SCIENCE CHINA
Chemistry

•REVIEWS•

April 2018 Vol.61 No. 4: 381–392
https://doi.org/10.1007/s11426-017-9186-y

Modificaomics: deciphering the functions of biomolecule modifications

Ting Liu, Cheng-Jie Ma, Bi-Feng Yuan* & Yu-Qi Feng

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China

Received October 11, 2017; accepted December 1, 2017; published online January 17, 2018

The spatiotemporal expression of genes is sophisticatedly controlled through three main layers: transcriptional, translational and post-translational. Now increasing chemical modifications are discovered on genomic DNA, RNA and proteins. These modifications are recognized as additional layer of regulatory mechanisms in controlling gene expression that defines cell status. So far, more than 150 chemical modifications are identified in nucleic acids, and more than 400 discrete types of modifications are identified in proteins. How these modifications are interpreted are fundamental questions to our understanding of living organisms. The omics sciences of systems biology, including genomics, transcriptomics, proteomics, and metabolomics, have been in existence for decades. Due to the large numbers of modifications occurring in DNA, RNA and proteins with regulatory roles, we propose the modificaomics from the words of modification and omics. Modificaomics mainly refers to the comprehensive study of the modifications on DNA, RNA and proteins. In this review, we conceive modificaomics by introducing the discovered modifications in DNA, RNA and proteins as well as summarizing their biological functions. We hope the proposed modificaomics can provide a whole picture of modifications of these biopolymers and simulate the study of the functions of the modifications on DNA, RNA and proteins.

modificaomics, DNA modifications, RNA modifications, protein modifications, functions

Citation: Liu T, Ma CJ, Yuan BF, Feng YQ. Modificaomics: deciphering the functions of biomolecule modifications. *Sci China Chem*, 2018, 61: 381–392, https://doi.org/10.1007/s11426-017-9186-y

1 Introduction

In a single living organism, all somatic cells have the same genetic information contained within the DNA sequence from the zygote although they differ greatly in their forms and functions [1]. The formation of various cell types is mainly due to the differentiated temporal and spatial expression of genes, which is precisely controlled through three main layers: transcriptional, translational and post-translational [2–4].

Genetic information flows from DNA to RNA and then to proteins in the central dogma of molecular biology. Modifications occurring on genomic DNA, RNA and proteins play important roles in controlling gene expression (Figure 1). The modifications generally do not change the sequence of these biopolymers, but modify their physical and biochemical properties, and eventually lead to multiple physiological functions [5].

So far, many chemical modifications of these three biopolymers have been described. In recent years, increasing modifications on DNA, RNA and proteins have been discovered. In 2009, 5-hydroxymethylcytosine (5hmC), an oxidized derivative of 5-methylcytosine (5mC), was discovered in mammalian genome and ten-eleven translocation (TET) proteins were identified as the enzymes for converting 5mC to 5hmC [6,7]. Subsequent studies revealed that TET

*Corresponding author (email: bfyuan@whu.edu.cn)

proteins can further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxycytosine (5caC) [8,9]. These studies established the biochemical mechanism of active DNA demethylation in mammals. In addition to DNA, the diverse functions of RNA are accompanied by more than 150 chemical modifications, although the functions of most of these RNA modifications remain elusive [10]. As for protein, more than 400 discrete types of modifications and more than 90000 individual modifications have been identified [11].

The large numbers of modifications discovered in DNA, RNA and proteins emerge for important roles. The omics sciences of systems biology, including genomics, transcriptomics, proteomics, and metabolomics, have been in existence for decades [12]. Here we propose the modificaomics from the words of modification and omics. In this review, we conceive modificaomics by introducing the discovered modifications in DNA, RNA and proteins as well as summarizing their biological functions.

2 DNA modifications

2.1 DNA cytosine methylation

DNA, RNA and proteins all can be chemically modified by methylation, which consists of the addition of a methyl group ($-CH_3$) to different acceptor sites. Methylation at the C5 position of cytosine to give 5mC is one of the best-characterized epigenetic modifications in DNA and is well conserved among fungus, plant and animal (Figure 2) [13,14].

5mC occurs primarily at CpG dinucleotides in vertebrates, which is established by two de novo methyltransferases, DNMT3A and DNMT3B, and is maintained by DNMT1 [15]. 5mC modification involves in diverse physiological functions including maintenance of chromosomal integrity,

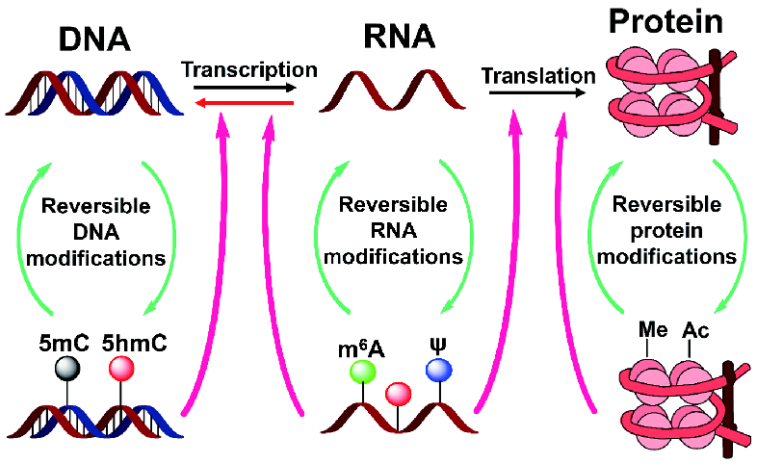

Figure 1 Reversible chemical modifications in DNA, RNA and proteins that regulate the flow of genetic information (color online).

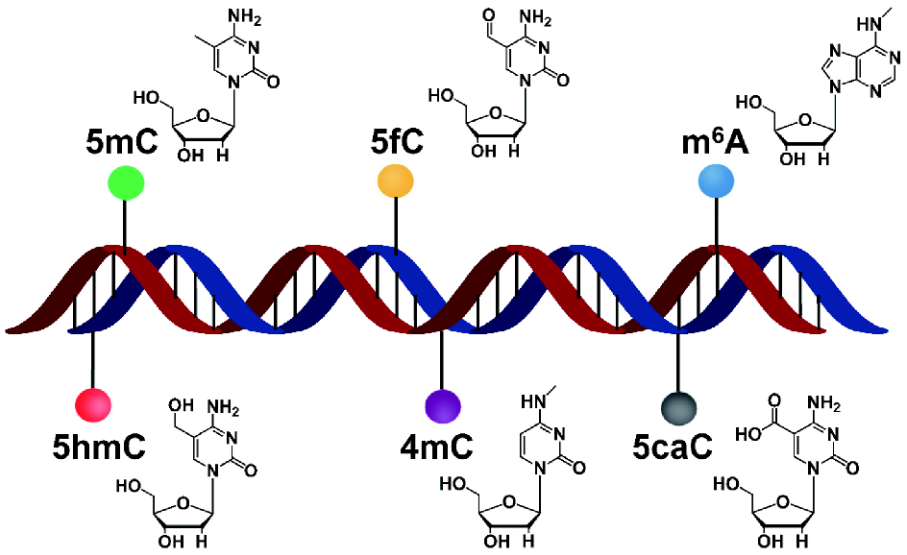

Figure 2 Schematic illustration of the modifications in DNA (color online).

transcriptional suppression of genes, and X-chromosome inactivation [15]. Aberrant DNA cytosine methylation is a well-recognized hallmark of many human diseases [16,17].

5mC modification is reversible and dynamic, however, the enzymes responsible for demethylation of 5mC were unknown until the recent discovery of TET family of dioxygenases [6–9]. It was found that the TET proteins were capable of converting 5mC to generate 5hmC, 5fC, and finally to 5caC (Figure 2) [6–9]. The 5fC and 5caC can be cleaved by thymine-DNA glycosylase followed by base-excision repair to restore unmethylated cytosine [9]. These identified novel DNA modifications were also found to have regulatory roles in multiple physiological processes [18–22]. Ficz *et al*. [18] found that declining levels of 5hmC at the promoters of embryonic stem cell-specific genes during differentiation. They then proposed that the balance between 5hmC and 5mC in the genome is tightly linked with the balance between pluripotency and differentiation. In addition, Kellinger *et al*. [19] observed that 5fC and 5caC can reduce the rate and substrate specificity of RNA polymerase II transcription, which provides new insights into potential functional interplay between cytosine modification status and transcription.

In addition to 5mC, N^4-methylcytosine (4mC) was also found to be present in bacterial genomic DNA [23], with more prevalent of 4mC occurring in thermophilic bacteria [24]. 4mC modification serves as the part of bacterial restriction-modification systems. Recently, Yu *et al*. [25] developed 4mC-Tet-assisted bisulfite-sequencing (4mC-TAB-seq) to map 4mC sites in bacterial genome. With 4mC-TAB-seq method, both cytosines and 5mCs are read out as thymines, whereas 4mCs are read out as cytosines, which then can be used to accurately locate the positions of 4mCs in genome. Three 4mC-containing motifs and six 5mC-containing motifs in *C. kristjanssonii* were identified by 4mC-TAB-seq in combination with MethylC-seq analysis [25], which may assist in genetic engineering of the bacteria and enhance the ability of bacteria to convert biomass into biofuels.

2.2 DNA adenine methylation

In addition to DNA cytosine methylation, DNA adenine methylation (N^6-methyladenine, m^6A) is another covalent modification of DNA that exerts essential roles for the viability of some bacteria (Figure 2) [26]. Reisenauer *et al*. [26] found that the status of DNA adenine methylation was important in control of cell cycle. They demonstrated that the pattern of m^6A in DNA affected the cell cycle by altering the expression of the CtrA response regulator. The function of m^6A is also associated with the protection of DNA from the cleavage of endonucleases via the restriction-modification system [27]. The presence of m^6A in the host prevents the digestion of its genome by DNA methylation-sensitive restriction enzymes. On the contrary, foreign unmethylated DNA will be readily degraded [27].

Accumulating data suggest that the presence of m^6A is not limited to bacterial DNA but also occurs in eukaryotic cells [28]. Recent studies further confirmed the presence of m^6A in various eukaryotic genomes including *Chlamydomonas reinhardtii*, *Caenorhabditis elegans*, *Drosophila melanogaster*, Zebrafish, and mammals with putative epigenetic functions [29–35]. These reports present evidence for the regulatory roles of m^6A during development, suggesting m^6A as an epigenetic mark in addition to 5mC. But Schiffers *et al*. [36] recently questioned the existence of m^6A in mammals and reported no evidence for m^6A in the genome of mouse embryonic stem cells and tissues. Due to the low *in-vivo* abundance of m^6A, development of improved technologies to characterize m^6A in different biological contexts will be necessary to fully elucidate the existence and functions of m^6A in DNA.

3 RNA modifications

RNA is the intermediate molecule that links genetic information from DNA to functional proteins. An additional regulatory layer of biology between DNA and proteins has been proposed with the advance on the functional studies of RNA modifications. Cellular RNA contains more than 150 structurally distinct post-transcriptional modifications [10], which are considered to be dynamic, reversible and can fine-tune the structures and functions of RNA to influence gene expression [37].

3.1 mRNA modifications

The recent discovery of reversible modifications on mRNA has opened a new realm of post-transcriptional gene regulation in eukaryotes. So far, more than 17 types of modifications were identified in eukaryotic mRNA, including m^6A, N^6,2′-*O*-dimethyladenosine (m^6Am), N^6,N^6,2′-*O*-trimethyladenosine ($m^6{}_2Am$), 7-methylguanosine (m^7G), N^2,7-dimethylguanosine ($m^{2,7}G$), N^2,N^2,7-trimethylguanosine ($m^{2,2,7}G$), inosine (I), 2′-*O*-methyladenosine (Am), 2′-*O*-methylcytidine (Cm), 2′-*O*-methylguanosine (Gm), 2′-*O*-methyluridine (Um), 3,2′-*O*-dimethyluridine (m^3Um), N^1-methyladenosine (m^1A), 5mC, 5hmC, pseudouridine (Ψ), and 3-methylcytidine (m^3C) (Figure 3(a)) [10,38–44].

3.1.1 RNA adenine methylation

m^6A is present in nearly all RNA types from bacteria to human, and it is considered to be the most abundant internal modification in eukaryotic mRNA [45,46].

Recent identification of RNA methylases and demethy-

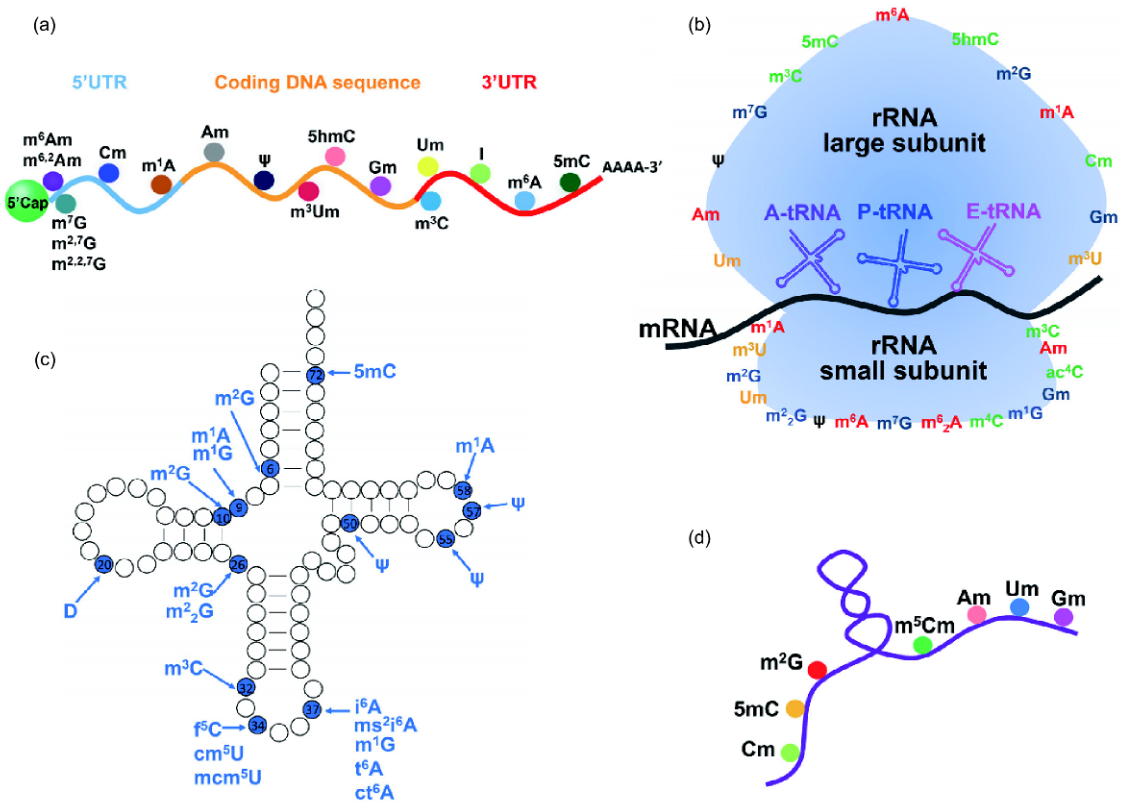

Figure 3 Schematic illustration of the modifications in mRNA (a), rRNA (b), tRNA (c), and small RNA (d) (color online).

lases further revealed the biological functions of m^{6}A. In 2011, He's group [47] found that fat mass and obesity associated (FTO) knockdown led to the increased content of m^{6}A in mRNA, and overexpression of FTO resulted in decreased content of m^{6}A. This study demonstrated that m^{6}A in nuclear RNA is the physiological substrate of FTO and m^{6}A modification is reversible and dynamic. In 2012, two groups combined anti-m^{6}A immunoprecipitation and deep-sequencing method and identified m^{6}A sites in mammalian mRNA [48,49]. Later, UV light-induced antibody-RNA cross-linking followed by reverse transcription enabled single-nucleotide-resolution mapping of m^{6}A in the human transcriptome [50]. m^{6}A was found in >25% of transcripts of human cells and these m^{6}A sites are mainly enriched in long exons, near stop codons and in 3′ untranslated regions (UTRs).

Functional studies on methyltransferases, demethylases and m^{6}A-binding proteins demonstrate that m^{6}A in mRNA is involved in multiple life processes [51]. Co-localization of m^{6}A methyltransferase components and RNA polymerase II suggested that m^{6}A occurred while pre-mRNA was being transcribed [52]. Knockdown of m^{6}A methyltransferase can cause alternative polyadenylation [53]. Decrease of m^{6}A methyltransferase component of METTL3 can delay mRNA nuclear export [54], whereas depletion of m^{6}A demethylase of ALKBH5 enhanced the transfer of mRNA from nuclear to cytoplasm [55], suggesting RNA adenine methylation can promote mRNA nuclear export. Collectively, m^{6}A has been demonstrated to influence mRNA mature, RNA folding and structure, nuclear export, mRNA decay, and translation [51].

Recent studies also demonstrated that the RNA adenine methylation status was critical for shaping cell states and appropriate distribution of m^{6}A was required for stem cells to properly differentiate to specific lineages [56]. In addition, emerging evidences showed that m^{6}A modification as well as its regulatory proteins (methyltransferases, demethylases and binding proteins) also played important roles in various cancers including leukemia, diabetes, brain tumor, and breast cancer [57,58]. For example, reduction of m^{6}A levels resulted in enhanced growth and promoted the ability of glioblastoma stem-like cells to form brain tumors [59]. m^{6}A demethylase of FTO has been demonstrated to play an oncogenic role in leukemia [60], and m^{6}A demethylase of ALKBH5 has been reported to exert a tumor-promoting function in glioblastoma and breast cancer [61,62]. Therefore, development of inhibitors for targeting m^{6}A regulatory proteins may provide an effective therapeutic strategy for cancers.

m^{6}A is also discovered to be closely related to circadian rhythm in mammals [54]. m^{6}A sites were found to be pre-

valent on transcripts of many clock genes. Inhibition of m^6A formation via knockdown of METTL3 led to delay of pre-mRNA processing and elongation of circadian period [54]. Similarly, the cell cycle is an oscillating process that is coupled with the circadian rhythm [63]. A notable shift in cell cycle duration following perturbation of m^6A in mRNAs was reported in mouse embryonic stem cells [64]. These studies demonstrated the essential roles of m^6A on the regulation of circadian clock.

m^1A, another RNA adenosine methylation, is recently discovered also present in eukaryotic mRNA [38,39]. Since m^1A contains a methyl group at N1 position that can disturb Watson-Crick base pairs, m^1A can cause reverse transcription stop and also cause misincorporation. Based on this, Dominissini *et al*. [39] and Li *et al*. [38] developed transcriptome-wide sequencing methods to identify and map m^1A in mRNA. These studies revealed that m^1A is reversible and mainly enriched around start codon in eukaryotic mRNA. Further identification and characterization of m^1A-binding proteins will facilitate the in-depth understanding of the functional roles of m^1A.

In addition to m^6A and m^1A, N^6,2′-*O*-dimethyladenosine (m^6Am) was recently identified to be a dynamic and reversible modification in the 5′ cap of mRNA and can influence cellular mRNA stability [65]. m^6Am-initiated transcripts are more stable than mRNAs that begin with other nucleotides, which is due to resistance to the mRNA-decapping enzyme DCP2. Moreover, FTO preferentially demethylates m^6Am rather than m^6A. Both m^6Am and m^6A in the 5′ UTR are related to increased translation, suggesting the location and specific combination of modified adenosine have distinct functional consequences on mRNA [65].

3.1.2 *RNA cytosine methylation*

5mC is well studied in DNA, while its existence in cellular RNA has been mainly confined to tRNA and rRNA. Recently, Squires *et al*. [66] developed an approach termed bsRNA-seq (sequencing of whole bisulfite converted transcriptomes) to map 5mC sites in HeLa cells. With this developed bsRNA-seq method, 10275 5mC sites were discovered in mRNAs and other non-coding RNAs. In addition, 5mC sites in mRNA were found to be mainly enriched in the untranslated regions and near Argonaute binding regions. This study provided the first global mapping of 5mC in the human transcriptome, suggesting broad roles of 5mC in post-transcriptional gene regulation and control of cellular RNA functions.

Expression of mRNA in cells was found to be affected by the presence of 5mC [67]. Warren *et al*. [68] established an efficient strategy to generate induced pluripotent stem cells by transfection of pseudouridine and 5mC-substituted mRNAs that encode the Yamanaka transcription factors, suggesting modifications in mRNA can trigger marked biological responses.

5mC in RNA can be further converted to 5hmC, 5fC, and 5caC by Tet family enzymes [42,43,69,70]. Fuks *et al*. [41] recently demonstrated that 5hmC existed in polyadenylated RNAs in Drosophila. They revealed 5hmC in the transcripts of many genes, notably in coding sequences, and identified consensus sites for 5hmC. 5hmC in RNA was considered to favor mRNA translation and played certain roles in brain development. These studies demonstrated the functional significance of 5mC as well as its oxidative products are critical for normal cellular processes.

In addition to 5mC, Xu *et al*. [44] recently reported the discovery and characterization of m^3C modification in mRNA of mice and human. They further found that methyltransferase-like (METTL) 8 was responsible for this m^3C modification in mRNA by biochemical and genetic analyses in Mettl8 null-mutant mice and two human METTL8 mutant cell lines. Their findings provide the evidence of the existence of m^3C modification in mRNA and more work is needed to reveal the functions of m^3C by investigating the distribution and dynamics of m^3C in mRNA.

3.1.3 *Pseudouridine*

Pseudouridine (Ψ), one of the most prevalent post-transcriptional RNA modifications, has been shown to increase the stability of tRNAs and ribosomal RNAs. Recently, the transcriptome-wide study revealed Ψ modification is highly abundant in mRNAs of yeast and human cells [40,71,72]. Ψ modification was found to be dynamic and reversible in mRNA. This modification acts to increase the diversity of the genetic code and can affect the translation, mRNA stability and RNA localization. However, the mechanism still requires further investigation.

RNA modifications can affect interactions between RNA-binding proteins and target RNA. RNA modifications have been found to be widely prevalent in the binding sites of many different RNA-binding proteins [73]. Recent study showed that Ψ and m^6A can weaken the binding of the human single-stranded RNA binding protein Pumilio 2 (hPUM2) by two- to three-fold per modification on average [74]. In addition, deLorimier *et al*. [75] also found that Ψ modifications can shift YGCY (Y represents pyrimidine) motifs into conformations with increased base stacking that reduce binding by MBNL (Muscleblind-like) RNA-binding proteins. These results demonstrated an additional layer of complexity in RNA-protein interaction networks and underscored the importance of RNA modifications on the regulation of gene expression by affecting the affinity between RNA and RNA-binding proteins.

3.1.4 *2′-O-methylation*

2′-*O*-methylated nucleosides are ubiquitous in rRNA, tRNA, mRNA, snRNA and microRNA, and 2′-*O*-methylation is

essential for the biogenesis, metabolism and functions of RNA [76]. RNA with 2′-*O*-methylation show increased resistance to degradation and stabilize helices [77]. Recently, He's group [78] developed a sensitive method for transcriptome-wide mapping of 2′-*O*-methylation with base precision by using the differential reactivity of 2′-*O*-methylated and 2′-hydroxylated nucleosides toward periodate oxidation. This study uncovered thousands of 2′-*O*-methylation sites in mammalian mRNA with most 2′-*O*-methylation sites being found in CDS.

3.2 rRNA modifications

Ribosomal RNAs constitute the structural framework of ribosomes and play indispensable roles in translation. Numerous modification types have been found to locate in rRNAs with different sequence and structural contexts (Figure 3(b)). In *E. coli*, 36 modified nucleosides were discovered in rRNA. And more than 100 and 200 modifications were identified in yeast and human rRNA, respectively [79]. 2′-*O*-methylation and Ψ are the most abundant rRNA modifications in eukaryotes. So far, 55 2′-*O*-methylation sites and 45 Ψ sites have been identified in the rRNAs of *S. cerevisiae* and about 100 of 2′-*O*-methylation and Ψ sites are reported in human rRNAs [80].

Most of these identified modifications in rRNA generally have conserved and distinctive spatial distributions [81]. Modifications in rRNA typically cluster in functionally important regions including the decoding and tRNA binding sites (the A-, P- and E-sites), the peptidyltransferase center and the interface of subunits of rRNA [82]. The modified rRNA motifs participate in various ligand interactions with RNA, proteins, and small molecules [80].

The natural modifications can impact thermal stability, dynamic behavior, and structures of RNA, suggesting rRNA modifications play important roles in regulating ribosome function [82]. Some modifications in rRNA serve to stabilize its secondary and tertiary structures [83]. In this respect, 2′-*O*-methylation can stabilize helices by increasing base-stacking. Similarly, Ψ modification offers greater hydrogen bonding interaction than uridine and increases the rigidity of the sugar-phosphate backbone [84]. *N*7-methylguanine modification carries positive charge thereby promoting ionic interactions between RNAs and RNA-binding proteins, and 5mC increases the stability of base-pairing with guanine [85].

Many rRNA modifications have fine-tuning roles in regulation of ribosomal activity [86]. In fact, some antibiotics exert their effects on protein synthesis by binding to modified regions of rRNAs [87]. Studies in *E. coli* and yeast showed that modifications in rRNA presented in functionally important regions with a critical role in the regulation of translation [81]. Alteration of Ψ modifications in rRNA has been reported to affect translation initiation on specific mRNAs by altering the affinity of the ribosome for these mRNA structures [88]. Methylation in rRNAs of plant can regulate rRNA stability by maintaining ribosomal function during temperature change [89]. However, the structural and biological consequences of many natural modifications, as well as the dynamic mechanism in rRNAs remain elusive and further in-depth investigation is required.

3.3 tRNA modifications

tRNAs have the highest density of modifications among all types of RNA [90]. Around 90 different types of modifications were found in various tRNAs (Figure 3(c)) [91]. Some modifications are present in nearly all tRNAs, such as dihydrouridine (D) and Ψ, whereas others are only present in a single tRNA [92].

Modifications in tRNA serve as important regulatory elements for the control of protein synthesis [93]. Some modifications in tRNA influence the codon-anticodon binding affinity, which regulates the speed and fidelity of translation [94]. Modification-mediated strengthening of codon-anticodon binding also prevents frame-shift errors [95]. In tRNA without modifications, the anticodon loop is typically flexible and collapses into a structure with unwanted base pairs. However, the collapse can be eliminated by modifications of the conserved purine residue at position 37, which reinforces the rigid and ordered functional anticodon conformation [96]. In fact, position 37 of tRNA is frequently modified, such as wybutosine (yW), threonylcarbamoyladenosine (t^6A), and 2-methylthio-N^6-isopentenyladenosine ($ms2i^6A$) [97]. In addition, modifications at position 37 of tRNA can improve the base stacking [98], which also contributes to the reduced structural flexibility and stabilizes the loop structure. Loss of stabilizing effect due to deficiencies in modifications can be pathologic and result in severe diseases, such as nonsyndromic X-linked intellectual disability and type II diabetes [99,100].

tRNA modifications were also demonstrated to control tRNA cleavage [101]. tRNA-derived fragments that play critical roles in regulation of various cellular functions [102], are the tRNA cleavage products [103]. Previous studies showed that tRNA modifications can regulate the formation of tRNA-derived fragments. For instance, 5mC38 in $tRNA^{Asp(GTC)}$, $tRNA^{Gly(GCC)}$ and $tRNA^{Val(AAC)}$ can protect tRNAs from cleavage, thus promoting global protein translation and differentiation [101]. In yeast cells, the 5-methylcarboxymethyluridine (mcm^5U) modification in tRNAs confers resistance to cleavage by PaT [104]. In mammalian cells, lack of methylation caused by UV light exposure resulted in cleavage of tRNA by angiogenin and the cleavage could be inhibited by tRNA methyltransferase NSun2 [105] or by DNA methyltransferase 2 (DNMT2) [106].

Modifications in and around the anticodon of tRNA can selectively alter the spectrum of proteins. Modifications at the wobble position 34 of tRNA increase the capabilities of tRNA to decode multiple synonymous mRNA codons differing by the third nucleoside [92]. And specific modifications on tRNAs have been reported to be closely related to cell growth rates [107], temperature [108], and oxidation [109]. Altering the frequency of tRNA modifications can change the stability of tRNAs, which induces tRNA to be degraded or stabilized under specific cellular conditions and allows the cell to rapidly response to environmental changes [110]. The abnormal tRNA modifications have been reported to be related to various diseases, including cancers and neurological disorders [99].

Mitochondrial tRNA also undergoes numerous post-transcriptional modifications [111]. A recent example of a newly discovered tRNA modification frequently found at position 37 is a cyclic form of N^6-threonylcarbamoyladenosine (ct^6A) [112], which is required for correct decoding of ANN codons. N^6-threonylcarbamoyladenosine (t^6A) might be an artifact resulting from hydrolysis of the native ct^6A in the conditions used for nucleoside isolation. In physiological conditions, ct^6A shows remarkable stability [113].

As for the tRNA modifications, many details remain to be understood, including the dynamics of tRNA modifications under physiological and pathological conditions. In addition, it will be important to reveal the dynamics and the molecular mechanisms of tRNA modifications. Integrative approaches including mass spectrometry and high-throughput sequencing may provide novel opportunities for interrogation of the mechanisms.

3.4 Small RNA modifications

Emerging evidence shows that small RNAs, such as microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs) and tRNA-derived small RNAs (tsRNAs), harbor a diversity of modifications (Figure 3(d)) [114]. Many modifications in small RNAs involve in the modulation of RNA stability and other complex physiological processes including metabolism, immunity, and epigenetic inheritance [114].

miRNAs are key players in RNA silencing pathways. 2′-*O*-methylation was first recognized in plants and found to protect miRNAs from decay [115,116]. Although mature miRNAs in animals lack 2′-*O*-methylation, other mammalian small RNAs such as siRNAs and piRNAs can be methylated by HUA enhancer 1 orthologs, which similarly protect these small RNAs from 3′-uridylation [116].

Recent discoveries have also revealed important roles for 5mC in RNA-mediated transgenerational epigenetic inheritance [117]. This study demonstrated 5mC in intergenerational epigenetic transmission of metabolic alterations arising from paternal dietary exposures. tsRNAs were shown to exhibit elevated 5mC and m^2G levels in high-fat diets sperm of mice compared with normal diets [117]. Compared to synthetic tsRNAs that lack RNA modifications, endogenous tsRNAs are more stable, suggesting possible roles of modifications in promoting the stability and transgenerational effects of tsRNAs.

Emerging evidence also indicates that modifications in small RNAs are important molecular markers that can alter the biogenesis, function, and informational capacity of small RNAs. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS) platform, Yan *et al*. [118] uncovered a diversity of modifications in small RNAs of mouse liver and revealed dynamic changes for many RNA modifications in mouse models of diabetes (Gm, m^5Cm, Cm, Am, Um). However, the study of small RNA modifications in biology and disease remains in its infancy. Elucidating the role of modified small RNAs in pathogeny will provide new opportunities for diagnosis of small RNA-associated diseases.

4 Protein modifications

Post-translational modifications (PTMs) of proteins that generally refer to the addition of a functional group to proteins are essential for the regulation of protein activity, folding, stability and binding partners [119]. Elucidating the roles of PTMs of proteins has had a major impact on the understanding of cellular signaling, apoptosis and cancer biology.

4.1 Histone modifications

Histones are highly modified by a variety of post-translational modifications, such as methylation, acetylation, phosphorylation, ubiquitination, sumoylation, butyrlyation and biotinylation (Figure 4) [4]. These PTMs on histones are important epigenetic marks and serve as dynamic control that participates in a wide range of biological development and differentiation processes [4,120].

Histone PTMs can perturb the interaction of histones with DNA or other chromatin factors, and change the affinity of binding proteins that recognize the modifications [120]. Methylation is the most prevalent modification on histones that typically occurs on lysine, arginine and histidine. Analysis of the histone methylation demonstrated that trimethyl H3K4, H3K36 and H3K79 typically correlated with transcriptionally active genes, and trimethyl H3K9, H3K27 and H4K20 normally correlated with transcriptionally inactive genes [121,122].

Histone modifications have been considered to involve in DNA repair, cellular reprogramming, differentiation, pluripotency, and cancers [120]. Recently it has been demonstrated that the DNA methylation and histone modifications

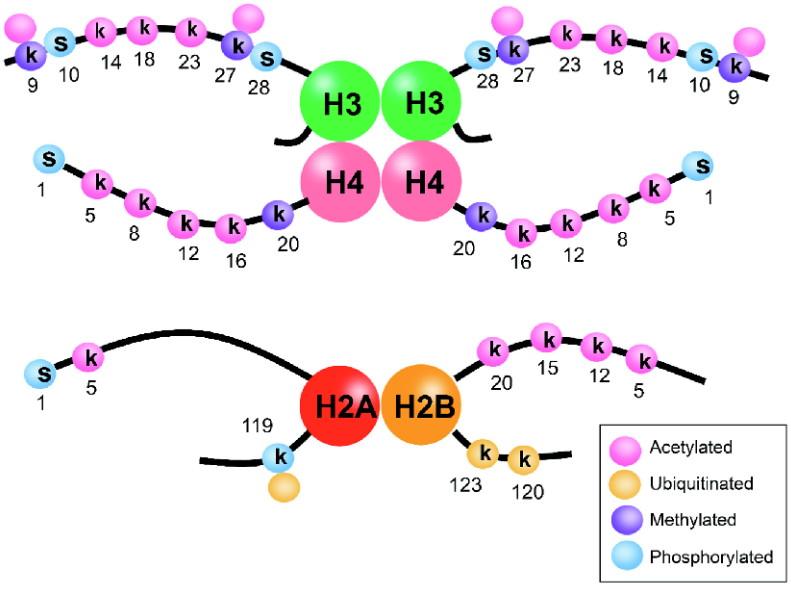

Figure 4 Schematic illustration of the modifications in histones (color online).

are highly interrelated for chromatin functions [123]. The accumulated knowledge on the more and more discovered histone modifications will further improve our understanding of the roles of histone modifications as well as their coordination with DNA modifications in chromatin compaction and transcription.

4.2 Other protein modifications

In addition to histones modifications, numerous other proteins also undergo various PTMs, which increas the functional diversity of the proteins. PTMs can occur on the amino acid side chains or at the C- or N- termini of proteins and extend the chemical repertoire of the 20 standard amino acids [124]. The identified PTMs on proteins include phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, and lipidation, which influence various aspects of normal cell biology and pathogenesis [124]. Sites that frequently undergo PTMs are those that have groups serving as a nucleophile, such as the hydroxyl groups of serine, threonine, and tyrosine; the amine groups of lysine, arginine, and histidine; the thiolate anion of cysteine; the carboxylates of aspartate and glutamate [125].

Most of the PTMs on proteins are dynamic and reversible. PTMs change local physical and chemical properties of proteins, which therefore alter the charge, hydrophobicity, and flexibility of proteins, eventually leading to the alteration of the functions of proteins. Phosphorylation is a very common mechanism for regulating the activities of many enzymes [126]. For example, kinases that phosphorylate proteins at certain amino acid side chains can activate or inactivate enzymes [127]. Conversely, phosphatases remove the phosphate group to reverse the biological activity [128]. Glycosylation refers to the attachment of carbohydrate molecules to proteins, which can promote protein folding and improve stability as well as serve as regulatory functions [129]. Addition of lipid molecules, known as lipidation, often targets proteins that are attached to cell membrane [130]. So far, many different types of lipids including fatty acids, isoprenoids, sterols, phospholipids, and glycosylphosphatidyl inositol, have been identified to be able to covalently attach to proteins [130].

PTMs can occur at any step in the life cycle of a protein. For example, some proteins are modified shortly after translation to mediate proper folding or stability or to guide the nascent protein to specific cellular compartments [131]. Some modifications occur after the folding and localization are completed to influence the biological activities of proteins [131]. Due to the important biological functions, protein PTMs need to be tightly controlled and aberrant modifications and alterations are closely associated with the pathogenesis of various human diseases [132].

5 Interplay among DNA, RNA and protein modifications

Accumulating evidences suggest close interplay among DNA, RNA, and protein modifications. Histone modifications and DNA methylation are highly interrelated and rely mechanistically on each other for the regulation of gene expression [133] (Figure 5(a)). Histone methylation facilitates the establishment of patterns of DNA methylation, and DNA methylation also helps to build the correct patterns of

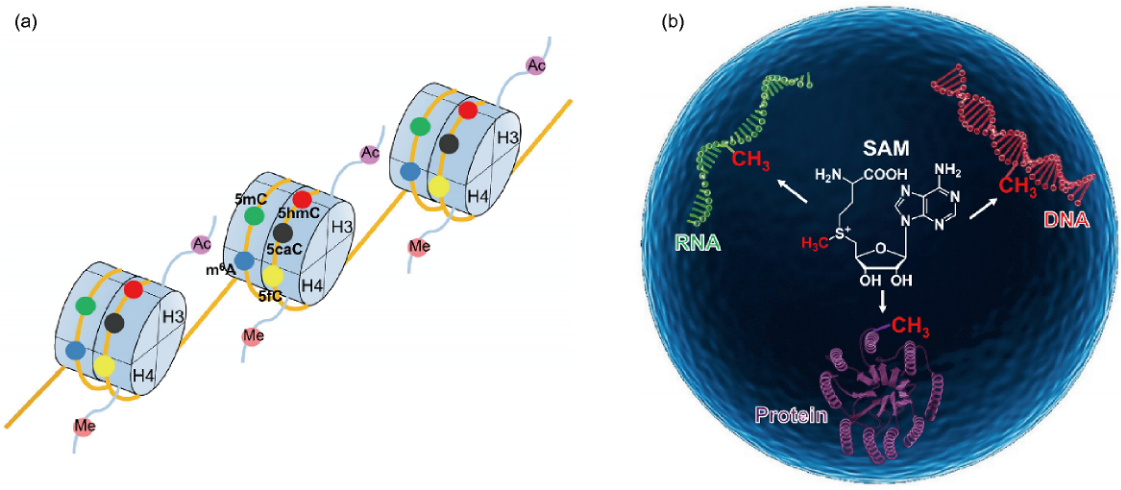

Figure 5 Schematic illustration of the interplay among DNA, RNA and protein modifications. (a) Interplay between DNA modifications and histone modifications; (b) S-adenosyl-*L*-methionine (SAM) is a universal methylating agent and provided methyl groups to DNA, RNA and histones (color online).

histone modifications following DNA replication [133].

Many modifications occurring in DNA and RNA also share the same enzymes. Demethylation of nucleic acid and proteins is generally achieved by 2-oxoglutarate (2-OG) and Fe(II) dependent oxygenases [134]. These enzymes share a common β-helix fold and highly conserved Fe(II) binding sites. *E. coli* AlkB was the first identified 2-OG oxygenase that is capable of demethylating DNA [135]. So far 9 human homologues of the *E. coli* AlkB have been identified (ALKBH1-8 and FTO) that have the potential to remove various DNA/RNA modifications, such as demethylation of m^1A and 5mC in tRNA and m^6A in DNA by ALKBH1 [32,136–138], demethylation of m^3C in both DNA and RNA by ALKBH1 [139], demethylation of m^1A in mRNA by ALKBH3 [38,39], demethylation of m^6A in mRNA by ALKBH5 [55], demethylation of mcm^5U in tRNA by ALKBH8 [140], demethylation of 3-methylthymine in DNA and m^3U and m^6A in RNA by FTO [47,141]. In addition, the TET family of 2-OG oxygenases (TET1-3) can catalyze demethylation of 5mC in both DNA and RNA [69]. Therefore, the expressions and activities changes of enzymes may simultaneously influence multiple modifications in both DNA and RNA, and DNA and RNA modifications may work synergistically to exert their biological functions.

It is worth noting that S-adenosyl-*L*-methionine (SAM) is a universal methylating agent, providing methyl groups to various acceptor substrates [142]. The methyltransferases of DNA, RNA and histones add methyl groups from SAM to DNA, RNA or lysine/arginine residues of histones, respectively [133] (Figure 5(b)). Although structurally diverse and high substrate specificities, these methyltransferases share similar reaction mechanism: transferring methyl group from SAM to the acceptors with the formation of S-adenosyl homocysteine (SAH). SAH is a typical inhibitor to these methyltransferases. Therefore, the complexity of SAM and SAH can affect the establishment of DNA, RNA and histone methylation, and the alteration of SAM *in vivo* can simultaneously affect the status of DNA, RNA and histone methylation.

6 Conclusions and perspective

The recent advances support a more dynamic view of biomolecule modifications, which substantially increases the complexity and diversity of biomolecule species in cells, and more importantly, add additional layers to the regulation of physiological processes. In this review, we propose modificaomics that refers to the comprehensive study of the functions and mechanisms of modifications on DNA, RNA and proteins.

Research on modificaomics is important not only for understanding the direct effects of modifications on the stability, structure, and function of biomolecules but also for aiding in the development of compounds that can specifically target these modifications to regulate the cellular states. Therefore, the subtle change of modifications induced by novel synthetic compounds will motivate continued development of new drugs that target these modifications.

Future work will pay attention to the in-depth investigation of the spatial and temporal characteristics of binding factors to biomolecule modifications. Structural alterations from site-specific modifications could affect biomolecules fate and activity through interactions with endogenous molecules. Identification of specific binding partners for the modifications represents a future research direction that is required to reveal their biological functions.

Continued and rapid improvements in technology make the study of modificaomics more accessible. New techniques including high-throughput LC-MS and sequencing-based

approaches using antibodies, enzymatic treatments, or chemical reaction hold great promise for elucidating the role of biomolecule modifications. High-throughput sequencing methods with base-level resolution are particularly urgent to precisely define various DNA and RNA modifications. However, there are still many challenges to the effective interrogation of biomolecule modifications. Improvements in technology may promote studies of the functions of the modifications that exist in low abundance. Systematic investigation of biomolecule modifications will provide valuable clues underlying association of these modifications with diseases. In this respect, the advancement of new technology may also lead to the discovery of novel modification-based biomarkers that will improve our understanding of the molecular biology of diseases.

Acknowledgements The work was supported by the National Key R&D Program of China (2017YFC0906800) and the National Natural Science Foundation of China (21522507, 21672166, 21635006, 21721005).

Conflict of interest The authors declare that they have no conflict of interests.

1 Cantone I, Fisher AG. *Nat Struct Mol Biol*, 2013, 20: 282–289
2 Smith ZD, Meissner A. *Nat Rev Genet*, 2013, 14: 204–220
3 Chen K, Zhao BS, He C. *Cell Chem Biol*, 2016, 23: 74–85
4 Zhao Y, Garcia BA. *Cold Spring Harb Perspect Biol*, 2015, 7: a025064
5 Fu Y, Dominissini D, Rechavi G, He C. *Nat Rev Genet*, 2014, 15: 293–306
6 Kriaucionis S, Heintz N. *Science*, 2009, 324: 929–930
7 Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A. *Science*, 2009, 324: 930–935
8 Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y. *Science*, 2011, 333: 1300–1303
9 He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, Ding J, Jia Y, Chen Z, Li L, Sun Y, Li X, Dai Q, Song CX, Zhang K, He C, Xu GL. *Science*, 2011, 333: 1303–1307
10 Machnicka MA, Milanowska K, Osman Oglou O, Purta E, Kurkowska M, Olchowik A, Januszewski W, Kalinowski S, Dunin-Horkawicz S, Rother KM, Helm M, Bujnicki JM, Grosjean H. *Nucleic Acids Res*, 2013, 41: D262–D267
11 Lothrop AP, Torres MP, Fuchs SM. *FEBS Lett*, 2013, 587: 1247–1257
12 Chuang HY, Hofree M, Ideker T. *Annu Rev Cell Dev Biol*, 2010, 26: 721–744
13 Feng S, Jacobsen SE, Reik W. *Science*, 2010, 330: 622–627
14 Yuan BF, Feng YQ. *TrAC Trends Anal Chem*, 2014, 54: 24–35
15 Jones PA. *Nat Rev Genet*, 2012, 13: 484–492
16 Robertson KD. *Nat Rev Genet*, 2005, 6: 597–610
17 Huang W, Qi CB, Lv SW, Xie M, Feng YQ, Huang WH, Yuan BF. *Anal Chem*, 2016, 88: 1378–1384
18 Ficz G, Branco MR, Seisenberger S, Santos F, Krueger F, Hore TA, Marques CJ, Andrews S, Reik W. *Nature*, 2011, 473: 398–402
19 Kellinger MW, Song CX, Chong J, Lu XY, He C, Wang D. *Nat Struct Mol Biol*, 2012, 19: 831–833
20 Tang Y, Chu JM, Huang W, Xiong J, Xing XW, Zhou X, Feng YQ, Yuan BF. *Anal Chem*, 2013, 85: 6129–6135
21 Tang Y, Zheng SJ, Qi CB, Feng YQ, Yuan BF. *Anal Chem*, 2015, 87: 3445–3452
22 Chen ML, Shen F, Huang W, Qi JH, Wang Y, Feng YQ, Liu SM, Yuan BF. *Clin Chem*, 2013, 59: 824–832
23 Janulaitis A, Klimašauskas S, Petrušyte M, Butkus V. *FEBS Lett*, 1983, 161: 131–134
24 Ehrlich M, Gama-Sosa MA, Carreira LH, Ljungdahl LG, Kuo KC, Gehrke CW. *Nucl Acids Res*, 1985, 13: 1399–1412
25 Yu M, Ji L, Neumann DA, Chung DH, Groom J, Westpheling J, He C, Schmitz RJ. *Nucleic Acids Res*, 2015, 43: e148
26 Reisenauer A, Shapiro L. *EMBO J*, 2002, 21: 4969–4977
27 Heyn H, Esteller M. *Cell*, 2015, 161: 710–713
28 Ratel D, Ravanat JL, Berger F, Wion D. *Bioessays*, 2006, 28: 309–315
29 Zhang G, Huang H, Liu D, Cheng Y, Liu X, Zhang W, Yin R, Zhang D, Zhang P, Liu J, Li C, Liu B, Luo Y, Zhu Y, Zhang N, He S, He C, Wang H, Chen D. *Cell*, 2015, 161: 893–906
30 Greer EL, Blanco MA, Gu L, Sendinc E, Liu J, Aristizábal-Corrales D, Hsu CH, Aravind L, He C, Shi Y. *Cell*, 2015, 161: 868–878
31 Fu Y, Luo GZ, Chen K, Deng X, Yu M, Han D, Hao Z, Liu J, Lu X, Dore LC, Weng X, Ji Q, Mets L, He C. *Cell*, 2015, 161: 879–892
32 Wu TP, Wang T, Seetin MG, Lai Y, Zhu S, Lin K, Liu Y, Byrum SD, Mackintosh SG, Zhong M, Tackett A, Wang G, Hon LS, Fang G, Swenberg JA, Xiao AZ. *Nature*, 2016, 532: 329–333
33 Liu J, Zhu Y, Luo GZ, Wang X, Yue Y, Wang X, Zong X, Chen K, Yin H, Fu Y, Han D, Wang Y, Chen D, He C. *Nat Commun*, 2016, 7: 13052
34 Koziol MJ, Bradshaw CR, Allen GE, Costa ASH, Frezza C, Gurdon JB. *Nat Struct Mol Biol*, 2016, 23: 24–30
35 Huang W, Xiong J, Yang Y, Liu SM, Yuan BF, Feng YQ. *RSC Adv*, 2015, 5: 64046–64054
36 Schiffers S, Ebert C, Rahimoff R, Kosmatchev O, Steinbacher J, Bohne AV, Spada F, Michalakis S, Nickelsen J, Müller M, Carell T. *Angew Chem Int Ed*, 2017, 56: 11268–11271
37 He C. *Nat Chem Biol*, 2010, 6: 863–865
38 Li X, Xiong X, Wang K, Wang L, Shu X, Ma S, Yi C. *Nat Chem Biol*, 2016, 12: 311–316
39 Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, Peer E, Kol N, Ben-Haim MS, Dai Q, Di Segni A, Salmon-Divon M, Clark WC, Zheng G, Pan T, Solomon O, Eyal E, Hershkovitz V, Han D, Doré LC, Amariglio N, Rechavi G, He C. *Nature*, 2016, 530: 441–446
40 Schwartz S, Bernstein DA, Mumbach MR, Jovanovic M, Herbst RH, León-Ricardo BX, Engreitz JM, Guttman M, Satija R, Lander ES, Fink G, Regev A. *Cell*, 2014, 159: 148–162
41 Delatte B, Wang F, Ngoc LV, Collignon E, Bonvin E, Deplus R, Calonne E, Hassabi B, Putmans P, Awe S, Wetzel C, Kreher J, Soin R, Creppe C, Limbach PA, Gueydan C, Kruys V, Brehm A, Minakhina S, Defrance M, Steward R, Fuks F. *Science*, 2016, 351: 282–285
42 Huber SM, van Delft P, Mendil L, Bachman M, Smollett K, Werner F, Miska EA, Balasubramanian S. *ChemBioChem*, 2015, 16: 752–755
43 Huang W, Lan MD, Qi CB, Zheng SJ, Wei SZ, Yuan BF, Feng YQ. *Chem Sci*, 2016, 7: 5495–5502
44 Xu L, Liu X, Sheng N, Oo KS, Liang J, Chionh YH, Xu J, Ye F, Gao YG, Dedon PC, Fu XY. *J Biol Chem*, 2017, 292: 14695–14703
45 Li X, Xiong X, Yi C. *Nat Meth*, 2016, 14: 23–31
46 Wang Y, Jia G. *Genomics Proteomics Bioinf*, 2016, 14: 172–175
47 Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG, He C. *Nat Chem Biol*, 2011, 7: 885–887
48 Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, Sorek R, Rechavi G. *Nature*, 2012, 485: 201–206
49 Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. *Cell*, 2012, 149: 1635–1646
50 Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE, Jaffrey SR. *Nat Meth*, 2015, 12: 767–772
51 Zhao BS, Roundtree IA, He C. *Nat Rev Mol Cell Biol*, 2016, 18: 31–42
52 Haussmann IU, Bodi Z, Sanchez-Moran E, Mongan NP, Archer N,

Fray RG, Soller M. *Nature*, 2016, 540: 301–304
53 Ke S, Alemu EA, Mertens C, Gantman EC, Fak JJ, Mele A, Haripal B, Zucker-Scharff I, Moore MJ, Park CY, Vågbø CB, Kusśnierczyk A, Klungland A, Darnell Jr. JE, Darnell RB. *Genes Dev*, 2015, 29: 2037–2053
54 Fustin JM, Doi M, Yamaguchi Y, Hida H, Nishimura S, Yoshida M, Isagawa T, Morioka MS, Kakeya H, Manabe I, Okamura H. *Cell*, 2013, 155: 793–806
55 Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, Vågbø CB, Shi Y, Wang WL, Song SH, Lu Z, Bosmans RPG, Dai Q, Hao YJ, Yang X, Zhao WM, Tong WM, Wang XJ, Bogdan F, Furu K, Fu Y, Jia G, Zhao X, Liu J, Krokan HE, Klungland A, Yang YG, He C. *Mol Cell*, 2013, 49: 18–29
56 Batista PJ, Molinie B, Wang J, Qu K, Zhang J, Li L, Bouley DM, Lujan E, Haddad B, Daneshvar K, Carter AC, Flynn RA, Zhou C, Lim KS, Dedon P, Wernig M, Mullen AC, Xing Y, Giallourakis CC, Chang HY. *Cell Stem Cell*, 2014, 15: 707–719
57 Deng X, Su R, Feng X, Wei M, Chen J. *Curr Opin Genet Dev*, 2017, 48: 1–7
58 Shen F, Huang W, Huang JT, Xiong J, Yang Y, Wu K, Jia GF, Chen J, Feng YQ, Yuan BF, Liu SM. *J Clin Endocrinol Metab*, 2015, 100: E148–E154
59 Cui Q, Shi H, Ye P, Li L, Qu Q, Sun G, Sun G, Lu Z, Huang Y, Yang CG, Riggs AD, He C, Shi Y. *Cell Rep*, 2017, 18: 2622–2634
60 Li Z, Weng H, Su R, Weng X, Zuo Z, Li C, Huang H, Nachtergaele S, Dong L, Hu C, Qin X, Tang L, Wang Y, Hong GM, Huang H, Wang X, Chen P, Gurbuxani S, Arnovitz S, Li Y, Li S, Strong J, Neilly MB, Larson RA, Jiang X, Zhang P, Jin J, He C, Chen J. *Cancer Cell*, 2017, 31: 127–141
61 Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, Chen Y, Sulman EP, Xie K, Bögler O, Majumder S, He C, Huang S. *Cancer Cell*, 2017, 31: 591–606
62 Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, He X, Semenza GL. *Proc Natl Acad Sci USA*, 2016, 113: E2047–E2056
63 Feillet C, van der Horst GTJ, Levi F, Rand DA, Delaunay F. *Front Neurol*, 2015, 6: 96
64 Aguilo F, Zhang F, Sancho A, Fidalgo M, Di Cecilia S, Vashisht A, Lee DF, Chen CH, Rengasamy M, Andino B, Jahouh F, Roman A, Krig SR, Wang R, Zhang W, Wohlschlegel JA, Wang J, Walsh MJ. *Cell Stem Cell*, 2015, 17: 689–704
65 Mauer J, Luo X, Blanjoie A, Jiao X, Grozhik AV, Patil DP, Linder B, Pickering BF, Vasseur JJ, Chen Q, Gross SS, Elemento O, Debart F, Kiledjian M, Jaffrey SR. *Nature*, 2017, 541: 371–375
66 Squires JE, Patel HR, Nousch M, Sibbritt T, Humphreys DT, Parker BJ, Suter CM, Preiss T. *Nucleic Acids Res*, 2012, 40: 5023–5033
67 Li B, Luo X, Dong Y. *Bioconjugate Chem*, 2016, 27: 849–853
68 Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ. *Cell Stem Cell*, 2010, 7: 618–630
69 Fu L, Guerrero CR, Zhong N, Amato NJ, Liu Y, Liu S, Cai Q, Ji D, Jin SG, Niedernhofer LJ, Pfeifer GP, Xu GL, Wang Y. *J Am Chem Soc*, 2014, 136: 11582–11585
70 Basanta-Sanchez M, Wang R, Liu Z, Ye X, Li M, Shi X, Agris PF, Zhou Y, Huang Y, Sheng J. *ChemBioChem*, 2017, 18: 72–76
71 Carlile TM, Rojas-Duran MF, Zinshteyn B, Shin H, Bartoli KM, Gilbert WV. *Nature*, 2014, 515: 143–146
72 Li X, Zhu P, Ma S, Song J, Bai J, Sun F, Yi C. *Nat Chem Biol*, 2015, 11: 592–597
73 Sun WJ, Li JH, Liu S, Wu J, Zhou H, Qu LH, Yang JH. *Nucleic Acids Res*, 2016, 44: D259–D265
74 Vaidyanathan PP, AlSadhan I, Merriman DK, Al-Hashimi HM, Herschlag D. *RNA*, 2017, 23: 611–618
75 deLorimier E, Hinman MN, Copperman J, Datta K, Guenza M, Berglund JA. *J Biol Chem*, 2017, 292: 4350–4357
76 Daffis S, Szretter KJ, Schriewer J, Li J, Youn S, Errett J, Lin TY, Schneller S, Zust R, Dong H, Thiel V, Sen GC, Fensterl V, Klimstra WB, Pierson TC, Buller RM, Gale Jr M, Shi PY, Diamond MS. *Nature*, 2010, 468: 452–456
77 Kumar S, Mapa K, Maiti S. *Biochemistry*, 2014, 53: 1607–1615
78 Dai Q, Moshitch-Moshkovitz S, Han D, Kol N, Amariglio N, Rechavi G, Dominissini D, He C. *Nat Meth*, 2017, 14: 695–698
79 Sergiev PV, Golovina AY, Prokhorova IV, Sergeeva OV, Osterman IA, Nesterchuk MV, Burakovsky DE, Bogdanov AB, Dontsova OA. *Ribosomes: Struct, Funct, Dyn*, 2011: 97–110
80 Sloan KE, Warda AS, Sharma S, Entian KD, Lafontaine DLJ, Bohnsack MT. *RNA Biol*, 2017, 14: 1138–1152
81 Decatur WA, Fournier MJ. *Trends Biochem Sci*, 2002, 27: 344–351
82 Jiang J, Seo H, Chow CS. *Acc Chem Res*, 2016, 49: 893–901
83 Helm M. *Nucleic Acids Res*, 2006, 34: 721–733
84 Ge J, Yu YT. *Trends Biochem Sci*, 2013, 38: 210–218
85 Behrmann E, Loerke J, Budkevich TV, Yamamoto K, Schmidt A, Penczek PA, Vos MR, Bürger J, Mielke T, Scheerer P, Spahn CMT. *Cell*, 2015, 161: 845–857
86 Chow CS, Lamichhane TN, Mahto SK. *ACS Chem Biol*, 2007, 2: 610–619
87 Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. *Nat Rev Micro*, 2015, 13: 42–51
88 Jack K, Bellodi C, Landry DM, Niederer RO, Meskauskas A, Musalgaonkar S, Kopmar N, Krasnykh O, Dean AM, Thompson SR, Ruggero D, Dinman JD. *Mol Cell*, 2011, 44: 660–666
89 Brown JWS, Echeverria M, Qu LH. *Trends Plant Sci*, 2003, 8: 42–49
90 Lorenz C, Lünse CE, Mörl M. *Biomolecules*, 2017, 7: 35
91 Duechler M, Leszczyńska G, Sochacka E, Nawrot B. *Cell Mol Life Sci*, 2016, 73: 3075–3095
92 Wilusz JE. *Wiley Interdiscip Rev RNA*, 2015, 6: 453–470
93 Björk GR, Hagervall TG. *EcoSal Plus*, 2014, : doi: 10.1128/ecosalplus.ESP-0007-2013
94 Manickam N, Joshi K, Bhatt MJ, Farabaugh PJ. *Nucleic Acids Res*, 2016, 44: 1871–1881
95 Hori H. *Front Genet*, 2014, 5: 144
96 Agris PF. *EMBO Rep*, 2008, 9: 629–635
97 Machnicka MA, Olchowik A, Grosjean H, Bujnicki JM. *RNA Biol*, 2014, 11: 1619–1629
98 Agris PF. *Prog Nucleic Acid Res Mol Biol*, 1996, 53: 79–129
99 Torres AG, Batlle E, Ribas de Pouplana L. *Trends Mol Med*, 2014, 20: 306–314
100 Guy MP, Shaw M, Weiner CL, Hobson L, Stark Z, Rose K, Kalscheuer VM, Gecz J, Phizicky EM. *Human Mutat*, 2015, 36: 1176–1187
101 Schaefer M, Pollex T, Hanna K, Tuorto F, Meusburger M, Helm M, Lyko F. *Genes Dev*, 2010, 24: 1590–1595
102 Anderson P, Ivanov P. *FEBS Lett*, 2014, 588: 4297–4304
103 Lee YS, Shibata Y, Malhotra A, Dutta A. *Genes Dev*, 2009, 23: 2639–2649
104 Klassen R, Paluszynski JP, Wemhoff S, Pfeiffer A, Fricke J, Meinhardt F. *Mol Microbiol*, 2008, 69: 681–697
105 Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, Humphreys P, Lukk M, Lombard P, Treps L, Popis M, Kellner S, Hölter SM, Garrett L, Wurst W, Becker L, Klopstock T, Fuchs H, Gailus-Durner V, Hrabě de Angelis M, Káradóttir RT, Helm M, Ule J, Gleeson JG, Odom DT, Frye M. *EMBO J*, 2014, 33: 2020–2039
106 Saikia M, Krokowski D, Guan BJ, Ivanov P, Parisien M, Hu G, Anderson P, Pan T, Hatzoglou M. *J Biol Chem*, 2012, 287: 42708–42725
107 Preston MA, D'Silva S, Kon Y, Phizicky EM. *RNA*, 2013, 19: 243–256
108 Han L, Kon Y, Phizicky EM. *RNA*, 2015, 21: 188–201
109 Chan CTY, Dyavaiah M, DeMott MS, Taghizadeh K, Dedon PC, Begley TJ. *PLoS Genet*, 2010, 6: e1001247
110 Tuorto F, Lyko F. *Open Biol*, 2016, 6: 160287
111 Suzuki T, Suzuki T. *Nucleic Acids Res*, 2014, 42: 7346–7357
112 Miyauchi K, Kimura S, Suzuki T. *Nat Chem Biol*, 2013, 9: 105–111
113 Matuszewski M, Sochacka E. *Bioorg Med Chem Lett*, 2014, 24:

2703–2706
114 Zhang X, Cozen AE, Liu Y, Chen Q, Lowe TM. *Trends Mol Med*, 2016, 22: 1025–1034
115 Yu B, Yang Z, Li J, Minakhina S, Yang M, Padgett RW, Steward R, Chen X. *Science*, 2005, 307: 932–935
116 Ji L, Chen X. *Cell Res*, 2012, 22: 624–636
117 Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, Feng G, Peng H, Zhang X, Zhang Y, Qian J, Duan E, Zhai Q, Zhou Q. *Science*, 2016, 351: 397–400
118 Yan M, Wang Y, Hu Y, Feng Y, Dai C, Wu J, Wu D, Zhang F, Zhai Q. *Anal Chem*, 2013, 85: 12173–12181
119 Breker M, Schuldiner M. *Nat Rev Mol Cell Biol*, 2014, 15: 453–464
120 Lawrence M, Daujat S, Schneider R. *Trends Genets*, 2016, 32: 42–56
121 Kouzarides T. *Cell*, 2007, 128: 693–705
122 Bannister AJ, Kouzarides T. *Cell Res*, 2011, 21: 381–395
123 Rose NR, Klose RJ. *Biochim Biophys Acta*, 2014, 1839: 1362–1372
124 Silva AMN, Vitorino R, Domingues MRM, Spickett CM, Domingues P. *Free Radical Biol Med*, 2013, 65: 925–941
125 Prabakaran S, Lippens G, Steen H, Gunawardena J. *WIREs Syst Biol Med*, 2012, 4: 565–583
126 Salovska B, Tichy A, Rezacova M, Vavrova J, Novotna E. *Rev Anal Chem*, 2012, 31: 29–41
127 Ubersax JA, Ferrell Jr JE. *Nat Rev Mol Cell Biol*, 2007, 8: 530–541
128 Sacco F, Perfetto L, Castagnoli L, Cesareni G. *FEBS Lett*, 2012, 586: 2732–2739
129 Moremen KW, Tiemeyer M, Nairn AV. *Nat Rev Mol Cell Biol*, 2012, 13: 448–462
130 Resh MD. *Curr Biol*, 2013, 23: R431–R435
131 Braakman I, Bulleid NJ. *Annu Rev Biochem*, 2011, 80: 71–99
132 Reimand J, Wagih O, Bader GD. *PLoS Genet*, 2015, 11: e1004919
133 Cedar H, Bergman Y. *Nat Rev Genet*, 2009, 10: 295–304
134 Walport LJ, Hopkinson RJ, Schofield CJ. *Curr Opin Chem Biol*, 2012, 16: 525–534
135 Falnes PØ, Johansen RF, Seeberg E. *Nature*, 2002, 419: 178–182
136 Liu F, Clark W, Luo G, Wang X, Fu Y, Wei J, Wang X, Hao Z, Dai Q, Zheng G, Ma H, Han D, Evans M, Klungland A, Pan T, He C. *Cell*, 2016, 167: 816–828.e16
137 Haag S, Sloan KE, Ranjan N, Warda AS, Kretschmer J, Blessing C, Hübner B, Seikowski J, Dennerlein S, Rehling P, Rodnina MV, Höbartner C, Bohnsack MT. *EMBO J*, 2016, 35: 2104–2119
138 Kawarada L, Suzuki T, Ohira T, Hirata S, Miyauchi K, Suzuki T. *Nucleic Acids Res*, 2017, 45: 7401–7415
139 Westbye MP, Feyzi E, Aas PA, Vågbø CB, Talstad VA, Kavli B, Hagen L, Sundheim O, Akbari M, Liabakk NB, Slupphaug G, Otterlei M, Krokan HE. *J Biol Chem*, 2008, 283: 25046–25056
140 van den Born E, Vagbo CB, Songe-Moller L, Leihne V, Lien GF, Leszczynska G, Malkiewicz A, Krokan HE, Kirpekar F, Klungland A, Falnes PO. *Nat Commun*, 2011, 2: 172
141 Jia G, Yang CG, Yang S, Jian X, Yi C, Zhou Z, He C. *FEBS Lett*, 2008, 582: 3313–3319
142 Landgraf BJ, McCarthy EL, Booker SJ. *Annu Rev Biochem*, 2016, 85: 485–514