Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Review

Histone-binding domains: Strategies for discovery and characterization☆

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ARTICLE INFO

Article history:
Received 7 January 2014
Accepted 17 January 2014
Available online xxxx

Keywords:
Methyl-lysine binding domain
Lysine methylation
Histone modification

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.

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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

☆ This article is part of a Special Issue entitled: Molecular mechanisms of histone modification function.

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1874-9399/$ – see front matter © 2014 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbagrm.2014.01.007

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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

Please cite this article as: A.W. Wilkinson, O. Gozani, Histone-binding domains: Strategies for discovery and characterization, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagrm.2014.01.007

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