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Expanding the Reader Landscape of Histone Acylation

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In this issue of *Structure,* Klein et al. (2017) expand our understanding of what reader domains bind to by showing that MORF, a double PHD domain containing lysine acetyltransferase, is a preferential reader of histone lysine acylation.

Histone post-translational modifications (PTMs) have been implicated in guiding a multitude of diverse cellular processes, including gene expression, DNA replication, chromosome segregation, and DNA repair. In particular, acetylation of histone lysine (K) residues—a chromatin mark first discovered by Vincent Allfrey in 1964—is known to regulate gene expression (Verdin and Ott, 2015). In the last few years, our understanding of how histone acetylation contributes to chromatin-based functions has greatly expanded, in part through the identification of reader domains that recognize K acetylation. The first identified K acetylation reader domain was the bromodomain, an ~100 amino acid module found in many chromatin-associated proteins (Dhalluin et al., 1999). For years, bromodomains remained in the spotlight as the only known reader of K acetylation. However, the list of K acetylation readers has grown with the discoveries that the double PHD finger (DPF) domain and the YEATS domain are also K acetylation readers (Zeng et al., 2010; Sabari et al., 2017). Bromo, DPF, and YEATS domains occur in diverse chromatin-associated proteins, including K acetyltransferases (KATs), ATP-dependent chromatin remodelers, and K methyltransferases. In this issue of *Structure,* Kutateladze and co-authors unveil how MORF, a double PHD-containing KAT, is a preferential reader of H3K14 acylation (Klein et al., 2017), thereby expanding our knowledge of the types of PTM landscapes reader domains interact with.

New technologies in mass spectrometry have dramatically enhanced our capabilities to detect novel yet related PTMs and to determine their locations in histones (Huang et al., 2015). Over the last few years, our knowledge regarding the locations and types of histone PTMs has rapidly expanded. Of the newly discovered PTM types, K acylations that include propionylation (pr), butyrylation (bu), crotonylation (cr), β-hydroxybutyrylation, succinylation, and malonylation, are garnering increasing attention, partly due to recent findings that several of these PTMs contribute to chromatin-based gene transcription (Sabari et al., 2017). K butyrylation (Kbu) and crotonylation (Kcr) particularly have been linked to the stimulation of gene transcription in yeast and mammals, and these PTMs exhibit similar genome-wide occupancy patterns with enrichment at promoter regions and enhancers (Sabari et al., 2017).

The advances mentioned above raise an intriguing question: how do histone acylations, such as Kbu and Kcr, cause downstream effects in chromatin? Although bromodomains might be likely suspects to bind these PTMs, these domains outside of a few exceptions prefer Kac and Kpr (Figure 1; Table 1) (Flynn et al., 2015; Andrews et al., 2016; Li et al., 2016). The answer to this puzzle lies in the discovery that the YEATS domain possesses acyl selectivity (Figure 1; Table 1). Structural studies revealed that the YEATS domains of Taf14, YEATS2, and AF9 bind to K acylation through an aromatic cage, with the highest affinity towards crotonylation followed by the other acyl marks approximately in order of the length of the fatty acid chain (Table 1) (Andrews et al., 2016; Li et al., 2016). Although these studies cracked one piece of the K acyl puzzle, the Li lab has added another K acyl reader

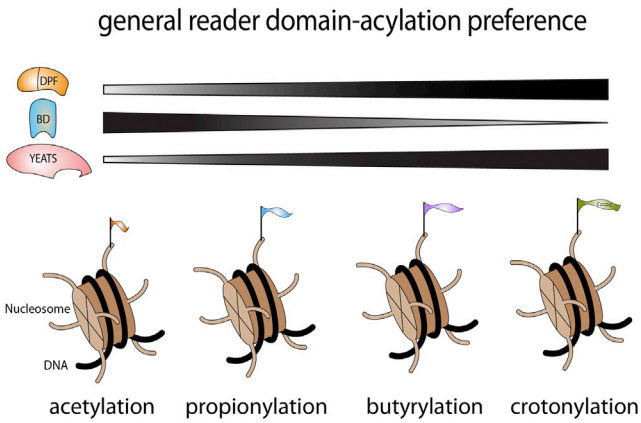

Figure 1. General Preferences of Histone Readers for the Distinct Histone Lysine Acylation States
Recent studies have uncovered the presence of additional lysine acylation states in histones, including lysine propionylation, butyrylation, crotonylation, β-hydroxybutyrylation, succinylation, and malonylation. Investigators are now unveiling how these distinct acylation "flavors" are "read" or interpreted in the context of chromatin. New studies from the Li and Kutateladze groups have added to the list of reader domains that bind the bulkier histone acyl forms that are largely "off limits" to most bromodomains (BD). In this issue of *Structure*, Klein et al. (2017) define the structural basis of the interaction of H3K14bu with the double PHD finger (DPF) of MORF, thus contributing to the finding that the DPF domain is a novel reader of lysine acylation. DPF domains, similarly to YEATS domains, largely prefer longer acyl forms of lysines. It will be of great interest to determine how DPF domains contribute to transcriptional regulation through their ability to be recruited to lysine acylation events in chromatin.

Table 1. Readers of Histone Acylation

Protein	Domain	Residue(s)	Acylation preference	Reference
BRD9	Bromo	H4 K5, K8	Pr>Bu>Ac	(Flynn et al., 2015)
CECR2	Bromo	H4 K5, K8	Bu>Ac>Pr	(Flynn et al., 2015)
TAF1	Bromo(2)	H4 K5, K8	Ac>Pr>Cr>Bu	(Flynn et al., 2015; Andrews et al., 2016)
AF9	YEATS	H3 K9	Cr>Pr>Bu>Ac	(Andrews et al., 2016; Li et al., 2016)
YEATS2	YEATS	H3 K27	Cr>Bu>Pr>Ac	(Andrews et al., 2016; Li et al. 2016)
ENL	YEATS	H3 K9, 27	Cr>Pr>Bu>Ac	(Andrews et al., 2016; Li et al., 2016)
GAS41	YEATS	H3 K9, 27	Cr>Pr>Bu>Ac	(Andrews et al., 2016; Li et al., 2016)
TAF14	YEATS	H3 K9	Cr>Pr>Bu>Ac	(Andrews et al., 2016; Li et al., 2016)
MOZ	DPF	H3 K14	Cr>Bu>Pr>Ac	(Xiong et al., 2016)
DPF2	DPF	H3 K14	Cr>Bu>Pr>Ac	(Xiong et al., 2016)
MORF	DPF	H3 K14	Bu>Ac	(Klein et al., 2017)

to the list with a recent report demonstrating that the DPF domains of MOZ and DPF2 are also selective readers of H3K14 acylation, notably H3K14cr (Xiong et al., 2016). Klein et al. (2017) now expand our basic understanding of K acyl reading by showing that the DPF module of the MORF KAT is also a selective reader of H3K14 acylation, with a preference for H3K14bu.

Similarly to the DPF domains of MOZ and DPF2, the DPF domain of MORF shows a slight preference for the longer butyryl-lysine compared with the well-characterized acetyl moiety at the same residue. A hydrophobic binding pocket in the first PHD finger that encapsulates the lipophilic butyryl-lysine dictates the specificity of the DPF domain for longer acylated lysine residues. This mechanism of recognition is distinct from that of the YEATS domain, which utilizes an aromatic cage to form a unique π-stacking interaction with the amide and alkene groups of the crotonyl-lysine, providing an increased affinity for crotonyl-lysine compared with other acyl-lysines. Another feature of the YEATS binding pocket is its openness, which prevents steric clashes with the longer acyl carbon chains (Andrews et al., 2016; Li et al., 2016).

As mentioned above, a select number of bromodomains can accommodate butyrl- and/or crotonyl-lysine (Flynn et al., 2015). BRD9 and CECR2 are two such bromodomains that can read butyryl-lysine; these bromodomains are characterized by a "tyrosine gatekeeper" and "methionine core" that provide increased flexibility in the binding pocket to accommodate the bulkier butyryl moiety. The second bromodomain of TAF1 can bind both butyryl- and crotonyl-lysine. Crotonyl recognition in the TAF1-2 bromodomain displaces water molecules in the binding pocket to allow a coplanar orientation of the crotonyl double bond with the amide group.

The distinct ways in which longer K acyl chains are bound by the different reader domain types argues for distinct physiological functions for the different acyl forms. Intriguingly, Klein et al. (2017) also showed that the bulkier K acyl forms (forms larger than acetylation and propionylation) are present at very low levels in vivo. Significantly, it has not been fully elucidated how these various acyl-CoAs end up on chromatin. Klein et al. (2017) began to address this knowledge gap by performing histone acyl-transferase assays in vitro with several well-studied KATs in the presence of the various acyl-CoAs. GCN5 and PCAF indiscriminately catalyzed the addition of acetyl, propionyl, and butyryl groups to H3K14; however, they did not add the other assayed acyl groups. MOF catalyzed butyrylation of H3K14, but to a lesser extent than it catalyzed acetylation of the same residue. It is of interest that none of the KATs tested robustly catalyzed the crotonylation of H3K14, although prior evidence has shown that p300 could catalyze crotonylation at H3K18 (Sabari et al., 2015). Recent studies of the DPF domains of MOZ and DPF2 indicate a DPF domain preference for H3K14cr (Xiong et al., 2016), but it has not been established which KAT installs this modification.

The discovery that the MORF DPF domain is an H3K14 acylation reader raises many exciting questions. For example, what is the biological relevance of MORF DPF binding in gene transcription? Why do the preferred acyl forms of MORF exist at such low levels in vivo? Perhaps answers to these questions will emerge by exploring the metabolism of the acyl-CoA forms. Under different nutrient conditions, humans possess vastly different levels of acyl-CoA (Sabari et al., 2017). During fasting when glucose levels are low, fatty acids are oxidized in the liver to generate an alternative source of acetyl-CoA. Propionyl-, butyryl-, and crotonyl-CoA are all generated during this process (Sabari et al., 2017). A portion of the acetyl-CoA derived from fatty acid oxidation is converted to β-hydroxybutyrate via ketogenesis. When mice were subjected to similar starvation conditions, the level of histone K-β-hyrodxybutyrylation was dramatically increased, and it correlated with activated transcription of starvation-induced stress response genes (Sabari et al., 2017). Clearly, future studies must examine the importance of the various acyl readers, writers, and erasers under different metabolic conditions and disease states, such as diabetic ketoacidosis in which levels of β-hyrodxybutyrate in blood can increase into the mM range (Sabari et al., 2017). The stoichiometry of the various histone K-acylations has also been demonstrated to change during cellular differentiation and development, such as in spermatogenesis (Sabari et al., 2017). The dynamic environment of histone acylation offers the possibility of an "acyl code" in which, during different metabolic or developmental states, readers of the bulkier acyl groups such as YEATS and DPF domain-containing proteins, stay bound

to chromatin, whereas most bromodomain-containing proteins are excluded.

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What's in an Average? An Ensemble View of Phosphorylation Effects

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The number of examples of functional post-translational modulation of disordered proteins rapidly grows. In a recent issue of *Structure*, Jie et al. (2017) show that phosphorylation of dynein intermediate chain alters partner binding but retains its dynamic, disorder character. An ensemble model offers an organizing framework to relate function, conformation, and phosphorylation in disordered and ordered proteins alike.

A characteristic feature of biological systems is that they are tightly regulated. Whether it is a metabolic process or a signaling cascade, biological systems have evolved a plethora of regulatory strategies to respond to environmental and metabolic cues to optimize the proteins responsible for a particular activity. In the vast majority of cases, regulation is mediated by a conformational change between an inactive and an active form of the molecule being regulated, and this conformational change is driven either by the binding of another molecule at an effector site (a process known as allostery) (Motlagh et al., 2014) or by a covalent modification (e.g., phosphorylation, methylation, sumoylation, acetylation, etc.). In either case, the modification (or the binding of the effector) will preferentially stabilize one state over the other, changing the relative population of active and inactive molecules and modulating the activity accordingly.

In many cases, the effects of phosphorylation and other modifications can be interpreted according to the "single structure model" of proteins (Figure 1A), wherein the active and inactive forms are adequately represented as single distinct structures. According to this view, phosphorylation induces a conformational change from one state to the other, and each state can be more or less represented by a single conformation. The tacit assumption of this model is that no molecules deviate significantly from the average in either state. In effect, one conformation is active and the other is inactive, and the structural change is the obligatory difference between the active and inactive (or less active) states. Numerous examples support this model, a classic being that of glycogen phosphorylase (GP) (Cori and Green, 1943; Johnson and Barford, 1993), which when phosphorylated undergoes domain rearrangements to remove steric occlusion, thus producing a shift to a more active state.

Over the past few decades, the picture has become more complex. Not only has phosphorylation been shown to modulate local flexibility in ostensibly folded structures (Garza et al., 2010), it has also been shown to modulate order-to-disorder transitions wherein a domain folds or unfolds upon phosphorylation, yielding a functional change (Figure 1B) (Bah et al., 2015; Mitrea et al., 2014). Furthermore, the discovery that more than 30% of eukaryotic protein sequences are intrinsically disordered (ID) (Ward et al., 2004) and that phosphorylation sites are found in hyper-abundance in these ID