. To be formally considered an epigenetic mark (as defined by Robin Holliday), the modification should be stably inherited through cell division in the absence of the initiating event [69]. However, dynamics through the cell cycle, in particular during replication (where there is a genome-wide transient disruption of the chromatin organization), challenges heritability. In the wake of the replication fork passage, parental histones are interspersed with newly synthesized histones, diluting parental marks (Figure 1) [70,71]. Notably, evicted parental H3-H4 tetramers are thought to remain intact and to randomly distribute to daughter cells in cultured cells. However, some tetramers could proceed through a dimeric state, and then either rapidly re-associate or remain dimeric and mix with new H3-H4 dimers. Several factors are implicated in these processes to ensure proper packaging of the duplicated DNA [72,73], in particular histone chaperones [74], which represent key players in epigenetic inheritance. Indeed, the histone chaperone anti-silencing function 1 (ASF1) is a candidate to coordinate the recycling of parental and new histones (and associated modifications). Through its connection with the MCM2 helicase [75], ASF1 handles parental H3-H4 dimers and can associate with new H3-H4 dimers, acting as a donor to hand off histones to another H3-H4 chaperone, chromatin assembly factor 1 (CAF-1). CAF-1 acts in a manner coupled to DNA synthesis to deposit the replicative H3.1 variant [65]. Failure in the deposition process can present gaps that are later filled by the histone regulator A (HIRA) chaperone to deposit H3.3 throughout the cell cycle [76]. Importantly, these dynamics may differ in various cellular contexts or cell types. In any event, it is interesting to consider the impact of recycling parental histones and associated marks where nucleosomes feature asymmetric modifications [77] and/or histone variant heterotypic particles [78].

Once deposited, the landscape of histone modifications and variants may be altered through replication-independent dynamics [76,79] and may have an impact on chromatin function. Indeed, H3.3 accumulates at transcription sites and regulatory elements [76] deposited by either the HIRA or DAXX (death associated protein) chaperones. Furthermore, a recent example in yeast shows that the Set2-mediated methylation of H3K36 impacts replication-independent dynamics that can alter the acetylation state and give rise to cryptic transcription. Perturbation of this methylation pathway, perhaps by altering the expression or activity of the Set2 methyltransferase or an H3K36 demethylase (KDM4A), promotes nucleosome turnover and results in an accumulation of acetylated histones at the coding regions of genes, allowing transcription factors access to cryptic sites [80]. Histone marks may also be maintained following replication due to histone-modifying enzymes (Figure 2A), some possibly associated with the replication fork [81]. Interestingly, the placement of variants can potentiate the action of enzymes, such as SUV39h1 (suppressor of variegation 3-9 homolog 1), an H3K9 methyltransferase [12]. Indeed, H3K9me1, more prevalent on soluble H3.1 compared to H3.3 [68], is a preferred substrate for SUV39h1 to establish H3K9me3. This leads to the binding of the heterochromatin protein 1 (HP1), a mark of centric heterochromatin [82]. Furthermore, existing modifications may recruit enzyme complexes to write modifications on neighboring newly synthesized histones. For example, the polycomb group (PcG) protein complex can recognize and bind to H3K27me3-modified histones. This suggests a model where the PcG complex can bind parental H3K27me3 histones, thereby recruiting enzymatic activity to newly synthesized histones to spread this mark (Figure 2B) [83]. Indeed, in Drosophila, a mutation in the PcG protein EED, which impairs its ability to bind H3K27me3, decreases global H3K27me3 levels [83]. While MS approaches support a model where H3K27me3 remains following replication [31,84,85], a study in Drosophila embryos (using the PLA assay) failed to detect H3K27me3 at replication foci but rather observed the responsible enzyme, enhancer of zeste (E(z)) [86]. It will be interesting to further test this effect with other assays and in different model organisms. Interestingly, recent work in plants shows that histone variants can also impact H3K27 methylation. Here, the enzymes ATXR (arabidopsis trithorax-related protein)5 and ATXR6 specifically monomethylate H3.1K27, as plant H3.3-specific T31 inhibits methylation, an effect not observed with other plant methyltransferases (Figure 2C) [87]. Together, these examples highlight the importance of assay choice and model systems when analyzing histone modifications and suggest that even conserved modifications may be subject to different regulatory mechanisms in various species. Notably, it is important to consider instances where histones do not have the capacity to bear the same modifications either due to mutations [88-91] or the presence of variants, such as the centromeric H3 variant
Histone chaperones participate in histone deposition during replication and are key candidates to regulate epigenetic inheritance. Parental H3-H4 histones evicted from chromatin are handled by the chaperone ASF1, which also associates with newly synthesized histones [75]. Parental histones and marks are recycled onto daughter strands along with newly synthesized histones with characteristic marks [68,107], the latter deposited by the chaperone CAF-1. Mixing of parental and new histones is rare [108] but may have functional consequences. Importantly, away from the fork, histones are susceptible to turnover, which can alter the modification landscape.

Abbreviations: ASF1, anti-silencing function 1; CAF-1, chromatin assembly factor 1; HIRA, histone regulator A; PTM, post-translational modification.
CenH3 (also known as CENP-A) [92], which lack the target residue and how this can impact chromatin integrity.

**Code red! Histone marks in cancer**

Genomic and epigenomic factors are now recognized as contributing to cancer [93-96]. Interestingly, recent evidence showcases links between the genome and epigenome, leading to new questions about how they cooperate in order to impact cancer progression and whether this can be exploited to better manage patient care. A pioneering study identified mutations in histone H3, including H3K27M, in pediatric glioblastoma [88], a finding recently supported in several new reports [89-91,97]. Biochemical evidence shows that even a fraction of histones presenting this mutation is sufficient to impact global H3K27 methylation levels [98]. Additionally, new data have shed light on how histone marks impact genome stability. The overexpression of KDM4A, which demethylates H3K9 and H3K36, leads to site-specific copy gain of regions often amplified in human tumors [99]. Interestingly, SUV39h1 or HP1γ (which binds H3K9me3) antagonizes this effect, highlighting the importance of balancing opposing enzyme activities [99].

Furthermore, a selective inhibitor for an H3K79 methyltransferase kills acute leukemia lines bearing mixed lineage leukemia (MLL) translocations [100], bringing hope of a therapy for this disease that has limited treatment options. It will be important to better understand the links between genome and epigenome as this may identify novel candidates for targeted therapeutic intervention, particularly in cancers with distinct genome anomalies.

The histone-modifying enzymes are common targets for identifying small molecule modulators. However, highly conserved active sites among similar enzymes have challenged the development of selective inhibitors. Interestingly, the sensitivity to histone deacetylase (HDAC) inhibitors varies depending on the protein complex [101], suggesting that altering the concentration of the inhibitor may target different HDAC complexes and thereby different groups of genes. An emerging strategy is to target the effector proteins, such as the bromo- and chromodomains, which bind acetylated and methylated lysines, respectively. Indeed, the demonstration that small molecule bromodomain inhibitors, such as JQ1 [102] and i-BET [103], can alter gene expression has motivated additional screens for other inhibitors. The Structural Genomics Consortium (SGC) has identified several potential epidrugs and has made them and corresponding structural information available to the research community (for more information see www.thescg.org), advancing our understanding of how epidrugs impact cell function. Another approach that has gained momentum is combining “epidrugs”, either with other “epidrugs” or with classical therapeutic...
regimes. Indeed, combining certain demethylase inhibitors with pan-HDAC inhibitors has a synergistic effect on cell death in glioblastoma cells, providing a proof-of-concept to test other combinations of epidrugs for enhanced sensitivity [104]. Interestingly, changing the chromatin structure through epidrugs also sensitizes cells to radiation [105]. This could enable lower doses and higher selectivity to cancer cells and minimize harmful side effects. Epidrugs have also shown promise in slowing the onset of resistance or reversing the resistant phenotype [106]. The future success of epidrugs in the clinic will depend on a better understanding of how these compounds function in cells, thus necessitating studies integrating several aspects of chromatin dynamics. Furthermore, identifying markers that could be exploited as companion diagnostics will help identify the patients that will most likely clinically benefit, improving overall patient care.

Conclusions and perspectives
The past few years have seen significant progress in our understanding of histone marks and their implications in diverse biological processes. However, several important questions remain. How can mutations in histones found in certain cancers alter the histone modification landscape and thus chromatin function? How do enzymes of opposing activities cooperate to establish a modification landscape with proper dynamics? Are histone modifications propagated through cell division and if yes is this inheritance due to the presence of recycled parental marks or a cellular memory that acts to re-establish the marks? Are variations in the dynamics of the chromatin landscape a cause or consequence of tumorigenesis? What is the impact of epidrugs on the global histone modification landscape, genome integrity, and on the proteome, featuring thousands of modification substrates? The answers to these questions should help place histone modifications within the network of other chromatin regulators and strengthen the link with both development and disease. In this respect, the histone variants deserve consideration in the histone code debate as the variants themselves impact the modification landscape as well as the dynamics of the genome itself, exemplified during DNA damage [79]. Code or not, the continuous development of new assays, including single-cell approaches, should help provide further insight into several aspects of histone modifications, such as cause or consequence of particular marks, and help identify novel biomarkers and targeted candidates for therapeutic intervention.

Abbreviations
ASF1, anti-silencing function 1; CAF-1, chromatin assembly factor 1; ChIP, chromatin immunoprecipitation; ChIP-seq, chromatin immunoprecipitation with next generation sequencing; HDAC, histone deacetylase; HIRA, histone regulator A; HP1, heterochromatin protein 1; MS, mass spectrometry; PcG, polycomb group protein; PLA, proximity ligation assay; PTM, post-translational modification; SGC, Structural Genomics Consortium; SUV39h1, suppressor of variegation 3-9 homolog 1.

Disclosures
The authors declare that they have no disclosures.

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References
1. Allfrey VG, Mirsky AE: Structural Modifications of Histones and their Possible Role in the Regulation of RNA Synthesis. Science 1964, 144:559.
2. Allfrey VG, Faulkner R, Mirsky AE: Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proc Natl Acad Sci USA 1964, 51:786-94.
3. Murray K: The occurrence of epsilon-N-methyl lysine in histones. Biochemistry 1964, 3:10-5.
4. Stevely WY, Stocken LA: Phosphorylation of rat-thymus histone. Biochem J 1966, 100:20C-1C.
5. Han M, Kim UJ, Kayne P, Grunstein M: Depletion of histone H4 and nucleosomes activates the PHOS gene in Saccharomyces cerevisiae. EMBO J 1988, 7:2221-8.
6. Johnson LM, Kayne PS, Kahn ES, Grunstein M: Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating loci in Saccharomyces cerevisiae. Proc Natl Acad Sci USA 1990, 87:6286-90.
7. Turner BM, Birley AJ, Lavender J: Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in Drosophila polytene nuclei. Cell 1992, 69:375-84.
8. Travis GH, Colavito-Shopanski M, Grunstein M: Extensive purification and characterization of chromatin-bound histone acetyltransferase from Saccharomyces cerevisiae. J Biol Chem 1984, 259:14406-12.
9. Kleff S, Andrulis ED, Anderson CW, Steenman R: Identification of a gene encoding a yeast histone H4 acetyltransferase. J Biol Chem 1995, 270:24674-7.
10. Brownell JE, Zhou J, Ranalli T, Kobayashi R, Edmondson DG, Roth SY, Allis CD: Tetrahymena histone acetyltransferase A:
a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell 1996, 84:843-51.

11. Taunton J, Hassig CA, Schreiber SL: A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science 1996, 272:608-11.

12. Rea S, Eisenhaber F, O’Carroll D, Strahl BD, Sun ZW, Schmid M, Grzelić S, Mechtler K, Ponting CP, Allis CD, Jenuwein T: Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 2000, 406:593-9.

13. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y: Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 2004, 119:941-53.

14. Turner BM: Acetylation and deacetylation of histone H4 continue through mitaphase with depletion of more-acetylated isoforms and altered site usage. Exp Cell Res 1989, 182:206-14.

15. Strahl BD, Allis CD: The language of covalent histone modifications. Nature 2000, 403:41-5.

16. Jenuwein T, Allis CD: Translating the histone code. Science 2001, 293:1074-80.

17. Turner BM: Histone acetylation and an epigenetic code. Bioessays 2000, 22:836-45.

18. Dhall A, Chatterjee C: Chemical approaches to understand the language of histone modifications. ACS Chem Biol 2011, 6:987-99.

19. Bojang P, Ramos KS: The promise and failures of epigenetic therapies for cancer treatment. Cancer Treat Rev 2014, 40:153-69.

20. Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ: How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat Struct Mol Biol 2012, 19:1025-40.

21. Szewczyk E, Ray-Gallet D, Almouzni G: The double face of the histone variant H3.3. Cell Res 2011, 21:421-34.

22. Filipescu D, Szenker E, Almouzni G: Developmental roles of histone H3 variants and their chaperones. Trends Genet 2013, 29:630-40.

23. Bannister AJ, Kouzarides T: Regulation of chromatin by histone modifications. Cell Res 2011, 21:381-95.

24. Rivera C, Gurard-Levin ZA, Almouzni G, Loyola A: Histone lysine methylation and chromatin replication. Biochim Biophys Acta 2014.

25. Fuchs SM, Strahl BD: Antibody recognition of histone post-translational modifications: emerging issues and future prospects. Epigenomics 2011, 3:247-9.

26. Barski A, Cuddapah S, Cui K, Roh T, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K: High-resolution profiling of histone modifications in the human genome. Cell 2007, 129:823-37.

27. Mikkelsen TS, Ku M, Jaffe DB, Isaac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim T, Koche RP, Lee W, Mendenhall E, O’Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE: Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature 2007, 448:553-60.

28. Johnson DS, Mortazavi A, Myers RM, Wold B: Genome-wide mapping of in vivo protein-DNA interactions. Science 2007, 316:1497-502.

29. Brinkman AB, Gu H, Bartels, Stefanie J J, Zhang Y, Matarese F, Simmer F, Marks H, Bock C, Gninke A, Meissner A, Stunnenberg HG: Sequential ChIP-bisulfite sequencing enables direct genome-scale investigation of chromatin and DNA methylation cross-talk. Genome Res 2012, 22:1128-38.

30. Byrum SD, Raman A, Taverna SD, Tackett AJ: ChAP-MS: a method for identification of proteins and histone posttranslational modifications at a single genomic locus. Cell Rep 2012, 2:198-205.

31. Alabert C, Bukowski-Wills J, Lee S, Kustatscher G, Nakamura K, de Lima Alves, Flavia, Menard P, Meijlink J, Rappsilber J, Groth A: Nascent chromatin capture proteomics determines chromatin dynamics during DNA replication and identifies unknown fork components. Nat Cell Biol 2014, 16:281-93.

32. Sirbu BM, McDonald WH, Dungrawala H, Badu-Nkansah A, Kavanaugh GM, Chen Y, Tabb DL, Cortez D: Identification of proteins at active, stalled, and collapsed replication forks using isolation of proteins on nascent DNA (iPOND) coupled with mass spectrometry. J Biol Chem 2013, 288:31458-67.

33. Maeder ML, Angstman JF, Richardson ME, Linder SJ, Cascio VM, Tsai SQ, Ho QH, Sander JD, Reyon D, Bernstein BE, Costello JF, Wilkinson MF, Joung JK: Targeted DNA demethylation and activation of endogenous genes using programmable TALE-TET1 fusion proteins. Nat Biotechnol 2013, 31:1137-42.

34. Mendenhall EM, Williamson KE, Reyon D, Zou JY, Ram O, Joung JK, Bernstein BE: Locus-specific editing of histone modifications at endogenous enhancers. Nat Biotechnol 2013, 31:1133-6.

35. Konermann S, Brigham MD, Trevino AE, Hsu PD, Heidenreich M, Le Cong, Platt RJ, Scott DA, Church GM, Zhang F: Optical control of mammalian endogenous transactivation and epigenetic states. Nature 2013, 500:472-6.

36. Fuchs SM, Krajewski K, Baker RW, Miller VL, Strahl BD: Influence of combinatorial histone modifications on antibody and effector protein recognition. Curr Biol 2011, 21:53-8.

37. Simon MD, Chu F, Racki LR, de la Cruz, Cecile C, Burlingame AL, Panning B, Narlikar GJ, Shokat KM: The site-specific installation of methyl-lysine analogs into recombinant histones. Cell 2007, 128:1003-12.

38. Neumann H, Hancock SM, Buning R, Rouh A, Chapman L, Somers J, Owen-Hughes T, van Noort J, Rhodes D, Chin JW: A method for genetically installing site-specific acetylation in recombinant
histones defines the effects of H3 K56 acetylation. Mol Cell 2009, 36:153-63.

39. 

Kee J, Villani B, Carpenter LR, Muir TW: Development of stable phosphohistidine analogues. J Am Chem Soc 2010, 132:14327-9.

40. 

Chatterjee C, McGinty RK, Fierz B, Muir TW: Disulfide-directed histone ubiquitylation reveals plasticity in hDot1L activation. Nat Chem Biol 2010, 6:267-9.

41. 

Rothbart SB, Krajewski K, Strahal BD, Fuchs SM: Peptide micro-arrays to interrogate the “histone code”. Meth Enzymol 2012, 512:107-35.

42. 

Bock I, Dhayalan A, Kudithipudi S, Brandt O, Rathert P, Jetsch A: Detailed specificity analysis of antibodies binding to modified histone tails with peptide arrays. Epigenetics 2011, 6:256-63.

43. 

Egelhofer TA, Minoda A, Klugman S, Lee K, Kosalsinska-Zwierz P, Alekseyenko AA, Cheung M, Day DS, Gadel S, Gorchakov AA, Gu T, Kharchenko PV, Kuan S, Lazarre I, Linden-Basso D, Luu Y, Ngo Q, Perry M, Rechtsteiner A, Riddlesteiner NC, Schwartz YB, Shanower GA, Vielle A, Ahringer J, Elgin SC, R, Kuroda MI, Pirrotta V, Ren B, Strome S, Park PJ, et al.: An assessment of histone-modification antibody quality. Nat Struct Mol Biol 2011, 18:91-3.

44. 

Gurard-Levin ZA, Kilian KA, Kim J, Bahr K, Mrksich M: Peptide arrays identify isoform-selective substrates for profiling endogenous lysine deacetylation activity. ACS Chem Biol 2010, 5:863-73.

45. 

Ibáñez G, McBean JL, Astudillo YM, Luo M: An enzyme-coupled ultrasensitive luminescence assay for protein methyltransferases. Anal Biochem 2010, 401:203-10.

46. 

Garske AL, Craciun G, Denu JM: A combinatorial H4 tail library for exploring the histone code. Biochemistry 2008, 47:8094-102.

47. 

Moyle PM, Muir TW: Method for the synthesis of mono-ADP-ribose conjugated peptides. J Am Chem Soc 2010, 132:15878-80.

48. 

Gurard-Levin ZA, Milne TA, Dewell S, McGinty RK, Yuen M, Ueberheide B, Dou Y, Muir TW, Patel DJ, Alles CD: Recognition of a mononucleosomal histone modification pattern by BPTF via multivalent interactions. Cell 2011, 145:692-706.

49. 

Xu C, Cole PA, Meyers DJ, Kormish J, Dent S, Zaret KS: Chromatin “prepattern” and histone modifiers in a fate choice for liver and pancreas. Science 2011, 332:963-6.

50. 

Zee BM, Young NL, Garcia BA: Quantitative proteomic approaches to studying histone modifications. Curr Chem Genomics 2011, 5:106-14.

51. 

Garcia BA: Mass spectrometric analysis of histone variants and post-translational modifications. Front Biosci (Schol Ed) 2009, 14:142-53.

52. 

Ong S, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M: Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics 2002, 1:376-86.

53. 

Su X, Ren C, Freitas MA: Mass spectrometry-based strategies for characterization of histones and their post-translational modifications. Expert Rev Proteomics 2007, 4:211-25.

54. 

Tessarz P, Santos-Rosa H, Robson SC, Sylvestersen KB, Nelson CJ, Nielsen ML, Kouzarides T: Glutamine methylation in histone H2A is an RNA-polymerase-I-dedicated modification. Nature 2014, 505:564-8.

55. 

Murphy PJ, Cipriani BR, Wallin CB, Ju CY, Szeto K, Hagarman JA, Benitez JJ, Craighead HG, Soloway PD: Single-molecule analysis of combinatorial epigenomic states in normal and tumor cells. Proc Natl Acad Sci USA 2013, 110:7772-7.

56. 

Gomez D, Shankman LS, Nguyen AT, Owens GK: Detection of histone modifications at specific gene loci in single cells in historial sections. Nat Methods 2013, 10:171-7.

57. 

Weibrich I, Leuchowius K, Clausson C, Conze T, Jarvis M, Howell WM, Kamali-Moghaddam M, Soederberg O: Proximity ligation assays: a recent addition to the proteomics toolbox. Expert Rev Proteomics 2010, 7:401-9.

58. 

Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y: Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways
dependent or independent of DNA synthesis. Cell 2004, 116:51-61.

66. Campos El, Fillingham J, Li G, Zheng H, Voigt P, Kuo WW, Seepany H, Gao Z, Day LA, Greenblatt JF, Reinberg D: The program for processing newly synthesized histones H3.1 and H4. Nat Struct Mol Biol 2010, 17:1343-51.

67. Loyola A, Bonaldi T, Roche D, Imhof A, Almouzni G: DNA methylation and epigenetic inheritance. F1000Prime Reports 2014, 6.

68. Lacoste N, Woolfe A, Tachiwana H, Garea AV, Barth T, Cantaloube S, Kurumizaka H, Imhof A, Almouzni G: Mislocalization of the centromeric histone variant H3.3/CENP-A in human cells depends on the chaperone DAXX. Mol Cell 2014, 53:631-44.

69. Voigt P, LeRoy G, Drury WJ, Zee BM, Son J, Beck DB, Young NL, Garcia BA, Reinberg D: Asymmetrically modified nucleosomes. Cell 2012, 151:181-93.

70. Lacoste N, Woolfe A, Tachiwana H, Garea AV, Barth T, Cantaloube S, Kurumizaka H, Imhof A, Almouzni G: Mislocalization of the centromeric histone variant H3.3/CENP-A in human cells depends on the chaperone DAXX. Mol Cell 2014, 53:631-44.

71. Adam S, Polo SE, Almouzni G: Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperone HIRA. Cell 2013, 155:94-106.

72. Voigt P, LeRoy G, Drury WJ, Zee BM, Son J, Beck DB, Young NL, Garcia BA, Reinberg D: Asymmetrically modified nucleosomes. Cell 2012, 151:181-93.

73. Jansen, Lars E T, Black BE, Foltz DR, Cleveland DW: Histone chaperones: some gap-filling mechanism for H3.3 to maintain chromatin integrity. Trends Biochem Sci 2010, 35:618-26.

74. Jacob Y, Bergamin E, Donoghue, Mark T A, Mongeon V, LeBlanc C, Schultz DC, Pchelintsev NA, Adams PD, Jansen, Lars E T, Almouzni G: Selective methylation of histone H3 variant H3.1 regulates heterochromatin replication. Science 2014, 343:1249-53.

75. Schwartzentruber J, Koehler AE, Tijssen J, de Lauor EV, Almouzni G: The program for processing newly synthesized histones H3.1 and H4. Nat Struct Mol Biol 2010, 17:1343-51.
94. Rius M, Lyko F. Epigenetic cancer therapy: rationales, targets and drugs. Oncogene 2012, 31:4257-65.
95. Rodriguez-Paredes M, Esteller M. Cancer epigenetics reaches mainstream oncology. Nat Med 2011, 17:330-9.
96. Best JD, Carey N. Epigenetic opportunities and challenges in cancer. Drug Discov Today 2010, 15:65-70.
97. Wu G, Diaz AK, Paugh BS, Rankin SL, Ju B, Li Y, Zhu X, Qu C, Chen X, Zhang J, Easton J, Edmonson M, Ma X, Lu C, Nagahawatte P, Hedlund E, Rusch M, Pounds S, Lin T, Onar-Thomas A, Huether R, Kriwacki R, Parker M, Gupta P, Becksfort J, Wei L, Mulder HL, Boggs K, Vadodaria B, Yergeau D, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. Nat Genet 2014, 46:444-50.
98. Lewis PW, Müller MM, Koletsky MS, Cordero F, Lin S, Banaszynski LA, Garcia BA, Muir TW, Becher OJ, Allis CD. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. Science 2013, 340:857-61.
99. Black JC, Manning AL, van Rechem C, Kim J, Ladd B, Cho J, Pineda CM, Murphy N, Daniels DL, Montagna C, Lewis PW, Glass K, Allis CD, Dyson NJ, Getz G, Whetstine JR. KDM4A lysine demethylase induces site-specific copy gain and rereplication of regions amplified in tumors. Cell 2013, 154:541-55.
100. Daigle SR, Olhava EJ, Therkelsen CA, Basavapathruni A, Jin L, Boriack-Sjodin PA, Allain CJ, Klaus CR, Raimondi A, Scott MP, Waters NJ, Chessworth R, Moyer MP, Copeland RA, Richon VM, Pollock RM. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. Blood 2013, 122:1017-25.
101. Bantscheff M, Hopf C, Savitski MM, Dittmann A, Grandi P, Michon A, Schlegl J, Abraham Y, Becher I, Bergamini G, Boesche M, Delling M, Dumpefeld B, Eberhard D, Huthmacher C, Mathiesen T, Poeck D, Reader V, Strunk K, Sweetman G, Kruse U, Neubauer G, Ramsden NG, Drewes G. Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. Nat Biotechnol 2011, 29:255-65.
102. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwarz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE. Selective inhibition of BET bromodomains. Nature 2010, 468:1067-73.
103. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewsell S, Chung C, Chandwani R, Marazzi I, Wilson P, Coste H, White J, Kirilovsky J, Rice CM, Lora JM, Prinjha RK, Lee K, Tarakhovsky A. Suppression of inflammation by a synthetic histone mimic. Nature 2010, 468:1119-23.
104. Singh MM, Manton CA, Bhat KP, Tsai W, Aldape K, Barton MC, Chandra J. Inhibition of LSD1 sensitizes glioblastoma cells to histone deacetylase inhibitors. Neuro-oncology 2011, 13:894-903.
105. Bar-Sela G, Jacobs KM, Gius D. Histone deacetylase inhibitor and demethylating agent chromatin compaction and the radiation response by cancer cells. Cancer J 2007, 13:65-9.
106. Sharma SV, Lee DY, Li B, Quindan MP, Takahashi F, Maheswaran S, McDermott U, Azizian N, Zou L, Fischbach MA, Wong K, Brandstetter K, Wittner B, Ramaswamy S, Classon M, Settleman J. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010, 141:69-80.
107. Sobel RE, Cook RG, Perry CA, Annunziato AT, Allis CD. Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. Proc Natl Acad Sci USA 1995, 92:1237-41.
108. Xu M, Long C, Chen X, Huang C, Chen S, Zhu B. Partitioning of histone H3-H4 tetramers during DNA replication-dependent chromatin assembly. Science 2010, 328:94-8.
109. Allis CD, Berger SL, Cote J, Dent S, Jenuwien T, Kozarides T, Pillus L, Reinberg D, Shi Y, Shiekhattar R, Shilatifard A, Workman J, Zhang Y. New nomenclature for chromatin-modifying enzymes. Cell 2007, 131:633-6.