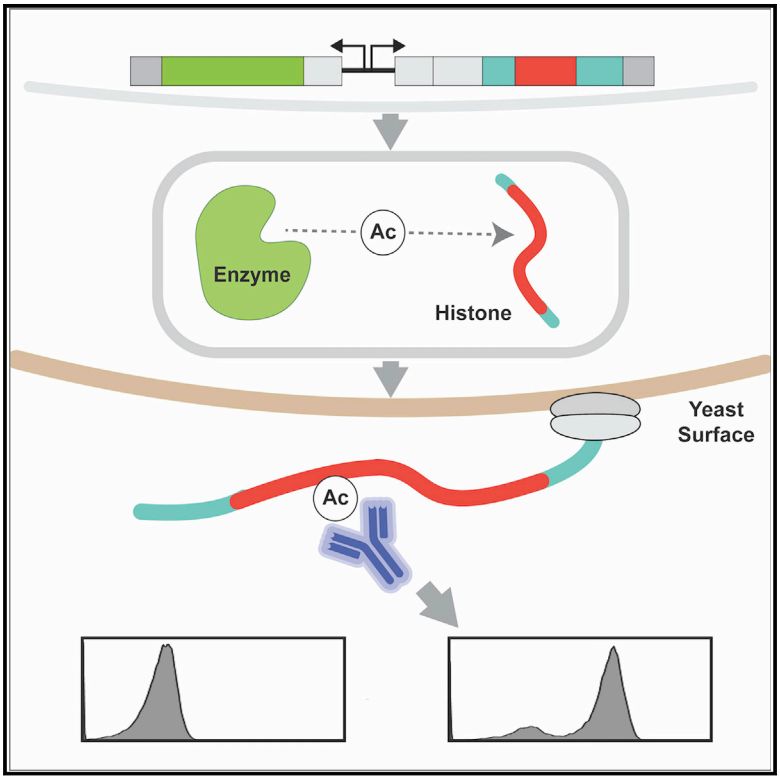
Resource

Cell Chemical Biology

Mapping the residue specificities of epigenome enzymes by yeast surface display

Graphical abstract



Highlights

- Yeast surface display was engineered to study histone modifications
- Crosstalk interactions on histones H3 and H4 were identified for p300

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In brief

Chromatin is numerically and combinatorially complex, with over 60 distinct histone modifications to date. Waldman et al. map the histone residue specificities and crosstalk of the histone acetyltransferase, p300, using yeast surface display.

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Mapping the residue specificities of epigenome enzymes by yeast surface display

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SUMMARY

Histone proteins are decorated with a combinatorially and numerically diverse set of biochemical modifications. Here, we describe a versatile and scalable approach which enables efficient characterization of histone modifications without the need for recombinant protein production. As proof-of-concept, we first use this system to rapidly profile the histone H3 and H4 residue writing specificities of the human histone acetyltransferase, p300. Subsequently, a large panel of commercially available anti-acetylation antibodies are screened for their specificities, identifying many suitable and unsuitable reagents. Furthermore, this approach enables efficient mapping of the large binary crosstalk space between acetylated residues on histones H3 and H4 and uncovers residue interdependencies affecting p300 activity. These results show that using yeast surface display to study histone modifications is a useful tool that can advance our understanding of chromatin biology by enabling efficient interrogation of the complexity of epigenome modifications.

INTRODUCTION

Chromatin is numerically and combinatorially complex with over 60 distinct biochemical histone modifications known to date. This complexity presents considerable challenges to understand the regulatory logic and function of chromatin as well as to simply map the properties of individual chromatin components. To address this complexity, new technologies have recently been developed, including libraries of DNA-barcoded nucleosomes that can rapidly map the binding specificities of protein domains to diverse histone modification patterns *in vitro* (Nguyen et al., 2014), genetic approaches, such as a yeast strain library that was used to assess the impact of histone H3 and H4 mutants on cell physiology (Dai et al., 2008), and large histone peptide arrays used to map the binding partners of different bromodomain families (Filippakopoulos et al., 2012).

Despite these and other advances, there remain several important gaps in our capabilities. *In vitro* methods provide exquisite control over residue specificities yet require recombinant production that often is refractory for many proteins or chemical syntheses and ligations that can become laborious to scale (Palomares et al., 2004). These limitations are particularly acute in investigating enzymatic activities, with no current high-throughput and easy-to-implement approach that can assess the specificities of epigenome "writers" for their histone substrates.

Here, we describe an approach for the efficient interrogation of epigenome modifications using yeast surface display. This platform bypasses the need for recombinant protein production or chemical ligation, preserves the ability to assess residue specificities as with *in vitro* biochemical assays, and is able to probe enzymatic activities of histone writers. In this system, the histone tail is co-expressed as a C-terminal fusion to the yeast cell wall protein Aga2p in the conventional yeast surface display system (Gera et al., 2013), along with the epigenome writer. Both the histone tail and the epigenome writer are targeted to the endoplasmic reticulum, where the histone tail is modified by the writer, and results in cell surface display of the modified histone tail (Figure 1A). Using this yeast surface display approach, we demonstrate histone acetylation by the histone acetyltransferase (HAT) p300 and map the specific histone H3 and H4 residues that are modified by p300. We further show how we can rapidly and rigorously assess the quality of commercial histone antibodies using this system. Finally, by comprehensively mapping binary crosstalk interactions between residues on histones H3 and H4, we demonstrate the scalability of this system that is dependent only on the construction of DNA expression libraries, not on recombinant protein production.

RESULTS

Our goal was to develop a platform to study epigenome writers that did not require recombinant protein production yet could also probe modifications to specific histone residues. We hypothesized that a yeast surface display system would be able to capture both of these requirements as it presents a hybrid intracellular and extracellular approach. A yeast-based system to map the residue specificities of epigenome enzymes will

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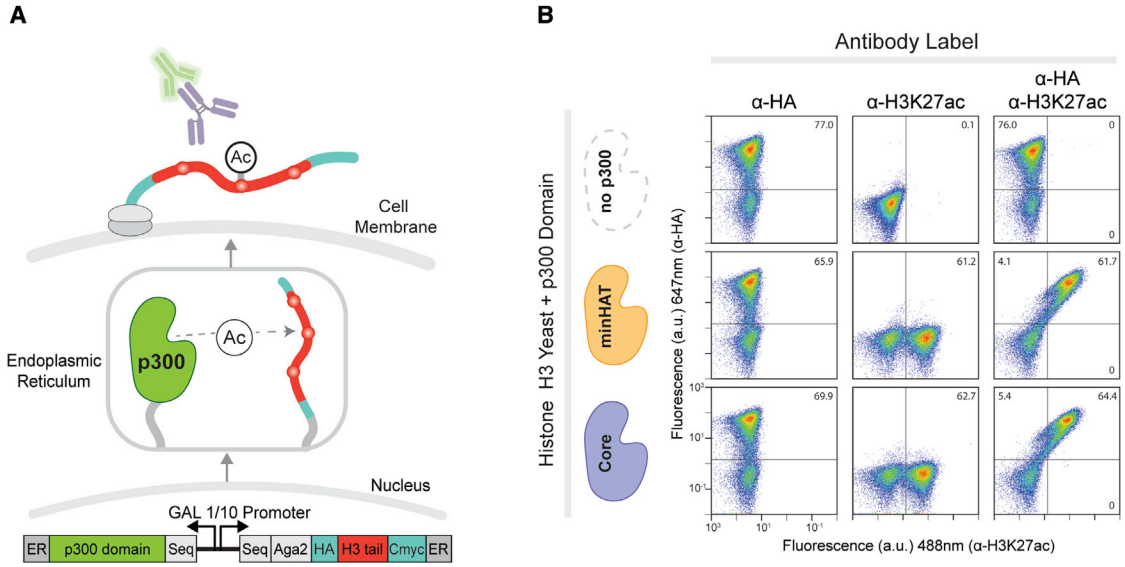

Figure 1. Histone peptides can be efficiently acetylated and then displayed on the surface of yeast

(A) A schematic of the endoplasmic sequestration and Aga1p-Aga2p yeast surface display method. Histone peptides have N-terminal HA tags and C-Myc tags that allow for quantification of their expression and display on a single-cell basis.

(B) Flow cytometry confirmation of histone tail acetylation only in the presence of p300. The HA tag measures the expression level of the histone H3 tail. The double-labeled plots show the high correlation between histone display levels and acetylation when p300 is present. Fluorescent gates were created based on unlabeled cells of the corresponding yeast strain per sample (not shown). The percentage of cells above each gate is recorded in the top corner of each plot. All plots contain 80,000 cells.

add an economical and rapid enzymatic assay to the toolbox of chromatin biologists and epigenome engineers.

Histone peptides can be efficiently acetylated and then displayed on the surface of yeast

We used yeast endoplasmic reticulum (ER) sequestration screening (Yi et al., 2013) to colocalize an epigenome enzyme with a histone tail peptide, allowing the peptide to be post-translationally modified. The histone peptide, fused to the Aga2p protein, and the epigenome writer are transcribed simultaneously by an inducible, bidirectional GAL1/10 promoter (Figure 1A). By tagging with ER sequestration and retention sequences, both proteins enter the ER and temporarily dock into the interior ER membrane. Here, the writer modifies the histone due to their high effective local concentration and proximity. Both proteins then continue through the secretory pathway where the writer is secreted into the supernatant and the modified histone is tethered to the yeast surface through the disulfide bond between the Aga1p and Aga2p proteins.

As a proof-of-principle, we chose the human HAT E1A binding protein p300 (p300 or KAT3A) as the writer and histones H3 and H4 as the substrates. p300 is a well-characterized HAT that acetylates histones and plays a significant role in chromatin regulation *in vivo* (Kurdistani et al., 2004; Fraga and Esteller, 2005; Marzi et al., 2018). Acetylation of lysine residues on histones H3 and H4 has been closely tied to activation of gene transcription (Struhl, 1998; Strahl and Allis, 2000; Bird, 2007; Rothbart and Strahl, 2014). Furthermore, transcription strength may be regulated by the number of acetylated residues on a histone tail (Dion et al., 2005) or preferential acetylation of specific lysine residues (Struhl, 1998; Dion et al., 2005).

We first investigated the acetylation of the lysine at the 27th residue on the histone H3 tail (H3K27ac); H3K27ac has been widely reported to be acetylated by p300 (Lasko et al., 2017; Klann et al., 2017). We used two different domains of p300, the core catalytic domain (Core, amino acids [aa]:1,048–1,664) (Hilton et al., 2015) and the minimal HAT domain (minHAT, aa: 1,284–1,669) (Bordoli et al., 2001). Labeling with antibodies against H3K27ac and HA showed that the acetylation signal (H3K27ac) correlated strongly with the amount of histone peptide displayed (HA) (Figure 1B). No acetylation was detected when p300 was absent. These results confirmed that histone peptides could be modified and displayed as cell surface fusions using this platform.

The specificities of anti-acetylation antibodies can be rapidly assessed by yeast-displayed histone peptides

Validation of the advertised specificities of commercially available antibodies is a significant challenge for the chromatin biology field (Rothbart et al., 2015). We investigated if our system could provide a general platform to test the specificity of commercial antibodies against histone modifications. To independently assess the antibody specificities of histone H3 and H4 acetylation antibodies for our specific study, we first used chemically defined histone peptides immobilized on the surface of yeast using the streptavidin-biotin linkage. In brief, a modified streptavidin monomer (mSA [Lim et al., 2011, 2013; Lim et al., 2012]) was expressed as a cell surface fusion using yeast surface display. We purchased biotinylated (but otherwise unmodified) histone H3 and H4 peptides as negative controls and biotinylated, singly acetylated peptides as positive controls (Table S1). These peptides were linked to the

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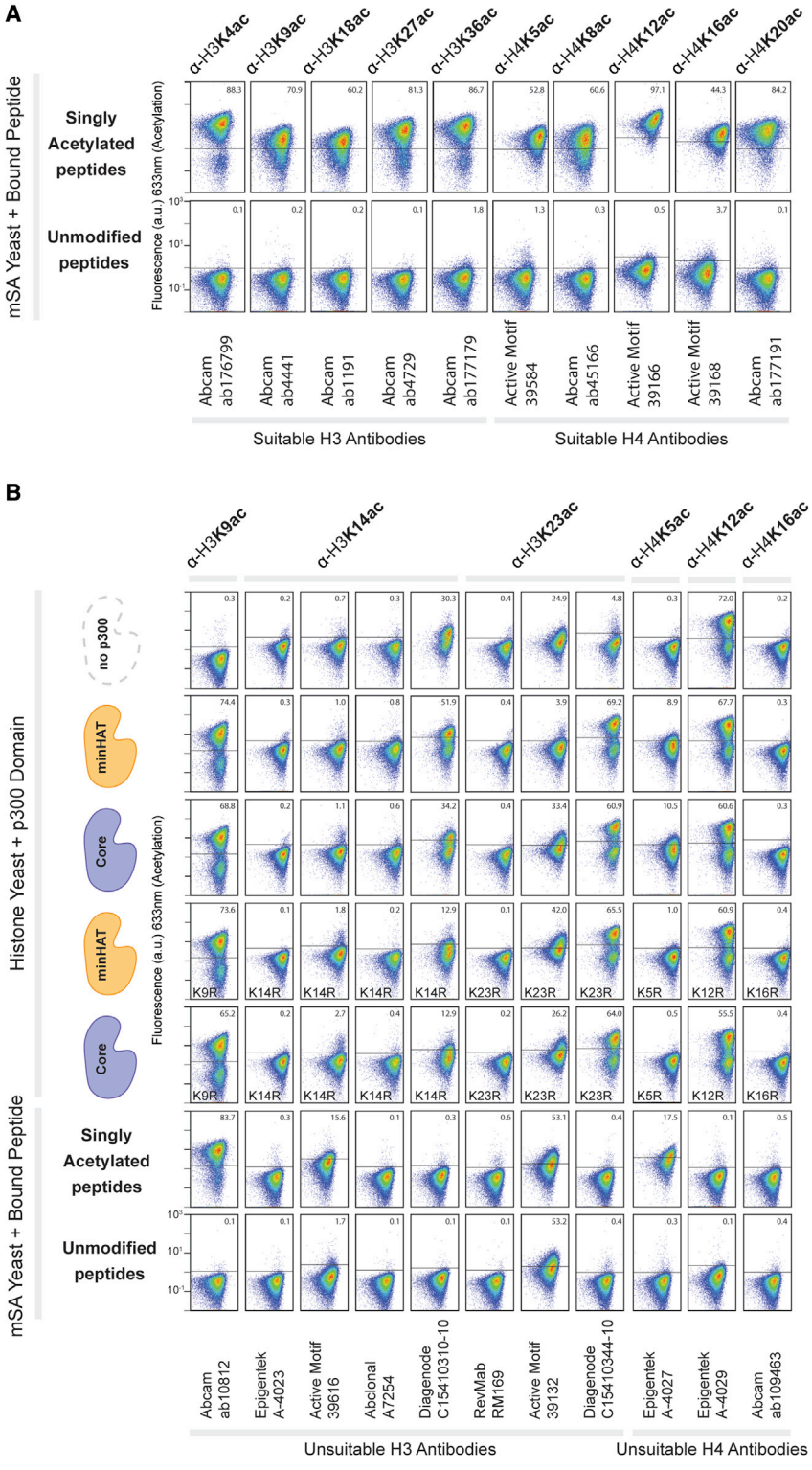

Figure 2. The specificities of anti-acetylation antibodies can be rapidly assessed by yeast-displayed histone peptides

(A) Primary antibodies against H3K4ac, H3K9ac, H3K18ac, H3K27ac, H3K36ac, H4K5ac, H4K8ac, H4K16ac, and H4K20ac that were selected to map the residue specificities of p300 domains (Table S2, in bold). These antibodies showed strong binding to their corresponding singly acetylated peptide when bound to the strep-displaying yeast and showed no binding to yeast displaying an unmodified histone peptide.

(B) Anti-acetylation antibodies not suitable for residue mapping. We were not able to identify suitable antibodies against H3K14ac or H3K23ac. 2E6 yeast cells were labeled with 1 μM peptide for each sample. Fluorescent gates were created based on unlabeled cells of the corresponding yeast strain per antibody (not shown). The streptavidin monomer has an N-terminal HA tag and a C-terminal FLAG tag, which were labeled to confirm high expression levels of mSA (data not shown).

surface of the mSA-displaying yeast. The specificities of an assortment of anti-acetylation antibodies targeting each of the available lysine residues on the N-terminal tails of histones H3 and H4 (Table S2) were tested by the ability of the antibody to specifically detect the appropriate acetylated peptides by flow cytometry (Figure 2).

In parallel, we investigated whether we could use our platform to assess the specificity of these antibodies. Toward this end, yeast cells expressing the histone peptides in the presence or absence of the p300 domains were labeled with the selected antibodies. Yeast cells expressing mutant histones with single arginine mutations at each of the lysine residues on the histone H3 and H4 tails were also generated (Table S3) and served as negative controls for the antibodies. The arginine mutant controls provided confidence that the antibodies were not binding other residues non-specifically that might be acetylated by p300 (Figures 2B and 3). Collectively, we confirmed the specificity of a set of primary antibodies against H3K4ac, H3K9ac, H3K18ac, H3K27ac, H3K36ac, H4K5ac, H4K8ac, H4K12ac, H4K16ac, and H4K20ac (Figure 2A), with both approaches yielding similar results (Table S2). However, despite testing multiple commercially available antibodies, we were unable to identify appropriate antibodies for H3K14ac and H3K23ac (Figure 2B). These results show that this yeast-display platform is a useful tool to efficiently assess the specificity of antibodies putatively recognizing histone modifications at specific residues. Antibodies found to be specific using both approaches were used in further experiments in this study.

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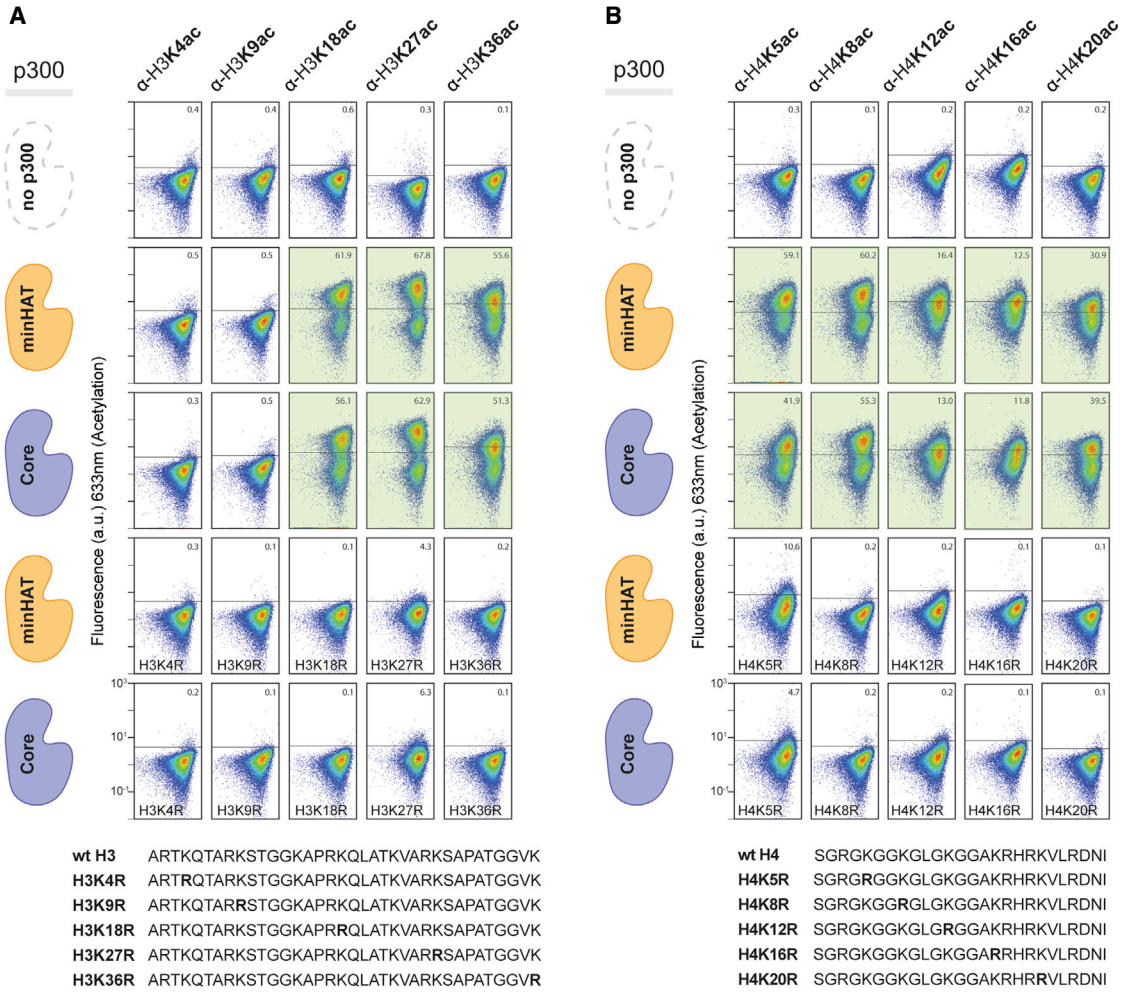

Figure 3. p300 residue specificities can be mapped by ER sequestration and yeast surface display

The y axes display the fluorescence due to binding of the corresponding anti-acetylation antibodies for the N-terminal tails of (A) histone H3 and (B) histone H4 expressed with or without p300 domains. The plots highlighted in green indicate which residues were acetylated by p300. The p300 domain expressed in each sample is indicated on the left. Lack of antibody binding on the different arginine-mutated histones indicate that the antibodies are not binding to acetyl groups deposited on off-target lysines. Fluorescent gates were created based on unlabeled cells of the corresponding yeast strain per antibody (not shown).

p300 residue specificities can be mapped by ER sequestration and yeast surface display

Mapping the residue specificities of epigenome writers is an important step in ultimately understanding their regulatory roles and functions. Using the set of suitable antibodies that we identified, we mapped the residue specificities of the minimal HAT and Core domains of p300 for the N-terminal tails of both histones H3 and H4 using our yeast platform and flow cytometry. We determined that the p300 minimal HAT and Core domains both acetylated histone H3 at K18, K27, and K36, and acetylated histone H4 at K5, K8, K12, K16, and K20 (Figure 3). We did not observe acetylation at H3K4 or H3K9. Notably, these acetylation patterns match what has been observed previously using biochemical assays (Schiltz et al., 1999; McManus and Hendzel, 2003; Kouzarides, 2007; Bedford et al., 2010; Henry et al., 2013; Dancy and Cole, 2015; Hilton et al., 2015) (Table 1). Furthermore, mutating each specific residue to arginine ablated labeling by the matching antibody. These results show that our system of displaying modified histone peptides on the yeast surface can be used to assess residue specificities of epigenome writers.

p300 exhibits crosstalk between modified histone residues

We used our platform to map crosstalk between modified histone residues, specifically the effect of histone modification at each specific residue on the enzymatic activity of p300 for all other residues. This form of crosstalk has been observed in several systems but is difficult to map comprehensively. For example, acetylation of the 14th lysine on histone H3 (H3K14ac) has been shown to enable the Rtt109-Vsp75 complex to acetylate the 56th lysine on the same histone (H3K56ac) (Cote et al., 2019); when histone H3 is not pre-acetylated at the 14th residue, H3K56 is not acetylated. In addition, some histone modifications have been found in pairs on histone tails, such as H4K5/K12ac and H4K8/K16ac (Makowski et al., 2001), suggesting a potential for crosstalk. Thus, understanding how writers

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Table 1. Previous studies that reported histone residues acetylated by p300

Reference	H2A and H2B residues	H3 residues	H4 residues
Schiltz et al., 1999	p300 acetylates all sites on H2A and H2B known to be acetylated in bulk chromatin: H2AK5 and H2BK5, K12, K15, K20	p300 prefers H3K14ac and H3K18ac over H3K4ac and H3K23ac. p300 did not acetylate H3K9 or H3K27	prefers H4K5ac and H4K8ac. Saw acetylation at H4K5 and K8, and low levels of acetylation at H4K12 and K16
McManus and Hendzel, 2003	p300 acetylates H2BK5, K12, K15, and K20 in COS-7, Hela, and IM cells	p300 acetylates H3K14, not H3K9, in COS-7 cells. p300 acetylates H3K9 and H3K14 in Hela and IM cells	p300 acetylates H4K5 and H4K8 in COS-7, Hela, and IM cells
Kouzarides 2007	CBP/p300 acetylates H2AK5, H2BK12, and H2BK15	CBP/p300 acetylates H3K14 and H3K18	CBP/p300 acetylates H4K5 and H4K8
Bedford et al., 2010		CBP/p300 is responsible for global H3K18ac and H3K27ac in mouse fibroblast cells	
Henry et al., 2013		p300 acetylates H3K9, K14, K18, and K23. Very low levels of H3K27ac	p300 acetylates H4K5, K8, K12, and K16
Dancy and Cole 2015			p300 prefers H4K5ac and H4K8ac. p300 knockout cells showed reduced acetylation at H4K5, K8, K12, and K16
Hilton et al., 2015		p300 Core domain acetylates H3K27	

Bedford et al., 2010; Dancy and Cole, 2015; Henry et al., 2013; Hilton et al., 2015; Kouzarides, 2007; Schiltz et al., 1999; McManus and Hendzel, 2003.

and histone modifications influence each other will be very important for understanding how chromatin states are regulated. The histone code hypothesis also supports the idea that histone modifications collectively contribute to transcriptional regulatory logic. However, these are all inherently combinatorial problems that exponentially increase the number of samples and experimental conditions to be tested. Furthermore, it is challenging to determine whether histone residue crosstalk could be achieved by just a single writer protein, as opposed to a situation where a complex of proteins is needed to recognize one modification and then recruit a writer to modify another residue. Here, we investigated whether this yeast surface display platform could map the crosstalk between all possible pairs of acetylated histone residues. In other words, does the absence of acetylation at one site affect the ability of p300 to acetylate another site?

To assess crosstalk between acetylated residues, we measured acetylation at all residues for yeast cells expressing distinct Lys-to-Arg mutants of histones H3 and H4, and p300. Each yeast sample was labeled with anti-C-Myc and residue-specific acetylation antibodies. Fluorescent gates were created using unlabeled cells of the same yeast strain for each sample (Figure S1). The relative acetylation level for each residue was calculated by quantifying the percentage of cells with signal for both the anti-C-Myc and residue-specific acetylation antibodies out of all cells with C-Myc signal. As expected, the acetylation signal for the arginine-mutated residue dropped sharply compared with the wild-type peptide (Figure 4).

Interestingly, we observed strong crosstalk between H4K20ac and both H4K8ac and H4K16ac. Mutating H4K20 to an arginine ablated the strong acetylation that was originally observed at H4K8 and H4K16. This crosstalk was slightly stronger for the min-HAT domain compared with the Core domain. Conversely, mutating H4K8 or H4K16 to an arginine did not change the acetylation levels of H4K20ac for either the p300 minimal HAT or Core domains. This observation indicates that H4K8ac and H4K16ac written by p300 relies on the presence of H4K20 being acetylated.

In addition to being dependent on H4K20ac, H4K16ac is also reduced when H4K12 is mutated to H4R12 and increased when H4K8 is mutated to H4R8. These crosstalk interactions are more pronounced when the p300 minimal HAT domain is present compared with the p300 Core domain. Mutation of H4K16 to H4R16 does not appear to change the levels of H4K8, H4K12, or H4K20 acetylation. Interestingly, H4K16 acetylation is generally associated with the MYST family acyltransferase KAT8 (Smith et al., 2005; Taipale et al., 2005; Li et al., 2012). However, there is evidence that p300 also acetylates this residue (Henry et al., 2013; Taylor et al., 2013), and there may be significance in p300 mediating a form of regulatory logic involving the crosstalk interactions of H4K16 and H4K20 acetylation.

Finally, on histone H3, there is also minor crosstalk between H3K36 and H3K14 and H3K23. When either is mutated to arginine, the relative level of acetylation at H3K36 is reduced by 31% and 47% when being written by the minimal HAT domain and the Core domain, respectively. These results collectively show that this platform is a powerful tool for efficient interrogation of crosstalk between histone residues.

DISCUSSION

We describe a facile, economical, and high-throughput approach for chromatin biology that enables post-translational modification and yeast surface display of histone tails. We have shown that this yeast surface display system can efficiently map the residue specificities of acetyltransferase domains, determine the specificities of commercially available anti-acetylation antibodies, and reveal crosstalk interactions between acetylated histone residues. Importantly, this system enables analysis of histone modifications without the need for chemical

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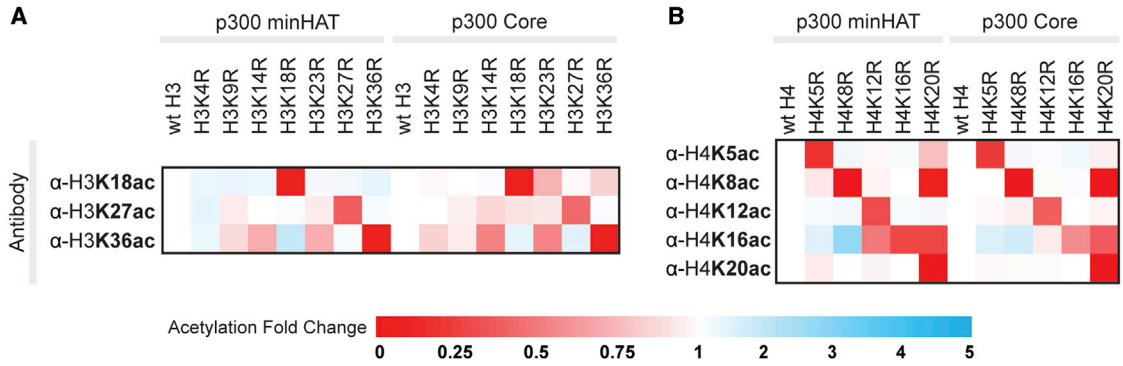

Figure 4. p300 exhibits crosstalk between modified histone residues

The yeast strain measured is listed across the top of each heatmap and the acetylation site probed by a specific antibody using flow cytometry is listed to the left of each row. Samples were double labeled with anti-C-Myc and anti-acetylation antibodies. An initial gate was created for cells showing C-Myc expression, indicating expression of the histone tail. Within this population, a secondary gate was created for cells also showing fluorescence from the anti-acetylation antibody. The number of cells in the double-labeled gate was divided by the number of cells in the anti-C-Myc gate to quantify the level of acetylation on each histone tail. Acetylation levels were normalized to the acetylation level of the wild-type histone tail for each acetylation site with the average acetylation level shown. Red is used to indicate a decrease, blue is used to indicate an increase, and white is used to show no change in relative acetylation level at each lysine site. No mutations established acetylation at H3K4 or H3K9 (data not shown).

peptide synthesis, chemical modification of enzymes, or production of recombinant proteins, thereby reducing experimental costs and expertise requirements. Histone residues identified as acetylated by p300 using yeast surface-displayed histone peptides are consistent with results from previous work, validating this approach (Table 1). Importantly, we also identified crosstalk between acetylation sites of histone H4.

This system has the potential to accelerate and augment the way we study chromatin biology and serves as a complement to fully *in vitro* and *in vivo* approaches. However, there are limitations that should be considered when using this system. Here, we discuss these considerations as well as potential future approaches to expand its utility. First, this system brings enzyme and substrate into artificial proximity within the ER environment with non-native conditions for many enzyme-substrate pairs. This could lead to promiscuous modifications that may not occur in a physiological context. Future work could focus on developing inducible systems that could control both the concentrations and timing of enzyme and substrate expression, allowing a more complete quantitative understanding of modification specificity. Further pairing this knowledge with intracellular experiments could potentially provide links between enzyme kinetics and specificity and different physiological conditions.

Another important consideration is that this system studies each histone tail independently. This has the advantage of providing tighter experimental control as well as the potential to study different segments of a histone tail in isolation, but at the cost of not having the full context of a nucleosome to study both enzyme specificity and crosstalk interactions as can be done in reconstitution assays. Future work could attempt to partially reconstitute histone interactions and octamers in yeast to provide this higher-order context. Relatedly, the use of lysine surrogates (i.e., arginine mutations) may not fully capture the nuances of crosstalk interactions. However, this issue remains in reconstitution assays; using semi-synthetic histones as writers, such as p300, could catalyze all residues necessary for crosstalk interactions even if not initially present on the synthesized histones. Future approaches could potentially bypass this challenge by the use of lysine analogs chemically more similar than arginine through the use of artificial amino acid systems. Finally, this system depends on the availability of sufficiently specific antibodies against modifications of interest. The reliability and specificity of affinity reagents have and continue to be a major issue in chromatin biology (Egelhofer et al., 2011; Fuchs and Strahl, 2011; Rothbart et al., 2015). Here, we demonstrated that this approach could be used to efficiently screen the suitability or specificity of antibodies. It could also be repurposed to generate new affinity reagents as well in the future as a platform for directed evolution.

In addition to these considerations, there are also exciting potential expansions of this system that could be pursued. One of the challenges of studying enzymes in native contexts is that they can exhibit considerable differences in molecular phenotypes. For example, the histone H3 residue specificity of p300 has been reported to be different in COS-7 (African green monkey kidney) cells, HeLa (human epithelioid cervical carcinoma) cells, and IM (male Indian Muntjac skin fibroblast) cells, while the histone H4 acetylation pattern remained the same across cell types tested (McManus and Hendzel, 2003). This yeast surface display approach provides the potential to rapidly alter microenvironmental conditions by co-expression of other proteins and cofactors to assess their potential impact on residue specificities (Voss and Thomas, 2018). Furthermore, this system could be expanded to other enzymes, including RNA polymerases, methyltransferases, kinases, and phosphatases. In these cases, it will be important to consider cofactor availability. In the case of p300, acetyl-CoA is naturally available in the lumen of the ER. If a cofactor is needed that is not already available in the yeast ER, that cofactor could be supplemented into the system.

In conclusion, this yeast surface display approach connects the powerful protein engineering and screening abilities of yeast surface display with chromatin biology. Only standard

molecular biology, yeast culture, and flow cytometry analyses are required. Since these are simple, robust, and automatable protocols, this system provides a potentially democratizing, cost-effective, and time efficient approach for studying chromatin biology.

SIGNIFICANCE

Histone proteins are decorated with a combinatorially and numerically diverse set of biochemical modifications. These modifications are of ubiquitous importance in normal and disease physiology across eukaryotic species. However, they are challenging and laborious to study due to their complexity. Here, we describe a platform to study epigenome writers that does not require recombinant protein production yet can probe modifications to specific histone residues. This platform is a hybrid intracellular and extracellular approach that leverages yeast surface display for rapid and cost-effective analyses. As proof-of-concept, we used this platform to rapidly profile the histone H3 and H4 residue writing specificities of the human histone acetyltransferase, p300. Subsequently, a large panel of commercially available anti-acetylation antibodies were screened for their specificities, identifying many suitable and unsuitable reagents. Furthermore, this platform enabled efficient mapping of the large binary crosstalk space between acetylated residues on histones H3 and H4 and uncovered residue interdependencies affecting p300 activity. These results present a yeast-based system to map the residue specificities of epigenome enzymes that will add an economical and rapid enzymatic assay to the toolbox of chromatin biologists and epigenome engineers.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.chembiol.2021.05.022.

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AUTHOR CONTRIBUTIONS

A.C.W., B.M.R., and A.J.K. conceived the study. A.C.W. planned and performed the wet lab experiments with guidance from B.M.R. and A.J.K. A.C.W., B.M.R., and A.J.K. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
HA	Thermo Fischer Scientific	Cat#PA1-985; RRID: AB_559366
C-Myc	Thermo Fischer Scientific	Cat# A-21281; RRID: AB_2535826
Flag	Thermo Fischer Scientific	Cat#MA1-91878; RRID: AB_1957945
His	Thermo Fischer Scientific	Cat#MA1-21315; RRID: AB_557403
H3K4ac	Abcam	Cat#ab176799; RRID: AB_2891335
H3K9ac	Abcam	Cat#ab10812; RRID: AB_297491
H3K9ac	Abcam	Cat#ab4441; RRID: AB_2118292
H3K14ac	Epigentek	Cat#A-4023; RRID: AB_2891336
H3K14ac	Abclonal	Cat#A7254; RRID: AB_2737401
H3K14ac	Active Motif	Cat#39616; RRID: AB_2793274
H3K14ac	Diagenode	Cat#C15410310-10; RRID: AB_2891338
H3K18ac	Abcam	Cat#ab1191; RRID: AB_298692
H3K23ac	RevMab	Cat#RM169; RRID: AB_2716400
H3K23ac	Active Motif	Cat#39132; RRID: AB_2891340
H3K23ac	Diagenode	Cat#C15410344-10; RRID: AB_2891341
H3K27ac	Abcam	Cat#ab4729; RRID: AB_2118291
H3K36ac	Abcam	Cat#ab177179; RRID: AB_2891342
H4K5ac	Epigentek	Cat#A-4027; RRID: AB_2891343
H4K5ac	Active Motif	Cat#39584; RRID: AB_2891344
H4K8ac	Abcam	Cat#ab45166; RRID: AB_732937
H4K12ac	Epigentek	Cat#A-4029; RRID: AB_2891345
H4K12ac	Active Motif	Cat#39166; RRID: AB_2891346
H4K16ac	Abcam	Cat#ab109463; RRID: AB_10858987
H4K16ac	Active Motif	Cat#39168; RRID: AB_2636968
H4K20ac	Abcam	Cat#ab177191; RRID: AB_2891348
Goat anti-Chicken IgY (H&L) DyLight®488 Conjugate	Immunoreagents	Cat#GtxCk-003-D488NHSX; RRID: AB_2891349
Donkey anti-Rabbit IgG (H&L) DyLight®633 Conjugate	Immunoreagents	Cat#DkxRb-003-E633NHSX; RRID: AB_2891350
Donkey anti-Mouse IgG (H&L) DyLight®633 Conjugate	Immunoreagents	Cat#DkxMu-003-G633NHSX; RRID: AB_2891351
Alexa Fluor 488 Donkey anti-mouse IgG H&L	Abcam	Cat#ab150105, RRID: AB_2732856
Goat anti-Chicken IgY (H&L) DyLight®488 Conjugate	Immunoreagents	Cat#GtxCk-003-D488NHSX; RRID: AB_2891349
Bacterial and virus strains		
Electrocompetent NovaBlue E.coli	This lab	n/a
NovaBlue E.coli	Millipore Sigma	(Cat#69825)
Chemicals, peptides, and recombinant proteins		
ARTKQTARKSTGGKAPRKQLA - GG - K(BIOTIN) - NH2	Anaspec	(Cat#AS-61702)
ATKAARKSAPATGGVKKPHRYRPG-GK(Biotin)	Anaspec	(Cat#AS - 64440-025)
ART-Kac-QTARKSTGGKAPRKQLA - GGYK(Biotin) - NH2 (1-21)	Active motif	(Cat#81039)
ARTKQTAR-Kac-STGGKAPRKQLA - GGYK(Biotin) - NH2 (1-21)	Active motif	(Cat#81044)
ARTKQTARKSTGG-K(Ac)-APRKQLA-GGK(Biotin)	Anaspec	(Cat#AS-64362-025)
ARTKQTARKSTGGKAPR - K(Ac) - QLA - GGK(Biotin) - NH2	Anaspec	(Cat#AS-64638-1)
AT-K(Ac)-AARKSAPATGGVKKPHRYRPGG-K(Biotin)	Anaspec	(Cat#AS-65241-1)

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
ATKAAR-KAc-SAPSTGGVKKPHRYRPG-GGK(Biotin)-NH2 (21-44)	Active motif	(Cat#81049)
ATKAARKSAPATGGV-Kac-KPHRYRPG-GGK(Biotin) - NH2 (21-44)	Active motif	(Cat#81054)
SGRGKGGKGLGKGGAKRHRKVLRGG-YK(Biotin)-NH2	Active motif	(Cat#81109)
SGRG - K(Ac) - GGKGLGKGGAKRHRKVLRDNGSGS - K(Biotin)	Anaspec	(Cat#AS-65229-1)
SGRGKGG - K(Ac) - GLGKGGAKRHRKVLRDNGSGS - K(Biotin)	Anaspec	(Cat#AS-65230-1)
SGRGKGGKGLG - K(Ac) - GGAKRHRKVLRDNGSGS - K(Biotin)	Anaspec	(Cat#AS-65208-1)
SGRGKGGKGLGKGGA - K(Ac) - RHRKVLRDNGSGS - K(Biotin)	Anaspec	(Cat#AS-65209-1)
SGRGKGGKGLGKGGAKRHR-Kac-VLRGG-YK(Biotin)-NH2	Active motif	(Cat#81110)
Experimental Models: Organisms/Strains		
EBY100 *Saccharomyces cerevisiae*	ATCC	MYA-4941
Oligonucleotides		
GCCTCCTCCACCGACGTC, GGTCGGCTAGCGCGC to amplify histone H3 inserts	Genewiz, custom oligos	n/a
GGTCGGCTAGCATGTCTGGTAGA, TCCTCCACCGACG TCTATGTTGTCA to amplify histone H4 inserts	Genewiz, custom oligos	n/a
AATTCGAATTCAACAAGGAATTCCTAG, GGTACCCTAT TATAATTCATCGTGTTCAAA to amplify p300 minimal HAT domain	Genewiz, custom oligos	n/a
CTCCGTAATCGCGAGCGTACTAGCCACCTGCAGGATT TTCAAACCAGAAGAACTACGAC, CTATTATAATTCATC GTGTTCAAACCATGGTGCGGCCGCGTCCTGGCTCTG CGTGTG to amplify p300 Core domain	Genewiz, custom oligos	n/a
Recombinant DNA		
pAW1	this manuscript	n/a
pAW1_minHAT	this manuscript	n/a
pAW1_Core	this manuscript	n/a
pAW1_R4_minHAT	this manuscript	n/a
pAW1_R4_Core	this manuscript	n/a
pAW1_R9_minHAT	this manuscript	n/a
pAW1_R9_Core	this manuscript	n/a
pAW1_R14_minHAT	this manuscript	n/a
pAW1_R14_Core	this manuscript	n/a
pAW1_R18_minHAT	this manuscript	n/a
pAW1_R18_Core	this manuscript	n/a
pAW1_R23_minHAT	this manuscript	n/a
pAW1_R23_Core	this manuscript	n/a
pAW1_R27_minHAT	this manuscript	n/a
pAW1_R27_Core	this manuscript	n/a
pAW1_R36_minHAT	this manuscript	n/a
pAW1_R36_Core	this manuscript	n/a
pAW2	this manuscript	n/a
pAW2_minHAT	this manuscript	n/a
pAW2_Core	this manuscript	n/a
pAW2_R5_minHAT	this manuscript	n/a
pAW2_R5_Core	this manuscript	n/a
pAW2_R8_minHAT	this manuscript	n/a
pAW2_R8_Core	this manuscript	n/a
pAW2_R12_minHAT	this manuscript	n/a
pAW2_R12_Core	this manuscript	n/a
pAW2_R16_minHAT	this manuscript	n/a

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
pAW2_R16_Core	this manuscript	n/a
pAW2_R20_minHAT	this manuscript	n/a
pAW2_R20_Core	this manuscript	n/a
pCTCon2 YESS	Rao Lab, NCSU	n/a
pRT2	Ryan Tam, Keung Lab, NCSU	n/a
Other		
Frozen-EZ Yeast Transformation II Kit	Zymo Research	(Cat# T2001)

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Albert Keung (ajkeung@ncsu.edu).

Materials availability

Plasmids generated in this study are available from the authors via contacting the lead contact.

Data and code availability

This study did not analyze datasets. This study did not generate code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Microbe strains

Electrocompetent NovaBlue *Escherichia coli* (haploid) were used for all cloning procedures to generate the plasmids used in this study. The *E.coli* were electroporated with the plasmid of interest and allowed to recover for 1 hour in a 1mL culture of LB media while shaking at 250rpm, 37°C before being plated onto LB + carbenicillin plates where they grew at 37°C for 16-24 hours. Colonies that appeared on these plates were transferred to 5mL LB + Ampicillin cultures and grown for 16 hours at 37°C, 250rpm. The plasmids were harvested from these 5mL cultures and sequenced to determine if they contained the correct sequences. NovaBlue cells were purchased from Millipore Sigma (Cat#69825) and prepared into electrocompetent stocks in the Rao Lab at NCSU.

Yeast strains

EBY100 (ATCC MYA-4941) *Saccharomyces cerevisiae* (MATa) were used to conduct all yeast-based experiments in this study. Frozen competent yeast were transformed with the plasmid of interest using the Zymo Research Frozen-EZ Yeast Transformation II Kit (Cat# T2001). Once transformed, the yeast were plated onto SDCAA -Tryptophan plates and grown for 3 days at 30°C. A colony was then picked from the plate and placed into a 5mL SDCAA -Tryptophan culture and grown for 2 days at 30°C, 250rpm. From this yeast stock, a fresh SDCAA -Tryptophan culture was seeded before each experiment. For an experiment, an SDCAA -Tryptophan culture of yeast was grown for 24 hours before passage into SGCAA -Tryptophan induction media where it was grown at 20°C, 250rpm for 16-24 hours. SGCAA -Tryptophan cultures were all seeded at an OD600nm of 1.

METHOD DETAILS

Creation of histone H3 and histone H4 plasmid families

Three gene fragments containing the N-terminal tail of histone H3 (aa1-45), the N-terminal tail of histone H4 (aa1-26), and the p300 minimal histone acetyltransferase (minHAT) domain along with the appropriate restriction sites were purchased from Genewiz. The histone gene fragments were cloned to an existing pCTCon2 YESS plasmid using XhoI and EcoRI restriction enzymes, allowing them to be transcribed by the GAL1 promoter. The histone tails were individually placed downstream of an endoplasmic reticulum sequence (MQLLRCFSIFSVIASVLA) and upstream of an endoplasmic reticulum retention sequence (FEHDEL). The resulting plasmids containing either the histone H3 tail or histone H4 tail without any acetyltransferase domain were named pAW1 and pAW2, respectively (Table S3). In both cases, the histone tail has an N-terminal HA tag and a C-terminal Myc tag to allow for quantification of expression levels.

The p300minHAT domain was cloned into the pCTCon2 YESS plasmid using NcoI and AvrII restriction enzymes, allowing it to be transcribed by the GAL10 promoter. The p300Core domain was amplified via PCR from pRT2, a plasmid previously used in the lab. The p300Core domain was inserted into the pCTCon2 YESS plasmid using CPEC cloning. The resulting plasmids were transformed

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into electrocompetent NovaBlue *E.coli* and grown on LB plates supplements with carbenicillin. Colonies were selected from these plates, grown in LB ampicillin liquid cultures, purified, and sent to Genewiz for Sanger sequencing. The Sanger sequencing confirmed the successful insertion of the histone tails and p300 domains into the pCTCon2 YESS backbone. The arginine mutations were introduced by purchasing gene fragments from Genewiz and inserting these new genes into the existing pAW1 and pAW2 plasmids using the NheI and AatII restriction sites inserted directly upstream and downstream of the histone substrates.

Plasmid transformation into EBY100 yeast

EBY100 *Saccharomyces cerevisiae* were transformed with the histone H3, histone H4, and pYD1-mSA(Lim et al., 2013) (Addgene plasmid #39865) plasmids using the Zymo Research Frozen-EZ Yeast Transformation II Kit (Catalog# T2001). Once transformed, the yeast were plated onto SDCAA -Tryptophan plates and grown for 3 days at 30°C. A colony was then picked from the plate and placed into a 5mL SDCAA -Tryptophan culture and grown for 2 days at 30°C, 250rpm to generate a saturated stock of yeast containing a target plasmid.

Yeast cell culturing

Yeast were grown in SDCAA -Tryptophan media containing dextrose, difco yeast nitrogen base, bacto casamino acids, Na_2HPO_4, and $NaH_2PO4 \cdot H_2O$ in ddH_2O. Yeast were grown in this media for 16-24 hours at 30°C shaking at 250 rpm. After this growth period, the yeast were induced by a passage into SGCAA -Tryptophan at an OD of 1 and kept in this induction media for 16-24 hours at 20°C shaking at 250 rpm before analysis. SGCAA -Tryptophan media contains galactose in a 10:1 ratio to dextrose. The galactose induces the expression of the target proteins under control of the GAL 1/10 bidirectional promoter. A fresh SDCAA – Tryptophan culture was seeded from a saturated yeast stock before each experiment.

Labeling mSA yeast with biotinylated peptides

EBY100 yeast containing the pYD1-mSA plasmid were cultured in SDCAA -Tryptophan media for two passages before induction in SGCAA – Tryptophan media supplemented with 1uM D-biotin for 16-24 hours. After induction, $2*10^6$ mSA yeast cells were labeled with 1uM of biotinylated peptide in 100uL 0.1% BSA PBS for 30 minutes, shaking at 500 rpm at 4C.

Flow cytometry analysis of induced yeast cultures

Freshly induced yeast cultures were processed for flow cytometry by aliquoting $2*10^6$ cells into individual wells of a 96-well plate for antibody labeling. Each acetylation site was labeled in separate samples to ensure that the antibodies did not occlude each other. 50uL of a primary antibody dilution (Table S2) was added to each well and allowed to incubate at 4C, shaking at 500-800 rpm for at least 20 minutes. The samples were then washed of unbound primary antibody by resuspension in 200uL of 0.1% BSA 1X PBS, centrifugation at 3000 G for 2 min, and aspiration. The samples were then labeled with a secondary antibody corresponding to the host animal of the primary antibody. The secondary antibodies were added in 50uL aliquots of 1:250 dilutions of their stock concentrations. The secondary antibodies were allowed to incubate with the samples for at least 15 minutes in the dark, at 4C. The secondary antibodies were washed in the same manner as the primary antibodies. The final sample pellets were resuspended in 200uL of 0.1% BSA 1X PBS when the plate was loaded into the flow cytometer (MACSQuant® VYB). Flow cytometry data was analyzed with FlowJo software. All mapping and binary crosstalk experiments were performed in triplicate with three biological replicates showing the same trend. All fluorescent gates were created based on an unlabeled sample of the same yeast strain (with the same bound peptide when applicable), measured on the same day, as the experimental samples. The percentage of cells above each gate is recorded in top corner of each plot. All plots contain 80,000 cells.

QUANTIFICATION AND STATISTICAL ANALYSIS

The mean fluorescent values from each sample of 80,000 cells was collected using FlowJo v10.6.1 software. No other statistical analyses were performed.

ADDITIONAL RESOURCES

There are no additional resources.