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Mechanisms of histone lysine-modifying enzymes: A computational perspective on the role of the protein environment

Wilian Augusto Cortopassi[a,b], Kiran Kumar[a,b], Fernanda Duarte[a,b], Andre Silva Pimentel[c], Robert S. Paton[a,b,*]

[a] Chemistry Research Laboratory, 12 Mansfield Road, Oxford OX1 3TA, UK
[b] Physical and Theoretical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, UK
[c] Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro, RJ 22451-900, Brazil

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ABSTRACT

Epigenetic pathways are involved in a wide range of diseases, including cancer and neurological disorders. Specifically, histone modifying and reading processes are the most broadly studied and are targeted by several licensed drugs. Although there have been significant advances in understanding the mechanistic aspects underlying epigenetic regulation, the development of selective small-molecule inhibitors remains a challenge.

Experimentally, it is generally difficult to elucidate the atomistic basis for substrate recognition, as well as the sequence of events involved in binding and the subsequent chemical processes. In this regard, computational modelling is particularly valuable, since it can provide structural features (including transition state structures along with kinetic and thermodynamic parameters) that enable both qualitative and quantitative evaluation of the mechanistic details involved. Here, we summarize knowledge gained from computational modelling studies elucidating the role of the protein environment in histone-lysine modifying and reading mechanisms. We give a perspective on the importance of calculations to aid and advance the understanding of these processes and for the future development of selective inhibitors for epigenetic regulators.

1. Introduction

Epigenetic modifications refer to changes in the structure of DNA and the associated histone-proteins, rather than in the DNA sequence itself (i.e. the genetic code). The result of these modifications is the activation or silencing of specific genes. These processes are highly dynamic, involving a number of proteins that add (*epigenetic writers*), recognize (*epigenetic readers*), or remove (*epigenetic erasers*) chemical modifications. Methyl groups are added to the nucleobases of DNA while histones can undergo several modifications including the addition and removal of methyl, acetyl and phosphoryl groups. The downstream effects of these chemical changes impact upon transcription, DNA repair and replication [1].

The first recognized epigenetic change in humans was DNA methylation, which is involved in transcriptional silencing, gene regulation, development and tumorigenesis [2]. Post-Translational Modifications (PTM), which broadly describe changes made to proteins following their synthesis by the ribosome are among the most studied epigenetic changes [3,4]. Histones consist of a globular octamer shaped core, around which DNA wraps, and an unstructured tail domain. Most Histone PTMs occur at the N-terminal tails, and less commonly on their globular domains [5]. To date, more than 85,000 Histone PTMs have been experimentally identified; involving the addition of over 25 different chemical groups, such as phosphoryl, acetyl, methyl and hydroxyl [6]. Among them, lysine acetylation/deacetylation and methylation/demethylation have been shown to be the most common.

* Corresponding author at: Chemistry Research Laboratory, 12 Mansfield Road, Oxford OX1 3TA, UK.
E-mail address: robert.paton@chem.ox.ac.uk (R.S. Paton).

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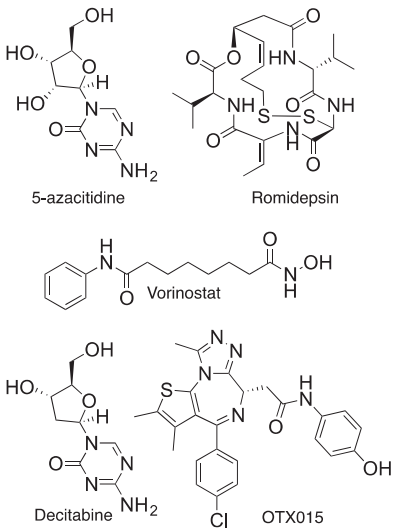


Fig. 1. Chemical structures of representative epigenetic FDA-approved drugs: 5-azacytidine, decibitabine, romidepsin, vorinostat and clinical-trial phase compound OTX015.

The emergence of small-molecule inhibitors targeting these epigenetic regulators has stimulated interest in understanding the molecular basis underlying epigenetic regulation, as well as in the design of more potent and selective inhibitors. Over the past few years, an increasing number of these compounds have undergone clinical trials [1], and some (Fig. 1) have already been approved for clinical use by the Food And Drug Administration (FDA). For example, cytosine analogues 5-azacytidine and decibitabine block DNA methylation (i.e. a writing inhibitor) and are used for the treatment of myelodysplastic syndromes [7,8], while romidepsin and vorinostat (both targeting epigenetic erasers) are used to treat cutaneous T-cell lymphoma [9–11]. Inhibitors of epigenetic-reader bromodomains are in an early-stage of drug development, including OTX015, currently in the initial phases of clinical trials as a haematological malignancy therapeutic [12]. However, despite these advances, determining at a molecular level how these inhibitors interrupt the native chemical reactions associated with epigenetic modifications remains challenging. A better understanding of the chemical events underlying substrate recognition and epigenetic modifications offers the potential to aid the design of more biologically active compounds with greater target specificity.

Computational modelling of epigenetic processes has been a topic of significant research in recent years. Important reviews in the field include Vellore and Baron [13], who have surveyed the work on histone deacetylases, demethylases and tail dynamics, giving valuable explanations of the background biology in each case; and Smith and Denu [14], who covered both experimental and computational studies on the chemical mechanisms of histone lysine and arginine modifications. In the present perspective, we will expand on these works by reviewing recent computational studies, using both Molecular Dynamics (MD) and Quantum Mechanics/Molecular Mechanics (QM/MM) techniques, on histone lysine modifying enzymes. These studies emphasise the role of the protein environment for the binding of reactants and stabilisation of transition states and intermediates. Our focus is on mechanistic work rather than non-covalent inhibition: we refer readers to recent excellent reviews concerning the development of epigenetic inhibitors [15–20] and recent works regarding accurate binding free energy predictions [21–23]. A valuable resource of publicly available experimentally determined binding sites of histone tails in complex with human proteins has been developed as an open access web server [24].

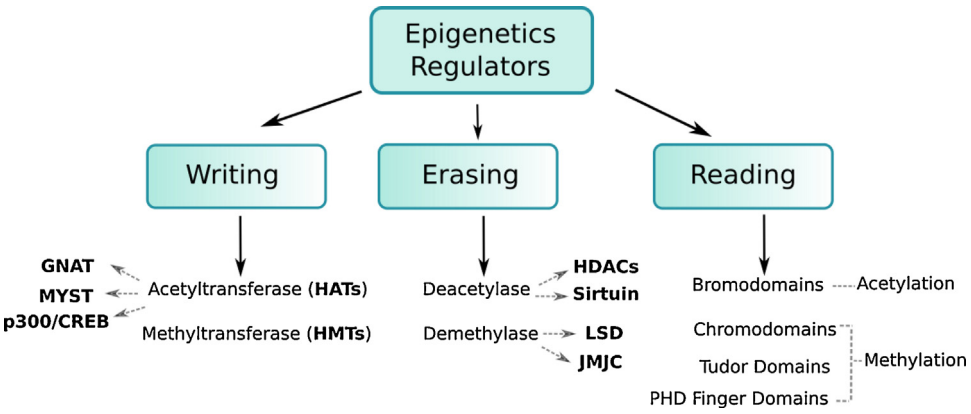
In this perspective, we begin by outlining the key chemical processes regulating histone writing, erasing and reading, as shown in Scheme 1. Following this, we will provide a brief overview of relevant computational approaches used to study these processes, highlighting recent advances and challenges. To illustrate this point, we will present key examples in the field. Finally, we conclude by discussing the future role of computational approaches in the continued study of these mechanisms.

2. Overview of epigenetic regulators

Epigenetic regulators can be divided into three main groups based on their functions: writers, erasers and readers (Scheme 1). Additionally, they can be grouped into families – usually structurally related – and according to the chemical process they catalyse. In this section we will briefly introduce the structural, kinetic and chemical features associated with the addition/removal of methyl and acetyl groups to histone lysines. A more detailed description on the chemical aspects of modifications to arginine residues, not discussed here, is presented in Ref. [14].

2.1. Epigenetic writing regulators

Histone acetylation and methylation are epigenetic writing modifications involved in a large number of druggable targets [19,25,26] and have been observed for a wide range of histones, such as the core histones H3, H4 and H2B, containing three alpha helices linked by two loops [27]. The writing process itself refers to the addition of an acetyl or methyl group to the ε-amino group of a lysine at the N-terminal tail of the histone, catalysed by Histone lysine acetyltransferases (HAT) and methyltransferases



Scheme 1. Schematic overview of the classification of epigenetic protein families and their function as presented in the main text.