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# Leading approaches in synthetic epigenetics for novel therapeutic strategies

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In recent years, our knowledge of the epigenetic functions regulated by post-translational modifications (PTMs) of histones, and their role in various diseases, has expanded rapidly, opening the way to novel therapeutic strategies that treat epigenetic abnormalities. Many of the current approaches have been focusing on the chemical inhibition of histone-modifying enzymes to modulate histone PTM states for attaining therapeutic effects. However, recent developments in chemistry and molecular biology have contributed to the emergence of new methods that introduce histone PTMs entirely through artificial means, without reliance on endogenous enzymes. In this review article, we summarize several state-of-the-art approaches for the introduction of synthetic epigenetic modifications in cells, and discuss both their therapeutic potential and the possible challenges in developing novel therapeutic strategies utilizing them.

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## Introduction

Our genetic information, encoded in DNA, is packaged in the cell nucleus in a polymeric complex called chromatin. The fundamental unit of chromatin is a nucleosome, composed of histone proteins (H2A, H2B, H3, and H4) and the DNA wrapped around them [1]. The function of the chromatin is regulated by chemical modifications of its histones and DNA, in the form of lysine acetylation, lysine methylation, and cytosine methylation, among others. These chemical modifications either directly affect the structure of the chromatin [2] or are recognized by other effector proteins [3], leading to the regulation of gene expression, which is a fundamental element of epigenetics [4].

The level of histone post-translational modification (PTM) in cells are tightly regulated by the balance between two different groups of enzymes [5]; writer enzymes promoting the introduction of the PTMs (e.g., histone acetyltransferase, HAT [6] and histone methyltransferase, HMT [7]) and eraser enzymes removing them (e.g., histone deacetylase, HDAC [8] and histone demethylase, HDM [9]). Abnormality of the histone PTM state is deeply involved in various diseases, such as cancer [10]. For example, small molecule inhibitors of HDACs are used as anticancer drugs [11] because they indirectly promote histone acetylation via endogenous HAT's activity and likely activate the transcription of a series of genes, including tumor suppressor genes, which are often inactivated in cancer cells (Figure 1a) [12]. On the other hand, the direct promotion of histone PTMs by an artificial means, bypassing endogenous enzymes, is a different and attractive therapeutic strategy as it would be particularly effective in treating diseases with little or no writer enzyme activity [13], or with mutations conferring resistance to inhibitors (Figure 1b) [14]. Such a therapeutic strategy, however, has not yet been achieved, despite its attractiveness.

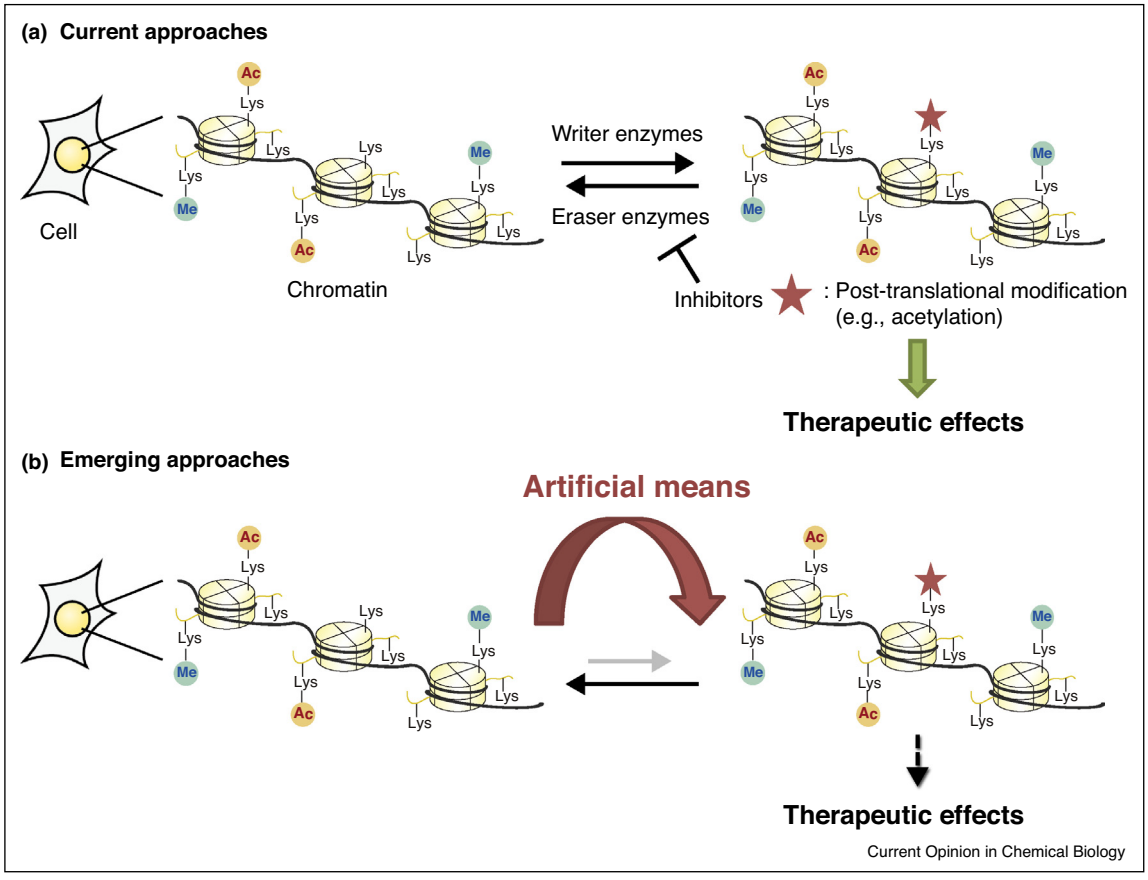
Many powerful techniques that allow *in vitro* introduction of a desired PTM into histones have been developed [15], such as protein semisynthesis using native chemical ligation [16•] and expressed protein ligation [17•], ribosome-mediated protein synthesis utilizing an expanded genetic code [18•], installation of a PTM mimic through cysteine alkylation [19], and chemistry-coupled post-translational mutagenesis [20•,21•]. In contrast, a method to artificially introduce histone PTMs in cells or *in vivo* has been lacking until recently. This review summarizes the recent progress in and challenges of approaches that introduce histone PTMs in cells by an artificial means with the goal of developing a new therapeutic paradigm.

## Approaches using genetic manipulation

### Protein *trans*-splicing approach

Although protein semisynthesis is a well-established method for producing a protein with a PTM *in vitro*, it had not been achieved in the histones of living cells until

**Figure 1**




Current and emerging approaches to introduce histone PTMs for therapeutic purposes. **(a)** A current approach to promote histone PTMs. Chemical inhibitors of histone-modifying enzymes are often used to indirectly promote histone PTMs. The efficacy is dependent on the ability of the enzymes catalyzing the installation of the PTMs. **(b)** An emerging approach to introducing histone PTMs. A direct introduction of the histone PTMs bypassing the endogenous enzymes is an attractive way to obtain therapeutic effects by treating epigenetic abnormalities in various diseases. This approach could be effective even for cells with inactive mutations in the histone-modifying enzymes or cells with resistance to enzyme inhibitors.

recently. In comparison, the natural world has had a method to ligate two polypeptides within the biological milieu; protein *trans*-splicing (PTS) by split inteins [22]. Muir and colleagues used the PTS strategy and reported in-cell protein semisynthesis able to produce site-specifically modified histones in 2015 (Figure 2a) [23••,24]. In this strategy, they used cells expressing a histone H2B fragment fused with the *N*-terminal fragment of the split-intein ($Int^N$). Upon treatment of the cells with the *C*-terminal fragment of the split-intein ($Int^C$) conjugated with the remaining H2B fragment, which contains the desired modification where specified, the $Int^C$-cargo enters the cells through endocytosis and subsequent endosomal escape owing to its conjugated cell-penetrating peptide (CPP). The $Int^C$-cargo associates with the H2B-$Int^N$ in chromatin, and undergoes PTS to generate a ligated H2B with a site-specific modification. They produced histone H2B ubiquitylated at lysine 120 (H2B-K120Ub) in isolated nuclei with this strategy, and observed a two-fold increase of H3K79Me2, which is a downstream cross-talk product of H2B-K120Ub [25]. These results suggest that the semisynthetic H2B-K120Ub is functional and able to induce chromatin signaling in at least the isolated nuclear environment.

### Genetic code expansion approach

The site-specific incorporation of unnatural amino acids, including natural amino acids with a PTM, into histones both *in vitro* as well as in cells has been achieved by a genetic code expansion approach using orthogonal aminoacyl-tRNA synthetase (aaRS)/tRNA pairs [26]. For example, unnatural amino acids with ultraviolet light-inducible cross-linkers were incorporated into histones in yeast cells to investigate the molecular details of the pathway downstream of H3 S10 phosphorylation during mitosis [27]. To generate histones with a desired PTM in