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Non-Enzymatic Covalent Modifications as a New Chapter in the Histone Code

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Abstract

The interior of the cell abounds with reactive species that can accumulate as non-enzymatic covalent modifications (NECMs) on biological macromolecules. These adducts interfere with many cellular processes, for example by altering proteins' surface topology, enzymatic activity or interactomes. Here, we discuss dynamic NECM modifications on chromatin, which serves as the cellular blueprint. We first outline the chemistry of NECM formation and then focus on the recently identified effects of their accumulation on chromatin structure and transcriptional output. We next describe the known cellular regulatory mechanisms that prevent or reverse NECM formation. Finally, we discuss recently developed chemical biology platforms for probing and manipulating these NECMs *in vitro* and *in vivo*.

Keywords

Acylation; glycation; lipidation; chromatin; epigenetics; chemical biology

Prevalent Chemical Reactions in Cells

Many cellular metabolites contain reactive moieties, such as aldehydes, that are key for their cellular function but are also capable of non-specifically modifying macromolecules to form non-enzymatic covalent modifications (NECMs) [1–3]. Some proteins have unique properties that render them susceptible to NECMs: namely a high abundance of accessible reactive functional groups on bases or side chains and comparatively long half-lives. Perhaps unsurprisingly, eukaryotic **chromatin** (see Glossary) - and in particular the DNA-packaging

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histone proteins - meet all these criteria and have recently been found to be rich with NECMs [4].

In chromatin, histones interact with DNA via electrostatic interactions between cationic histone tails which are rich in arginine and lysine residues, and the anionic DNA phosphodiester backbone [5]. Modulation of this interaction is key for regulating gene accessibility and is implemented directly or indirectly through various **post-translational modifications (PTMs)** on the histone tails [6–8]. These modifications, which include acetylation, methylation, and ubiquitination, occur on the highly abundant lysine and arginine residues of the histone tails [4]. The influence of histone PTM patterning on gene expression is the basis of the “**Histone Code**” hypothesis, wherein combinatorial histone modifications form a code that is interpreted by effector proteins to control gene expression [9].

While many of the modifications on histones are introduced by enzyme “writers”, an increasing number of modifications are being discovered that are derived through non-enzymatic origins [10]. Although, by comparison, many of these PTMs are poorly characterized, and many tend to be deleterious through several mechanisms. Since NECMs react with amine side chains present on lysine and arginine, they can directly disrupt the electrostatic interaction with DNA or block canonical modifications on the same sites. Since **epigenetic** information needs to be retained accurately across multiple cell divisions, the accumulation of adducts on genome-associated macromolecules may be extremely harmful for the cell (Figure 1) [11].

Cells have evolved several mechanisms to counter the threat of accumulated NECMs causing damage: 1) metabolite efflux, 2) metabolite scavenging, 3) adduct reversal or editing, and 4) global cellular signaling response [12]. While these mechanisms prevent NECM accumulation and systemic damage, they are undoubtedly vital in reducing the negative effects of histone NECMs in particular. In metabolically aberrant cells, such as cancer cells where anaerobic **glycolysis** rates are increased (i.e. the **Warburg effect**), these defense systems are upregulated, most likely to attenuate the toxicity which accompanies their higher metabolic state [13]. Uncovering the mechanisms surrounding this dynamic nature of NECMs could be leveraged to understand the fitness of metabolically dysregulated cells. Here, we describe in detail the mechanism of formation of the three best-characterized classes of histone NECM types: **glycation, acylation, and lipidation** (Table 1). We then summarize how these are regulated in a cellular context and provide discussion of recent technological advances to track and understand the consequences of these notoriously elusive modifications.

NECMs that Affect Chromatin Physiology

Glycation

Sugars, their derivatives, and metabolic by-products can often exist within the cell for extended periods of time and at substantial concentrations, facilitating their chemical reaction with proteins [14]. The metabolite’s carbonyl is sufficiently electrophilic to react with and modify nucleophiles in the cell, including small molecules, protein side chains (e.g.

lysine, arginine), or nucleobases, a process termed glycation. The initial Schiff base adduct can undergo rapid rearrangement to form more stable “Amadori” ketoamine intermediates which can thereafter react with other species, degrade, or rearrange into a plethora of modifications called **advanced glycation end-products (AGEs)** [15]. Glycation has been shown to interfere with a number of metabolic and epigenetic processes through the modification of key amino acid side chains on enzymes and histones, as well as the direct modification of DNA [16].

Not all metabolites are equally reactive in glycating histones. It is thought that the rate at which a glycation agent is capable of modifying its substrate is dependent on the amount of time the reacting aldehyde is available [17]. Therefore, glycolysis byproducts such as glyoxal or methylglyoxal (MGO) are extremely reactive, whereas cyclized sugars such as glucose, which primarily exists as a hemi-acetal, are more inert (Figure 2A). Indeed, MGO was recently shown to modify arginine and lysine on histones at key regulatory positions at levels comparable to the endogenous, enzymatically installed modifications, such as methylation and acetylation [18]. The modifications were detected on all four core histones at critical residues involved in both nucleosome stability and “reader” domain binding. **RNA-seq** data revealed that MGO can alter gene transcription, most notably in cells lacking the major detoxifying enzyme, glyoxalase 1 (GLO1), which normally eliminates MGO. In the same cells, MGO treatment resulted in marked disruption of H2B acetylation and ubiquitination without affecting H2A, H3, and H4 modifications [18]. Altogether, histone MGO modification was found to have global ramifications on histone enzymatic PTMs, the assembly and stability of nucleosomes, and chromatin architecture. The resulting impacts on chromatin structure affect gene accessibility via a two-step mechanism. As lower levels of MGO modifications accumulate, through low concentration or short exposure, chromatin decompacts and gene accessibility is increased due to the disruption of the electrostatic interactions between DNA and histones. By contrast, increased levels of MGO adduct accumulation, through high concentration or long exposure, results in **cross-links** and hyper compaction of chromatin, leading to a decrease in gene accessibility [19].

While glucose glycation in general is slow, it does occur in low quantities in the cell to form **Maillard products** [17]. Subsequent glucose, and more broadly cyclic sugar glycation adducts, can degrade into ketohexones such as 3-deoxyglucosone (3-DG) which are much more reactive and have been demonstrated to modify histones [20]. However, the relatively more labile ribose, as well as its derivatives such as the ubiquitous ADP-ribose, have been shown to glycate all four core histones, as well as the linker histone H1 [21, 22]. In solution, the core histones have been shown to react faster with ribose than the linker histone H1; however, in a chromatin context, where H1 is loosely bound to the periphery of the nucleosome and is more accessible, the reverse trend is observed as ribose modifies the linker histone faster than the core histones. In endothelial cells, high concentrations of ribose can generate protein crosslinks and ultimately cause apoptotic cell death. As with MGO, ribose disrupts chromatin PTM patterning and specifically reduces H3 and H4 acetylation; however, without tracking the specific sites and adducts on the site, it remains unclear if this is a direct competition or the result of a more convoluted mechanism [23]. Despite impressive innovations in the field and the discovery of broad epigenetic phenotypes,

uncovering a link between site-specific histone glycation and an epigenetic effect remains elusive.

Acylation

While carboxylate groups alone at physiological pH are poor **electrophiles**, they can be enzymatically converted into thioesters or even more labile anhydrides, capable of acylation [24]. Advances in mass spectrometry (MS) have revealed a variety of such metabolites including lipids, amino acids, CoA, acetate, malonate, and lactate that can be readily activated and subsequently react with cellular components to form easily hydrolysable esters and thioesters or stable amides (Figure 2B) [25–27]. The simplest forward acylation (acetylation) can be performed enzymatically by histone acetyltransferases, which specifically deposit the mark, or non-enzymatically, resulting in non-directed localization [26]. Conversely, the reverse deacylation process does not occur spontaneously and requires enzymatic regulation, primarily by histone deacetylases (HDACs) or members of the Sirtuin family [28, 29].

In the decades since the discovery of non-enzymatic histone acetylation by acetyl-CoAs, several other histone acylations NECMs have been identified [27, 30]. Generally, acylation occurs on lysine side chains and removes the charge on their substrate amine. The resulting loss of electrostatic binding energy between DNA and the histone tail leads to chromatin decompaction and gene expression [31]. Due to the breadth of reactive molecules capable of non-enzymatic acylation, including crotonylation, butyrylation, or succinylation, the degree to which acylation NECMs are expected to alter histone structure or function varies greatly depending on the chemical structure of the acyl-donor [27]. For example, histone benzoylation, the first described modification by an aromatic acyl group, was identified on 22 lysine residues in cancer cells. The mark was stimulated using the common food preservative and FDA-approved drug sodium benzoate, which is converted into active benzoyl-CoA intracellularly. Notably, **ChIP-seq** and RNA-seq studies of this mark uncovered substantial physiological consequences of the mark compared to acetylation [32]. Similarly, increased cellular concentrations of lactate in M1 macrophages due to increased glycolysis following hypoxia or bacterial challenge stimulates histone **lactylation** (also referred to as lactoylation) via a later detected CoA intermediate [33]. Lactylation was identified at 28 sites on core histones in human and mouse cells, and displayed different temporal dynamics from acetylation. Ultimately, in late phase of M1 macrophage polarization it induced homeostatic genes such as *Arg1*, which is involved in wound healing [34].

The discovery of histone lactylation suggests a potential crosstalk between glycation and acylation, as the latter can also occur in response to cellular upkeep and maintenance through the glyoxalase system. **Dicarbonyls** such as MGO and glyoxal react with glutathione to form S-D-lactoylglutathione (LGSH), a reaction catalyzed by GLO1. This intermediate can then be hydrolyzed with the assistance of GLO2 to complete the cycle by regenerating glutathione and forming lactate. Alternatively, it can spontaneously react with nearby lysine residues to form lactylLys. The activity of lactylLys depends on GLO2 amounts, as GLO2 knockdown leads to marked increases in LGSH and the subsequent

increases in lactylLys levels [34, 35]. Additionally, through the use of an alkyne-tagged MGO analog, these modifications were shown to be enriched on glycolytic enzymes and cause a global decrease in glycolytic output [36].

Whereas the acylations discussed above rely on an acyl-donor being activated by common cellular cofactors like glutathione or CoA, other mechanisms of acylation have also been identified [10, 37]. For example, during error editing in protein biosynthesis, methionyl-tRNA synthetases generate homocysteine **thiolactone**. The labile thiolactone ring is hydrolyzed and opened by lysine side chains, generating a novel thiol on the surface of the protein, a process termed homocysteinylation (hcy) [38]. Increased hcy levels have been associated with suppressed DNA damage repair as well as neural tube defects. Specifically, 39 histone hcy sites have been identified in samples from human embryonic brain tissues. Additionally, ChIP-Seq and RNA-seq experiments have demonstrated that an increase in H3K79hcy levels down regulates the expression of selected neural tube closure genes and induces the onset of neural tube defects [39]. However, as with most acylations, it remains a challenge to uncover the extent to which these modifications are enzymatic or non-enzymatic in nature without knowledge of existing “writer” enzymes.

Lipidation

Lipid peroxidation in cells occurs in response to oxidative stress and the breakdown of peroxides leads to the formation of diverse aldehydes (Figure 2C) [40]. Two of these aldehydes, 4-hydroxynonenal (4-HNE) and 4-oxo-2-nonenal (4-ONE) have been extensively studied and found to chemically modify a number of proteins and DNA [41, 42]. Mildly cross-linked HNE-modified proteins are preferentially degraded by the proteasome but extensive cross-linking results in protein aggregation and proteasome inhibition. This is thought to be a defense mechanism to oxidative stress as mild damage can be reversed but extensive damage requires full cellular turnover [43]. Furthermore, stable 4-HNE and 4-ONE adducts on histones have established a potential link between oxidative stress and epigenetics [44]. 4-HNE was initially shown to react with histones using electron paramagnetic resonance in conjunction with protein spin label experiments [45]. While both 4-HNE and 4-ONE have since been shown to form adducts on histones, 4-ONE only attaches to lysine residues while 4-HNE also targets cysteines. Thus, cysteine-depleted histones are more susceptible to 4-ONE over 4-HNE lipidation as the latter will additionally be consumed by the cysteine proteome [44, 46]. The resulting lipid NECMs are much larger than most small molecule PTMs, such as methyl or acetyl marks. This, combined with the fact that both 4-ONE and 4-HNE have been shown to also modify DNA directly, reduce histone-DNA binding as well as a number of other chromatin effector proteins [44, 46]. Together, these may define an additional mechanism behind the observed epigenetic effect of lipidation.

Cellular Defense Mechanisms Against NECMs

Due to the deleterious nature of most NECMs, cells must maintain firm control on the extent of adduct accumulation. This is especially true for metabolically challenged cells, like in the cases of cancer or diabetes [13, 47]. Cells are equipped with a 3-pronged NECM

regulatory network that consists of 1) scavenging or deactivating reactive metabolites to prevent the NECM reaction, 2) reversing the adducts or converting them into more tolerable forms, and 3) detecting the damage and activating signaling responses for either repair or induction of cell death. Below we discuss the first two mechanisms as this review centers around histone NECMs, but for detailed reviews on cellular recognition of AGEs and the subsequent cellular response, see [48–50].

Discouraging NECM Formation

Although histones are translated in the cytosol, they are captured by chaperones as they emerge from ribosomes and are immediately escorted to the nucleus where they are deposited into chromatin. Their fleeting cytosolic half-lives, together with the fact that they are mostly masked, reduces the possibility of them accumulating NECMs in the cytosol. Indeed, histones spend most of their lifespan within the nuclear compartment where reactive small molecules, via the large nuclear pores, can freely access and ultimately modify them as well as DNA, and other chromatin-effector proteins.

Several protein systems have previously been described as being capable of intercepting reactive metabolites and byproducts both in the cytosol and the nucleus before they can reach the histones (Figure 3A). In addition to the glyoxalase cycle mentioned above, aldehyde and lactate dehydrogenases utilize NAD^+ as a cofactor and directly oxidize aldehyde and lactate molecules into their respective less deleterious carboxylates [51, 52]. Conversely, aldo-keto reductases use NADPH as a cofactor to reduce aldehydes into alcohols, which are more easily managed [53]. Carnosine synthase 1 (CARNS1) catalyzes the ATP-dependent formation of the dipeptide carnosine from L-histidine and β -alanine, which then scavenges both reactive oxidative and carbonyl species [54, 55].

Amending NECMs

In addition to the preventative measures discussed above, cells have evolved various enzymatic mechanisms to remove NECMs from proteins and DNA at both early and late stages (Figure 3B). One example is the deglycase DJ-1/Park7, which hydrolyzes early MGO glycation adducts from both proteins and DNA to release lactate [18, 19, 56]. In another case, fructosamine 3-kinase (FN3K) catalyzes the removal of glycation adducts by phosphorylating the C-3 hydroxyl on the sugar, leading to the subsequent spontaneous degradation of the adduct, producing a deoxyketone byproduct as well as the regenerated amine [57]. Alternatively, protein arginine deiminase 4 (PAD4) has been shown to convert arginine side chains, including those that have been glycated with MGO, into citrulline. Though it does not regenerate the unmodified substrate, it does stall the glycation cascade to prevent the formation of AGEs as well as protect the arginine from being a glycation substrate again [58].

Several deacylases have been implicated in the repair of non-specific acylation including HDACs and SIRT3 for acetylation, SIRT5 that can remove succinyl acyl groups (see references [59, 60] for summary of the epigenetic roles of Sirtuins), and HDAC11 which acts as a general fatty acid deacylase [32, 61–65]. In addition, SIRT2 wash shown to remove both acetylation as well as histone 4-ONE and lacyllys modifications, which

were themselves recently shown to also be removed by HDACs 1-3 [66]. The general low selectivity of these enzymes permits the accommodation of an array of adducts and substrates of differing sizes and shapes. This attribute may be evolved as a fundamental cellular mechanism to prevent the accumulation of non-enzymatic and non-specific acylation.

The activities of the repair enzymes are notably restricted to their cellular localization. So, while FN3K can deglycate histones and attenuate their glycation damage during and immediately after translation, the kinase has no effect on histone chronic NECM accumulation. Conversely, pervasive DJ-1 and PAD4 can affect the accumulated nuclear histone NECM damage as they are present in the nucleus. Interestingly, several of the above enzymes (DJ1, FN3K, and PAD4) are overexpressed in many cancers and have been associated with cancer cell proliferation [67–69]. Consequently, small molecule campaigns have been conducted against DJ-1 and PADI4 to leverage their deglycation activity as a new therapeutic avenue and the resulting molecules may be promising starting points as therapeutic agents [70–72].

Novel Tools Developed for Tracking NECMs

Traditional methods to track NECMs have relied on biophysical approaches including spectroscopic, colorimetric, and chromatographic methods. These methods focused on physical characteristics that changed as NECMs formed, for example the formation of aromatic AGEs, such as pentosidine, which allowed for visualization of glycation on proteins. Additionally, accumulation of many adducts cause physical distortions of protein structure which were detected through spectroscopic analyses. Furthermore, global changes in protein structure were detectable via circular dichroism and various chromatographies. Finally, modifications resulting in cross-links were identified through protein laddering after separation by gel electrophoresis [15]. While effective, these approaches have low sensitivity and thus require extensive accumulation of adducts under extremely harsh and non-physiological conditions, and the scope of identifiable modifications is extremely narrow. There was thus a need for the development of more sensitive and broadly applicable strategies.

Immunoassays

Antibodies were and remain very powerful tools utilized to uncover and study specific protein adducts. Indeed, they have been extensively used in the context of NECMs, particularly in glycation discoveries. However, antibodies have substantial limitations in studying protein glycation. Primarily, glycation adducts are unstable and frequently rearrange to form an array of chemical structures, while antibodies require stable modifications on peptides inoculated in animals for weeks. Using a mixture of adducts as an antigen results in the generation of a less specific antibody [15]. Despite these limitations, powerful and specific antibodies have been commercially developed for certain species, such as pan antibodies against MGO, carboxymethyl lysine, or carboxyethyl lysine. Moreover, chemical synthesis of peptides with the relatively stable MGO arginine modifications, MG-

Hs, were used to produce effective antibodies. The success of this pipeline suggests that additional antibodies against other stable NECMs can be similarly generated [73].

Mass Spectrometry

Much of the pioneering work in understanding the diversity of enzymatic histone PTMs came through innovations in MS sample preparations, physical instrumentation, and data analysis including top-down and PTM scanning approaches among others [74–76]. For instance, trypsin digestion limitations on histones, due to an overabundance of lysine and arginine on histone tails, was overcome through the chemical **propionylation** of these residues prior to digestion [77]. MS analysis of NECMs on histones presents even greater challenges. For example, the acid-based histone extraction and propionylation could alter the structures of NECMs generating additional complexity that is difficult to account for. Additionally, some adducts are constitutional isomers and cannot be distinguished by mass spectrometry. Moreover, many glycation pathways pass through or result in identical structures, thus preventing identification of the initial glycating reagent. Several NECMs also form on lysine and arginine side chains, occluding sites of enzymatic cleavage during sample preparation resulting in peptides that are too long for chromatographic separation, and subsequently pass undetected. During the MS analysis itself, NECMs decrease the net charge on these residues, further reducing their likelihood of detection by ion activation methods. Finally, cross-linking adds an almost insurmountable complexity barrier without prior knowledge of the peptides being cross-linked in addition to the added difficulty of generating quality MS/MS data.

Several of these limitations have been overcome through technical and analytical innovations. Firstly, alternate sample preparations, including a high salt isolation strategy, have been described and recently used to analyze MGO and ribose glycation NECMs on histones while avoiding extreme pH reagents [19, 58, 77, 78]. Secondly, a label-free approach for the quantitative analysis and discovery of Lys and Arg modifications (QuARKMod) for complex biological samples, was developed (Figure 4A). The method involves digestion of protein samples down to individual amino acids followed by collision induced dissociation (CID) MS to determine the absolute concentrations of each amino acid species, with or without modification [79]. This method has since been successfully applied in the study of novel histone NECMs such as lactoylysine and riboLys, as well as MGO adducts [18, 23, 36, 44]. While MS-based methods to study histone NECMs face limitations, the continued development of such approaches will undoubtedly yield further important discoveries in the future.

Chemical Probes

Chemical biology approaches have offered a powerful alternative strategy through the development of chemical analogs of the desired reactive molecules (Figure 4B, Table 2). The analogs contain appended **enrichment moieties** and have been successfully applied toward the study of a broad range of histone NECMs. For example, two alkynyl-modified MGO probes have been synthesized and used for visualization and enrichment of MGO adducts. The longer of the two was used to characterize the blood MGO proteome, whereas the shorter was used to visualize and enrich histone MGO-glycation NECMs [78, 80,

81]. Similarly, an azidoribose probe was recently synthesized and applied to track histone ribose glycation. Its enrichment handle was utilized to isolate the ribose glycation nuclear proteome, revealing that in addition to histones, several other key nuclear systems, such as those involved in DNA repair, are susceptible to ribose glycation [23].

Additionally, a biotinylated non-specific thioester probe was developed and used in a competition assay with acyl-CoAs to characterize non-enzymatic acylation targets. Applying the method to cancer cell lines uncovered numerous candidate targets of non-enzymatic acylation, including several enzymes in glycolysis [25]. 4-ONE lipidation histone NECMs have also been tracked using an alkynyl analog of the peroxide by-product and this probe was instrumental in the discovery of SIRT2 as an “eraser” of this modification [63, 64]. Although they provide temporal control and useful enrichment capabilities, these probes are limited in that they are by definition non-endogenous and their distinction from the native molecules, though minor, could be crucial. Alternatively, homocysteinylation adducts can be labeled by an alkynyl-fused thioester probe via a biotin switch assay. This probe targets the thiol of the homocysteine, then an S-to-N acyl shift yields the alkynyl modified adduct. By labeling the adduct rather than mimicking it, this probe does not face the same limitations as the chemical analogs discussed above [82]. The development of such technologies will facilitate the advancement of histone NECM research from searching for modifications, into understanding the mechanisms of action linking these NECMs, cellular physiology, and pathology.

Concluding Remarks

Due to the diverse natures of the cellular metabolome, there are a wide range of NECMs that can affect macromolecules within the proteome. Through their roles in chromatin regulation, the long-lived and arginine- and lysine-rich histone proteins are extremely susceptible to accumulate these NECMs. The resulting modifications can alter their stability, three-dimensional structure, and their functional interactions. These disruptions can lead to several cellular pathophysiological outcomes, including aging and death. As such, multiple layers of regulation including protein scavenging, enzymatic attenuation, and cellular recognition respond to these stresses. These forces of modification and regulation compete, and the resultant has a powerful influence on the cell's fate and lifespan.

Further development of tools to track, predict and perturb histone NECMs and the systems that surveil them will continue to broaden our knowledge of their effect on cellular function. Although tremendous progress has already been made within the field, much work remains to be done (see Outstanding Questions). We envision that as a new family of modifications, NECMs on histones continue to emerge and further regulatory systems and mechanisms linking them to chromatin dynamics will be established. Finally, the continued development of chemical and biological tools will prompt exciting opportunities for discovery in the field.

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Glossary

Acylation

the spontaneous adduction of a small molecule onto the lysine ϵ -amine or N-terminal amine via an acyl linkage.

Advanced glycation end products (AGEs)

the resulting diverse group of products in a glycation chemical reaction cascade.

ChIP-seq

Chromatin Immunoprecipitation-sequencing; the process of identifying the regions where a specific mark is located in terms of genomic position. Involves the cross-linking of DNA and proteins, shearing chromatin, antibody enrichment, reverse cross-linking, digestion, and next-generation sequencing of the isolated DNA fragments.

Chromatin

the physiologically relevant protein-DNA complex for storage and access of genetic information in eukaryotes. The fundamental unit of this complex is a nucleosome, which is comprised of about 150 DNA base pairs wrapped approximately 1.65 times around a histone octamer. The histone octamer itself contains two copies of each of the four core histones H2A, H2B, H3, and H4.

Cross-links

covalent bonds linking one polymer chain to another.

Dicarbonyls

molecules containing two carbonyl groups.

Electrophiles

chemical species that accept electron pairs to form bonds with nucleophiles.

Enrichment moieties

functional groups that confer specific purification advantages.

Epigenetics

the study of phenotypic changes caused by alterations in gene expression rather than the genetic code.

Glycation

the non-enzymatic modification of biological macromolecules by a reducing sugar or aldehyde-containing sugar derivative via the Mailiard reaction.

Histone Code

the hypothesis that transcription is partly regulated by chemical modifications, primarily on unstructured histone tails.

Lactylation

the incorporation of a lactyl moiety onto an ϵ -amine or N-terminal amine via an acyl linkage.

Lipidation

the incorporation of a fatty chain as a modification on a protein.

Glycolysis

the metabolic pathway that describes the breakdown of glucose by enzymes into pyruvate, releasing energy in the process.

Maillard products

The plethora of species formed from a chemical reaction between amino acids and reducing sugars giving browned food its distinctive color and flavor.

Propionylation

the reaction of propionyl groups onto the ϵ -amine or N-terminal amine via an acyl linkage that can be used to remove the ability of trypsin to recognize lysine and arginine residues in mass spectrometry sample preparation.

Post-translational modifications (PTMs)

the covalent modification of proteins following their biosynthesis.

RNA-seq

the characterization of the genetic sequences of the RNA in a sample, cell, or tissue.

Thiolactone

a cyclic chemical compound with a characteristic sulfur adjacent to a carbonyl.

Warburg effect

a modified form of cellular metabolism found in cancer cells whereby a specialized mixture of mostly anaerobic glycolysis is preferred to aerobic glycolysis.

References

1. Harmel R and Fiedler D (2018) Features and regulation of non-enzymatic post-translational modifications. *Nat Chem Biol* 14, 244–252 [PubMed: 29443975]
2. DeBerardinis RJ and Thompson CB (2012) Cellular metabolism and disease: what do metabolic outliers teach us? *Cell* 148, 1132–1144 [PubMed: 22424225]
3. Cloos PA and Christgau S (2002) Non-enzymatic covalent modifications of proteins: mechanisms, physiological consequences and clinical applications. *Matrix Biol* 21, 39–52 [PubMed: 11827791]
4. Erler J, et al. (2014) The role of histone tails in the nucleosome: a computational study. *Biophys J* 107, 2911–2922 [PubMed: 25517156]
5. Luger K, et al. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 389, 251–260 [PubMed: 9305837]
6. Bannister AJ and Kouzarides T (2011) Regulation of chromatin by histone modifications. *Cell Res* 21, 381–395 [PubMed: 21321607]

7. Allfrey VG, et al. (1964) Acetylation and Methylation of Histones and Their Possible Role in the Regulation of Rna Synthesis. *Proc Natl Acad Sci U S A* 51, 786–794 [PubMed: 14172992]
8. Phillips DM (1963) The presence of acetyl groups of histones. *Biochem J* 87, 258–263 [PubMed: 13943142]
9. Jenuwein T and Allis CD (2001) Translating the histone code. *Science* 293, 1074–1080 [PubMed: 11498575]
10. Chan JC and Maze I (2020) Nothing Is Yet Set in (Hi)stone: Novel Post-Translational Modifications Regulating Chromatin Function. *Trends Biochem Sci* 45, 829–844 [PubMed: 32498971]
11. Probst AV, et al. (2009) Epigenetic inheritance during the cell cycle. *Nat Rev Mol Cell Biol* 10, 192–206 [PubMed: 19234478]
12. Zheng Q, et al. (2020) Non-enzymatic covalent modifications: a new link between metabolism and epigenetics. *Protein Cell* 11, 401–416 [PubMed: 32356279]
13. Liberti MV and Locasale JW (2016) The Warburg Effect: How Does it Benefit Cancer Cells? *Trends Biochem Sci* 41, 211–218 [PubMed: 26778478]
14. Ahmad S, et al. (2018) Oxidation, glycation and glycooxidation-The vicious cycle and lung cancer. *Semin Cancer Biol* 49, 29–36 [PubMed: 29055529]
15. Hellwig M and Henle T (2014) Baking, ageing, diabetes: a short history of the Maillard reaction. *Angew Chem Int Ed Engl* 53, 10316–10329 [PubMed: 25044982]
16. Thornalley PJ (2008) Protein and nucleotide damage by glyoxal and methylglyoxal in physiological systems--role in ageing and disease. *Drug Metabol Drug Interact* 23, 125–150 [PubMed: 18533367]
17. Tessier FJ (2010) The Maillard reaction in the human body. The main discoveries and factors that affect glycation. *Pathol Biol (Paris)* 58, 214–219 [PubMed: 19896783]
18. Galligan JJ, et al. (2018) Methylglyoxal-derived posttranslational arginine modifications are abundant histone marks. *Proc Natl Acad Sci U S A* 115, 9228–9233 [PubMed: 30150385]
19. Zheng Q, et al. (2019) Reversible histone glycation is associated with disease-related changes in chromatin architecture. *Nat Commun* 10, 1289 [PubMed: 30894531]
20. Ashraf JM, et al. (2015) Glycation of H1 Histone by 3-Deoxyglucosone: Effects on Protein Structure and Generation of Different Advanced Glycation End Products. *PLoS One* 10, e0130630 [PubMed: 26121680]
21. Cervantes-Laurean D, et al. (1996) Glycation and glycooxidation of histones by ADP-ribose. *J Biol Chem* 271, 10461–10469 [PubMed: 8631841]
22. Talasz H, et al. (2002) Nonenzymatic glycation of histones in vitro and in vivo. *J Cell Biochem* 85, 24–34 [PubMed: 11891847]
23. Maksimovic I, et al. (2020) An Azidoribose Probe to Track Ketoamine Adducts in Histone Ribose Glycation. *J Am Chem Soc* 142, 9999–10007 [PubMed: 32390412]
24. Li LO, et al. (2010) Acyl-CoA synthesis, lipid metabolism and lipotoxicity. *Biochim Biophys Acta* 1801, 246–251 [PubMed: 19818872]
25. Kulkarni RA, et al. (2017) Discovering Targets of Non-enzymatic Acylation by Thioester Reactivity Profiling. *Cell Chem Biol* 24, 231–242 [PubMed: 28163016]
26. Olia AS, et al. (2015) Nonenzymatic Protein Acetylation Detected by NAPPA Protein Arrays. *ACS Chem Biol* 10, 2034–2047 [PubMed: 26083674]
27. Simithy J, et al. (2017) Characterization of histone acylations links chromatin modifications with metabolism. *Nat Commun* 8, 1141 [PubMed: 29070843]
28. Cress WD and Seto E (2000) Histone deacetylases, transcriptional control, and cancer. *J Cell Physiol* 184, 1–16 [PubMed: 10825229]
29. Sauve AA, et al. (2006) The biochemistry of sirtuins. *Annu Rev Biochem* 75, 435–465 [PubMed: 16756498]
30. Paik WK, et al. (1970) Nonenzymatic acetylation of histones with acetyl-CoA. *Biochim Biophys Acta* 213, 513–522 [PubMed: 5534125]
31. Chang L and Takada S (2016) Histone acetylation dependent energy landscapes in tri-nucleosome revealed by residue-resolved molecular simulations. *Sci Rep* 6, 34441 [PubMed: 27698366]

32. Huang H, et al. (2018) Lysine benzylation is a histone mark regulated by SIRT2. *Nat Commun* 9, 3374 [PubMed: 30154464]
33. Varner EL, et al. (2020) Quantification of lactoyl-CoA (lactyl-CoA) by liquid chromatography mass spectrometry in mammalian cells and tissues. *Open Biol* 10, 200187 [PubMed: 32961073]
34. Zhang D, et al. (2019) Metabolic regulation of gene expression by histone lactylation. *Nature* 574, 575–580 [PubMed: 31645732]
35. Rabbani N, et al. (2014) Activity, regulation, copy number and function in the glyoxalase system. *Biochem Soc Trans* 42, 419–424 [PubMed: 24646254]
36. Gaffney DO, et al. (2020) Non-enzymatic Lysine Lactoylation of Glycolytic Enzymes. *Cell Chem Biol* 27, 206–213 e206 [PubMed: 31767537]
37. Diehl KL and Muir TW (2020) Chromatin as a key consumer in the metabolite economy. *Nat Chem Biol* 16, 620–629 [PubMed: 32444835]
38. Jakubowski H (1999) Protein homocysteinylation: possible mechanism underlying pathological consequences of elevated homocysteine levels. *FASEB J* 13, 2277–2283 [PubMed: 10593875]
39. Zhang Q, et al. (2018) Elevated H3K79 homocysteinylation causes abnormal gene expression during neural development and subsequent neural tube defects. *Nat Commun* 9, 3436 [PubMed: 30143612]
40. Skrzydlewska E, et al. (2005) Lipid peroxidation and antioxidant status in colorectal cancer. *World J Gastroenterol* 11, 403–406 [PubMed: 15637754]
41. Esterbauer H, et al. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 11, 81–128 [PubMed: 1937131]
42. West JD and Marnett LJ (2005) Alterations in gene expression induced by the lipid peroxidation product, 4-hydroxy-2-nonenal. *Chem Res Toxicol* 18, 1642–1653 [PubMed: 16300372]
43. Castro JP, et al. (2017) 4-Hydroxynonenal (HNE) modified proteins in metabolic diseases. *Free Radic Biol Med* 111, 309–315 [PubMed: 27815191]
44. Galligan JJ, et al. (2014) Stable histone adduction by 4-oxo-2-nonenal: a potential link between oxidative stress and epigenetics. *J Am Chem Soc* 136, 11864–11866 [PubMed: 25099620]
45. Drake J, et al. (2004) 4-Hydroxynonenal oxidatively modifies histones: implications for Alzheimer's disease. *Neurosci Lett* 356, 155–158 [PubMed: 15036618]
46. Sun R, et al. (2017) Chemoproteomics Reveals Chemical Diversity and Dynamics of 4-Oxo-2-nonenal Modifications in Cells. *Mol Cell Proteomics* 16, 1789–1800 [PubMed: 28814509]
47. Wellen KE and Thompson CB (2010) Cellular metabolic stress: considering how cells respond to nutrient excess. *Mol Cell* 40, 323–332 [PubMed: 20965425]
48. Schmidt AM, et al. (2000) The biology of the receptor for advanced glycation end products and its ligands. *Biochim Biophys Acta* 1498, 99–111 [PubMed: 11108954]
49. Palanisami G and Paul SFD (2018) RAGE and Its Ligands: Molecular Interplay Between Glycation, Inflammation, and Hallmarks of Cancer—a Review. *Horm Cancer* 9, 295–325 [PubMed: 29987748]
50. Bierhaus A, et al. (2005) Understanding RAGE, the receptor for advanced glycation end products. *J Mol Med (Berl)* 83, 876–886 [PubMed: 16133426]
51. Zheng Q, et al. (2019) (De)Toxifying the Epigenetic Code. *Chem Res Toxicol* 32, 796–807 [PubMed: 30839196]
52. Bommer GT, et al. (2020) Metabolite Repair Enzymes Control Metabolic Damage in Glycolysis. *Trends Biochem Sci* 45, 228–243 [PubMed: 31473074]
53. Chang KC and Petrash JM (2018) Aldo-Keto Reductases: Multifunctional Proteins as Therapeutic Targets in Diabetes and Inflammatory Disease. *Adv Exp Med Biol* 1032, 173–202 [PubMed: 30362099]
54. Drozak J, et al. (2010) Molecular identification of carnosine synthase as ATP-grasp domain-containing protein 1 (ATPGD1). *J Biol Chem* 285, 9346–9356 [PubMed: 20097752]
55. Cripps MJ, et al. (2017) Carnosine scavenging of glucolipotoxic free radicals enhances insulin secretion and glucose uptake. *Sci Rep* 7, 13313 [PubMed: 29042678]

56. Richarme G, et al. (2015) Parkinsonism-associated protein DJ-1/Park7 is a major protein deglycase that repairs methylglyoxal- and glyoxal-glycated cysteine, arginine, and lysine residues. *J Biol Chem* 290, 1885–1897 [PubMed: 25416785]
57. Swzergold BS, et al. (2001) Human fructosamine-3-kinase: purification, sequencing, substrate specificity, and evidence of activity in vivo. *Diabetes* 50, 2139–2147 [PubMed: 11522682]
58. Zheng Q, et al. (2020) Protein arginine deiminase 4 antagonizes methylglyoxal-induced histone glycation. *Nat Commun* 11, 3241 [PubMed: 32591537]
59. Jing H and Lin H (2015) Sirtuins in epigenetic regulation. *Chem Rev* 115, 2350–2375 [PubMed: 25804908]
60. Kosciuk J, et al. (2019) Updates on the epigenetic roles of sirtuins. *Curr Opin Chem Biol* 51, 18–29 [PubMed: 30875552]
61. Seto E and Yoshida M (2014) Erasers of histone acetylation: the histone deacetylase enzymes. *Cold Spring Harb Perspect Biol* 6, a018713 [PubMed: 24691964]
62. Kutil Z, et al. (2018) Histone Deacetylase 11 Is a Fatty-Acid Deacylase. *ACS Chem Biol* 13, 685–693 [PubMed: 29336543]
63. Jin J, et al. (2016) SIRT2 Reverses 4-Oxononanoyl Lysine Modification on Histones. *J Am Chem Soc* 138, 12304–12307 [PubMed: 27610633]
64. Cui Y, et al. (2017) Histone Ketoamide Adduction by 4-Oxo-2-nonenal Is a Reversible Posttranslational Modification Regulated by Sirt2. *ACS Chem Biol* 12, 47–51 [PubMed: 28103679]
65. Jennings EQ, et al. (2021) Sirtuin 2 Regulates Protein LactoylLys Modifications. *Chembiochem*
66. Moreno-Yruela C, et al. (2021) Class I Histone Deacetylases (HDAC1–3) are Histone Lysine Delactylases. *BioRxiv*
67. Cao J, et al. (2015) DJ-1 as a human oncogene and potential therapeutic target. *Biochem Pharmacol* 93, 241–250 [PubMed: 25498803]
68. Sanghvi VR, et al. (2019) The Oncogenic Action of NRF2 Depends on De-glycation by Fructosamine-3-Kinase. *Cell* 178, 807–819 e821 [PubMed: 31398338]
69. Yuzhalin AE (2019) Citrullination in Cancer. *Cancer Res* 79, 1274–1284 [PubMed: 30894374]
70. Lewis HD, et al. (2015) Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. *Nat Chem Biol* 11, 189–191 [PubMed: 25622091]
71. Drechsel J, et al. (2018) Chemical Probe To Monitor the Parkinsonism-Associated Protein DJ-1 in Live Cells. *ACS Chem Biol* 13, 2016–2019 [PubMed: 30011180]
72. Tashiro S, et al. (2018) Discovery and Optimization of Inhibitors of the Parkinson's Disease Associated Protein DJ-1. *ACS Chem Biol* 13, 2783–2793 [PubMed: 30063823]
73. Wang J, et al. (2015) Generation and characterization of antibodies against arginine-derived advanced glycation endproducts. *Bioorg Med Chem Lett* 25, 4881–4886 [PubMed: 26117561]
74. Arnaudo AM and Garcia BA (2013) Proteomic characterization of novel histone post-translational modifications. *Epigenetics Chromatin* 6, 24 [PubMed: 23916056]
75. Brunk E, et al. (2018) Characterizing posttranslational modifications in prokaryotic metabolism using a multiscale workflow. *Proc Natl Acad Sci U S A* 115, 11096–11101 [PubMed: 30301795]
76. Tran JC, et al. (2011) Mapping intact protein isoforms in discovery mode using top-down proteomics. *Nature* 480, 254–258 [PubMed: 22037311]
77. Shechter D, et al. (2007) Extraction, purification and analysis of histones. *Nat Protoc* 2, 1445–1457 [PubMed: 17545981]
78. Zheng Q, et al. (2020) Synthesis of an Alkynyl Methylglyoxal Probe to Investigate Nonenzymatic Histone Glycation. *J Org Chem* 85, 1691–1697 [PubMed: 31875401]
79. Galligan JJ, et al. (2017) Quantitative Analysis and Discovery of Lysine and Arginine Modifications. *Anal Chem* 89, 1299–1306 [PubMed: 27982582]
80. Sibbersen C, et al. (2018) Profiling of Methylglyoxal Blood Metabolism and Advanced Glycation End-Product Proteome Using a Chemical Probe. *ACS Chem Biol* 13, 3294–3305 [PubMed: 30508371]
81. Sibbersen C, et al. (2013) Development of a chemical probe for identifying protein targets of alpha-oxoaldehydes. *Chem Commun (Camb)* 49, 4012–4014 [PubMed: 23563755]

82. Chen N, et al. (2018) Chemical proteomic profiling of protein N-homocysteinylation with a thioester probe. *Chem Sci* 9, 2826–2830 [PubMed: 29732068]
83. Mir AR, et al. (2014) Methylglyoxal mediated conformational changes in histone H2A-generation of carboxyethylated advanced glycation end products. *Int J Biol Macromol* 69, 260–266 [PubMed: 24879922]
84. Arakawa S, et al. (2020) Mass spectrometric quantitation of AGEs and enzymatic crosslinks in human cancellous bone. *Sci Rep* 10, 18774 [PubMed: 33139851]

Outstanding Questions

1. The cellular metabolome is extremely dense and diverse, consisting of thousands of different reactive molecules. Relative to the surfeit of species, only a handful of non-enzymatic covalent modifications (NECMs) have been identified on histones and their effect on chromatin structure and function remain murky. This apparent contradiction raises the question: are there other uncharacterized NECMs that exist on histones and can they play a role in linking metabolism and cell fate through epigenetic mechanisms?
2. Are all NECMs enzymatically reversible? What other mechanisms exist to regulate NECMs broadly in the cell and more specifically on histones?
3. What combination of chemical, proteomics, and genetics tools can be utilized to uncover and characterize new NECMs on histones as well as the cellular mechanisms that regulate them?
4. What chromatin-associated events do NECMs regulate? Are certain histone NECMs recognized and “read” by chromatin associated proteins and are they part of the so-called “histone code”? Furthermore, are some histone NECMs associated with a specific epigenetic state? Can they induce the genetic expression/repression of specific genes resulting in a cellular phenotypic outcome?
5. Are putative NECM surveillance factors and oncoproteins (including DJ-1, FN3K, PADI4, and GLO1/2) new therapeutic targets in cancer?
6. Can the chemical composition of NECMs be linked and then calibrated as biomarkers for cell state, age, or disease?

Highlights

- Post-translational modifications (PTMs) of proteins are introduced either by the specific activity of “writer” enzymes or the spontaneous chemical reaction with reactive small molecules forming non-enzymatic covalent modifications (NECMs).
- Histones are particularly susceptible to accumulate NECMs due to their long half-lives, unstructured tails, and abundance of accessible reactive arginine and lysine residues that are key sites for regulatory enzymatic PTMs.
- NECMs on histones compete with enzymatic PTMs as well as alter chromatin structure, subsequently affecting tightly regulated genetic processes, and ultimately resulting in global cellular phenotype changes.
- NECMs can be enzymatically “erased” as well as prevented by scavenger systems including both small molecules and proteins.
- Advances in biophysical methods, immunoassays, mass spectrometry, chemical probe development, and chemoproteomics have established a platform which can be leveraged to predict, track, manipulate, and study histone NECMs.

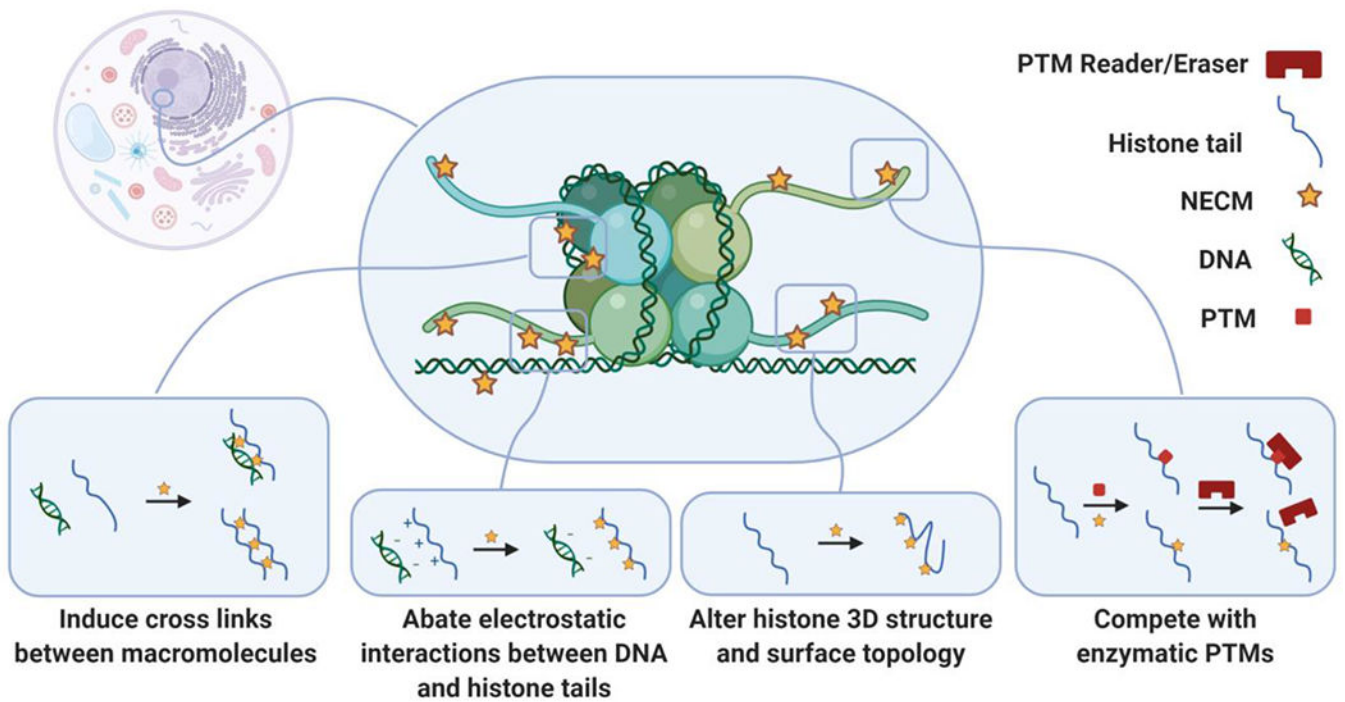


Figure 1. Effects of histone NECMs on Chromatin.

Non-enzymatic chemical modifications (NECMs) can induce cross-links, abate interactions between DNA and histones, alter histone three-dimensional structure and surface topology, and compete for sites with enzymatic post-translational modifications (PTMs).

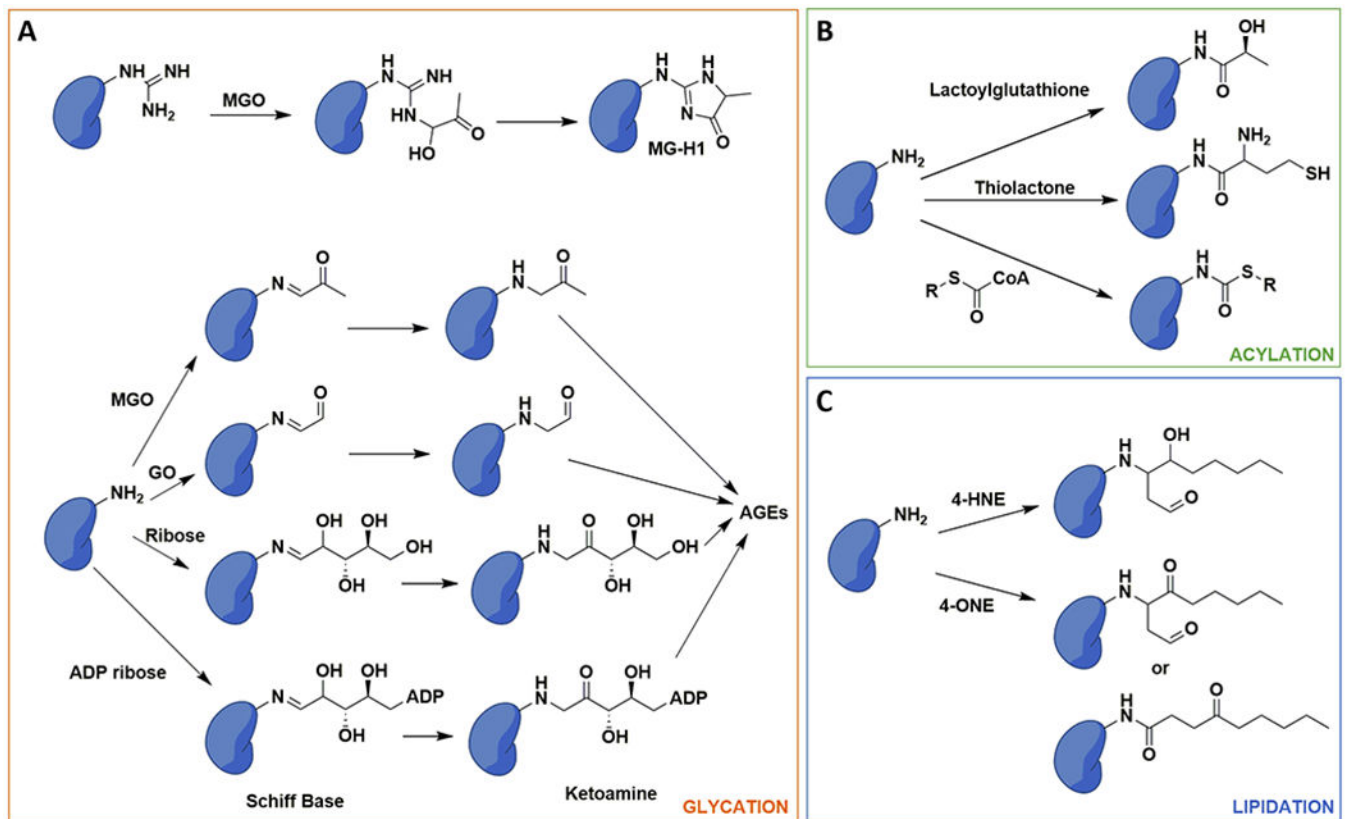


Figure 2. Chemical nature of histone glycation, acylation and lipidation NECMs.

A) Glycation adducts go through stabilized intermediates but can eventually form advanced glycation end products (AGEs). MGO: methylglyoxal; MG-H1: methylglyoxal hydroimidazolone 1; GO: glyoxal. B) Activated acyl groups such as the glyoxalase 1 (GLO1) product lactoylglutathione or metabolic cofactors can modify histone lysine ϵ -amines. C) Products of lipid peroxidation, including 4-hydroxynonenal (4-HNE) and 4-oxo-2-nonenal (4-ONE) can react with and modify histone amines.

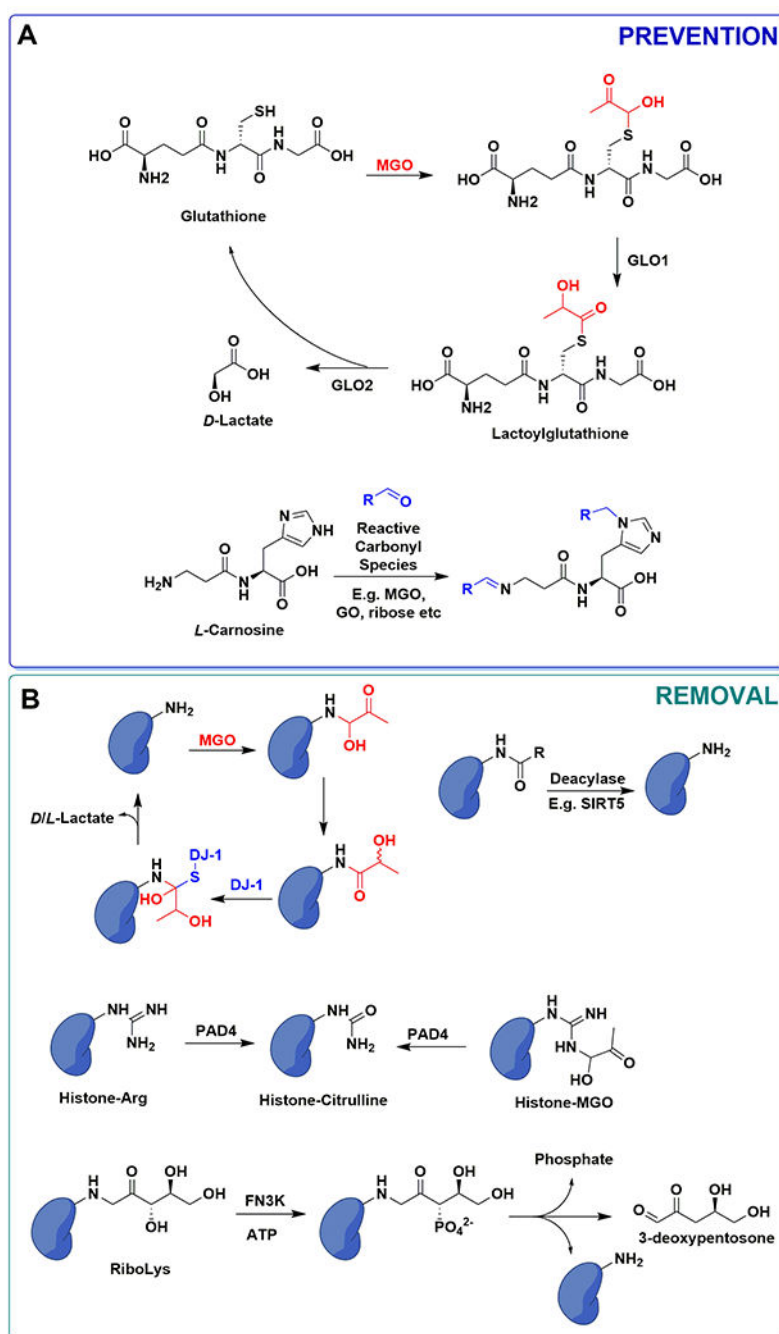


Figure 3. Cellular housekeeping mechanisms of NECMs.

A) GLO1/GLO2 scavenger system (top). GLO1 can catalyze the reaction between glutathione and methylglyoxal (MGO), forming lactoylglutathione which can then be hydrolyzed by GLO2 to release *D*-lactate and regenerate glutathione. Small molecule nucleophiles like *L*-Carnosine (bottom) can scavenge reactive species like MGO by reacting with them and diminishing the pool of reactive small molecule. B) Enzymatic systems can remove the adducts (DJ-1, deacylases, or FN3K) or modify the early intermediates (PAD4) to reduce the likelihood of further damage and downstream consequences.

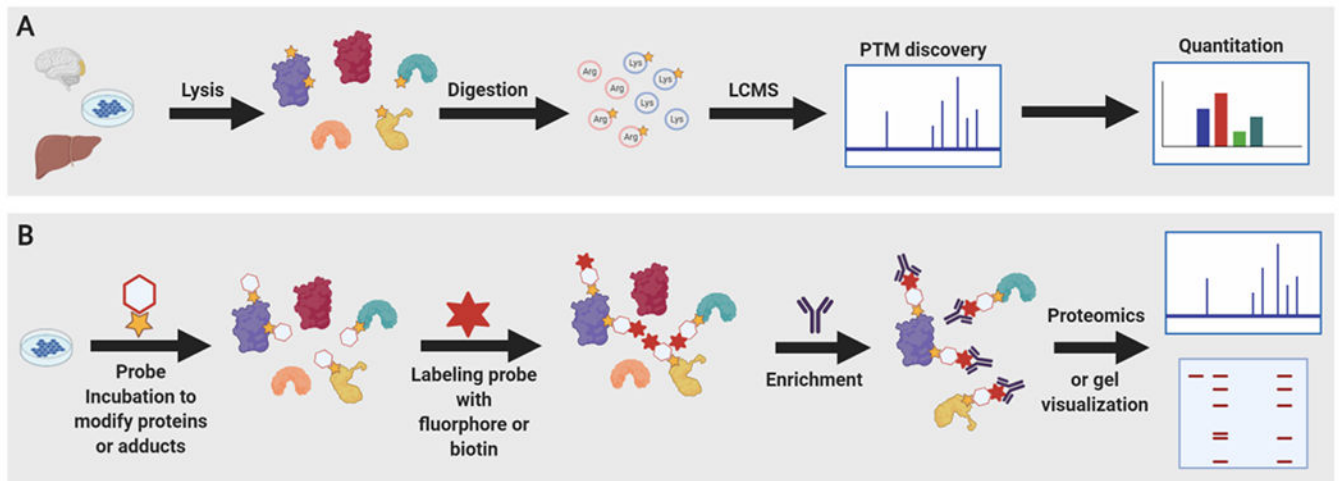


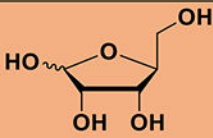
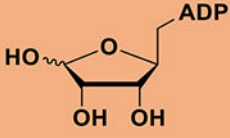
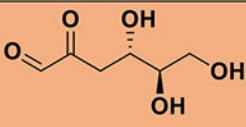
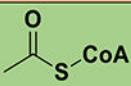
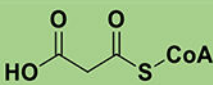
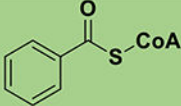


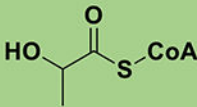
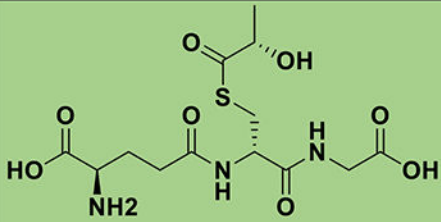
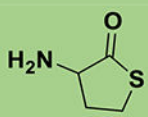
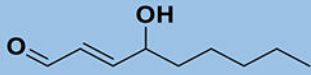
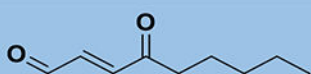
Figure 4. New strategies to uncover and study histone NECMs.

A) Quantitative Analysis and Discovery of Arginine and Lysine Modifications (QuARKMod) is a label-free method to discover and quantitate new modifications on lysine and arginine residues from live cells. Relevant cells or tissues are lysed, proteins are isolated, digested, and analyzed by liquid chromatography mass spectrometry (LCMS), before predicted adduct masses are searched and quantitated. B) General strategy for chemical probe application to reveal or characterize non-enzymatic chemical modifications (NECM) targets. Probes either modify existing NECMs or introduce trackable NECM mimics in cells. The introduced probe can itself be labeled with fluorophore or biotin which are used for detection and enrichment for western blot or proteomic analysis.

Table 1.

Structures of molecules that form histone non-enzymatic chemical modifications (NECMs), as well as their detected effects on chromatin physiology.

	Reactant	Structure	Effect(s) Detected	Ref(s)
Glycation	Methylglyoxal (MGO)		Forms AGEs ^a , alters gene accessibility, transcription, and PTM landscape ^b , modifies DNA, and induces crosslinks	[16, 18, 19, 56, 80, 83]
	Glyoxal (GO)		Forms AGEs, alters 3-dimensional histone structure, modifies DNA, and induces crosslinks	[16, 56]
	Ribose		Forms AGEs, alters PTM landscape, and induces crosslinks	[21-23]
	ADP-Ribose		Forms AGEs, and induces crosslinks	[21]
	3-Deoxyglucosone (3-DG)		Forms AGEs, alters 3-dimensional histone structure, and induces crosslinks	[20]
Acylation	Acetyl CoA		Alters PTM landscape	[26, 30]
	Malonyl CoA		Alters PTM landscape and introduces new 3-dimensional topology	[25, 27]
	Benzoyl CoA		Alters PTM landscape and introduces new 3-dimensional	[32]

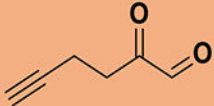
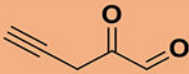
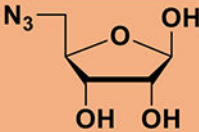
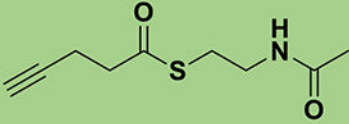
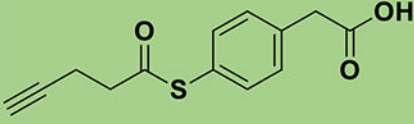
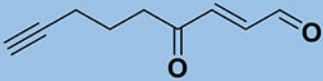
Author Manuscript			topology	
	Lactyl CoA		Alters PTM landscape and introduces new 3-dimensional topology	[34]
	Lactoylglutathione		Alters PTM landscape and introduces new 3-dimensional topology	[36]
Author Manuscript	Thiolactone		Alters gene expression and PTM landscape, introduces new 3-dimensional topology	[84]
Lipidation	4-Hydroxynonenal (4-HNE)		Alters PTM landscape, introduces new 3-dimensional topology, and induces crosslinks	[41-43, 45]
	4-Oxo-2-nonenal (4-ONE)		Alters PTM landscape and introduces new 3-dimensional topology	[44, 46]

^a AGEs: Advanced Glycation End products are the resulting diverse group of products in a glycation chemical reaction cascade.

^b Alters PTM landscape refers to affecting the patterning of enzymatically introduced PTMs by competitive or other means.

Table 2.

Structures of chemical probes used to characterize and study histone non-enzymatic chemical modifications (NECMs) as well as their reported uses.

	Probe	Application	Ref(s)
Glycation		Adduct biotin or fluorescent labeling, site determination, enrichment for proteomics	[80, 81]
		Adduct biotin or fluorescent labeling, and subsequent enrichment of modified proteins	[78]
		Adduct fluorescent labeling and subsequent enrichment for proteomics	[23]
Acylation		Adduct biotin or fluorescent labeling, and subsequent enrichment of modified proteins for proteomics	[25]
		Capturing and fluorescently visualizing or enriching homocysteine adducts for proteomics or site determination	[82]
Lipidation		Adduct biotin or fluorescent labeling, and subsequent enrichment of modified proteins	[63, 64]