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Review

Histone-binding domains: Strategies for discovery and characterization ☆

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ABSTRACT

Chromatin signaling dynamics fundamentally regulate eukaryotic genomes. The reversible covalent post-translational modification (PTM) of histone proteins by chemical moieties such as phosphate, acetyl and methyl groups constitutes one of the primary chromatin signaling mechanisms. Modular protein domains present within chromatin-regulatory activities recognize or "read" specifically modified histone species and transduce these modified species into distinct downstream biological outcomes. Thus, understanding the molecular basis underlying PTM-mediated signaling at chromatin requires knowledge of both the modification and the partnering reader domains. Over the last ten years, a number of innovative approaches have been developed and employed to discover reader domain binding events with histones. Together, these studies have provided crucial insight into how chromatin pathways influence key cellular programs. This article is part of a Special Issue entitled: Molecular mechanisms of histone modification function.

1. Introduction

Chromatin signaling dynamics fundamentally regulate eukaryotic genomes. The reversible covalent post-translational modification (PTM) of histone proteins by chemical moieties such as phosphate, acetyl and methyl groups constitutes one of the primary chromatin signaling mechanisms. Modular protein domains present within chromatin-regulatory activities recognize or "read" specifically modified histone species and transduce these modified species into distinct downstream biological outcomes. Thus, understanding the molecular basis underlying PTM-mediated signaling at chromatin requires knowledge of both the modification and the partnering reader domains. Over the last ten years, a number of innovative approaches have been developed and employed to discover reader domain binding events with histones. Together, these studies have provided crucial insight into how chromatin pathways influence key cellular programs.

Here, we discuss approaches and limitations of the main methods currently used to define interactions between reader domains and histone post-translational modifications. We focus on lysine methylation as a model chromatin modification that can be used to illustrate the successes and challenges in the field. However, the principles of these approaches can be applied to study other modification systems. Lysine residues can be mono-, di- or tri-methylated, with the potential for at least one unique activity being coupled to the specific lysine residue and extent of methylation on that residue. Thus, methylation of lysine residues on a target protein can increase the signaling potential of the modified protein and as such lead to complex downstream signaling. The principal mechanism by which lysine methylation acts on histones is by mediating modular protein–protein interactions via reader proteins that are sensitive to methylated lysine. In this regard, the proteins that recognize a methylated lysine within a specific sequence context define the outcome of a lysine methylation event. To date, the dozens of methyl-lysine readers that have been discovered fall within ten distinct protein domain families: Chromodomain (CD), Plant Homeodomain (PHD) finger, Tudor, Malignant Brain Tumor (MBT), Proline–Tryptophan–Tryptophan–Proline (PWWP), Bromo Adjacent Homology (BAH), Ankyrin repeats, WD40 repeats, ATRX–DNMT3A–DNMT3L (ADD), and zn-CW. Given the number of potential methylation sites and states on histone proteins and non-histone proteins and the observation that typically several readers exist for a single histone PTM site [1], it is virtually certain that large numbers of readers with important biological behaviors remain to be discovered.

Currently, there are three principal ways to screen for binding of a particular protein domain to a desired histone modification: 1) Hypothesis-driven pairwise screening between protein domains and methylated peptides, 2) high-throughput array-based screening where many protein domains or modified peptides can be probed in a single experiment, and 3) identification of binding proteins isolated from nuclear extract by quantitative mass spectrometry. Each of these techniques has been utilized to characterize or identify binding interactions with varying degrees of success. Drawing on notable successful

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examples in the literature, we review the strengths and weakness of these approaches in their ability to identify and define the interaction between a protein domain and its associated methylated lysine.

2. Pairwise screening of protein domains or histone marks

The existence of methylated lysines on histones has been known for many decades [2]. However, until the discovery of the enzymes that modify histones, the function associated with this modification was largely unknown. The discovery in 2000 that SUV39H1 catalyzes H3K9 methylation fueled our understanding of the role of lysine methylation in the formation of heterochromatin and more broadly in regulating chromatin organization and function [3]. SUV39H1 interacts with the heterochromatin-associated protein HP1, which contains a CD module. Observations, including the proposal that recognition of acetylated lysine by bromodomain-containing proteins recruit the transcriptional machinery to target genes [4,5] and the localization and activity of SUV39H1, HP1, and H3K9 methylation at heterochromatin, led the Kourzarides and Jenuwein labs to postulate that the CD of HP1 is a candidate H3K9 methyl-lysine binding domain. To test this hypothesis, peptides of the N-terminal H3 tails were synthesized incorporating various modifications including methylation at lysine 9. Peptide-binding assays with these reagents established a direct interaction between the HP1 CD and H3K9me3 peptides [6,7]. These studies provided a paradigm for how methylated lysine acts at the molecular level and showed HP1 CD to be the first of many protein domains that function by binding to methylated lysines. Moreover, these two publications established a robust, productive, and straightforward method that has served as a blueprint for candidate-based testing of interactions between chromatin-associated domains and distinct modified histone peptides, of which several examples are described below.

The chromodomain is present in dozens of other proteins including polycomb group proteins. The finding that the HP1 CD can recognize H3K9me3 suggested that other CDs like the one in polycomb might share a similar function. For example, direct peptide-binding assays were performed to demonstrate that *Drosophila* Polycomb protein could bind H3K27me3 [8]. This work was further expanded to mammalian proteins where many orthologs of the *Drosophila* Polycomb exist. For example, mouse orthologs present in the PRC1 complex, which include CBX2 and CBX7, are able to bind H3K27me3 [9]. The crystal structure of the unrelated PRC2 component EED led to the hypothesis that its WD40 propeller domain binds to H3K27me3, which was experimentally validated by candidate-based screening using peptide-binding assays [10,11].

The chromodomain constitutes one of a few domain families that share structural homology. Other domains within this 'Royal Family' include the PWWP, MBT, agenet, and tudor domain. Among the proteins that contain the latter tudor domain, 53BP1 served as an early example of its capability as a methyl-lysine binding module. Our understanding of 53BP1 binding to H4K20me1/2 is rooted in genetic information from *Schizosaccharomyces pombe*. Specifically, the recruitment of the 53BP1 ortholog Crb2, a DNA damage response and tudor domain-containing protein, to double strand breaks was found to be dependent upon the H4K20 methyltransferase spSet9 [12]. From these data and protein array work from the Bedford lab, it was postulated that the tandem tudor domain of 53BP1 could bind methylated H4K20 [12,13]. Further structural and biochemical data provided the support necessary to directly determine that the 53BP1 tandem tudor domain bound H4K20me1/2 [14].

Another example for how candidate-based screening of modified peptides was used to identify new methyl-lysine binding modules comes from the example of the PHD finger from the ING family of chromatin-regulatory proteins. The PHD finger of the ING family member ING2 was found to bind to nucleosomes purified from HeLa cells but not to recombinant nucleosomes, where the histones are individually expressed in bacteria and lack PTMs. This finding suggested that the ability of ING2 to interact with nucleosomes is dependent upon a PTM present on HeLa-purified nucleosomes. To determine the responsible modification, the ING2 PHD finger was screened against a large panel of modified histone peptides. This analysis revealed that the ING2 PHD finger was both necessary and sufficient for high affinity and specific binding to H3K4me3-containing peptides [15]. Several other PHD fingers from the ING family (ING1, ING3, ING4, ING5 and yeast YNG1, YNG2, and PHO23) were shown to have the same property [15]. In simultaneous work, the PHD finger of BPTF was also found to bind to H3K4me3 [16]. This study used H3K4me3 peptides to extract candidate domains from cellular extract rather than screening a domain against a panel of modified peptides. Ultimately in both cases, direct peptide pulldowns encompassing many methylated histone residues demonstrated the specificity of the PHD fingers from the INGs and BPTF for H3K4me3. The molecular and biophysical bases for this specificity were elucidated in accompanying publications describing the crystal structures of BPTF and ING2 complexed with H3K4me3 peptides [17,18].

Each of the discoveries mentioned above provided great insight into our current understanding of protein methylation biology. However, in the absence of a clear and specific hypothesis to be tested, new high-throughput approaches have recently been developed to facilitate identification of novel reader domains and reader domain interactions with methylated proteins.

3. Array-based high-throughput screening

Advances in technology have allowed for higher throughput methods for screening domains and peptides against one another. Both modified peptides and protein domains of chromatin-associated proteins have been printed onto slide array platforms for screening. Each of these techniques has been useful in the discovery and definition of new protein interactions. Generally, these array platforms contain immobilized peptides or proteins upon which a query protein or peptide can be exposed (Fig. 1). Common immobilization methods include direct peptide synthesis onto a substrate, biotin-streptavidin affinity, and glutathione-GST affinity, although theoretically any covalent or high affinity interaction could be utilized. Typical arrays can contain hundreds to thousands of individual spots that provide broad accessibility to comprehensive peptide and domain libraries that would otherwise be burdensome to test.

3.1. Peptide arrays

Synthesis of biotinylated peptides followed by high performance liquid chromatography (HPLC) purification allows for the production of high quality and pure peptides carrying a diverse set of histone modifications that can be immobilized onto streptavidin-coated slides. In the most straightforward form, a single protein domain can be incubated on a peptide array to act as an initial discovery tool (Fig. 1A–D). To date, this approach has been highly productive and has led to the discovery of dozens of novel reader domains, including recent identification of tudor domain proteins that specifically bind to H3K36me3 to regulate PRC2 function and two that directly link disruption of the histone modification readout to human disease [19–24]. For example, a peptide array revealed that the non-canonical PHD finger of RAG2, an essential component of the RAG1/2V(D)J recombinase that mediates antigen receptor gene assembly, could bind H3K4me3 peptides with great specificity [23]. This interaction was demonstrated to be critical for V(D)J recombination *in vivo*. Moreover, a residue essential for the interaction is mutated in patients suffering from Omenn's syndrome, an immunodeficiency disease, providing a molecular explanation for the mutation [23]. An array-based approach also led to the discovery that the BAH domain of ORC1 is a novel binding domain with specificity and affinity for H4K20me2 [24]. In this case, the ORC1 BAH domain bound to H4K20me2 peptides but not sixty other methylated peptides

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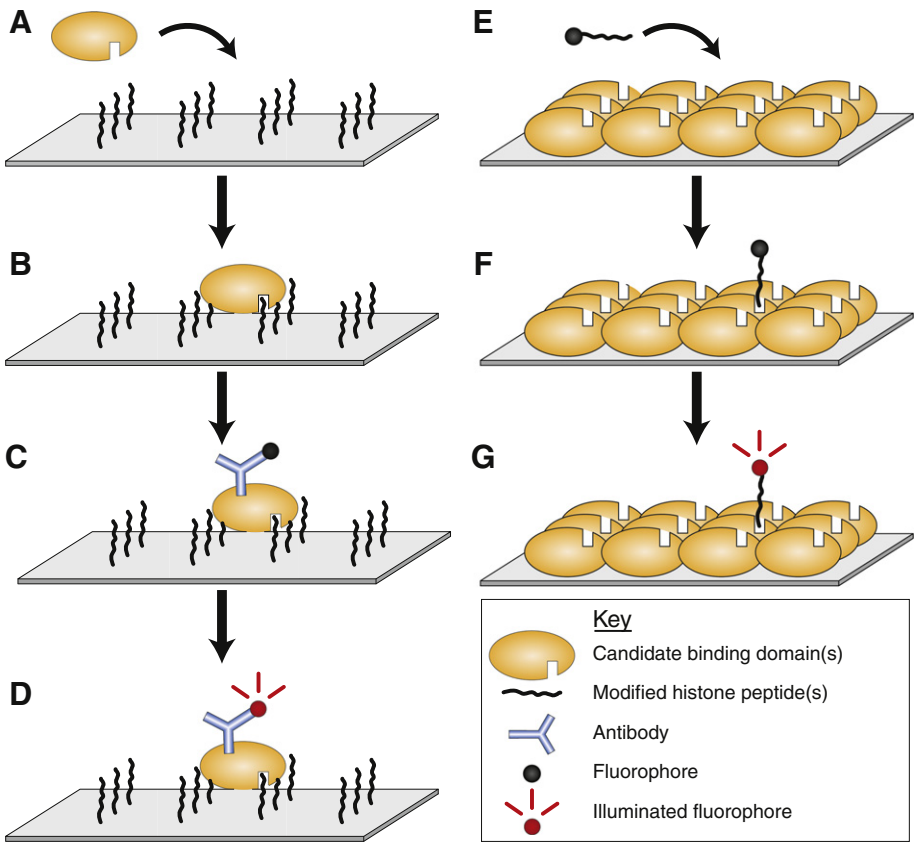

Fig. 1. Array-based screening of candidate binding domains and modified peptides. Peptide arrays contain many unique peptides that are probed with a candidate binding domain. A–B) Domains are incubated on the array surface and allowed to interact with the peptides on the array. C) Antibodies are used to detect the domain either directly using fluorescently labeled anti-epitope antibody or indirectly through a fluorescently labeled secondary antibody. D) Binding interactions are visualized using a fluorescent array scanner. Conversely, protein domain arrays are spotted with several unique recombinantly expressed candidate protein domains to be probed with a query modified peptide. E) Fluorescently labeled peptides are applied to the immobilized protein domains. F) The query modified peptide is allowed to bind to the candidate domains. G) Peptides are then visualized as with a peptide array. The visualization schemes for both techniques are not limited to fluorescent visualization as techniques such as enhanced chemiluminescence have also been applied with effective results.

on the arrays. Thus, the approach of screening a candidate domain on peptide arrays had uncovered new mechanisms by which chromatin signaling impacts biology and human disease.

Array technology allows for the definition of a single protein domain in a very comprehensive manner. However, one can also perform the same analysis on a large number of domains to define characteristics of a larger protein family previously determined to have potential to recognize modified histones. One such study took on this challenge to examine several domains within the Royal Family of chromatin-associated domains as well as MRG, SWIRM, and BRK domains [25]. Many existing domain–peptide interactions were corroborated using this approach such as the HP1 CD interaction with H3K9me, but new interactions were also found such as the CD of MPP8 binding to methylated H3K9 [25,26]. Interestingly, an independent peptide array experiment showed that MPP8 and HP1β could bind H3K9me and H3K23me, an interaction not seen in the previous array study [27]. While the array platforms and methodology were similar, the most likely cause of discrepancy between these experiments is the peptides on the array. In our experience, peptides are the most effective binding substrates when the modification of interest is centered within the peptide, which emphasizes the importance of peptide choices when defining binding events to modified histones.

In addition to individually synthesizing and purifying peptides for spotting on arrays with robots, a second approach of peptide array production referred to as SPOT synthesis (synthetic peptide arrays on membrane supports) has been used by multiple labs. On SPOT arrays, peptides are directly synthesized on cellulose membranes, anchored to the membrane by a chemical linker. Employing SPOT arrays has helped define the potential consensus sequence of certain lysine methyltransferases as well as characterize reader domains [28,29]. For instance, the Royal Family member PWWP domain was found to bind H3K36me3 peptides present by SPOT array [30]. The advantage of SPOT arrays versus individual synthesis of peptides is the cost and versatility of this approach. The main disadvantage is that the integrity, purity, and spatial orientation of the peptides cannot be determined and/or controlled for in the same manner as with the individual synthesis approach.

The interplay between different histone modifications has proven to be an important consideration with regard to functional readout. To address combinatorial analysis, peptide arrays provide a powerful platform to perform this type of screening because of the large number of distinct peptides that can be tested simultaneously. This is highlighted by a recent elegant study dissecting the binding profile of the tandem tudor domain (TTD) of the E3 ubiquitin ligase UHRF1. The TTD of UHRF1 was known to be sufficient to specifically bind H3K9me2/3 [31,32]. Notably, combinatorial peptides on an array revealed that the H3K9me3 binding occurs independent of phosphorylation at H3S10, a canonical chromatin signature of mitosis that excludes other H3K9me2/3 effectors [33]. UHRF2, the homolog of UHRF1, shares similar domain structure and *in vitro* functionality. However, defects in DNA methylation derived from mice lacking UHRF1 cannot be rescued by UHRF2 suggesting a non-redundant function for UHRF2 [34]. The UHRF1 TTD, therefore, provides a mechanism by which H3K9 methylation permits the faithful propagation of DNA methylation

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through mitosis [33]. The UHRF proteins contain a domain structure where its TTD is adjacent to a PHD finger. A larger construct spanning both the TTD and the PHD fingers therefore represents a more complete binding module of UHRF1. Structural and molecular data indicated that the PHD finger binds unmodified H3 tails with most sensitivity to H3R2 methylation [35,36]. When combined, both the TTD- and PHD finger-mediated binding events are required for efficient H3K9me2/3 recognition on a select number of peptides [37,38]. Consistent with these data, a more exhaustive analysis of peptides further showed that a non-functional PHD finger was able to abrogate the many binding interactions of UHRF TTD–PHD [34,39].

The expansive number of peptides on arrays can also enlighten more general phenomena described in previously published work. For example, the MBT domains of L3MBTL1 were found to directly interact with methylated p53K382, RbK860, and H4K20 [13,40–44]. Structural analysis of these domains revealed that the middle MBT domain of the 3xMBT repeat contained a canonical aromatic pocket that was capable of binding methylated lysines in a manner that had little requirement for the surrounding sequence context [43,44]. When probed on peptide arrays, the 3xMBT domain showed broad specificity for mono- and dimethylated lysines irrespective of the peptide sequence [45,46]. The binding promiscuity of this naturally occurring domain allowed it to be utilized as a pan-specific mono- and dimethyl-lysine affinity reagent for pull-down and far western applications [45].

Another powerful application of peptide arrays is to characterize modification state-specific histone antibodies, which are extensively used in chromatin research. In an analysis of several commonly used antibodies, many antibodies were found to lack the advertised specificity [25,47–49]. Inconsistency exists in the quality of modification-specific antibodies that are critical for understanding chromatin biology; therefore, care must be taken when interpreting data using these reagents. However, antibody quality can easily be determined using array technology.

Peptide arrays serve as a versatile and comprehensive approach for both identifying and defining interactions with modified histones, including antibody specificity. Nonetheless, like all techniques, peptide array platforms have limitations and drawbacks that should be taken into account. The majority of histone modifications occur within the N-terminus, which can be reasonably mimicked by an unstructured peptide, but some reader domain interactions with histone modifications are highly enhanced when presented in the native nucleosome form [50]. In addition, the finite number of peptides spotted on the surface of arrays naturally limits their power. While many different interactions and combinations can be spotted, not all interactions can feasibly be represented. This drawback is especially true for long-range interactions that are made difficult by the limitations of peptide synthesis.

3.2. Protein domain arrays

Peptide arrays are useful when asking whether a particular domain binds one of many histone peptides. Protein domain arrays can be used to perform the converse experiment where a modified peptide is allowed to bind to immobilized protein domains (Fig. 1E–G). Many epigenetic binding domain families have been defined, which provide starting points for defining proteins with potential reader-type binding capacity. These types of domains have been expressed and immobilized onto an array to be probed with a peptide [13]. Protein domain arrays have been useful in defining binding partners on a broad scale for H3K4me, H3K9me, and H4K20me [13]. More targeted experiments helped identify 53BP1 tudor domain to dimethylated p53 at lysine 372 and the ankyrin repeats of GLP as a binding domain that recognized the NF-κB subunit RelA when mono-methylated at lysine 310 [51,52]. While modifications of p53 and RelA are not histone modifications, the principles used to define these interactions apply to histone modifications such as H3R17me2a and H4R3me2a recognition by the TDRD3 tudor domain [53].

4. Peptide screening by SILAC

Candidate domain screening relies mostly on homology to previously characterized binding domains or directed experiments that are based on preliminary evidence and specific hypotheses. However, these criteria are not always met when beginning to characterize a binding event to methylated lysine. Thus, relatively unbiased methods have been developed to take advantage of SILAC (stable isotope labeling of amino acids in cell culture) and quantitative mass spectrometry to identify protein–protein interactions from cellular extracts. These extracts contain far greater numbers of proteins with potential to bind a particularly modified peptide. SILAC provides one with the ability to enrich for proteins from the human proteome rather than a defined set of proteins. Pulldown experiments can then be performed with "heavy" and "light" isotopically labeled nuclear extracts and analyzed by quantitative mass spectrometry (Fig. 2). Comparison of relative heavy-to-light ratios is used to identify proteins that are enriched in a modification-dependent manner.

The method of using SILAC-based quantitative mass spectrometry to identify methyl-lysine binding domains was validated when previously characterized binding domains were enriched from HeLa nuclear extracts: H3K4me3 interactions with BPTF, PHF8, and TFIID complex, H3K9me3 interactions with HP1α/β/γ, H3K27me3 interactions with CDYL1/2, [54] and 53BP1 with H4K20me1 [55]. Additionally, proteins

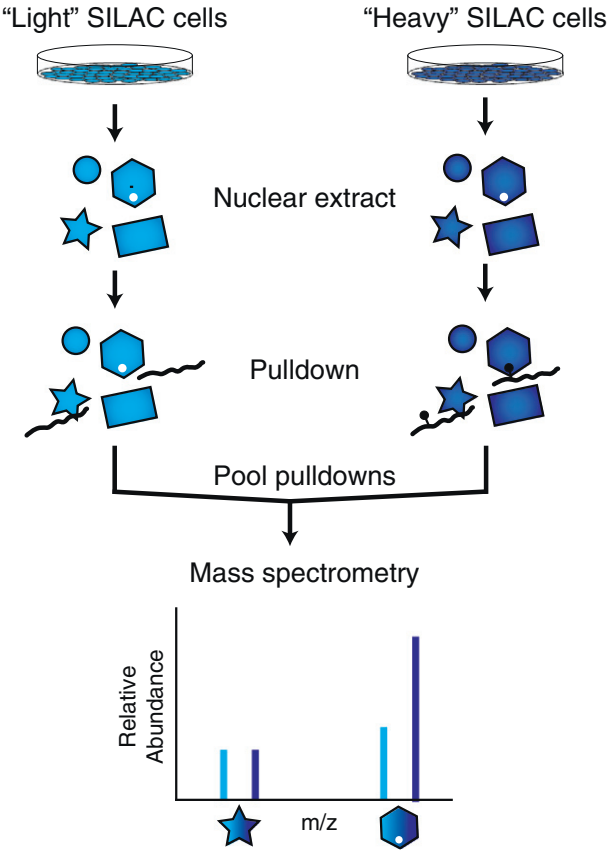

Fig. 2. Peptide screening by SILAC. Cells are grown in tissue culture media that is isotopically labeled with "light" or "heavy" amino acids. Nuclear extracts are prepared from these cells to enrich for nuclear proteins that may bind histone modifications. Unmodified and modified peptides are used as bait in pulldowns from the light and heavy nuclear extracts, respectively. Beads containing the immobilized peptides and any bound proteins are pooled and boiled. The bound proteins can then be digested and analyzed by mass spectrometry. Heavy-to-light ratios are then compared to find proteins that are enriched or excluded due to the presence of the modification of interest.

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containing a PWWP domain, a Royal family member, were enriched in pulldowns for H3K36me3 [54]. Subsequent experiments showed that the interaction between MSH6 PWWP domain and H3K36me3 as important for recruitment of DNA mismatch repair machinery to chromatin [56]. Similarly, the PWWP domain of PSIP1/LEDGF was also shown to bind H3K36me3 [50]. SILAC pulldowns followed by mass spectrometry are biased toward higher abundance proteins because these binding events are easier to detect. Modifications to this procedure have been made to enrich for lower affinity or lower abundance interactions. For example, photo-crosslinking was effective at identifying the binding event of a lower abundance protein such as ING2 to H3K4me3 [57].

5. Beyond defining histone interactions with peptides

Peptides serve as a convenient proxy to characterize binding interactions with the histone tails of the more physiologically relevant nucleosome. However, many modifications of importance occur within the globular domain of the nucleosome, such as H3K56 acetylation, H3K79 methylation, and H2BK120 ubiquitination. Even modifications on the histone tail, such as H3K36me, are located close to the nucleosome core where they may be influenced by the nucleosome structure as a whole. Evidence is beginning to support the idea that more physiologically relevant substrates may increase affinity or change identified substrates, even for residues toward the N-terminus of histones [50,58–60].

A primary challenge for assembly of designer chromatin is the homogenous incorporation of post-translational modifications. Histones are too large to make using traditional peptide-synthesis methods, so other chemical techniques must be applied to get a modified substrate. These chemical manipulations can be broken down into two main categories: Methyl-lysine analogs (MLAs) and protein ligation.

5.1. Methyl-lysine analog (MLA) technology

MLAs take advantage of the scarcity of cysteine resides in histones to perform alkylation reactions on a cysteine sulfhydryl group. *Xenopus* nucleosome core particles contain only a single cysteine residue at H3C110. H3C110A mutations do not severely impact DNA alignment around the nucleosome core particle and were used in the determination of the nucleosome core particle crystal structure [61,62]. In this system, a lysine-to-cysteine mutation is made at the site of a desired MLA in a histone already lacking cysteines (e.g. an H3C110A background for H3 modifications). Subsequently, an alkylation reaction is performed on the cysteine sulfhydryl group of this lysine-to-cysteine mutant protein to make unmodified, mono-, di-, or trimethyl-lysine analogs (Fig. 3A) [63]. MLA technology provides a relatively accessible and semi-genetically encoded method for representing a specific methyl modification within a whole protein. MLA histones can be used individually as free histones or can be incorporated into higher

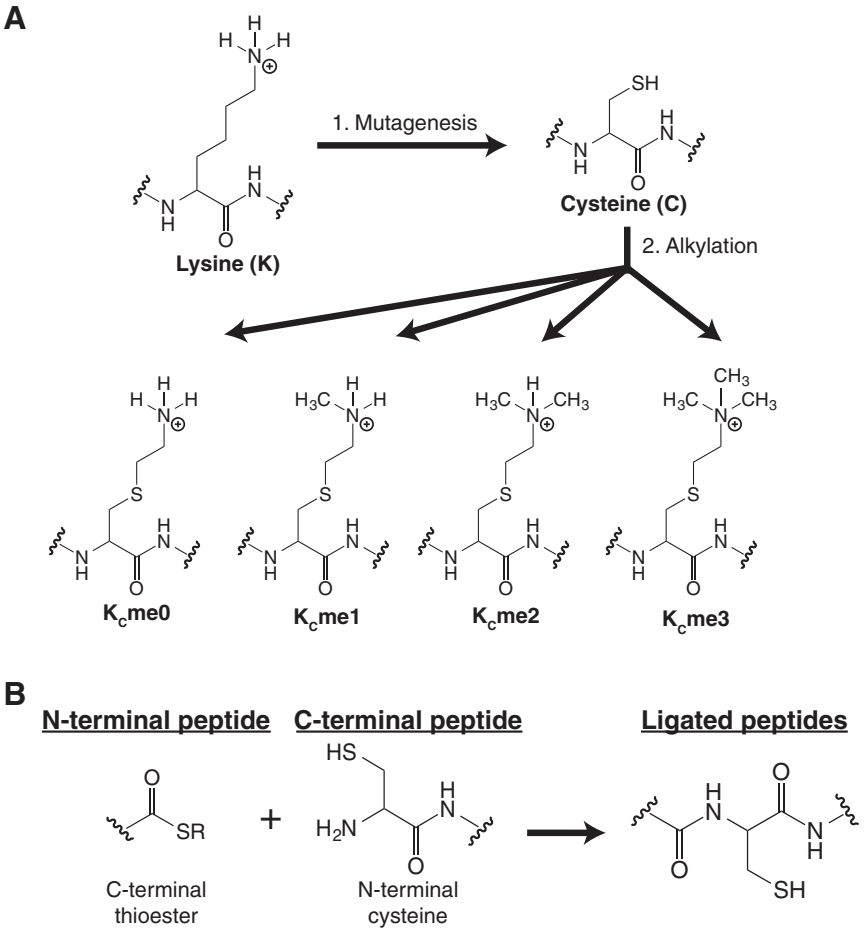

Fig. 3. Chemical strategies to generate specifically modified histone proteins. A) Modified lysine analog construction. All cysteines must be removed from a candidate protein, such as making an H3C110A mutation. The desired modified lysine is then mutated to cysteine and expressed in *Escherichia coli*. Following expression and purification, alkylating reagents are applied to the cysteines to generate a specific methyl lysine state at that particular residue. B) Protein ligation. Two peptides can be ligated if they contain the correct chemical signatures. The N-terminal peptide must contain a C-terminal thioester moiety that can either be generated by peptide synthesis or by using natural inteins. The C-terminal peptide must contain an N-terminal cysteine residue, which can be installed by peptide synthesis or molecular techniques such as protease cleavage. The chemoselective reaction will occur spontaneously to generate a ligated protein through a native peptide bond.

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ordered structures such as octamers and nucleosomes. Nucleosome-incorporated MLAs of H3K36me3 were instrumental to the characterization of PWWP domains binding to nucleosomes, which indicated that the nucleosome DNA is important for their physiological affinity [50,56].

Despite the promise and accessibility of this technique, MLAs may not behave exactly like a native methylated lysine because of the thioether generated from the alkylation reaction. For example, the TTD of 53BP1, CD of HP1β, and MBT domains of L3MBTL1 had notably decreased affinity for their cognate MLA-containing peptide relative to their affinity for the native methyl-lysine peptide [64]. Although, this phenomenon is not uniformly the case as the ING1 PHD finger and the UHRF1 TTD domain bound their MLA and native methyl-lysine substrates with comparable affinity [64]. Therefore when using MLAs as binding substrates, the structural changes caused by the thioether should be taken into consideration.

MLAs are also limited in their ability to test multiple different modifications in *cis*. In order to obtain a homogenous substrate, the same degree of methylation must be applied to all cysteines within a single alkylation reaction. If multiple methyl modifications are to be incorporated within the same histone, then they are required to have the same degree of methylation. Overall, MLA technology is a very powerful and accessible method that has been tremendously helpful in dissecting methyl-lysine biology.

5.2. Protein ligation technology

Protein ligation takes advantage of the ability to incorporate component histone peptides into full-length histones. Native chemical ligation (NCL) of two synthetic peptides can generate a native peptide bond between a peptide with a C-terminal thioester and a peptide with an N-terminal cysteine (Fig. 3B) [65]. The related process of expressed protein ligation (EPL) uses the same chemical functionality except that one of the peptides is obtained from a biological source [66]. The flexibility of peptide synthesis allows for the potential to include a wide range of combinatorial modifications across the entire protein. Additionally, native modifications are preserved, which eliminates potential affinity issues sometimes observed with MLAs [64]. In the simplest usage of EPL to generate a modified histone, a synthesized, modified peptide can be ligated to an unmodified portion expressed in bacteria. SILAC-based proteomics and EPL-generated mono-nucleosomes containing H3K4me3, H3K9me3, and H3K27me3 modifications were combined to identify canonical proteins associated with these marks [60]. More complicated syntheses have been implemented to make H3K56ac in a three-part ligation [67]. A residual cysteine will be created at any peptide junction, potentially resulting in structural problems. However, strategically placed cysteines can be desulfurized to alanine to get a native sequence if peptide junctures fall on an alanine [68].

Native chemical and expressed protein ligation provide the benefits of including native modifications within whole histone proteins. These reagents provide the most physiologically relevant substrates to define a binding event to histone modifications, especially when considering the potential importance of having a complete nucleosome structure. However, depending on the resources and expertise of the lab, these methods can be expensive, time consuming, and technically challenging to execute. Together, MLA technology and protein ligation technology have had a transformative impact on the chromatin biology and methyl lysine-signaling fields.

6. Conclusion

Although many protein modules have been identified to directly bind modified histones, our continued understanding of chromatin dynamics will be aided by the tools available to discover new binding proteins. Direct peptide pulldowns have had great historical significance in our foundational understanding of chromatin biology and will continue to be the most accessible method to test binding interactions. The popularization of designer chromatin reagents has provided a new perspective to ask outstanding questions, old and new. Integration of these reagents as time progresses will only expedite progress in identifying and characterizing reader domains. For example, modified chromatin reagents are also compatible with existing technologies such as slide arrays. Our ability to represent more specific chromatin environments and our ability to make these reagents will evolve. Work has been done to test the ability of proteins to bind trimethylated nucleosomes in the context of DNA methylation [60]. Also, nonsense-mediated suppression systems to incorporate modified, unnatural amino acids can be developed and improved [69]. Fully genetically encoded modifications will only facilitate the production and usage of customized chromatin. All of the aforementioned technologies have both strengths and weaknesses, which must be carefully considered when choosing and using reagents to interrogate the biology. Some of the most significant discoveries were made using the simplest methods. In the future, greater accessibility and development of these technologies will be a boon to the work defining interactions with modified histones.

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