Revealing chromatin-specific functions of histone deacylases

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Abstract

Histone deacylases are erasers of N^{ε}-acyl-lysine post-translational modifications and have been targeted for decades for the treatment of cancer, neurodegeneration and other disorders. Due to their relatively promiscuous activity on peptide substrates *in vitro*, it has been challenging to determine the individual targets and substrate identification mechanisms of each isozyme, and they have been considered redundant regulators. In recent years, biochemical and biophysical studies have incorporated the use of reconstituted nucleosomes, which has revealed a diverse and complex arsenal of recognition mechanisms by which histone deacylases may differentiate themselves *in vivo*. In this review, we first present the peptide-based tools that have helped characterize histone deacylases *in vitro* to date, and we discuss the new insights that nucleosome tools are providing into their recognition of histone substrates within chromatin. Then, we summarize the powerful semi-synthetic approaches that are moving forward the study of chromatin-associated factors, both *in vitro* by detailed single-molecule mechanistic studies, and in cells by live chromatin modification. We finally offer our perspective on how these new techniques would advance the study of histone deacylases. We envision that such studies will help elucidate the role of individual isozymes in disease and provide a platform for the development of the next generation of therapeutics.

Introduction

Histones are proteins that scaffold the packing of DNA into chromatin and serve as control hubs for the regulation of DNA transcription, replication, and repair. The basic unit of

chromatin is the nucleosome, formed by a core octamer of histones H2A, H2B, H3 and H4, around which 147 base pairs of DNA are wrapped [1]. Each of the eight histones consists of a globular domain which forms part of the nucleosome core, as well as both N- and C-terminal tails that protrude from the nucleosome structure. These tails can interact with the DNA, neighboring nucleosomes, and other components of chromatin. Moreover, histone tails and, to a lesser extent, the structured domains, are the subject of combinations of numerous post-translational modifications (PTMs) [2, 3]. Lysine residues are a common target of PTMs: via the action of specific 'writer' enzymes, lysines can be methylated to different degrees, or acylated with an arsenal of groups derived from the cell metabolism (*e.g.* acetylated, succinylated, lactylated) [4, 5]. Conversely, these modifications are reversible through the action of 'eraser' enzymes, namely histone demethylases and histone deacylases, which serves to control the impact of each PTM on the structure and function of chromatin [6].

Two families of histone deacylases exist in humans: the Zn^{2+}-dependent histone deacetylases (HDACs) and the NAD^{+}-consuming sirtuins. Specifically, the members of these two families that are consistently found in the cell nucleus and have robust deacylase activity on histones are the class I HDACs: HDAC1–3 and HDAC8 [7], and members of the class I and class IV sirtuins: SIRT1, SIRT2, SIRT6 and SIRT7 [8]. All of these enzymes remove N^{ε}-acetyl-lysine (Kac) modifications, and each of them exhibits specific eraser activities against additional acyl PTMs, such as N^{ε}-crotonyl-lysine [9], N^{ε}-lactyl-lysine [10], and long-chain N^{ε}-acyl modifications [11]. Through these activities, histone deacylases control gene expression in a broad sense, including key biological processes like development, cell cycle and differentiation, and also DNA repair and aging [7, 8]. Due to these functions, histone deacylases are regarded as therapeutic targets against many diseases, and inhibitors of the class I HDACs are used clinically for the treatment of hematologic cancers [6].

The nuclear histone deacylases have been studied extensively *in vitro*, most often as isolated enzymes or catalytic domains, and using peptide-based substrates and tools of study. However, HDACs and sirtuins have conserved catalytic sites with relatively promiscuous substrate recognition capabilities and rely on neighboring domains or interaction with chromatin-interacting factors to direct their activity [12, 13]. Moreover, recent research suggests that substrate recognition within chromatin is determined by interactions with DNA and other histones in the nucleosome (Figure 1) [13-16]. These regulatory mechanisms are not contemplated when characterizing substrates and modulators using peptides, which can hamper the translation to a biological setting. In this review, we summarize how current

peptide-based technologies have advanced HDAC and sirtuin research, and present recent insights provided by nucleosome-based functional studies. We then propose further chromatin-based methods to dissect histone deacylase function, which we believe will be essential to understanding their dynamics and regulatory mechanisms. These tools will help bridge the gap between current *in vitro* approaches and the complex environment of native chromatin.

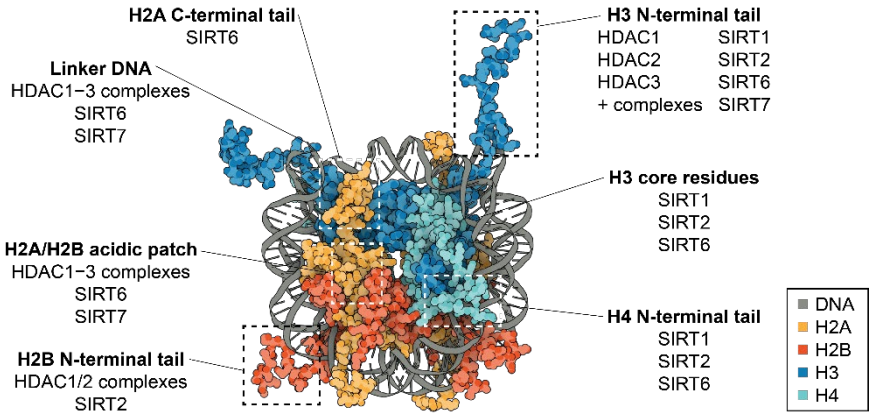

Figure 1. Summary of known nucleosome interaction hotspots for the histone deacylases. These include binding interactions (*e.g.* DNA, H2A C-terminus) and substrate positions (*e.g.* H3 and H4 N-termini) (PDB 1KX5). Structure representations throughout the article were created with Protein Imager [17].

Peptide-based tools for the study of histone deacylases

The biochemical characterization of HDACs and sirtuins up to date has relied on peptides carrying acylated lysine or lysine-derivatives as substrate mimics. Facilitated by advances in peptide chemistry and chemical biology, these studies have provided widely accessible tools, *e.g.*, for high-throughput screening approaches, and helped to characterize the *in vitro* behaviour of HDACs and sirtuins [18-20].

Initial deacetylation assays were performed on histones and histone peptides containing radiolabelled Kac, allowing to quantify the reaction progress via the released radioactivity. These experiments provided a means to characterize the first isolated enzymes (HDAC1 and Sir2) and their inhibitors [21-23]. However, due to the use of radioactivity for detection, these tools were not easily accessible to the community. To address this limitation, researchers applied fluorescent peptide labels, which enabled experiments to be resolved by HPLC (Figure 2a) [24, 25]. Shortly after, in 2003, a development step was added. Digestion by the protease trypsin was used to release a 7-amino-4-coumarin (AMC) label from the C-terminus of the deacetylated lysine, whereas the acetylated starting material remained inert. This

coupled assay format enabled in-situ activity measurements, since the fluorescence spectrum of AMC shifts upon release (Figure 2b) [26]. Such an experimental setup provided the means for high-throughput screening and became commercially available [27]. In addition, it served as platform to probe substrate requirements and to find lysine PTMs other than acetylation which were regulated by HDACs [9, 10, 28-30] and sirtuins [11, 31, 32]. The first continuous assays to measure enzyme and inhibitor kinetics efficiently were also based on in-situ AMC release [33, 34]. However, these experiments require co-incubation with trypsin, which is not compatible with all enzyme preparations. Thus, the most recent studies focus on the development of uncoupled in-situ substrates. These substrates rely on Förster resonance energy transfer (FRET) quenching and fluorescence release (Figure 2c) [35-37], or on fluorophore uncaging by the free lysine product [38-41].

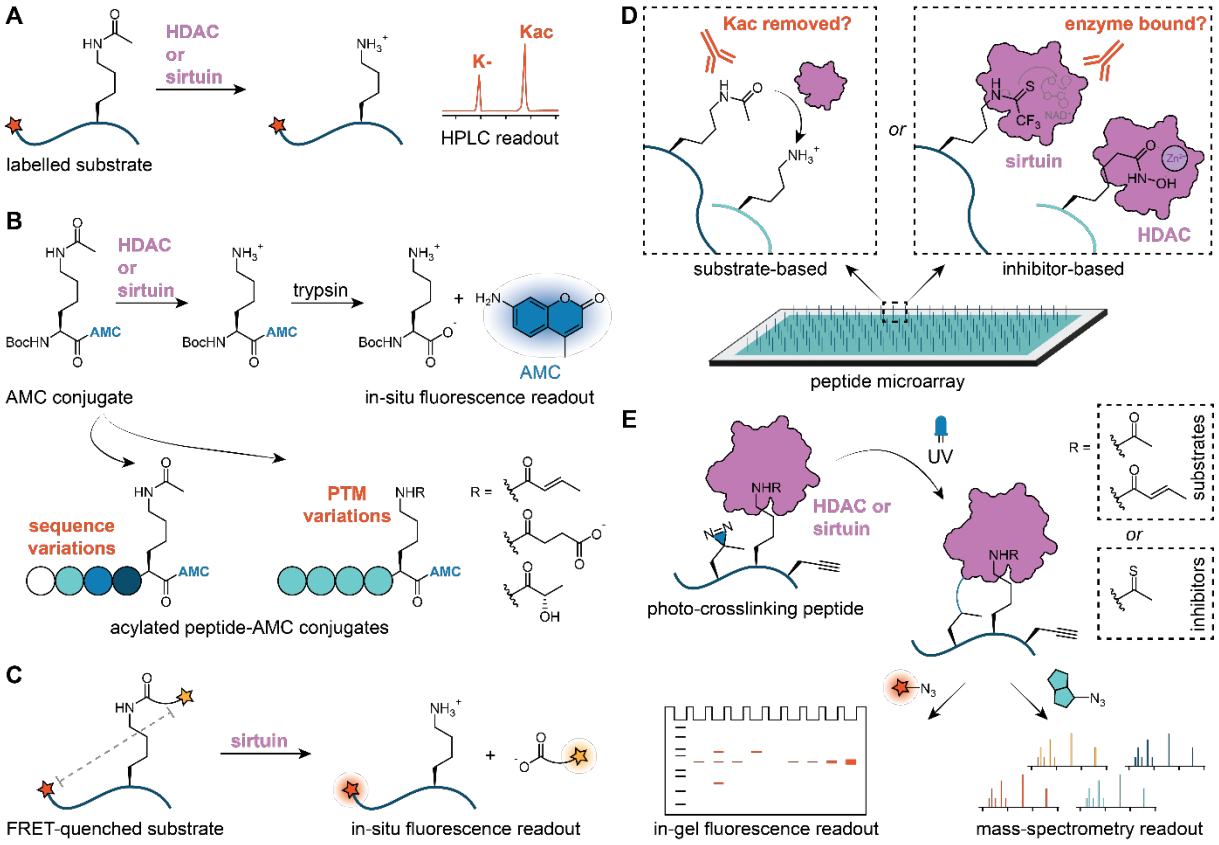

Figure 2. Peptide-based methods to decipher the sequence and modification preference of histone deacylases. (**A**) Fluorophore and chromophore-labelled peptides. (**B**) Fluorogenic 7-amino-4-methylcoumarin (AMC)-conjugated substrates. (**C**) FRET-based fluorogenic peptides. (**D**) Substrate and inhibitor-based peptide microarrays. (**E**) Substrate and inhibitor-based photo-crosslinking peptide probes.

Peptide-based substrates can inform on substrate PTM and sequence preferences, but experiments using individual peptides suffer from two major limitations for such studies: they

do not report on the full substrate scope of a given HDAC or sirtuin, and they are not compatible with studies in a cellular context. To address the first of these two drawbacks, researchers harnessed the experimental throughput of peptide microarrays (Figure 2d). These are libraries of immobilized acetylated peptides covering a wide sequence space, where histone deacylase activity can be probed with cocktails of anti-Kac antibodies [42, 43]. Alternatively, peptides bearing enzyme inhibitor-derived warheads to capture the peptide-enzyme interaction, such as hydroxamic acids for HDACs and thioamides or thioureas for sirtuins (Figure 2d), allow for HDAC- and sirtuin-specific readouts via antibody detection [44, 45]. Although limited to short sequences, microarray technologies serve to determine the sequence preferences of specific HDACs and sirtuins, enabling the identification of novel protein targets. Moreover, they are useful for probe- and inhibitor development, as they enable the optimization of peptide sequences in high throughput. Finally, microarray technologies have also been applied to the study of histone deacylases in cell lysates [43]. The second limitation, assessing deacylase activity in a cellular context, has been addressed better with the use of pull-down probes. These tools consist of a substrate-like or inhibitor-derived warhead typically surrounded by a photo-activated reactive group and a handle for click chemistry (Figure 2e) [46]. Incubating the probe in cell lysate allows to trap any interacting proteins. Subsequent biorthogonal functionalization with a fluorophore reveals targeted enzymes, while biotinylation enables the purification of whole enzyme complexes and thus identification of associated regulatory complexes by tandem mass spectrometry [47-50]. Moreover, recent developments in probe design permit the identification of crosslinks to map the substrate binding region of the enzyme or enzyme complex [51].

Together, peptide-based tools have helped answer many questions in HDAC and sirtuin research, but they cannot inform on the chromatin context of the histone substrates, which has become more and more relevant to explain the different mechanisms of the HDAC and sirtuin regulatory functions.

Histone deacylases use different nucleosome recognition mechanisms

In recent years, the ability to produce precisely modified histones and nucleosomes has provided new tools to characterize HDACs and sirtuins, and revealed specific mechanisms of chromatin substrate recognition.

Preparation of nucleosomes with defined modifications

The most common methods to generate modified histones are functionalization of cysteines, genetic code expansion, and protein semi-synthesis [52]. The first two are useful technologies that permit installing various modifications at selected residues, and genetic code expansion has been especially useful for the study of lysine acetylation [53, 54]. Arguably, the most versatile tool is however semi-synthesis, which can generate histones with multiple modifications of various sizes and positioned both at the tails and at the histone core [55].

Histone semi-synthesis entails the ligation of one or more synthetic peptides carrying the modifications of interest to an expressed portion of the histone, to generate the native full-length protein sequence. This can be achieved via native chemical ligation reaction, where a peptide or protein fragment carrying a C-terminal thioester reacts with a fragment carrying an N-terminal cysteine [56, 57]. C-terminal thioesters can be generated directly via peptide synthesis, or in expressed protein fragments via the action of inteins [58]. Inteins are protein segments that can ligate flanking regions, or exteins, in a splicing reaction. Some intein sequences are found split into two parts, which serve to ligate two separate fragments into a single protein through a thioester intermediate [59]. Alternatively, enzymes such as the sortase transpeptidases can be employed to connect recombinant and synthetic peptides and proteins. These enzymes cut and reconnect at distinct recognition sequences, which means that they may require the introduction of tags (Figure 3a) [60]. Thanks to the most recent developments, semi-synthetic methods can generate modified histones without ligation-derived sequence mutations or “scars” [55]. Semi-synthetic histones can then be incorporated into nucleosomes or nucleosome arrays *in vitro*, and used to probe the binding and activity of the protein of interest [52]. Importantly, nucleosomes are amenable to strategies established with peptides such as the introduction of inhibitor moieties [16, 61] and photo-crosslinkers [62, 63]. In analogy with peptide libraries, in order to expedite screening, the Muir lab implemented a multiplexing platform where nucleosomes with different modifications were labelled using DNA barcodes (Figure 3b) [64], which enabled the study of chromatin remodelers in high throughput [65]. In addition, both the Fierz and the Muir laboratories have developed synthetic strategies to install different modifications at each of the copies of a set histone within the nucleosome (asymmetric nucleosomes, Figure 3c) [66-68]. This is also possible with certain histone mutations that prevent symmetry [69]. Thus,

semi-synthetic nucleosomes are becoming a versatile platform for studies *in vitro*, including for HDACs and sirtuins as discussed below.

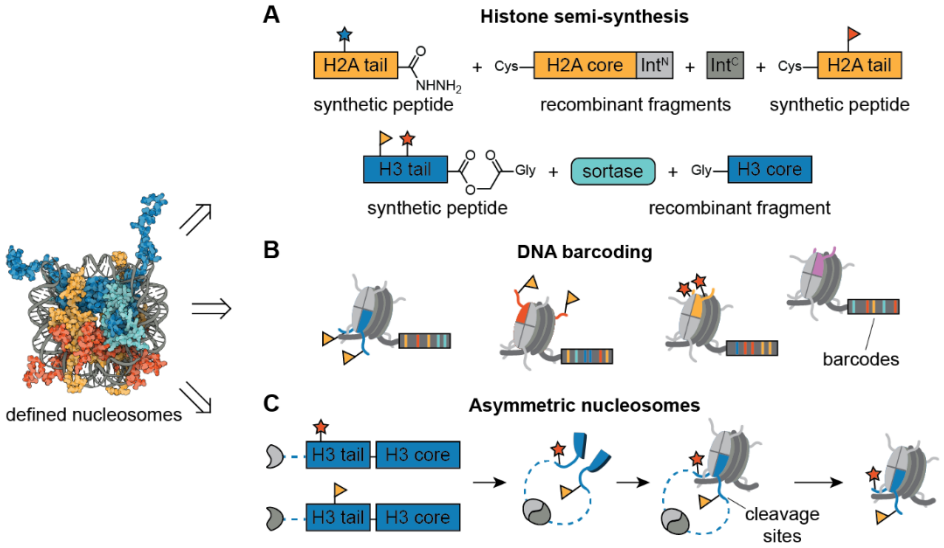

Figure 3. Methods to prepare nucleosomes with defined modifications. (**A**) Chemical and enzymatic histone semi-synthesis to introduce PTM combinations [61, 70]. (**B**) Nucleosome multiplexing using DNA barcodes [64]. (**C**) Use of semi-synthetic histones to generate asymmetric nucleosomes [66, 68]. Int^N and Int^C represent N-terminal and C-terminal split intein fragments, respectively.

Zn^{2+}-dependent HDACs interact with nucleosomes via complex partners

The nuclear class I HDACs have been studied extensively due to their involvement in cancer progression and the success of HDAC inhibitors in the clinic [6]. However, most of their investigation has focused on individual isozymes and peptide substrates, which is not representative of their main state in cells.

The first detailed study of mammalian HDAC activity using histones and nucleosomes was published in 2017 by Fierke and coworkers, focusing on HDAC8 [71]. This report showed a striking increase in catalytic activity towards histone tetramers and octamers compared to peptide substrates and, more importantly, a change in the site selectivity of the enzyme. Interestingly, the formation of nucleosomes abolished activity, which indicates that HDAC8 is most likely unable to deacylate histones within chromatin. In this regard, HDACs 1–3 appear to be the predominant Zn^{2+}-dependent nucleosome deacylases.

HDACs 1–3 form part of multiprotein complexes that often share the deacylase module but differ entirely in their function and interaction with chromatin [12]. HDACs 1 and 2 both take part of CoREST, MiDAC, NuRD and Sin3 complexes among others, while HDAC3 is mainly associated with the NCoR complex [12]. Within the last decade, the Schwabe laboratory and

others have resolved the molecular structure of several complexes and showed how HDACs are positioned within each of these assemblies (Figure 4a) [14, 72-76]. In all these complexes, HDACs coexist with proteins that interact with DNA, histones, or both, allowing multivalent engagement of nucleosomes for deacylation [14]. Indeed, such an interaction mode was recently shown for the yeast Rpd3S deacetylase complex [77-79]. Interestingly, complexes such as MiDAC and NuRD are oligomeric in nature and orient HDAC molecules in multiple directions simultaneously (Figure 4a) [75].

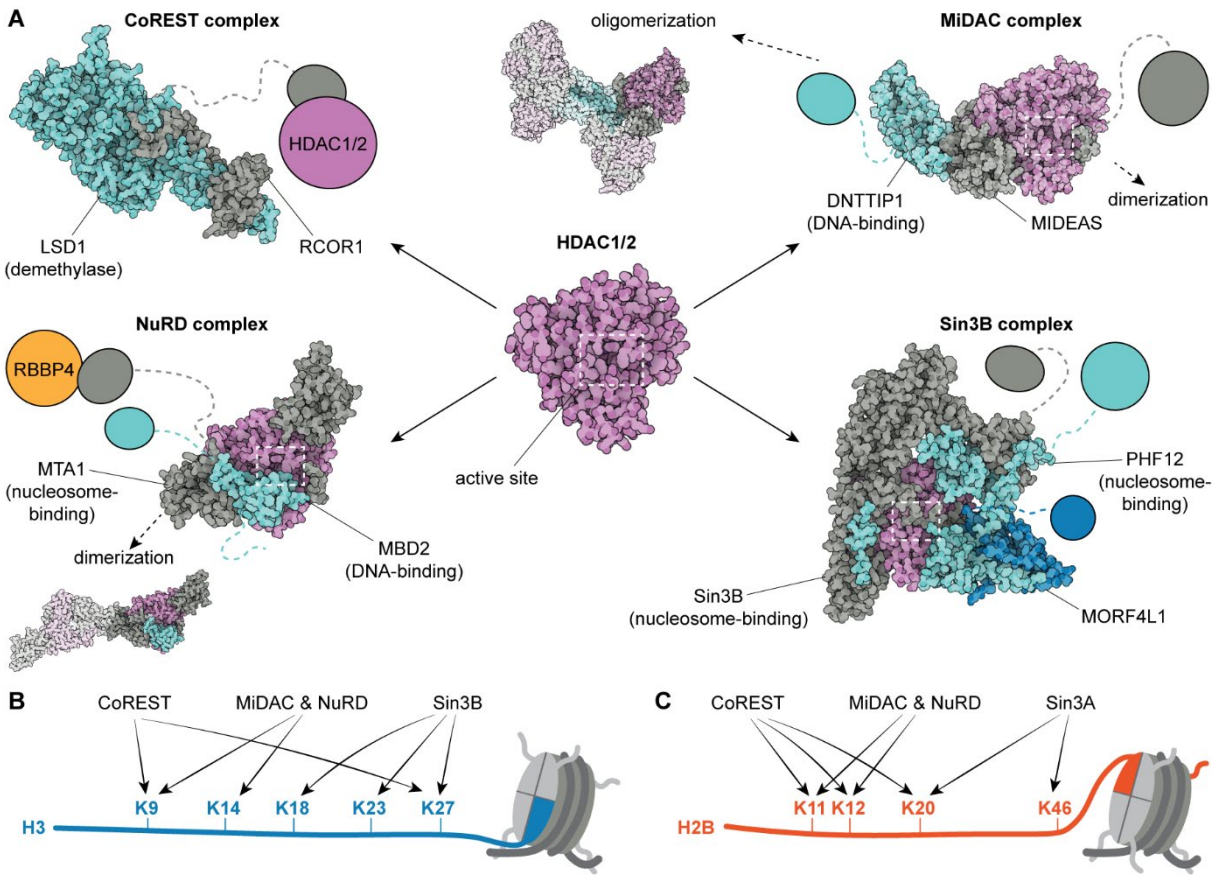

Figure 4. Recognition of nucleosome substrates by HDAC-containing complexes. (**A**) Examples of the core of HDAC1/2 complexes (HDAC2 PDB: 7ZZP, CoREST PDB: 2IW5 [80], MiDAC PDB: 6Z2J and 6Z2K [75], NuRD PDB: 7AO8 [14], Sin3B PDB: 8C60 [76]), and reported interactions with DNA and nucleosomes. (**B**) Preferred H3Kac nucleosome substrates for the HDAC1 deacylase core of the indicated complexes *in vitro* [15]. (**C**) Preferred H2BKac nucleosome substrates for the HDAC1 deacylase core of the indicated complexes *in vitro* [81].

Since class I HDACs themselves lack domains responsible for nucleosome engagement, substrate recognition is provided by other factors within complexes. In addition, complex partners create a binding pocket for inositol phosphates, enabling allosteric regulation of enzyme activity [72, 82]. Conversely, access to the HDAC active site can also be restricted

in certain complexes, as shown for Sin3B (Figure 4a) [76]. Thus, the regulation of HDAC activity is finetuned by the complex partners beyond recruitment to chromatin substrates.

Nucleosome deacetylation by the core of HDAC1-containing complexes has been tested *in vitro* by Cole and coworkers using with H3- and H2B-acetylated nucleosomes. Thanks to sortase-mediated histone semi-synthesis, the reconstituted nucleosomes were modified at defined sites and permitted precise activity measurements [61, 81]. With these nucleosomes in hand, it was shown how the substrate selectivity of HDAC1 is dictated by the complex partners (Figure 4b,c), and how specific substrate requirements are unique to certain complexes [15, 81]. Further modification of the nucleosomes permits probing the effect of simultaneous PTMs, and was used to show how the H3 demethylase activity of CoREST is inhibited by H3K14 acetylation [61].

Together, studies on reconstituted chromatin substrates, containing distinct PTM patterns and potentially taking advantage of DNA barcoding, will be of high importance to understand the combined effects of chromatin-binding modules in the regulation of HDAC activity in cells. In particular, since HDAC complexes are often multimeric and expected to target several nucleosomes at the same time [12, 14, 75], techniques involving defined oligo-nucleosomes or chromatin fibers will be needed for in-depth characterization.

Sirtuins directly bind nucleosomes and deacylate histones at specific positions

Based on their activity *in vitro*, sirtuins have been considered rather promiscuous deacylases, both in terms of substrate sequence and acyl structure. However, studies with nucleosomes are revealing recognition mechanisms that appear specific for each sirtuin and may explain better the activities found in cells.

The most characterized sirtuin-nucleosome interaction is that of SIRT6. Using nucleosomes reconstituted with purified chicken histone octamers, the Cohen laboratory found that SIRT6 shows poor activity on histone octamers, and that it is a much more active deacetylase when its histone substrates form part of nucleosomes [83]. This stimulatory effect was observed for deacetylation of H3K9ac and H3K56ac, both substrate PTMs of SIRT6 in cells. This was later validated by Liu and coworkers using chemically defined nucleosomes acylated at individual H3 positions. Here, they used genetic code expansion to install the acyl substrates at distinct positions within the histone sequence, carrying a handle for biorthogonal functionalization. Then, a click reaction with a fluorophore enabled visualization of the remaining acyl substrate by in-gel fluorescence without the need for specific antibodies [84].

To explain why SIRT6 is more active on nucleosomes, the Denu lab performed a mechanistic study that showed that SIRT6 interacts with an acidic patch on the nucleosome surface, a critical protein-protein interaction hub exploited by many chromatin-modifying enzymes (*e.g.* PRC1, BRCA1) [1]. Moreover, SIRT6 uses its intrinsically-disordered C-terminal region to bind DNA further enhancing its activity [13]. These findings provide a mechanistic explanation why SIRT6 requires a multivalent engagement of both the nucleosomal surface and DNA to efficiently deacetylate substrates.

In 2023, several groups reported cryoEM structures of SIRT6 bound to nucleosomes [16, 85, 86], which revealed that binding to the acidic patch relies on a key arginine residue, similar to most acidic patch-binding enzymes [1]. This arginine is part of a SIRT6-specific loop, and it is therefore not translatable to how other sirtuins might recognize nucleosomes (Figure 5a). The C-terminus is not resolved in these structures, which indicates that it retains a level of structure heterogeneity even when contacting DNA. The flexibility in these DNA contacts could further allow SIRT6 to bind neighbouring nucleosomes or linker DNA further away from the substrate dyad. Unexpectedly, the C-terminal tail of H2A was shown in these structures to interact with the catalytic domain of SIRT6 and inhibit its activity [85], although this interaction is also present when SIRT6 is trapped in a catalytic pose via mechanism-based inhibition with a thiourea warhead [16]. In addition to regulating activity, binding to H2A appears to close an allosteric pocket used for pharmacological activation of SIRT6 (Figure 5a) [87], which could have implications for differential activation towards specific substrates. Thus, studying SIRT6 with nucleosome-derived tools has revealed new regulatory mechanisms with important implications for functional studies and drug design.

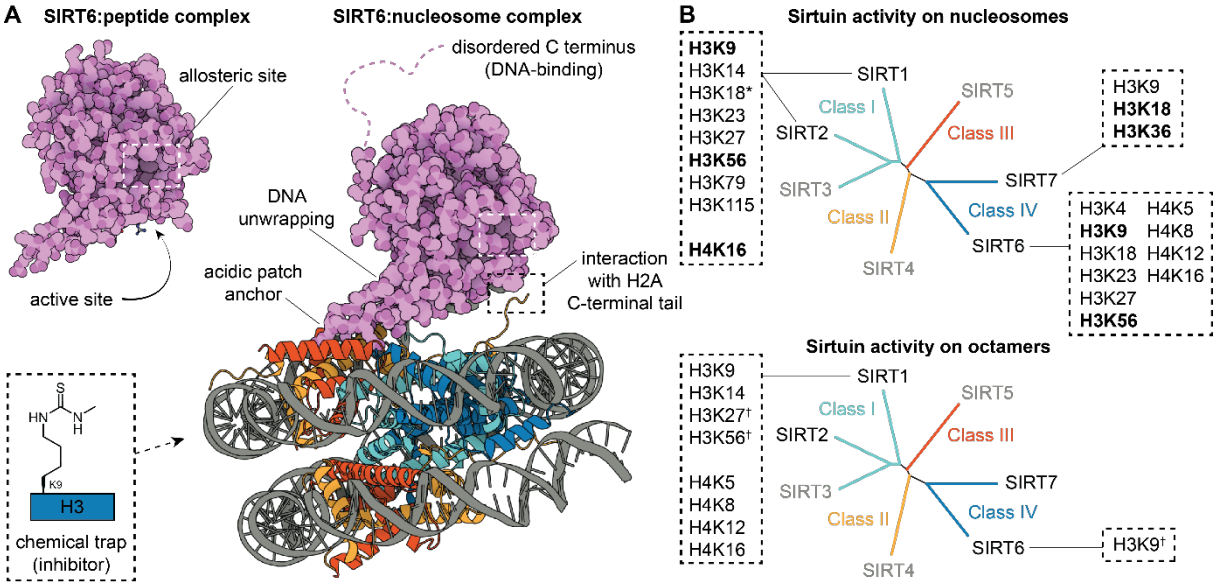

Figure 5. Recognition of nucleosome substrates by SIRT6 and activity of the nuclear sirtuins. (**A**) Comparison of the structure of SIRT6 bound to a histone peptide (PDB 7CL0) [88] and to a nucleosome (PDB 8F86) [16], both depicting the active conformation of H3K9 deacylation. (**B**) Summary of sirtuin activity on nucleosome substrates *in vitro* (bold residues indicate substrates strongly regulated in cells [8], *H3K18 is a cellular target of SIRT2 only), and on histone octamers (†activity found with H3-H4 tetramers).

SIRT7, the closest homolog of SIRT6, is also reported to bind DNA and other nucleic acids [89, 90], and shows a striking increase in deacylase activity towards nucleosome substrates [91]. These effects seem to be mediated by the lysine and arginine-rich terminal regions of SIRT7, although no detailed biophysical studies have been reported to date. Interestingly, SIRT7 is much more selective towards H3 substrates, and only shows robust deacylation of H3K18 and H3K36 *in vitro* and in cells (Figure 5b) [92, 93]. This selectivity likely indicates that the binding of SIRT7 to nucleosomes is more restrained conformationally than that of SIRT6.

Out of the five remaining sirtuins, only SIRT1 and SIRT2 are consistently found in the cell nucleus (although others are able to deacylate nucleosomes *in vitro* as well) [8]. SIRT1 prefers histone multimer substrates rather than nucleosomes for modifications on the H2B, H3 and H4 tails [81, 83, 94], while activity of SIRT2 does not seem to depend on the nucleosome structure as markedly (Figure 5b) [81, 94]. Both of these enzymes present robust deacylase activity towards multiple histone substrates [92, 95] and might even be able to reach residues buried within the octamer core [83, 96]. Future work using nucleosomes tools will reveal whether SIRT1 and SIRT2 are indeed able to distort the nucleosome structure and reach these core modifications. In addition, their substrate selectivity likely indicates they possess individual recognition mechanisms yet to be defined [92, 94, 95].

Chromatin-based approaches to further unravel the mechanisms of histone deacylation

Nucleosome substrates have already revealed unexpected site selectivities for HDAC-containing complexes and sirtuins [15, 84, 93, 94], and the introduction of inhibitory warheads has helped characterize the interaction with chromatin in higher detail [16, 61]. These tools permit studying the activity of these enzymes and the effect of modulators in a more biologically relevant context. However, the field has only taken advantage of a small portion of the techniques that are overall accelerating chromatin research. We believe that histone deacylase research would benefit greatly from novel approaches to directly investigate dynamic enzyme function within in a chromatin context *in vitro* or in living cells.

Determination of substrate search dynamics and activity within chromatin

Histone deacylases are sensitive to the chromatin environment and present DNA- and histone-dependent regulation. However, little is known about the mechanism and dynamics of substrate recognition within specific chromatin environments, such as gene promoters or heterochromatin. Reconstituted chromatin containing defined histone variants and PTM combinations, as well as specific DNA sequences, mimic the native chromatin environment and are amenable for detailed studies *in vitro*. Methods providing insight into the dynamic interplay between chromatin structure and the binding and function of histone-modifying enzymes include single-molecule Förster resonance energy transfer (smFRET) detection, and observing co-localization via single-molecule total internal reflection fluorescence microscopy (smTIRFM). On the one hand, strategic placing of a pair of fluorophores at different positions of the chromatin structure or the protein of interest permits measuring chromatin remodeling [97-99] or the dynamics of specific interactions by smFRET *in vitro* [100]. On the other hand, dynamic enzyme binding can be studied in great detail by having fluorescent labels on both the chromatin and the protein of interest and tracking the co-localization of single molecules *in vitro* by smTIRFM (Figure 6a) [98, 101, 102]. Co-localization can also be used to measure protein diffusion along DNA, by placing DNA or chromatin substrates parallel to the imaging plane using hydrodynamic forces (Figure 6a) [103] or double tethering [102]. In addition, two strategies have been applied to increase the measurement throughput of smTIRFM: the incorporation of a mixture of barcoded nucleosomes (multiplexed single-molecule detection of chromatin association, XSCAN)

[104], and the spatiotemporally-controlled generation of enzyme co-substrates (local generation of nucleoside triphosphates, LAGOON) [105].

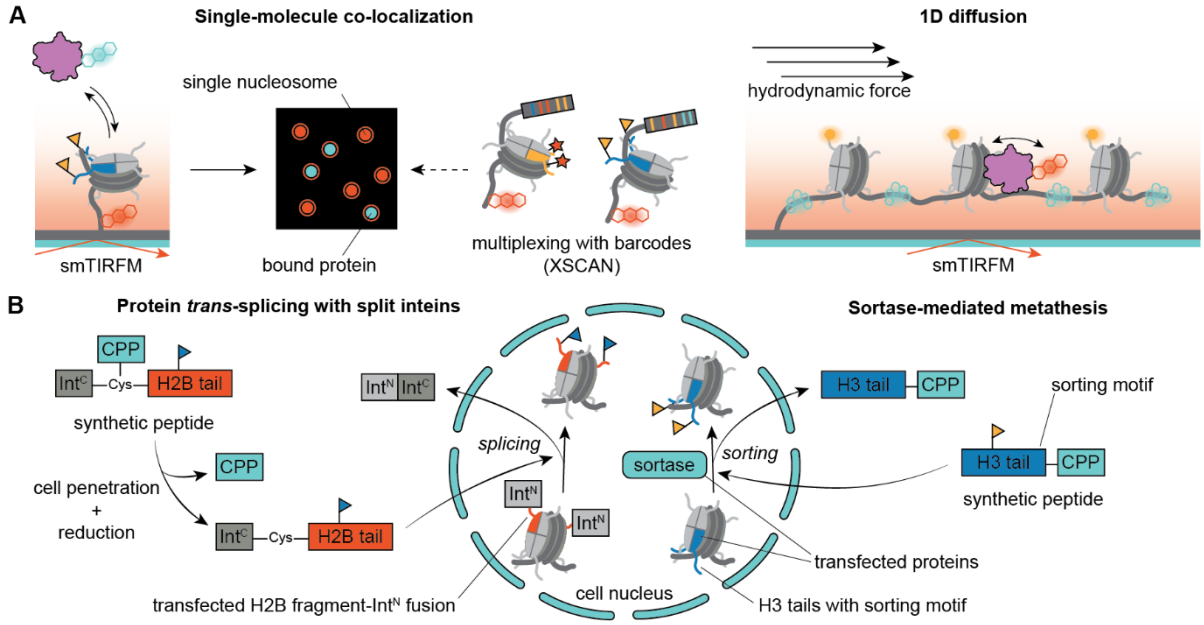

Figure 6. Methods to further dissect the mechanisms of chromatin-associated proteins. (**A**) Single-molecule fluorescence microscopy techniques to characterize binding, activity and diffusion within defined chromatin states [101, 103, 104]. (**B**) Preparation of semi-synthetic chromatin in live cells [106, 107]. CPP: cell penetrating peptide.

Many chromatin-modifying enzymes sense histone PTMs, including histone methyltransferases, demethylases, ubiquitylases and deubiquitylases, but this PTM cross-talk has not been explored to the same extent for histone deacylases. HDAC-containing complexes appear to be primed for this type of regulation, due to the reader domains present in some complex partners [12], and sirtuins may also sense PTMs [85]. Single-molecule fluorescence microscopy experiments with modified chromatin will help identify cross-talk and characterize the changes in binding dynamics due to pre-existing PTMs. Histone acetylation is considered a transient and highly dynamic modification, but the dynamic turnover depends on HDAC activity. Co-existing PTMs within chromatin that reduce the HDAC activity site-specifically may therefore generate long-lived acyl marks resistant to deletion. Defined nucleosome substrates *in vitro*, in combination with highly sensitive dynamic detection methods down to the single-molecule level, will allow the testing of these hypotheses.

Introduction of precise chromatin modifications in live cells

An important problem to address is how the different *in vitro* activities of histone deacylases translate to chromatin in living cells. This is especially challenging to dissect due to overlaps

in site selectivity and compensatory mechanisms that often counteract genetic perturbations. To enable the study of defined chromatin modifications in cells, Muir and coworkers developed a technique to modify histone tails using split inteins [106, 108]. One fragment of the split intein is fused to the core of the histone of interest, which can still be incorporated into chromatin, and the synthetic histone tail is linked to the remaining split intein peptide (Figure 6b) [106]. This method has already been used successfully to modify the tails of H2A and H3 histones in live cells or cell nuclei [106, 108], and even to introduce warheads for photo-proximity labelling studies [109]. A second strategy developed by Wu and coworkers introduces a sortase recognition sequence into the H3 tail, so that the unmodified tail of an expressed histone can be switched with a synthetic, modified H3 tail via a sortase-mediated metathesis reaction (Figure 6b) [107]. Unlike intein splicing, sortase-mediated metathesis is reversible and can suffer from lower yields, but the expressed histone required for this method is closer to the native sequence and potentially easier to incorporate into chromatin in living cells.

Native chromatin editing through these two methods can be useful to introduce specific histone deacylase substrates into native chromatin, or to insert PTM combinations that potentially regulate histone deacylase function [12, 16]. In addition, these techniques would permit the introduction of imaging labels or histone deacylase sensors into chromatin of living cells, which may be used to study deacylation dynamics by live-cell fluorescence microscopy. These approaches can serve to dissect histone deacylase function in their native environment. More importantly, such studies may help in the difficult quest to determine which PTMs regulate disease pathways, and what are the specific enzymes or complexes that target them. Current pharmacological modulation of histone deacylase activity focuses on individual enzymes, which can affect the dynamics of multiple histone PTMs and pathways [8, 12]. In contrast, detailed studies of deacylase chromatin recognition mechanisms may afford strategies to target single substrate interactions and, potentially, design more specific modulators. Taken together, we believe that the growing repertoire of chromatin technologies will be of great assistance in histone deacylase research.

Perspectives

- Histone lysine deacylases (HDACs and sirtuins) are broad epigenetic regulators involved in the progression of human diseases and studied as therapeutic targets

- Studies *in vitro* using peptide-based tools have been instrumental in the field but lack crucial contextual information needed to understand the behavior of these enzymes in the cell. This would be important to identify specific regulators in each pathway and disease, and to design therapies better poised for translation
- Advances in DNA and protein chemistry enable the use of designer chromatin for the detailed investigation of HDAC and sirtuin function, therefore incorporating the missing biological context

Keywords

Histone acetylation, sirtuin, HDAC, nucleosome, substrate, structure, fluorescence microscopy

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author contributions

Conceptualization: C.M.-Y. and B.F., Writing – original draft: C.M.-Y., Writing – review and editing: C.M.-Y. and B.F., Project administration & Funding acquisition: C.M.-Y. and B.F.

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