Influences of DNA Methylation and Histone Acetylation on Gene Expressions

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Abstract. Epigenetics is the concept of modifying the chromatin to achieve the different levels of transcription. This paper mainly focuses on two well-discovered mechanisms of epigenetics: DNA methylation and histone acetylation. While both affect chromatin assembly, the former acts on cytosine in the DNA strand, while the latter mostly acts on the amino acid lysine in the protein histones. Upon methylation and acetylation, the winding of chromatin is either strengthened or loosened. In addition to their actions, this paper illustrated the chemistry bases of DNA methyltransferases (DNMTs), histone acetylases (HATs), and histone deacetylases (HDACs), including the inhibitors that act against them. The discovery of these factors gives insights into clinical adaptations in analyzing pathologies of complex diseases. DNA methylation is largely being explored in neuropathic pain for diagnostic purposes and intensity detection. This paper also stresses the onerous evaluations taken to draw a conclusion in the perspective of epigenetics and the possible attention to be drawn when contradictive patterns are detected.

Keywords: DNA methylation, Histone Acetylation, Nucleosomes, HATs, HDACs, DNMTs, Imprinting Gene, Neuropathic Pain.

1. Introduction

The significance of Epigenetics in recent days is widely accepted. Not only did its past investigations uncover a comprehensible image for contemporary scientists, but it also paved the way for identifying the hallmarks of specific diseases once the findings are applied. Epigenetics is commonly known as a way of modifying gene expression in the sense that it does not alternate the intrinsic DNA sequence [1]. These various phenotypes are achieved concerning major processes like DNA methylation, Histone modifications, and the production of Long non-coding RNA. Through increasing or decreasing the attractive forces between histone and the winding DNA sequence around them or physically blocking the transcription of chromatin, various profiles of gene expression patterns can be exhibited. In this paper, I focus on DNA methylation and Histone modifications. Their mechanisms and relative disease pathologies will also be explored.

2. DNA methylation

2.1. Methylating the Cytosine Residue

DNA methylation, by name, is simply adding a methyl group to the nucleotides that constitute the chromatin. Usually, methylation happens on the residue cytosine before the guanine in a region abundant in cytosines and guanines [1]. This region is called the CpG island and is often unmethylated to allow subsequent gene transcription to perform [1]. The methyl group is obtained from S-adenyl methionine (SAM) and added to the 5'C of that cytosine, forming 5-methylcytosine (5mC) [1]. The process of moving the methyl group onto and away from the cytosine residue depends on the utilization of DNA methyltransferases (DNMTs) [1,2]. The three members of the DNMT family, DNMT1, DNMT3a, and DNMT3b, are enzymes that interact with the nucleotide through covalent bonding [2,3]. While all three of the enzymes are responsible for the maintenance of the DNA methylation state [2], the discrepancies among them involve the following: First, DNMT1 methylates the hemimethylated DNA to modify the double helix to become symmetrical again [1,4]. Hemimethylated DNA means that only the parental strand of DNA is being methylated at its CpG

sites [4]. Thus, methylating the complementary cytosine on the unmethylated daughter strand recovers the conservatory symmetric pattern [2,4] (Figure 1a). Second, DNMT3a and DNMT3b do not restrict their acts on hemimethylated DNA. Instead, they can carry out de novo methylation on unmethylated naked DNA and hemimethylated DNA [1, 4] (Figure 1b).

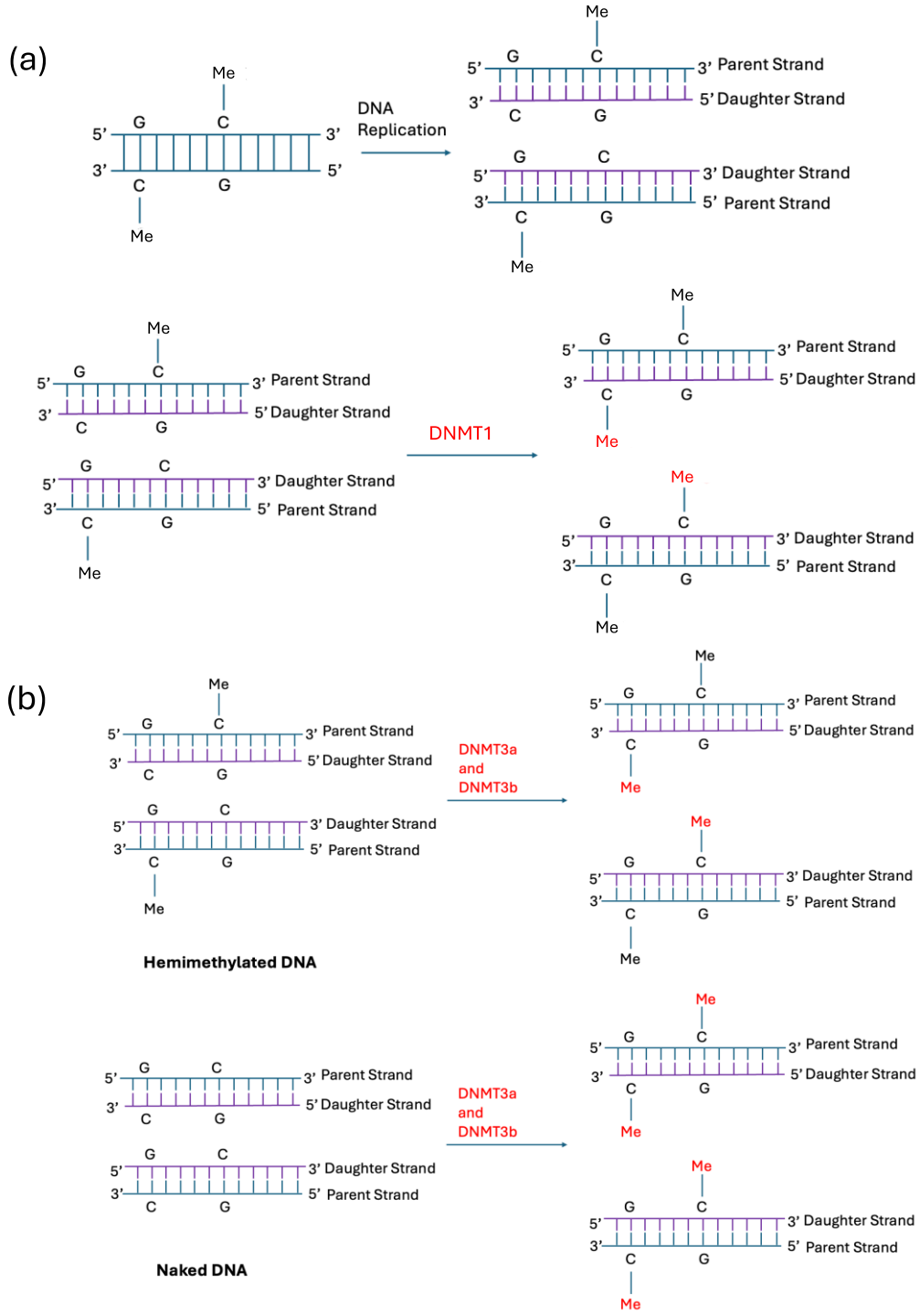
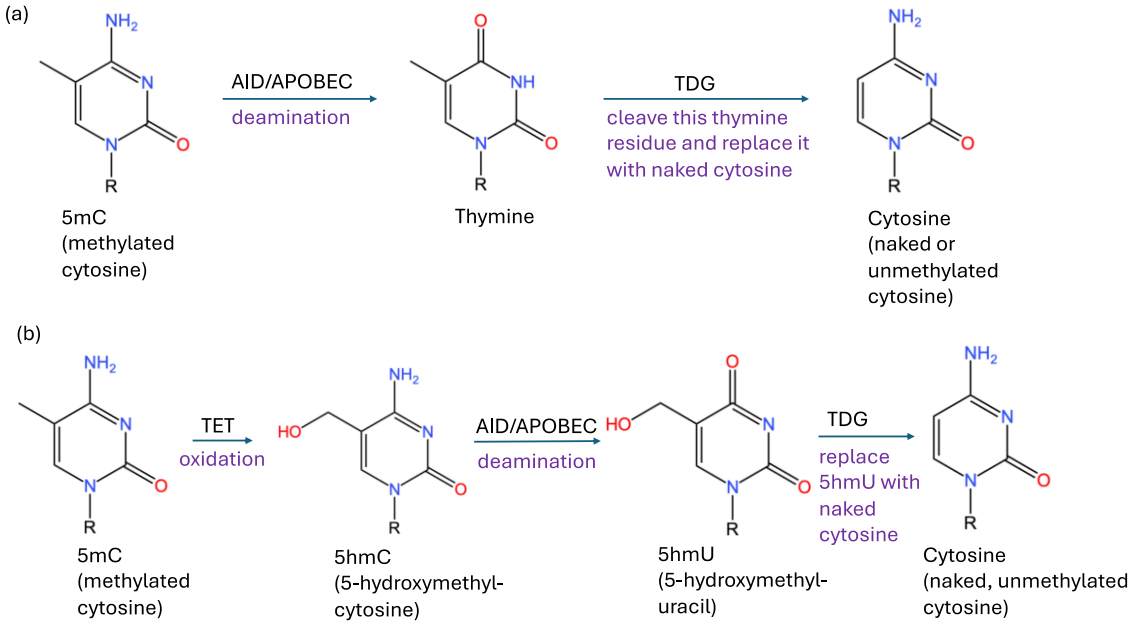


Figure 1. [1, 4] Targets of DNMTs

Fig.1 (a) Presents the function of DNMT1, in which, after DNA replication, usually the hemimethylated DNA is obtained. DNMT1 adds the methyl group, shown in red, to the cytosine in the complementary daughter strand (the purple strand), and the maintenance of the DNA methylation pattern is obtained. (b) Presents the function of DNMT3a and DNMT3b, in which they can methylate both hemimethylated DNA and unmethylated DNA, methyl groups shown in red are the ones being transferred by the DNA methyltransferases.

2.2. DNA Demethylation

Based on the 'reversibility' of Epigenetic mechanisms, if a methyl group can be attached to the nucleotide cytosine by DNMTs, that methyl group is capable of being removed by other factors. To simply stress the chemistry underpins this pathway, Figure 2 demonstrates four ways for demethylation to occur. Pathway 1 is to convert 5mC (the methylated form of cytosine) to thymines directly by AID/APOBEC (Activation-induced Cytidine Deaminase/Apolipoprotein B mRNA-editing Enzyme Complex) [1], it is a polynucleotide cytidine deaminase [5], an enzyme that removes the amine group and attaches an oxygen with double bond instead. Subsequently, an enzyme called TDG (Thymine DNA Glycosylase) removes this thymine from G/T mispairing and replaces it with naked cytosine, illustrated by RefSeq, Jul 2008 [1,6]. The process illustrated in the precedent sentence about how 5mC is turned into unmethylated cytosine residue is called the active DNA demethylation pathway [6]. Pathway 2 involves the enzyme TETs (Ten-eleven translocation family protein), which is a deoxygenase that oxidizes 5mC to form 5-hydroxymethyl-cytosine (5hmC) [7], which means one of the hydrogen atoms within the methyl group is replaced by a hydroxyl group [1]. The second step within this pathway utilizes AID/APOBEC again, undergoing deamination [5], turning 5hmC into 5hmU (5-hydroxymethyl-uracil) [1]. The last step is to convert 5hmU into the naked cytosine, adopting the enzyme TDG [1,7]. This is because TDG is not only capable of cleaving off the mispaired thymine, but it can also cleave off 5hmU and replace it with cytosine [1]. Not only one paper suggests that TETs' ability is not restricted to oxidizing 5mC to 5hmC [1,7,8]. Thus, it leads to pathway 3. As discussed above, TET is capable of oxidizing 5mC into 5hmC, which was the first step within pathway 2. Moreover, TET can further oxidize 5hmC into 5-formyl-cytosine (5fC), which then 5fC could be cleaved and replaced with cytosine by TDG [1,8]. Pathway 4 extends from pathway 3, in which 5fC is again further oxidized by TET into 5-carboxy-cytosine (5caC) [1,7,8]. 5caC is then identified and replaced with naked cytosine by TDG [1]. Hence, it is warranted to expect TET to be a powerful oxidizing agent.



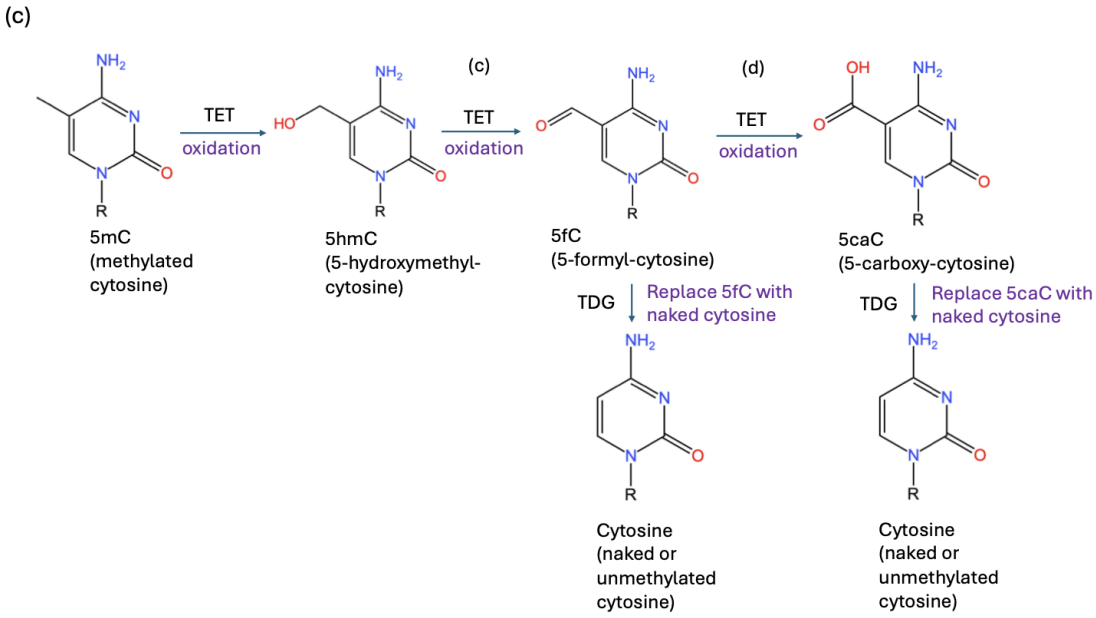


Figure 2. [1] The Four Pathways for Demethylation

Fig.2 Pathway 1 is matched with (a), in which 5mC is deaminated by AID/APOBEC, forming thymine and then being replaced with naked cytosine by TDG. Pathway 2 is matched with (b), in which oxidation happens first by TET, forming the first product 5hmC, and then deamination is carried out by AID/APOBEC, forming 5hmU, lastly TDG function to substitute cytosine in place of 5hmU. Pathways 3 and 4 can be illustrated together while exhibited by (c) and (d). Both start with the first oxidation by TET, turning 5mC into 5hmC. (c) is stressing only one more oxidation on 5hmC, producing 5fC, which can then be replaced with naked cytosine by TDG. (d) is the subsequent oxidation on 5fc, producing 5caC, which can also be replaced with naked cytosine by TDG.

2.3. Results of DNA Methylation

DNA methylation results in the silence of gene expression. Alternatively, it can be expected that demethylation will retrieve the active state of gene transcription. It was said that the methylations on cytosines at the promoter regions of inactive genes would stabilize the nucleosomes to prevent that piece of gene from being transcribed, as transcription factors (TFs) cannot bind to those promoters anymore [9]. Although it was discovered that the CpG island methylation level indicates the extent to which TFs will bind, most CpG islands that are associated with promoters remain rarely methylated [1]. Here, the structures of the chromatin and nucleosomes will be illustrated. The DNA sequence is made up of phosphate, pentose sugar, and bases, with that phosphate group, it is acidic and negatively charged. Histones are small proteins that the DNA sequence is winded onto; they are strongly alkaline because of those basic amino acids and slightly positive because of lysine (K) and arginine (R) residues within their amino acid sequences [10] (Figure 3). Thus, it explains the stable structure that chromatin has formed. The small repeated units that constitute the chromatin are called the nucleosomes [11]. Therefore, the stronger the attractive forces between the DNA and the histone, the more tightly the nucleosomes are packed, the less likely that the DNA sequence will be exposed to transcription factors, and reduces the possibility of gene expression. DNA methylation is the process that causes the DNA to wrap around the histone more tightly and inhibits transcription [1]. Inversely, unmethylated DNA will be normally and successfully transcribed (Figure 4).

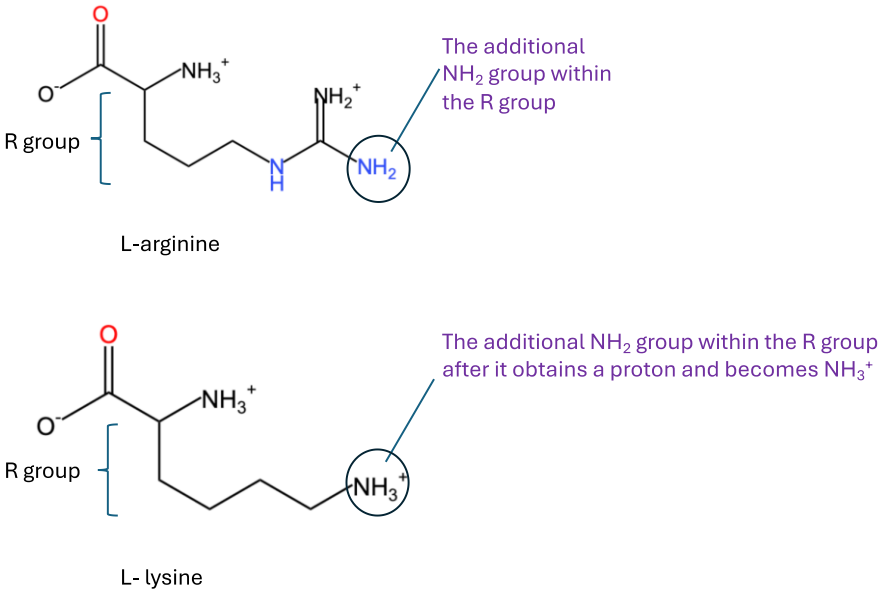


Figure 3. [10] The Lysine and Arginine Residues Making Up the Histone Tails

Fig.3 Both the lysine and arginine contain an R group with an additional NH2 group. This NH2 group is responsible for leading K and R to be basic. Furthermore, once the NH2 group accepts one more proton, which is H+, the NH2 group becomes NH3+. This also makes K and R a bit positive. Thus, attracting the acidic and negatively charged naked DNA sequence to wind around them.

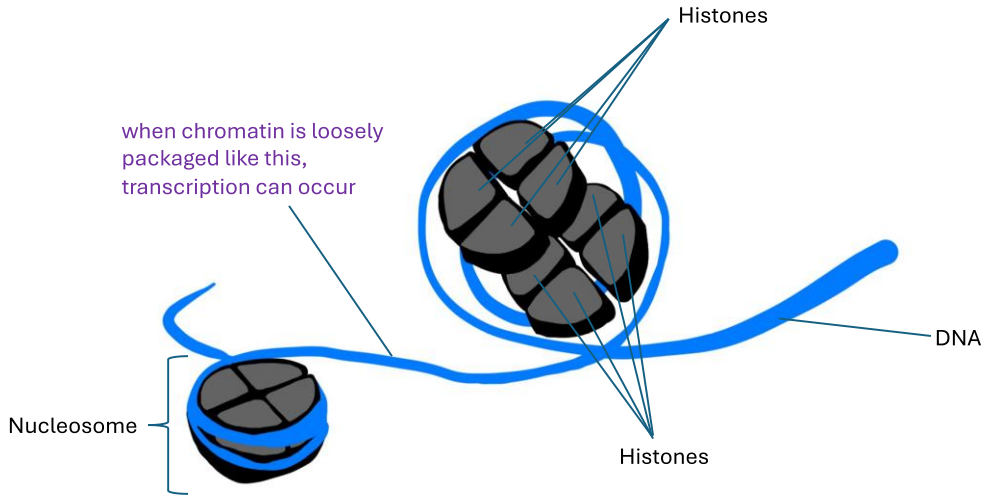


Figure 4. [1, 9, 11] The Zoomed-in Structure of Chromatin

Fig. 4 Each nucleosome consists of 8 histone proteins (shown in black and grey) that are wrapped around the DNA sequence (shown in blue). How tightly the chromatin is winded indicates the possibility of transcription to occur.

2.4. Genomic Imprinting

Talking about DNA methylation must touch on imprinted genes. Genomic imprinting, by definition, is the expression of only one copy of the genes in both maternal and paternal chromosomes while the other copy is being methylated and silenced [1,12]. Thus, taking a set of two chromosomes as an example, although they are identical in sequence, some genes are only expressed in the paternal chromosomes, while some other genes are only expressed in the maternal chromosome [13]. Linking these silenced genes to epigenetics, they are caused by DNA methylation. In addition, this DNA methylation pattern is directly obtained from the parent the offspring inherited from and exhibits the same gene expression brought by that parent [13, 14]. Epigenetic factors in the context of genomic imprinting can be either maintained or erased and rewritten throughout the offspring's embryonic

development in 2 cycles; this is the explanation of Epigenetic reprogramming [14]. Nevertheless, imprinted genes can only maintain their DNA methylation pattern, passing cycle 1, which is when the zygote exists as a blastocyst [14]. Cycle 2, when primordial germ cells start to develop, erasing and reprogramming of those existing epigenetic markers begin [14]. Therefore, another description of epigenetics is revealed: the changes in gene expression are not due to genetic mutation but can be maintained and passed on to the next generation [15].

3. Histone Acetylation

3.1. Histone Modifications Basic Background

Histone modification stands for the modifications on the N-terminal tails of the amino acids that constitute the histones. Extending from the picture of nucleosomes I demonstrated previously, these N-terminal tails would extend out of each nucleosome to interact with the neighboring nucleosomes, thus altering the tightness of the chromatin [16]. Any modifications on the residues of that N-terminal tail post-translationally, for example, acetylation, phosphorylation, and methylation, would affect the stability of chromatin as the neutral status would be influenced [10,16]. First, I shall elaborate on the structure of histones within the nucleosomes (Figure 5). Each nucleosome consists of an octamer of histones in the center, with two of each H2A, H2B, H3, and H4 histone arranged in that octamer shape, and they are held together by a strand of DNA [17]. The octamer was made step by step. Two H3 histones and two H4 histones assemble to form a H32-H42 tetramer. This tetramer is first bound to the naked DNA strand. Second, an H2A and an H2B histone bind together to form an H2A-H2B dimer. Subsequently, two of these dimers are added to the DNA-bound tetramer, forming an octameric structure [17]. Each histone contains two tails: one is the amino group-end tail (N-terminal tail), and the other is the carboxyl group-end tail (C-terminal tail) [19]. Hence, in total, for an octamer of histones, 16 tails are presented. Nevertheless, while the N-terminus tail of each histone will protrude out of the nucleosome, the C-terminus tail of each histone H3 and H4 will remain inside the histone core, and only the C-terminal tail of each H2A and H3B will extend out of the nucleosome and are subject to modifications. Between adjacent nucleosomes is the linker DNA, including the section of DNA just starting to wind around that octamer and the section of DNA just finished winding around that octamer [17]. In addition, H1 anchors the histone octamer with the DNA surrounding it and is removed during transcription to open the chromatin loosely [17, 18].

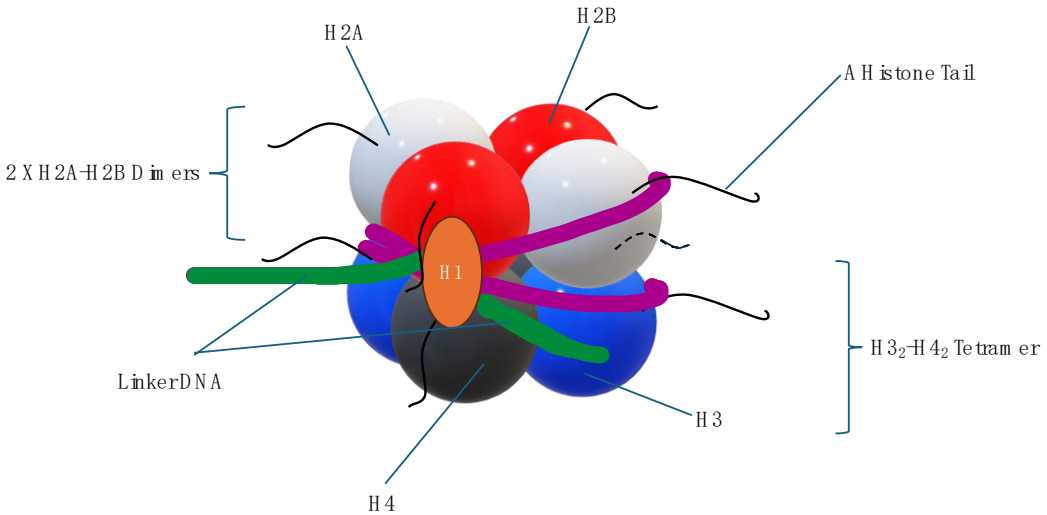


Figure 5. [17] The Core Octameric Histones Within a Nucleosome

Fig. 5 Showing N-terminal tails only. Two H2A histones shown in white, two H2B histones shown in red, two H3 histones shown in blue, and two H4 histones shown in black are arranged in an octameric shape. Each histone has an N-terminal tail extending out of the nucleosome. The linker DNA shown in green is anchored by histone H1 (shown in orange).

It was found that the attractions between the histone and the DNA sequence through various forms of chemical bonding. The amino acids from both the core histones and the histone tails will interact with the surrounding DNA, including the amino acid backbone of histones and their side chains [14, 20]. While the amino acids making up the core histones usually function to stabilize the nucleosome, the amino acids in histone tails are subject to modifications mentioned above to intervene in the assembly of chromatin [17]. The amide linkage within the histone backbone will interact strongly with the phosphate group of DNAS through hydrogen bonding [17, 20]. As mentioned above, the DNA and the histone are also attracted through electrostatic forces. It was found that although lysine and arginine perform positive charges on tails, the contact surfaces between the naked DNA and the core histone were revealed to be negative [17]. The negatively charged surface was proved to attract the positive histone tails protruding from the neighboring nucleosomes [17], thus assisting the linkage between adjacent nucleosomes and the tightness of the chromatin. Therefore, it can be predicted that any epigenetic post-translational modifications will result in either loosening or tightening of the chromatin through alterations of the charged states to expose the winded portion of DNA.

3.2. Mechanisms of HATs and HDACs

Histone acetylation, just like the mechanism of DNA methylation, involves factors transferring that acetyl group onto the residues of histone tails or removing that acetyl group away from the histone. From all the residues that constitute the histone N-terminal tail, lysine and arginine present extra NH2 groups that will capture a proton to become positive NH3+. In the case of histone acetylation, the targeted residue is lysine. A family of enzymes, the histone acetyltransferases (HATs), will act together with acetyl CoA to transfer an acetyl group to the NH3+ group in lysine's side chain and reduce the positiveness of lysine [16]. Supplement to the previous sentence, the acetyl group is donated from the enzyme acetyl CoA (Figure 6a). Once the positiveness of the histone tail is reduced, the attraction between the histone and the DNA will be influenced, and the chromatin will be loosened.

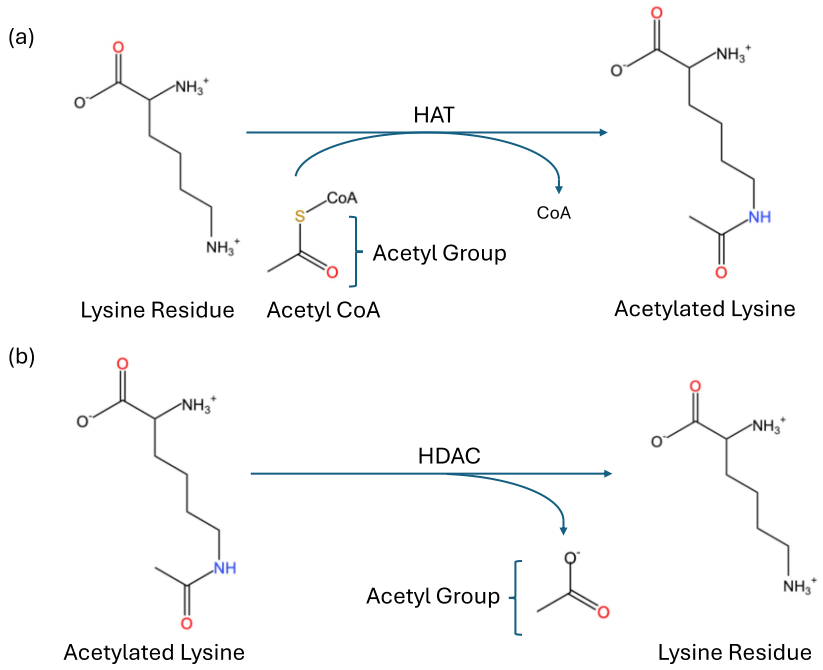


Figure 6. [2, 16, 21] The Lysine Acetylation and Deacetylation

Fig.6 (a) Histone Acetylation: demonstrates the process when HATs obtain the acetyl group from the acetyl CoA enzyme and add it to lysine. (b) Histone Deacetylation: HDACs remove the acetyl group from the acetylated lysine and return the original lysine residue.

The HATs family is categorized mainly into five groups: HAT1, Gcn5/PCAF (KAT2A/KAT2B, where KAT stands for lysine acetyltransferase [22]), MYST (KAT5, KAT5A, KAT6B, KAT7, KAT8), p300/CBP (KAT3B/KAT3A), and Rtt109 (KAT11) [16, 23]. These five subfamilies share

similar cores that are structured to bind acetyl CoA, and around the core, the surrounding C-terminals and N-terminals function to the binding of histone [23]. Classifying these groups into two larger types, type-A and type-B HATs [16]. The difference between the two would be the location of their targeted histone [16]. HAT1 and Rtt109 should belong to type-B HATs [24, 25]. Gcn5/PCAF, MYST, and p300/CBP belong to type-A HATs [22, 26]. Type-B HATs primarily acetylate histones that have not been incorporated into the core histone octamer, they stay in the cytoplasm [16, 27]. They are responsible for acetylating H4 within the cytoplasm at the place of lysine number 5 (K5) and at lysine number 12 (K12) [16]. On the other hand, type-A HATs mainly function in the nucleus and acetylate chromosomal histones [28]. This paper targets the histone acetylation patterns that result in gene transcription involving type-A HATs. As illustrated above, histone acetylation affects the winding of the chromatin and the establishment of its euchromatin and heterochromatin areas [29]. Thus, maneuvering gene expression through targeting the patterns of histone tail acetylation is a goal for modern scientific research. This wish is founded on the discovery of the difference in acetylation patterns between histone H4 in the euchromatin and the heterochromatin region, where the former exhibited four sites of lysine residues on H4 that are acetylated while the latter region lacks these acetylations [29]. This, in turn, proves that the euchromatin region is usually active during interphase to duplicate DNA information. These four sites on H4, K5, K8, K12, and K16 are also being interpreted using strategies such as deacetylation from histone deacetylase (HDACs) to shift acetylation sites [29]. This technique is also used on H3, and both experiments aim to find the primary factors controlling the assembly and disassembly of nucleosomes [29].

The ‘reversibility’ characterized by epigenetics is also exhibited by histone tail deacetylation. While HATs carry out acetylation, HDACs undergo deacetylation (Figure 6b) [30]. To describe HDAC actions in brief. A zinc ion is responsible for polarizing the C=O double bond within the acetyl group connected to the N-terminal of the lysine residue, and this prepares for the nucleophilic attack brought by the surrounding water [31]. The two residues of HDAC, tyrosin, and histidine act together to withdraw that acetyl group away from lysine [31]. This is the main mechanism carried out by most classes of HDACs. In mammals, the total number of HDACs is 18, and these 18 HDACs are classified into four groups: Class I HDACs (HDAC1, 2, 3, 8), Class II HDACs (HDAC4, 5, 6, 7, 9, 10), Class III HDACs (SIRT1, 2, 3, 4, 5, 6, 7), and Class IV HDACs (HDAC11) [30]. Class III HDACs’ nomenclature is because they have amino acid sequences like the yeast Sir2 protein [30]. Furthermore, Class III HDACs are different from the HDACs from the other three classes because they require additional NAD+ to assist their actions [30, 32]. This paper focuses on Class I and II HDACs as they are the ones that deacetylate the acetyl group of lysine residue on histone N-terminal tails [32].

3.3. HATs and Transcription Activation

The basic mechanisms of HATs are known, and their participation in transcription should be discussed. Some HATs not only unwind the chromatin by acetylation, but they also participate as co-factors incorporated within the transcription machinery [33]. Take the type-A HAT p300/CBP as an example; these two proteins perform as co-factors for transcription that assist activators in binding to the promoter region of a gene [29, 34]. For instance, the nuclear hormone receptor, which is activated by the binding of the ligands within the cytosol, will then be translocated from the cytosol into the nucleus to start the transcription after binding to the regulatory sequence of the DNA [35]. It was discovered that this process needs the participation of p300/CBP to promote the recruitment of RNA polymerase II to activate transcription (Figure 7) [36]. Another factor, the p300/CBP associated factor (P/CAF), also acts as a HAT that acetylates H4 and H3 histones [35]. In addition to its acetylation activity, experimental findings suggest that P/CAF is required to activate nuclear hormone receptors through its binding to specific promoter regions and carry out acetylation on that part of the chromatin, together with p300/CBP [29, 35, 37].

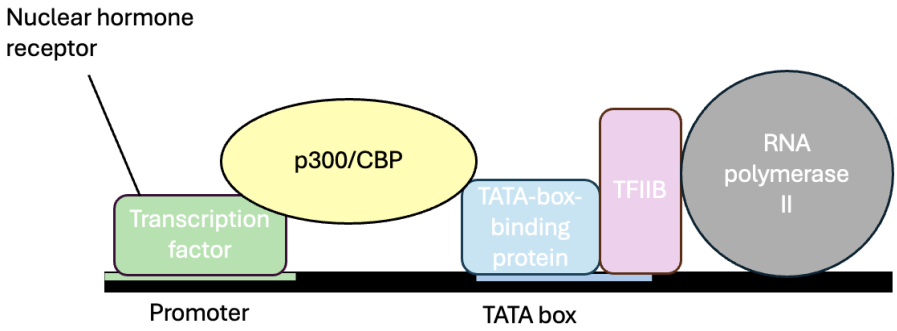


Figure 7. [36] The Recruitment of p300/CBP for Transcription

Fig. 7 For a transcription process initiated by the nuclear hormone receptor to occur, p300/CBP is there to help attract RNA polymerase II (shown in grey) to bind to the DNA sequence. The transcription factor, in this case, is the nuclear hormone receptor shown in green, which is bound to the promoter. The TATA box region is bound by TATA-box-binding protein and Transcription factor IIB (TFIIB). Altogether, it is ready to start transcription.

3.4. HDAC and HAT Inhibitors

Some drugs aim to reverse the actions of HATs and HDACs by identifying proteins and enzymes with felicitous functions. The first class of agents illustrated here are histone deacetylase inhibitors (HDACis). Different types of HDACis, based on their structural differences, may target different HDACs [38]. It is important to stress that HDACs do not always specify their targets to acetylated histone residues, residues that make up the non-histone proteins can also be their sites of action [30]. One example relevant to HDACs targeting the deacetylation of non-histone proteins would be the inhibition of HDAC6 [38]. For instance, experimental results revealed that the overexpression of HDAC6 was found in colon cancerous tissue, and the knockdown of HDAC6 led to a decreased level of cancer cell formation and motility [39]. Hence, discovering agents composed of HDACis that act against HDAC6 would be an ideal approach. Findings suggest that HDAC6 inhibition decreased the phosphorylation level of serine and threonine kinase AKT (also called protein kinase B- PKB) [40, 41]. Thus, less AKT is being activated to monitor the amplified expression of AKT in cancers [41]. Indeed, HDAC6 inhibition does not directly restrict the activation of AKT [40]. HDAC6, together with phosphatase PP1, form a complex called HDAC6-PP1 that captures free PP1 in the cytosol to prevent their actions of dephosphorylating AKT [40]. In other words, HDAC6 promotes AKT phosphorylation and thereby increases AKT activation. Upon the addition of HDACi (S)-8, the HDAC6-PP1 complex dissembles, releasing PP1 to dephosphorylate ATK, hence controlling ATK overexpression in cancerous tissues [38, 40]. On the other hand, the circumstance of HDACs targeting the deacetylation of histone proteins through alterations in chromatin structure is more common as an HDACi target. The cyclin-dependent kinase inhibitor p21, p21 (WAF1/CIP1), is a protein that terminates tumor cell divisions by cell cycle arrest depending on stimuli like HDACis [38, 42]. HDACis inhibit HDAC1 and HDAC2 and increase the acetylation level of the promoter region of the p21 gene to enhance p21 protein synthesis [38].

HATs are associated with the acetylation of histone and non-histone proteins, as discussed. While acetylation increases the level of gene transcription, overexpression of proto-oncogenes will lead to cancer [27]. Therefore, the optimum drug target is to inhibit the hyperactive HATs using the concept of HAT inhibitors. Unfortunately, the lucid chemical mechanisms of how HAT inhibitors act have not been fully discovered [43]. Different families of HATs and their molecular properties are well-defined [43]. The most well-explored HAT inhibitor would be the first one called bi-substrate inhibitors [44]. Bi-substrates of CoA have been found to inhibit HAT activity [44]. Types of CoA bi-substrates targeting p300 include Lys-CoA and H4K16CoA, where Lys-CoA showed a selective inclination towards p300 by inhibiting the transcriptions utilizing p300 (Figure 8) [44,45]. To elaborate on Lys-CoA, the basic characteristic of this bi-substrate inhibitor is that it imitates acetyl-coenzyme As and lysine residues. As mentioned before, the enzyme and the amino acid are targeted

by HATs to bring about acetylation [27]. Thus, Lys-CoA is composed of peptides like lysine on one side and CoA on the other side; in between, they are linked by a space or linker of different lengths [27, 44]. Although the skeletal formula and proposed functions of HAT inhibitors were established, further investigations that elucidate their mechanisms into steps should still be encouraged.

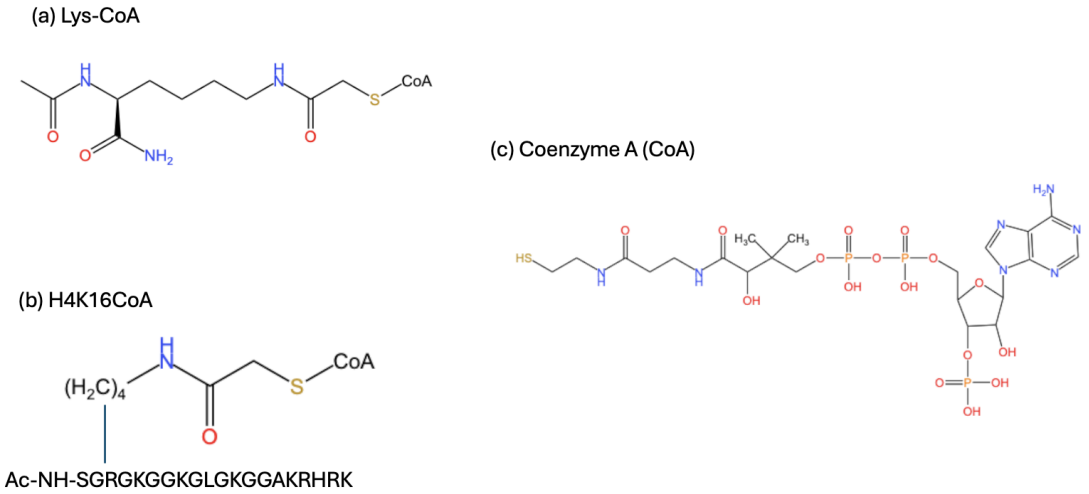


Figure 8. [27, 44, 46] The Structures for Lys-CoA, H4K16CoA, and Coenzyme A

Fig. 8 (a) shows the structure of Lys-CoA with the S of coenzyme A attached to the peptide assembling the amino acid lysine. (b) shows the structure of H4K16CoA, which is like Lys-CoA, but S is attached to histone four at the lysine 16 residue. (c) this is the chemical structure of the coenzyme A, which in both (a) and (b) is abbreviated into S-CoA.

4. Disease Pathology Involving Epigenetics

4.1. DNA Methylation Patterns in Neuropathic Pain

Neuropathic pain, determined simply by name, is the pain generated when neuronal lesions happen, which in turn affects the structure and function of neurons, leading to responses to harmful and non-harmful stimuli [47]. The nervous system is divided into two branches: the central nervous system (CNS) and the peripheral nervous system (PNS). Both CNS and PNS are vulnerable to injuries. Studies revealed that the diagnosis and engendering of nerve pain are closely related to DNA methylation levels [48]. Because DNA methylation mainly happens on the 5'C of the cytosine base within the CpG island and some gene promoter's resident in the CpG region, alterations of methylation levels of those promoter regions should indicate variations in the expressions of relevant genes associated with neurons [1, 48]. It was reported that in the case of global DNA methylation, mouse models of chronic back pain are related to DNA methylation level changes, and injuries of the periphery have something to do with the low methylation status within the prefrontal cortex [49]. Nevertheless, specific changes in DNA methylation patterns upon neuropathic pain should further be investigated and classified. Attempts can be concluded as an overview of the participation of DNMTs within the pathways of neuropathic pain. Experiments proved that DNMTs do not associate with the causation of neuronal pain but rather influence the transcription of pain-related genes [48]. For example, after spinal nerve ligation (SNL), in dorsal root ganglion (DRG) neurons, the methylation level of the *GPR151* (G-protein coupled receptor 151) gene promoter is downregulated due to the decreased expression of DNMT3b [48, 50]. Hence, with the lack of activity from DNMT3b, the restricted methylation encourages *GPR151* expression level and subsequently enhances the pain state induced by SNL [48]. Furthermore, chronic pain as a type of neuropathic pain brought insight to scientific investigations. One paper investigated the similarities and differences in the DNA methylation profiles of the stress driver gene *Fkbp5* between chronic pain generated by ankle inflammation and nerve injury using mouse models [51]. It underpins chronic pain diagnostic

strategies by discovering that DNA methylation level alterations induced by both stimuli in a specific CpG region in the hippocampus can be elicited across different tissues, including blood samples, for efficient diagnostic purposes [51]. Nevertheless, the importance here is the understanding of the dynamic increase and decrease of different types of DNMTs, which results in the increase and decrease of DNA methylation at different CpG sites upon one nerve injury [52]. Establishing a concept of only one DNMT entailed DNA methylation imbalance is insufficient to consider the pathology. Thus, numerous pathways played by a variety of DNMTs should be extensively investigated. Results of DNA methylation profiles in DRG neurons neighboring the site of SNL found not only a decreased level of DNMT3b but also an increased level of DNMT3a [52]. While an increased amount of DNMT3a will induce hypermethylation at the promoters of the Oprm1 (opioid receptor mu 1) gene, leading to gene silencing, the *GPR151* gene would be actively expressed based on a decreased level of DNMT3b transcripts, which leads to its hypomethylation state [48, 52]. In addition, the *Fkbp5* gene DNA methylation level decline was accompanied by an increased *Fkbp5* gene expression [51]. This finding should give insights into other mechanisms that overweighted the action of demethylation upon trauma.

5. Summary

From the above discussion, it is concluded that the basic mechanisms of DNA methylation and histone acetylation are already widely acknowledged, including their biochemistry, which is efficiently being tested and utilized in numerous biomedical realms. The molecular bases of DNMTs, HATs, and HDACs were explored, and their involvements not only in the facet of chromosome assembly but also in transcription and factor recruitment were discussed. Even the chemistry of their inhibitors was well established. However, the importance should be their applications in the clinical analysis of complex diseases. Although epigenetic markers are being observed in inheritable diseases, they still need efforts to localize sites where specific alterations happen rather than globally summarizing the results. Understandably, syndromes often exhibit different patterns of certain downregulated or upregulated genes, like the *Fkbp5* gene, as this paper has quoted. Nevertheless, the difficulties in discovering distinct steps that resulted in their changes in expression still require nowadays scientists to overcome. To sum up, epigenetics opened the horizon of modifying gene expressions. It influences protein synthesis not by generating mutations in the DNA like deletions, inversions, substitutions, etc., but by engendering reversible changes to the chromatin that might be less harmful than those induced permanent DNA damages listed as the former. Hopefully, soon, the pathology of epigenetic origin can be sufficiently established, and the inhibitor drugs will be used more readily.

References

[1] Moore, L. D., Le, T., & Fan, G. (2012). DNA methylation and its basic function. Neuropsychopharmacology, 38 (1), 23 – 38.

[2] Castillo-Aguilera, O., Depreux, P., Halby, L., Arimondo, P. B., & Goossens, L. (2017). DNA methylation targeting: The DNMT/HMT crosstalk challenge. Biomolecules, 7 (1), 3.

[3] Lyko, F. (2018). The DNA methyltransferase family: A versatile toolkit for epigenetic regulation. Nature Reviews Genetics, 19 (2), 81 – 92.

[4] Sharif, J., & Koseki, H. (2018). Hemimethylation: DNA's lasting odd couple. Science, 359 (6380), 1102 – 1103.

[5] Pecori, R., Di Giorgio, S., Paulo Lorenzo, J., et al. (2022). Functions and consequences of AID/APOBEC-mediated DNA and RNA deamination. Nature Reviews Genetics, 23, 505 – 518.

[6] Wang, G., Zhou, Y., Yi, B., Long, Y., Ma, B., & Zhang, Y. (2023). Comprehensive analysis of the prognostic value and biological function of TDG in hepatocellular carcinoma. Cell Cycle, 22 (12), 1478 – 1495.

[7] Zhang, X., Zhang, Y., Wang, C., et al. (2023). TET (ten-eleven translocation) family proteins: Structure, biological functions and applications. Signaling Transduction and Targeted Therapy, 8, 297.

[8] Linowiecka, K., Guz, J., Dziaman, T., Urbanowska-Domańska, O., Zarakowska, E., Szpila, A., Szpotan, J., Skalska-Bugała, A., Mijewski, P., Siomek-Górecka, A., Różalski, R., Gackowski, D., Oliński, R., & Foksiński, M. (2024). The level of active DNA demethylation compounds in leukocytes and urine samples as potential epigenetic biomarkers in breast cancer patients. Scientific Reports, 14 (1), 6481.

[9] Developmental Biology (6th ed.). (2000). Methylation pattern and the control of transcription. Sinauer Associates, Inc.

[10] Lorch, Y., Kornberg, R. D., & Maier-Davis, B. (2023). Role of the histone tails in histone octamer transfer. Nucleic Acids Research, 51 (8), 3671 – 3678.

[11] Annunziato, A. (2008). DNA packaging: Nucleosomes and chromatin. Nature Education, 1 (1), 26.

[12] Genetic imprinting. (n.d.). Genome.gov. https://www.genome.gov/genetics-glossary/Genetic-Imprinting#:~:text=%E2%80%8BGenetic%20Imprinting&text=Genomic%20imprinting%20is%20the%20process,the%20other%20copy%20is%20suppressed.

[13] Mattei, A. L., Bailly, N., & Meissner, A. (2022). DNA methylation: A historical perspective. Trends in Genetics, 38 (7), 676 – 707.

[14] Elhamamsy, A. R. (2017). Role of DNA methylation in imprinting disorders: An updated review. Journal of Assisted Reproduction and Genetics, 34 (5), 549 – 562.

[15] Fernandes, M. T., Almeida-Lousada, H., & Castelo-Branco, P. (2020). Histone modifications in diseases. Histone Modifications in Therapy, 20, 1 – 15.

[16] Bannister, A., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. Cell Research, 21, 381 – 395.

[17] Mariño-Ramírez, L., Kann, M. G., Shoemaker, B. A., & Landsman, D. (2005). Histone structure and nucleosome stability. Expert Review of Proteomics, 2 (5), 719 – 729.

[18] Vicent, G. P., Nacht, A. S., Font-Mateu, J., Castellano, G., Gaveglia, L., Ballaré, C., & Beato, M. (2011). Four enzymes cooperate to displace histone H1 during the first minute of hormonal gene activation. Genes & Development, 25 (8), 845 – 862.

[19] Ouellette, R. J., & Rawn, J. D. (2018). Amino acids, peptides, and proteins. In Organic Chemistry (pp. 929 – 971).

[20] Luger, K., Mäder, A., Richmond, R., et al. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature, 389, 251 – 260.

[21] Mu, A., Latario, C., Pickrell, L., & Higgs, H. (2020). Lysine acetylation of cytoskeletal proteins: Emergence of an actin code. The Journal of Cell Biology, 219.

[22] Fournier, M., Orpinell, M., Grauffel, C., et al. (2016). KAT2A/KAT2B-targeted acetylome reveals a role for PLK4 acetylation in preventing centrosome amplification. Nature Communications, 7, 13227.

[23] Marmorstein, R., & Zhou, M. M. (2014). Writers and readers of histone acetylation: Structure, mechanism, and inhibition. Cold Spring Harbor Perspectives in Biology, 6 (7), a018762.

[24] Ruiz-García, A. B., Sendra, R., Galiana, M., Pamblanco, M., Pérez-Ortín, J. E., & Tordera, V. (1998). HAT1 and HAT2 proteins are components of a yeast nuclear histone acetyltransferase enzyme specific for free histone H4. Journal of Biological Chemistry, 273 (20), 12599 – 12605.

[25] Wang, Y., Miao, X., Liu, Y., Li, F., Liu, Q., Sun, J., & Cai, L. (2014). Dysregulation of histone acetyltransferases and deacetylases in cardiovascular diseases. Oxidative Medicine and Cellular Longevity.

[26] Herrera, J. E., Bergel, M., Yang, X. J., Nakatani, Y., & Bustin, M. (1997). The histone acetyltransferase activity of human GCN5 and PCAF is stabilized by coenzymes. The Journal of Biological Chemistry, 272 (43), 27253 – 27258.

[27] Wapenaar, H., & Dekker, F. J. (2016). Histone acetyltransferases: Challenges in targeting bi-substrate enzymes. Clinical Epigenetics, 8, 59.

[28] Guo, S.-W. (2012). The epigenetics of endometriosis. In Epigenetics in Human Disease (pp. 443–469).

[29] [29] Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. Nature, 389, 349 – 352.

[30] Seto, E., & Yoshida, M. (2014). Erasers of histone acetylation: The histone deacetylase enzymes. Cold Spring Harbor Perspectives in Biology, 6 (4), a018713.

[31] Milazzo, G., Mercatelli, D., Di Muzio, G., Triboli, L., De Rosa, P., Perini, G., & Giorgi, F. M. (2020). Histone deacetylases (HDACs): Evolution, specificity, role in transcriptional complexes, and pharmacological actionability. Genes, 11 (5), 556.

[32] Park, S. Y., & Kim, J. S. (2020). A short guide to histone deacetylases including recent progress on class II enzymes. Experimental & Molecular Medicine, 52, 204 – 212.

[33] Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., & Nakatani, Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell, 87 (5), 953 – 959.

[34] Iyer, N., Özdag, H., & Caldas, C. (2004). p300/CBP and cancer. Oncogene, 23, 4225 – 4231.

[35] Cathcart, P., Lucchesi, W., Ottaviani, S., De Giorgio, A., Krell, J., Stebbing, J., & Castellano, L. (2015). Noncoding RNAs and the control of signalling via nuclear receptor regulation in health and disease. Best Practice & Research Clinical Endocrinology & Metabolism, 29 (4), 529 – 543.

[36] Janknecht, R., & Hunter, T. (1996). A growing coactivator network. Nature, 383, 22 – 23.

[37] Yang, X. J., Ogryzko, V., Nishikawa, J., et al. (1996). A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature, 382, 319 – 324.

[38] Xu, W., Parmigiani, R., & Marks, P. (2007). Histone deacetylase inhibitors: Molecular mechanisms of action. Oncogene, 26, 5541 – 5552.

[39] Zhang, S. L., Zhu, H. Y., Zhou, B. Y., Chu, Y., Huo, J. R., Tan, Y. Y., & Liu, D. L. (2019). Histone deacetylase 6 is overexpressed and promotes tumor growth of colon cancer through regulation of the MAPK/ERK signal pathway. OncoTargets and Therapy, 12, 2409 – 2419.

[40] Balliu, M., Guandalini, L., Romanelli, M. N., D'Amico, M., & Paoletti, F. (2015). HDAC-inhibitor (S)-8 disrupts HDAC6-PP1 complex prompting A375 melanoma cell growth arrest and apoptosis. Journal of Cellular and Molecular Medicine, 19 (1), 143 – 154.

[41] He, Y., Sun, M. M., Zhang, G. G., et al. (2021). Targeting PI3K/Akt signal transduction for cancer therapy. Signaling Transduction and Targeted Therapy, 6, 425.

[42] Abbas, T., & Dutta, A. (2009). p21 in cancer: Intricate networks and multiple activities. Nature Reviews Cancer, 9 (6), 400 – 414.

[43] Cole, P. (2008). Chemical probes for histone-modifying enzymes. Nature Chemical Biology, 4, 590 – 597.

[44] Huang, M., Huang, J., Zheng, Y., Sun, Q. (2019). Histone acetyltransferase inhibitors: An overview in synthesis, structure-activity relationship and molecular mechanism. European Journal of Medicinal Chemistry, 178, 259 – 286.

[45] Lys-COA. Bio. (n.d.). https://www.bio-techne.com/p/small-molecules-peptides/lys-coa_5103#product-documents.

[46] Barritt, S. A., DuBois-Coyne, S. E., & Dibble, C. C. (2024). Coenzyme A biosynthesis: Mechanisms of regulation, function and disease. Nature Metabolism, 6, 1008 – 1023.

[47] Costigan, M., Scholz, J., & Woolf, C. J. (2009). Neuropathic pain: A maladaptive response of the nervous system to damage. Annual Review of Neuroscience, 32, 1 – 32.

[48] Jiang, W., Tan, X. Y., Li, J. M., Yu, P., & Dong, M. (2022). DNA methylation: A target in neuropathic pain. Frontiers in Medicine, 9, 879902.

[49] Massart, R., Dymov, S., Millecamps, M., et al. (2016). Overlapping signatures of chronic pain in the DNA methylation landscape of prefrontal cortex and peripheral T cells. Scientific Reports, 6, 19615.

[50] Bielczyk-Maczynska, E., Zhao, M., Zushin, P. J. H., et al. (2022). G protein-coupled receptor 151 regulates glucose metabolism and hepatic gluconeogenesis. Nature Communications, 13, 7408.

[51] Maiarù, M., Acton, R. J., Woźniak, E. L., Mein, C. A., Bell, C. G., Géranton, S. M. (2023). A DNA methylation signature in the stress driver gene Fkbp5 indicates a neuropathic component in chronic pain. Clinical Epigenetics, 15 (1), 155.

[52] Guo, X., Yao, Y., & Tao, Y.-X. (2019). Role of DNA methylation in chronic pain. In Translational Epigenetics (Vol. 7, pp. 99-110). Academic Press.