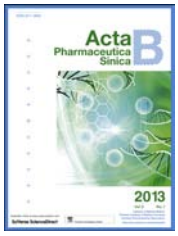
Acta Pharmaceutica Sinica B 2013;3(3):141–149

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

Epigenetic modification enzymes: catalytic mechanisms and inhibitors

Duo Lu*

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

Received 10 April 2013; accepted 12 April 2013

KEY WORDS

Epigenetic modification;
Histone acetylation;
Histone methylation;
DNA methylation;
Catalytic mechanism;
Inhibitor

Abstract Epigenetic modifications alter chromatin structures and consequently affect transcription and cellular functions. Major epigenetic markers include DNA methylation and histone acetylation and methylation. The modifications are reversible and are achieved in aid of relative enzymes. Much effort has been directed at the understanding of the chemical mechanisms of individual catalytic reactions, which can serve as a foundation for inhibitor development. Among the many methods deployed, structural studies have proven the most effective for understanding enzyme-mediated modifications and have provided support for the development of lead-candidate drug inhibitors. This review briefly summarizes the existing knowledge on the catalytic mechanisms of the major epigenetic modification enzymes, with an emphasis on the structural information and inhibitors of these enzymes.

*Corresponding author.
E-mail address: luduo@imm.ac.cn (Duo Lu).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

2211-3835
http://dx.doi.org/10.1016/j.apsb.2013.04.007

1. Introduction

Transcriptional regulation governs gene expression, and consequently defines the fate of individual cells. Early studies in the field of transcriptional regulation have been focussed on the identification of key factors that influence the expression of certain genes, which led to the discovery of transcription factors and their correlate DNA elements, such as promoters and enhancers. The attempt to explain the mechanism of these regulators has revealed the importance of the accessibility of DNA promoter regions for RNA polymerase. For a simple linear model, transcription factors bind their DNA elements and recruit RNA polymerase to the gene for the transcription initiation, or in the case of repression, exclude the polymerase from the gene.

A gene is, however, never in a straight line. In eukaryotic cells, genomic DNA is packed into the nucleus in the form of chromosome. The nucleosome is the packing unit for the chromosome, which comprises of 147 bp of DNA in 1.7 left-handed superhelical turns wrapping around a histone octamer (Fig. 1). Two sets of the four core histones, H2A, H2B, H3 and H4, build up the octamer, and the linker histone H1/H5 binds an additional 20 bp DNA at the start and end of the nucleosome. An array of nucleosomes is further folded into a 30 nm fibre and possibly other high-order structures.

The high-order structure is regulated by the modifications on the histones and DNA, which are generated by various modification enzymes. The most common modifications are histone acetylation/deacetylation, histone methylation/demethylation, and DNA methylation/demethylation. These modifications are believed to be inheritable, and therefore called epigenetic markers. Alterations in the epigenetic markers have been associated with many diseases. For example, hypomethylation of CpG sites has been related to tumour genesis[1]. Small molecule inhibitors have been developed against the modification enzymes, in an attempt to affect the structure of the chromosome and consequently regulate transcription and cell function.

A full understanding of the chemical mechanisms of individual enzymes is essential to inhibitor development, and structural studies have been shown to be an efficient means to explore enzyme mechanisms and inhibitor design. After a few decades efforts, our knowledge of epigenetic modification has been greatly enriched. This review attempts to briefly summarize the mechanisms of the most common modification enzymes and the enzyme inhibitors.

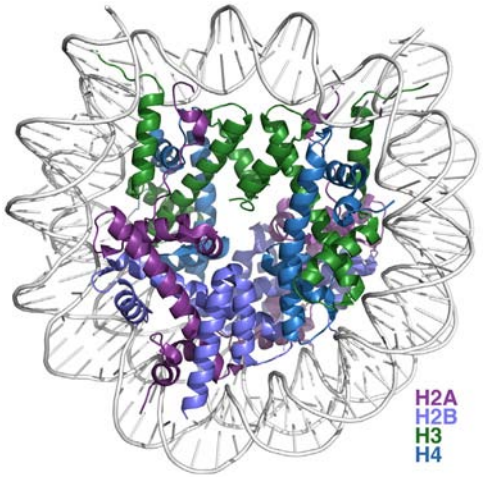


Figure 1 Nucleosome structure. Ribbon diagram of nucleosome core structure. The DNA double helix is in grey, and 4 core histones are coloured differently: H2A in purple, H2B in light purple, H3 in green, and H4 in blue.

2. Histone acetylation

N-terminal tails of core histones are subject to reversible post-translational acetyl modification. Taking acetyl-coenzyme A (AcCoA) as a cofactor, histone acetyltransferases (HATs) attach an acetyl group to the ε-amino group of substrate lysine residues. HATs have been grouped into 5 major families: HAT1, Gcn5/PCAF, MYST, p300/CBP, and Rtt109, and some additional proteins also display HAT activity.

HATs in the 5 major families share the structural feature of a 3-stranded anti-parallel β-sheet with an α-helix spanning the length of sheet at one side. This structural motif contributes to the binding of cofactor AcCoA, and its flanking regions contribute to substrate specificity, which vary greatly between the different HAT families (Fig. 2a).

The catalytic mechanism of Gnc5/PCAF and MYST HATs has been extensively studied. It involves the deprotonation of substrate lysine ε-amino group, which is achieved through a water molecule-mediated contact to a structurally conserved glutamate residue (Fig. 2b and c). Subsequently, the lysine residue makes a nucleophilic attack on the acetyl moiety of AcCoA, through a putative tetrahedral intermediate, resulted in an acetylated lysine[2]. Alternatively, a ping-pong mechanism has been proposed for MYST HATs, which included an additional middle step of MYST auto-acetylation on a conserved cysteine residue (Fig. 2c), before the acetyl group was transferred to the substrate lysine[3].

A hit-and-run (Theorell–Chance) mechanism has been proposed for p300/CBP HATs, based on the crystal structure of p300 HAT complexed with a bi-substrate inhibitor, Lys–CoA[4]. The highly dynamic mechanism was proposed based on the fact that a more authentic bi-substrate analogue inhibited p300 activity much less effectively than Lys–CoA, which argued against a standard sequential mechanism. No general base was identified for deprotonation of the substrate ε-amino group (Fig. 2c). Only a hydrogen bond was found between the ε-amino group and the main-chain carbonyl group of a tryptophan residue in p300. A tyrosine residue was thought to stabilize the leaving group, CoA.

The mechanisms for HAT1 and Rtt109 classes HATs are not clear. A crystal structure is available for the ternary complex of HAT1, cofactor AcCoA, and a histone H4 peptide containing residue K12[5]. Superimposing HAT1 with the Gcn5 active site has shown a glutamate residue located at the similar position of the one in Gcn5, which has been proven functionally important (Fig. 2c). Mutation at this residue greatly impairs the catalytic ability of HAT1. However, the ordered water molecule in Gcn5 has not been detected in HAT1 structures.

Rtt109 by itself has a very weak catalytic ability to acetylate histone. The activity is, however, elevated upon association with its chaperones such as yeast Vps75 and Asf1. The structure of Rtt109 complexed with Vps75 reveals little conformational difference from its isolated form (Fig. 2c), which implies that the enhanced activity is possibly due to an increase in substrate binding[6]. Yet mutations at the Rtt109–Vps75 interface do not affect Asf1–Rtt109 activity, suggesting a different mechanism for Asf1 stimulation on Rtt109.