Curr Pharmacol Rep (2017) 3:286–293
DOI 10.1007/s40495-017-0103-4

EPIGENETICS (ATY LAU, SECTION EDITOR)

Discovering Epimodifications of the Genome, Transcriptome, Proteome, and Metabolome: the Quest for Conquering the Uncharted Epi(c) Territories

Yan-Ming Xu[1] · **Fei-Yuan Yu**[1] · **Andy T. Y. Lau**[1]

Published online: 22 August 2017

Abstract

Purpose of Review In this review, we would like to present the overall concepts of "-omes–epi-omes" interactions, i.e., the interactions among the four most noticeable "-omes" (genome, transcriptome, proteome, and metabolome) to the four "epi-omes" (epigenome, epitranscriptome, epiproteome, and epimetabolome) as well as discussing the recently identified epimodifications in humans.

Recent Findings With the advancement of mass spectrometry and sequencing technologies, novel epimodifications/epi-marks are gradually revealed in recent years. Nowadays, it is becoming clear that all the constituents of the genome, transcriptome, proteome, and even the metabolome can further be modified/decorated with various epi-marks. Given the fact that a variety of modifications can occur in DNA/RNA, proteins, and metabolites, it is possible that an unknown number of epimodifications/epi-marks might exist and are yet to be discovered.

Summary The ability to decipher and manipulate the epi-omes might present new avenues in drug design for procuring better treatment of various human diseases.

Keywords Epimodifications · Epigenome · Epitranscriptome · Epiproteome · Epimetabolome

This article is part of the Topical Collection on *Epigenetics*

✉ Andy T. Y. Lau
andytylau@stu.edu.cn

[1] Laboratory of Cancer Biology and Epigenetics, Department of Cell Biology and Genetics, Shantou University Medical College, Shantou 515041, Guangdong, People's Republic of China

Introduction

The term "-omics" is now a very familiar term especially in the field of life sciences. This suffix seems to be first coined in the 1980s, to describe the global analysis of biological molecules. Since then, an explosion of -omics approaches has been observed in the last few decades. Although the study of a particular area suffices to constitute an "-omics" approach, e.g., phosphoproteomics, which is the systematic/global study of phosphoproteins [1]. However, most of these new "-omics" approaches are actually stemmed from the four main noticeable "-omics" approaches, i.e., genomics, transcriptomics, proteomics, and metabolomics. As from the concept of central dogma, it is not difficult to anticipate the gradual evolvement of genomics to transcriptomics and to proteomics. For metabolomics, although it is regaining attention lately, this approach could never attain such high throughput competency without the recent advancement of tandem mass spectrometry (MS/MS) [2]. Even for that, because of the complex diversity of known and unknown metabolites that might actually be present, therefore, various MS-based (e.g., GC-MS, LC-MS/MS) as well as non-MS-based methodologies (e.g., NMR-based) are still required to fulfill the objectives in each specific need in metabolomic study [3•]. Moreover, although each main approach alone is powerful, throughout these years, it becomes evident for us to realize that no single omics approach would be indeed comprehensively/conclusive enough to understand/solve/explain a particular biological problem. Therefore, in the future, it is expected that the combined utilization of multiple approaches in one study would be deemed necessary to get an even more global/comprehensive picture and facilitate in deciphering the complex biological phenomenon [4•].

Epimodifications of the Fantastic Four "-omes"

In the past half century, DNA methylation and protein phosphorylation are among the most common modifications discovered. It has been a while when histone proteins have been regarded as just the bulk materials or inert building blocks for organizing the eukaryotic genome [5], and gene expressions are primarily controlled by the degree of DNA methylation in the genome [6]. However, this concept has been changed dramatically later when histone H3 was demonstrated to be able to undergo phosphorylation and acetylation (which are closely related to cell division and participated in cellular gene regulation) [7, 8]. Since then, DNA methylation and histone modifications appear to layout the foundation of epigenetics. Indeed, it is now becoming clear that post-translational modification (PTM) of proteins happens not just in histones but virtually in every protein [9]. In here, we would like to strengthen the notion that although there should presumably still exist an unknown number and type of post-synthetic modifications on a variety of biomolecules, however, only those modifications that exert heritable changes should be considered as epimodifications, whether decorated in DNA, RNA, or proteins (although it is not clear if epimetabolites would have heritable changes to the epigenetic landscape for the moment). For instance, histone protein modifications are considered as epiproteomic modifications. Other proteins we think that they should be categorized within the epiproteome are the chromatin-associated enzymes/factors that can write, read, or erase DNA, RNA, or histone protein epi-marks [such as DNMTs (DNA writers), METTL3-METTL14-WTAP-KIAA1429 complex (RNA writers), and Aurora kinases (histone writers); methyl-CpG-binding proteins (DNA readers), YTH family proteins (RNA readers), and 14-3-3 proteins (histone readers); TETs (DNA/RNA erasers), FTO (RNA eraser), and PP1 (histone eraser)] [10••, 11, 12, 13••], as their activities could lead to chromatin remodeling/alteration of DNA methylation, histone modifications, and ncRNA expression, which subsequently influent the dynamics and operations of cellular genetics. Epimodifications of RNA and metabolites are the more recently studied field and it is intriguing to discover that RNA, similar to DNA, can also be methylated and hydroxymethylated [10••, 14]. Likewise, the modification of metabolites is gradually appreciated in the past few years and it is believed that with the use of MS/MS technology, more and more epimetabolites/oncometabolites will be identified and their functions deciphered in the near future. Nowadays, MS/MS technology is a powerful tool for the de novo identification and quantitation of epimodifications in diverse biomolecules in the field of life sciences [15, 16].

In this article, we would like to present the overall concepts of "-omes−epi-omes" interactions, i.e., the relationships among the four most noticeable "-omes" (genome, transcriptome, proteome, and metabolome) to the four "epi-omes" (epigenome, epitranscriptome, epiproteome, and epimetabolome) as well as discussing the recently identified epimodifications in humans. Lastly, the recent advances in targeting the epi-omes by various epi-drug strategies will be discussed.

The Cellular Regulatory Paradigm of "-omes −epi-omes" Interactions

We would like to present the following models to demonstrate the gradual shaping of "-omes−epi-omes" interactions (the four -omes and four epi-omes) (Fig. 1). First, we start with the four noticeable "-omes", i.e., genome (G), transcriptome (T), proteome (P), and metabolome (M) (Fig. 1a). The identification of DNA methylation in the genome has initially shaped the concept of epigenome (Epi-G), while the discovery of histone phosphorylation and acetylation in chromatin has founded the epiproteome (Epi-P) (Fig. 1b). Transcripts of the genome, whether the coding and non-coding RNA messages, constitute the transcriptome and are also subjected to various post-transcriptional modifications, which encompassed the epitranscriptome (Epi-T) (Fig. 1b). Within the constituents of the proteome (i.e., enzymes), they can perform various catabolic and anabolic reactions to produce diverse kinds of biomolecules and metabolites; the global study of all the metabolites inside the cells constitute the metabolome (Fig. 1a). Very recently, it has been proposed that the modification of metabolome can give rise to epimetabolome (Epi-M) (the dark matter of metabolism) (Fig. 1b). Since a variety of metabolites like amino acids, carbohydrates, hydroxy acids, lipids, peptides, purines, pyrimidines, and sterols can potentially be undergoing diverse epimodifications, it is highly possible that a great deal of novel epimetabolites exist and are waiting to be discovered. From this point, all eight players are now clearly emerged (Fig. 1b).

As a matter of fact, the transitions of the four -omes to four epi-omes are largely catalyzed by various enzymes/protein complexes of the proteome. For example, the modification of G by P created the Epi-G (genomic DNA being cytosine methylated by DNMTs). Indeed, constituents of P can modify itself to become Epi-P; this is not difficult to imagine, for instance, histone protein kinases phosphorylate histone proteins. Therefore, the transition of G to Epi-G and P to Epi-P is indeed a very common phenomenon in the cell. Recently, RNA was demonstrated to be modified by a variety of RNA modifying enzymes in the cells [17], which confirmed the transition of T to Epi-T. Finally, several metabolites can be further modified by some metabolic enzymes in the cells [18, 19], which also supported the M to Epi-M transition.

Inside the cells, -ome(s)/epi-ome(s) and -ome(s)/epi-ome(s) are closely interacted. We would like to cite a few examples here: (1) Epi-P→G (Ser10-phosphorylated histone H3 leads to chromatin DNA condensation) [20], (2) P↔Epi-

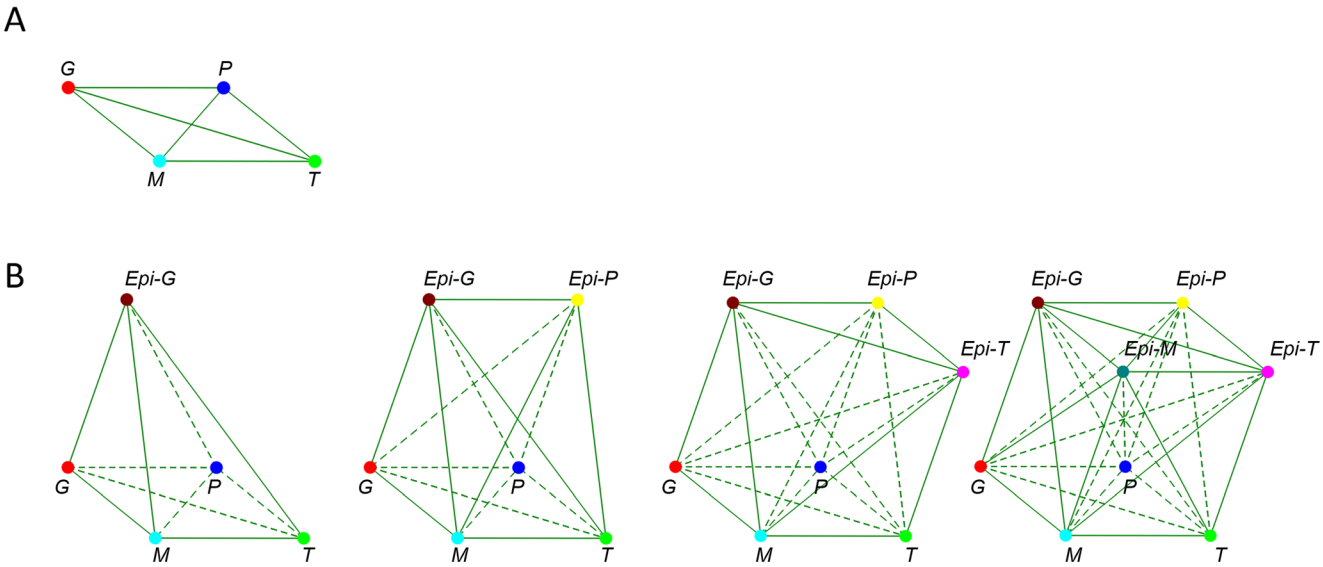

Fig. 1 The cellular regulatory paradigm of "-omes−epi-omes" interactions. **a** The possible interactions among the four most noticeable "-omes": genome (G), transcriptome (T), proteome (P), and metabolome (M). **b** All the possible interactions among the four "-omes" to the four "epi-omes": epigenome (Epi-G), epitranscriptome (Epi-T), epiproteome (Epi-P), and epimetabolome (Epi-M)

P→G↔Epi-G (deacetylation or acetylation of chromatin-associated enzyme DNMT1 can enhance or reduce its stability, and as a result increasing or decreasing the level of methylated genomic DNA) [21], (3) T↔Epi-T→P (N^6-adeonine methylated mRNA which affects its stability and translation efficiency, and as a result influent the level of protein translated) [22], and (4) T and M interaction (the discovery of metabolite-binding RNA domains in eukaryotes, in which cells are capable of using RNA for metabolite-binding and to sense cellular metabolism) [23]. Besides of the above, we think it would be tempting to investigate other novel interactions between Epi-M and the other players in this model. Suffice to say, all the four -omes to the four epi-omes are dynamically interrelated and all the possible direct/indirect effects could happen, which may ultimately explain why the cellular physiology is so complicated.

Novel Epimodifications/Epi-marks in Humans

Epimodifications are largely in part identified by MS/MS discovery/untargeted mode of identification. It is becoming usual for the identification of new epi-marks nowadays. In here, we would like to discuss some of the latest epi-marks reported (as shown in Table 1).

Epigenomic Modifications

It is generally accepted that DNA methylation by DNMTs promotes chromatin condensation and transcriptional repression, whereas DNA demethylation by DNA demethylases is associated with gene/transcriptional activation. In mammalian cells, DNA methylation is faithfully maintained and written by three major DNMTs, including DNMT1 (for maintenance of methylation), DNMT3a, and DNMT3b (for de novo methylation) [24, 25]. On the other hand, active DNA demethylation is carried by a class of enzymes called ten-eleven translocations (TETs), including TET1, TET2, and TET3, which are methylcytosine dioxygenases that convert methylated cytosine to hydroxymethylated cytosine [26].

Similar to nuclear DNA (nDNA), mitochondrial DNA (mtDNA) can also be subjected to cytosine methylation (m^5C)/hydroxymethylation (hm^5C) [27]. Lately, it has also been reported that instead of CpG methylation, mitochondrial DNA can undergo GpC methylation (mediated by a mitochondria-targeted DNMT1 transcript variant, mtDNMT1) as potential regulator of mitochondrial gene expression [28•]. Moreover, recent findings also indicate that dysfunctional mtDNA methylation could underlie aging and diseases [29].

Besides of the above, other less familiar DNA epimodifications can also occur, including cytosine formylation (5fC, a demethylation intermediate and epigenetic mark), cytosine carboxylation (5caC, a demethylation intermediate and possible epigenetic mark), and N^6-adeonine methylation (m^6A, a potential epigenetic mark) [14, 30]. For a more detailed perspective of the related information of other DNA epimodifications, we refer the reader to the following online DNA modification database (DNAmod) [31•].

Table 1 An update on epimodification of biomolecules

Type of "-ome"	Biomolecules	Epimodifications [ref]
Genome	Nuclear DNA (nDNA) Mitochondrial DNA (mtDNA)	• Cytosine methylation (m^5C) [25, 27, 28•] • Cytosine hydroxymethylation (hm^5C) [26, 27] • Cytosine formylation (5fC) [14] • Cytosine carboxylation (5caC) [14] • N^6-adeonine methylation (m^6A) [30]
Transcriptome	mRNAs tRNAs rRNAs snRNAs ncRNAs • lncRNAs • miRNAs • snoRNAs	• Cytosine methylation (m^5C) [10••, 14, 33–35, 36•] • Cytosine hydroxymethylation (hm^5C) [10••, 14, 33–35, 36•] • N^6-adeonine methylation (m^6A) [32–35, 36•] • N^1-adeonine methylation (m^1A) [10••, 33–35, 36•, 37–39] • Pseudouridylation (Ψ) [10••, 33–35, 36•, 37–39] • 2′-O-methylation (2′-O-Me) [10••, 33-35, 36•, 37–39]
Proteome	Proteins such as • Histones • Histone writers/readers/erasers • Chromatin-associated enzymes/factors	• Serine/threonine/tyrosine phosphorylation (Sph/Tph/Yph) [13••, 45••] • Lysine acylation [acetylation (Kac), β-hydroxybutyrylation (Kbhb), crotonylation (Kcr), 2-hydroxyisobutyrylation (Khib), malonylation (Kma), and succinylation (Ksu)] [13••, 45••, 46–49] • Lysine methylation (Kme/Kme2/Kme3) [13••, 45••, 57] • Arginine methylation (Rme/Rme2s/Rme2a) [13••, 45••, 58] • Ubiquitylation (ub) (at M1/K6/K11/K27/K29/K33/K48/K63 position of ubiquitin, also can undergo monoubiquitylation, multi-monoubiquitylation, or homotypic/heterotypic polyubiquitylation) [45••, 53••, 54–56] • O-glcNAcylation (O-glc) [50] • Cysteine glutathionylation (Cglu) [51]
Metabolome	Metabolites such as • Amino acids • Carbohydrates • Hydroxy acids • Lipids • Peptides • Purines • Pyrimidines • Sterols	Modification of metabolites or repairing of damaged metabolites but without creating new metabolic pathways, e.g., • Fatty esters of monohydroxy fatty acids (FAHFAs) [18] • Methylated epimetabolites such as N-methylglycine [60], 1-methylnicotinamide (1MNA) [61], and symmetric and asymmetric dimethylarginine (SDMA and ADMA) [19] • Isomeric variants of epimetabolite such as *L*- or *D*-enantiomer forms of 2-hydroxyglutarate (2HG) [62]

Epitranscriptomic Modifications

Since the first discovery of N^6-adeonine methylation (m^6A) as the most abundant reversible post-transcriptional modification on mRNAs and lncRNAs in eukaryotes [32], an explosion of over 100 different types of chemical modifications have been found in coding and non-coding RNAs [including mRNAs, tRNAs, rRNAs, snRNAs, and ncRNAs (lncRNAs, miRNAs, and snoRNAs)] so far with the use of the latest MS and sequencing technologies [10••, 33–35, 36•]. As mentioned, similar to DNA, RNA can also be cytosine methylated/hydroxymethylated. In addition, other less familiar RNA epimodifications can also occur, including N^1-adeonine methylation (m^1A), pseudouridylation (Ψ), and 2′-O-methylation (2′-O-Me) [All these epimodifications are closely related to RNA stability, structure, translational efficiency, and viral replication] [10••, 37–39]. More recently, it has been suggested the important role of reversible RNA modifications in meiosis and pluripotency [40] as well as in memory formation [41]. For a more detailed perspective of the related information of other RNA epimodifications, we refer the reader to the following online RNA modification databases (RMBase, MODOMICS, and RNAMDB) [42, 43, 44•].

Epiproteomic Modifications

Acetylation, phosphorylation, methylation, and ubiquitylation are among the most abundant and well-known reversible histone PTM marks [45••]. Recently, diverse histone acyl lysine modifications that use intermediates in metabolism have been gradually reported, including lysine β-hydroxybutyrylation (bhb) [46], crotonylation (cr) [47], 2-hydroxyisobutyrylation (hib) [48], malonylation (ma) [49], and succinylation (su) [49] (which are closely related to cellular metabolism and played important roles in regulating histone structure and functions). Other less familiar epiproteomic modifications include histone O-glcNAcylation (O-glc) (important for cell cycle transition) [50], cysteine glutathionylation (glu) (responsible for redox sensing and regulation of chromatin structure) [51], and the latest lysine deacetylimination, which converts the acetyllysine to allysine (as firstly demonstrated in transcription factor STAT3) [52]. As mentioned earlier, besides histones, chromatin-associated enzymes/factors that can write, read, or erase DNA, RNA, or histone protein epi-marks are likely exerting epigenetic effects; therefore, the epimodification status (such as ubiquitylation) and turnover of these chromatin-associated enzymes/factors would likely influent the cellular genetics. Here, we want to address on the complexity of protein ubiquitylation that at such not only eight different types of ubiquitin (ub) linkage can occur (at M1, K6, K11, K27, K29, K33, K48, and K63 position of ub), but the degree of ub-conjugation can also be variable (i.e., whether the target molecule is monoubiquitylated, multi-monoubiquitylated, or homotypically/heterotypically polyubiquitylated) [53••]. For example, the K48 homotypic polyubiquitylation, the most studied type of ub-conjugation, targets protein to proteasomal degradation [54], while K63 homotypic polyubiquitylation is linked to DNA damage/stress response, translation, and lysosomal targeting [55, 56]. Likewise, although not as complex as protein ubiquitylation, lysine can undergo mono/di/tri-methylation (Kme/Kme2/Kme3) [57]; while arginine can undergo mono/dimethylation (Rme/Rme2). Arginine dimethylation can occur in a symmetrical (Rme2s) or asymmetrical (Rme2a) manner [58]. Therefore, close and cautious examination should be undertaken for epiproteomic studies involving ubiquitylation and methylation.

Epimetabolomic Modifications

More recently, studies of identification and elucidation of the biological functions of epimetabolites are emerging. Epimetabolites are produced by modification of metabolites or repairing of damaged metabolites but without creating new metabolic pathways [59]. For instance, several categories of epimetabolites were discovered, including lipid epimetabolites [fatty esters of monohydroxy fatty acids (FAHFAs) with anti-diabetic and anti-inflammatory effects], methylated epimetabolites [N-methylglycine, an oncometabolite; 1-methylnicotinamide (1MNA), acts as a methylation sink in naive embryonic stem cells preventing deposit of H3K27me3 marks; symmetric and asymmetric dimethylarginine (SDMA and ADMA), in which SDMA can be used as a urine biomarker for renal insufficiency, while ADMA levels are significantly associated with an increased risk of coronary artery disease], and isomeric variants of epimetabolite 2-hydroxyglutarate (2HG), an oncometabolite, which exists in *L*- or *D*-enantiomer forms [18, 19, 60–62].

From the above, although the presence of diverse epimodifications/epi-marks have been proven to be existing in each of the four -omes, however, intensive researches would still be needed to dissect the inter-omes–epi-omes relationship as well as their biological function and significance. Since epimodification of the constituents within a particular -ome might directly/indirectly affect the constituents of the other -ome(s)/epi-ome(s), therefore, it is deemed necessary to have the whole epimodification profile of the four -omes in order for us to be able to comprehend the physiological status of the cells at a particular time/event.

Rise of the Epi-drugs

Drugs that target the epi-omes have potential over conventional cancer therapeutic approaches. Many epi-drugs are being developed and undergoing clinical trials. It can be seen that drugs that target the DNMT and various epigenetic modifiers (HDAC, Aurora B, EZH2, IDH1/2, LSD1, SETD2, NSD2, SWI/SNF complex, SMARCA2/4, BRD4, and DOT1L) are being deployed as a strategy for battling against various human diseases [63]. Although genome editing by zinc-finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), or a (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated 9) system can remove or insert genetic elements within the genome [64••], however, the effect is somewhat permanent and is difficult to be reverted. Therefore, epigenetic editing by artificial transcription factors (such as zinc finger-based artificial transcription factors or fusion of designer DNA binding domains to epigenetic writers/erasers) have been examined as novel therapeutics in killing of cancer cells as in general the genomic DNA sequence in these cells are not altered but only the epimodifications/epi-marks in the target chromatin region are manipulated which ultimately fine-tune the gene expression levels by the self, endogenous gene promoters (i.e., whether re-expressing of selected epigenetically silenced tumor suppressor genes or silencing of pro-metastatic genes/oncogenes) [65–67, 68•]. Moreover, natural dietary compounds such as astaxanthin (AST) [69•], phenethyl

isothiocyanate (PEITC) [70], curcumin (CUR) [71], sulforaphane (SFN) [72], resveratrol (RSVL) [73], and epigallocatechin-3-gallate (EGCG) [74] have also been demonstrated to have the ability for epigenetically suppressing cancer cell invasion or proliferation, reactivating tumor suppressors, and enhancing cellular anti-oxidative stress responses, which suggested the beneficial role of dietary intake of these phytochemicals. Last but not the least, epi-drugs in combination with immunotherapeutics has been proposed as a new avenue to improve anticancer efficacy [75]. To sum up, all these studies have provided the potentials of epi-drugs that laid out the foundation and paved the way for future epi-drug research and development.

Conclusions

With the advancement of MS and sequencing technologies, novel epimodifications/epi-marks are gradually revealed in recent years. Given the fact that a variety of epimodifications can occur in DNA/RNA, proteins, and metabolites, it is possible that an unknown number of epimodifications/epi-marks might exist and are yet to be discovered. Nevertheless, it will be an exciting quest for scientists for the ongoing discovery of novel epimodifications and hopefully one day we will conquer these uncharted epi(c) territories and be able to monitor the global epi-omic signature of individuals. In this regard, the ability to decipher and manipulate the epi-omes might present new avenues in drug design for procuring better treatment of various human diseases. Suffice to say, the era of epi-omics research and epi-drug development is on its way.

Acknowledgments This work was supported by the grants from the National Natural Science Foundation of China (Nos. 31170785 and 31271445), the Science and Technology Planning Project of Guangdong Province of China (No. 2016A020215144), the Guangdong Natural Science Foundation of China (No. S2012030006289), "Thousand, hundred, and ten" project of the Department of Education of Guangdong Province (No. 124), the Department of Education, Guangdong Government under the Top-tier University Development Scheme for Research and Control of Infectious Diseases, and the Colleges and Universities Innovation Project of Guangdong Province of China (Nos. 2016KTSCX041 and 2016KTSCX042). We would like to thank members of the **L**au **A**nd **X**u laboratory for critical reading of this manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors have declared no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Salih E. Phosphoproteomics by mass spectrometry and classical protein chemistry approaches. Mass Spectrom Rev. 2005;24(6):828–46. doi:10.1002/mas.20042.
2. Fischer R, Bowness P, Kessler BM. Two birds with one stone: doing metabolomics with your proteomics kit. Proteomics. 2013;13(23–24):3371–86. doi:10.1002/pmic.201300192.
3.• Jordan KW, Cheng LL. NMR-based metabolomics approach to target biomarkers for human prostate cancer. Expert Rev Proteomics. 2007;4(3):389–400. doi:10.1586/14789450.4.3.389. **An expert review on NMR-based metabolomic approaches to target biomarkers for human prostate cancer**.
4.• Bhat AR, Gupta MK, Krithivasan P, Dhas K, Nair J, Reddy RB, et al. Sample preparation method considerations for integrated transcriptomic and proteomic analysis of tumors. Proteomics Clin Appl. 2017;11(3–4) doi:10.1002/prca.201600100. **A technical brief on the choice of the method for optimal extraction of analytes from the clinical tissue specimens for integrated transcriptomic and proteomic analyses**.
5. Carter CW Jr. Histone packing in the nucleosome core particle of chromatin. Proc Natl Acad Sci U S A. 1978;75(8):3649–53.
6. Cedar H. DNA methylation and gene activity. Cell. 1988;53(1):3–4.
7. Mahadevan LC, Willis AC, Barratt MJ. Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. Cell. 1991;65(5):775–83.
8. Simpson RT. Structure of chromatin containing extensively acetylated H3 and H4. Cell. 1978;13(4):691–9.
9. Khoury GA, Baliban RC, Floudas CA. Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. Sci Rep. 2011;1 doi:10.1038/srep00090.
10.•• Roundtree IA, Evans ME, Pan T, He C. Dynamic RNA modifications in gene expression regulation. Cell. 2017;169(7):1187–200. doi:10.1016/j.cell.2017.05.045. **A detailed perspective of the information of the dynamic chemical modifications of coding and non-coding RNA with a focus on introducing the underlying regulatory mechanisms and their biological consequences**.
11. Hamidi T, Singh AK, Chen T. Genetic alterations of DNA methylation machinery in human diseases. Epigenomics. 2015;7(2):247–65. doi:10.2217/epi.14.80.
12. Hu X, Lu X, Liu R, Ai N, Cao Z, Li Y, et al. Histone cross-talk connects protein phosphatase 1alpha (PP1alpha) and histone deacetylase (HDAC) pathways to regulate the functional transition of bromodomain-containing 4 (BRD4) for inducible gene expression. J Biol Chem. 2014;289(33):23154–67. doi:10.1074/jbc.M114.570812.
13.•• Lall S. Primers on chromatin. Nat Struct Mol Biol. 2007;14(11):1110–5. doi:10.1038/nsmb1107-1110. **A detailed summary of histone-modifying enzymes, histone recognition domains, and chromatin remodeling complexes as well as the functions associated with covalent histone modifications**.
14. Chen K, Zhao BS, He C. Nucleic acid modifications in regulation of gene expression. Cell Chem Biol. 2016;23(1):74–85. doi:10.1016/j.chembiol.2015.11.007.
15. Seidler J, Zinn N, Boehm ME, Lehmann WD. De novo sequencing of peptides by MS/MS. Proteomics. 2010;10(4):634–49. doi:10.1002/pmic.200900459.

16. Zheng Y, Huang X, Kelleher NL. Epiproteomics: quantitative analysis of histone marks and codes by mass spectrometry. Curr Opin Chem Biol. 2016;33:142–50. doi:10.1016/j.cbpa.2016.06.007.
17. Gilbert WV, Bell TA, Schaening C. Messenger RNA modifications: form, distribution, and function. Science. 2016;352(6292):1408–12. doi:10.1126/science.aad8711.
18. Yore MM, Syed I, Moraes-Vieira PM, Zhang T, Herman MA, Homan EA, et al. Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell. 2014;159(2):318–32. doi:10.1016/j.cell.2014.09.035.
19. Xuan C, Tian QW, Li H, Zhang BB, He GW, Lun LM. Levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, and risk of coronary artery disease: a meta-analysis based on 4713 participants. Eur J Prev Cardiol. 2016;23(5):502–10. doi:10.1177/2047487315586094.
20. Wei Y, Yu L, Bowen J, Gorovsky MA, Allis CD. Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. Cell. 1999;97(1):99–109.
21. Scott A, Song J, Ewing R, Wang Z. Regulation of protein stability of DNA methyltransferase 1 by post-translational modifications. Acta Biochim Biophys Sin Shanghai. 2014;46(3):199–203. doi:10.1093/abbs/gmt146.
22. Mauer J, Luo X, Blanjoie A, Jiao X, Grozhik AV, Patil DP, et al. Reversible methylation of m6Am in the 5′ cap controls mRNA stability. Nature. 2017;541(7637):371–5. doi:10.1038/nature21022.
23. Sudarsan N, Barrick JE, Breaker RR. Metabolite-binding RNA domains are present in the genes of eukaryotes. RNA. 2003;9(6):644–7.
24. Jurkowska RZ, Jurkowski TP, Jeltsch A. Structure and function of mammalian DNA methyltransferases. Chembiochem. 2011;12(2):206–22. doi:10.1002/cbic.201000195.
25. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;99(3):247–57.
26. Kohli RM, Zhang Y. TET enzymes, TDG and the dynamics of DNA demethylation. Nature. 2013;502(7472):472–9. doi:10.1038/nature12750.
27. Shock LS, Thakkar PV, Peterson EJ, Moran RG, Taylor SM. DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria. Proc Natl Acad Sci U S A. 2011;108(9):3630–5. doi:10.1073/pnas.1012311108.
28.•• van der Wijst MG, van Tilburg AY, Ruiters MH, Rots MG. Experimental mitochondria-targeted DNA methylation identifies GpC methylation, not CpG methylation, as potential regulator of mitochondrial gene expression. Sci Rep. 2017;7(1):177. doi:10.1038/s41598-017-00263-z. **The first study which directly addresses the functionality of mtDNA methylation and identifies GpC methylation, not CpG methylation, as potential regulator of mitochondrial gene expression**.
29. van der Wijst MG, Rots MG. Mitochondrial epigenetics: an overlooked layer of regulation? Trends Genet. 2015;31(7):353–6. doi:10.1016/j.tig.2015.03.009.
30. Heyn H, Esteller M. An adenine code for DNA: a second life for N6-methyladenine. Cell. 2015;161(4):710–3. doi:10.1016/j.cell.2015.04.021.
31.• Sood AJ, Viner C, Hoffman MM. DNAmod: the DNA modification database. bioRxiv. 2016; doi:10.1101/071712. **The first online database to comprehensively catalogue DNA modifications**.
32. Liu N, Pan T. N6-methyladenosine-encoded epitranscriptomics. Nat Struct Mol Biol. 2016;23(2):98–102. doi:10.1038/nsmb.3162.
33. Song J, Yi C. Chemical modifications to RNA: a new layer of gene expression regulation. ACS Chem Biol. 2017;12(2):316–25. doi:10.1021/acschembio.6b00960.
34. Shafik A, Schumann U, Evers M, Sibbritt T, Preiss T. The emerging epitranscriptomics of long noncoding RNAs. Biochim Biophys Acta. 2016;1859(1):59–70. doi:10.1016/j.bbagrm.2015.10.019.
35. Saletore Y, Meyer K, Korlach J, Vilfan ID, Jaffrey S, Mason CE. The birth of the epitranscriptome: deciphering the function of RNA modifications. Genome Biol. 2012;13(10):175. doi:10.1186/gb-2012-13-10-175.
36.• Li X, Xiong X, Yi C. Epitranscriptome sequencing technologies: decoding RNA modifications. Nat Methods. 2016;14(1):23–31. doi:10.1038/nmeth.4110. **A special review on the sequencing technologies used to profile the major mRNA modifications in the transcriptome of eukaryotic cells.**
37. Zhao BS, Roundtree IA, He C. Post-transcriptional gene regulation by mRNA modifications. Nat Rev Mol Cell Biol. 2017;18(1):31–42. doi:10.1038/nrm.2016.132.
38. Li X, Ma S, Yi C. Pseudouridine: the fifth RNA nucleotide with renewed interests. Curr Opin Chem Biol. 2016;33:108–16. doi:10.1016/j.cbpa.2016.06.014.
39. Pereira-Montecinos C, Valiente-Echeverria F, Soto-Rifo R. Epitranscriptomic regulation of viral replication. Biochim Biophys Acta. 2017;1860(4):460–71. doi:10.1016/j.bbagrm.2017.02.002.
40. Klungland A, Dahl JA, Greggains G, Fedorcsak P, Filipczyk A. Reversible RNA modifications in meiosis and pluripotency. Nat Methods. 2016;14(1):18–22. doi:10.1038/nmeth.4111.
41. Walters BJ, Mercaldo V, Gillon CJ, Yip M, Neve RL, Boyce FM, et al. The role of the RNA demethylase FTO (fat mass and obesity-associated) and mRNA methylation in hippocampal memory formation. Neuropsychopharmacology. 2017;42(7):1502–10. doi:10.1038/npp.2017.31.
42. Sun WJ, Li JH, Liu S, Wu J, Zhou H, Qu LH, et al. RMBase: a resource for decoding the landscape of RNA modifications from high-throughput sequencing data. Nucleic Acids Res. 2016;44(D1):D259–65. doi:10.1093/nar/gkv1036.
43. Machnicka MA, Milanowska K, Osman Oglou O, Purta E, Kurkowska M, Olchowik A, et al. MODOMICS: a database of RNA modification pathways—2013 update. Nucleic Acids Res. 2013;41(Database issue):D262–7. doi:10.1093/nar/gks1007.
44.• Cantara WA, Crain PF, Rozenski J, McCloskey JA, Harris KA, Zhang X, et al. The RNA modification database, RNAMDB: 2011 update. Nucleic Acids Res. 2011;39(Database issue):D195–201. doi:10.1093/nar/gkq1028. **A useful online database to comprehensively catalogue RNA modifications**.
45.•• Xu YM, Du JY, Lau AT. Posttranslational modifications of human histone H3: an update. Proteomics. 2014;14(17–18):2047–60. doi:10.1002/pmic.201300435. **A detailed perspective of the information of the overall concepts of histone H3 PTMs as well as discussing all the recently identified yet less well-known PTMs on human histone H3**.
46. Xie Z, Zhang D, Chung D, Tang Z, Huang H, Dai L, et al. Metabolic regulation of gene expression by histone lysine beta-hydroxybutyrylation. Mol Cell. 2016;62(2):194–206. doi:10.1016/j.molcel.2016.03.036.
47. Sabari BR, Tang Z, Huang H, Yong-Gonzalez V, Molina H, Kong HE, et al. Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. Mol Cell. 2015;58(2):203–15. doi:10.1016/j.molcel.2015.02.029.
48. Dai L, Peng C, Montellier E, Lu Z, Chen Y, Ishii H, et al. Lysine 2-hydroxyisobutyrylation is a widely distributed active histone mark. Nat Chem Biol. 2014;10(5):365–70. doi:10.1038/nchembio.1497.
49. Xie Z, Dai J, Dai L, Tan M, Cheng Z, Wu Y, et al. Lysine succinylation and lysine malonylation in histones. Mol Cell Proteomics. 2012;11(5):100–7. doi:10.1074/mcp.M111.015875.
50. Fong JJ, Nguyen BL, Bridger R, Medrano EE, Wells L, Pan S, et al. Beta-N-acetylglucosamine (O-GlcNAc) is a novel regulator of

mitosis-specific phosphorylations on histone H3. J Biol Chem. 2012;287(15):12195–203. doi:10.1074/jbc.M111.315804.
51. Garcia-Gimenez JL, Olaso G, Hake SB, Bonisch C, Wiedemann SM, Markovic J, et al. Histone h3 glutathionylation in proliferating mammalian cells destabilizes nucleosomal structure. Antioxid Redox Signal. 2013;19(12):1305–20. doi:10.1089/ars.2012.5021.
52. Ma L, Huang C, Wang XJ, Xin DE, Wang LS, Zou QC, et al. Lysyl oxidase 3 is a dual-specificity enzyme involved in STAT3 deacetylation and deacetylimination modulation. Mol Cell. 2017;65(2):296–309. doi:10.1016/j.molcel.2016.12.002.
53.•• Kulathu Y, Komander D. Atypical ubiquitylation—the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. Nat Rev Mol Cell Biol. 2012;13(8):508–23. doi:10.1038/nrm3394. **A detailed discussion of the mechanistic insights into linking-specific ubiquitin chain assembly, disassembly, and binding by ubiquitin-binding proteins as well as describing the physiological roles of atypical ubiquitin chains from recent studies**.
54. Kravtsova-Ivantsiv Y, Sommer T, Ciechanover A. The lysine48-based polyubiquitin chain proteasomal signal: not a single child anymore. Angew Chem Int Ed Engl. 2013;52(1):192–8. doi:10.1002/anie.201205656.
55. Sun L, Chen ZJ. The novel functions of ubiquitination in signaling. Curr Opin Cell Biol. 2004;16(2):119–26. doi:10.1016/j.ceb.2004.02.005.
56. Duncan LM, Piper S, Dodd RB, Saville MK, Sanderson CM, Luzio JP, et al. Lysine-63-linked ubiquitination is required for endolysosomal degradation of class I molecules. EMBO J. 2006;25(8):1635–45. doi:10.1038/sj.emboj.7601056.
57. Martin C, Zhang Y. The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol. 2005;6(11):838–49. doi:10.1038/nrm1761.
58. Bedford MT, Richard S. Arginine methylation an emerging regulator of protein function. Mol Cell. 2005;18(3):263–72. doi:10.1016/j.molcel.2005.04.003.
59. Showalter MR, Cajka T, Fiehn O. Epimetabolites: discovering metabolism beyond building and burning. Curr Opin Chem Biol. 2017;36:70–6. doi:10.1016/j.cbpa.2017.01.012.
60. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature. 2009;457(7231):910–4. doi:10.1038/nature07762.
61. Sperber H, Mathieu J, Wang Y, Ferreccio A, Hesson J, Xu Z, et al. The metabolome regulates the epigenetic landscape during naive-to-primed human embryonic stem cell transition. Nat Cell Biol. 2015;17(12):1523–35. doi:10.1038/ncb3264.
62. Intlekofer AM, Dematteo RG, Venneti S, Finley LW, Lu C, Judkins AR, et al. Hypoxia induces production of L-2-hydroxyglutarate. Cell Metab. 2015;22(2):304–11. doi:10.1016/j.cmet.2015.06.023.
63. Salarinia R, Sahebkar A, Peyvandi M, Mirzaei HR, Jaafari MR, Riahi MM, et al. Epi-drugs and Epi-miRs: moving beyond current cancer therapies. Curr Cancer Drug Targets. 2016;16(9):773–88.
64.•• Gaj T, Gersbach CA, Barbas CF 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013;31(7):397–405. doi:10.1016/j.tibtech.2013.04.004. **A brief overview of the therapeutic potential of ZFNs, TALENs, and CRISPR/Cas-based RNA-guide DNA endonucleases**.
65. Stolzenburg S, Rots MG, Beltran AS, Rivenbark AG, Yuan X, Qian H, et al. Targeted silencing of the oncogenic transcription factor SOX2 in breast cancer. Nucleic Acids Res. 2012;40(14):6725–40. doi:10.1093/nar/gks360.
66. Huisman C, van der Wijst MG, Falahi F, Overkamp J, Karsten G, Terpstra MM, et al. Prolonged re-expression of the hypermethylated gene EPB41L3 using artificial transcription factors and epigenetic drugs. Epigenetics. 2015;10(5):384–96. doi:10.1080/15592294.2015.1034415.
67. Chen H, Kazemier HG, de Groote ML, Ruiters MH, Xu GL, Rots MG. Induced DNA demethylation by targeting ten-eleven translocation 2 to the human ICAM-1 promoter. Nucleic Acids Res. 2014;42(3):1563–74. doi:10.1093/nar/gkt1019.
68.• de Groote ML, Verschure PJ, Rots MG. Epigenetic editing: targeted rewriting of epigenetic marks to modulate expression of selected target genes. Nucleic Acids Res. 2012;40(21):10596–613. doi:10.1093/nar/gks863. **A survey and summary on epigenetic editing by targeted DNA methylation editors and targeted repressive/activating histone modifying enzymes**.
69.• Yang Y, Fuentes F, Shu L, Wang C, Pung D, Li W, et al. Epigenetic CpG methylation of the promoter and reactivation of the expression of GSTP1 by astaxanthin in human prostate LNCaP cells. AAPS J. 2017;19(2):421–30. doi:10.1208/s12248-016-0016-x. **This study demonstrates the role of astaxanthin in restoring the expression of Nrf2 and GSTP1 through epigenetic modification in human prostate LNCaP cells**.
70. Zhang C, Shu L, Kim H, Khor TO, Wu R, Li W, et al. Phenethyl isothiocyanate (PEITC) suppresses prostate cancer cell invasion epigenetically through regulating microRNA-194. Mol Nutr Food Res. 2016;60(6):1427–36. doi:10.1002/mnfr.201500918.
71. Guo Y, Shu L, Zhang C, Su ZY, Kong AN. Curcumin inhibits anchorage-independent growth of HT29 human colon cancer cells by targeting epigenetic restoration of the tumor suppressor gene DLEC1. Biochem Pharmacol. 2015;94(2):69–78. doi:10.1016/j.bcp.2015.01.009.
72. Zhang C, Su ZY, Khor TO, Shu L, Kong AN. Sulforaphane enhances Nrf2 expression in prostate cancer TRAMP C1 cells through epigenetic regulation. Biochem Pharmacol. 2013;85(9):1398–404. doi:10.1016/j.bcp.2013.02.010.
73. Dhar S, Kumar A, Rimando AM, Zhang X, Levenson AS. Resveratrol and pterostilbene epigenetically restore PTEN expression by targeting oncomiRs of the miR-17 family in prostate cancer. Oncotarget. 2015;6(29):27214–26. doi:10.18632/oncotarget.4877.
74. Borutinskaite V, Virksaite A, Gudelyte G, Navakauskiene R. Green tea polyphenol EGCG causes anti-cancerous epigenetic modulations in acute promyelocytic leukemia cells. Leuk Lymphoma. 2017:1–10. doi:10.1080/10428194.2017.1339881.
75. Mazzone R, Zwergel C, Mai A, Valente S. Epi-drugs in combination with immunotherapy: a new avenue to improve anticancer efficacy. Clin Epigenetics. 2017;9:59. doi:10.1186/s13148-017-0358-y.