Cross-talk between histone H3 tails produces cooperative nucleosome acetylation

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Acetylation of histone proteins by the yeast Spt-Ada-Gcn5-acetyltransferase (SAGA) complex has served as a paradigm for understanding how posttranslational modifications of chromatin regulate eukaryotic gene expression. Nonetheless, it has been unclear to what extent the structural complexity of the chromatin substrate modulates SAGA activity. By using chromatin model systems, we have found that SAGA-mediated histone acetylation is highly cooperative (cooperativity constant of 1.97 ± 0.15), employing the binding of multiple noncontiguous nucleosomes to facilitate maximal acetylation activity. Studies with various chromatin substrates, including those containing novel asymmetric histone octamers, indicate that this cooperativity occurs only when both H3 histone tails within a nucleosome are properly oriented and unacetylated. We propose that modulation of maximal SAGA activity through this dual-tail recognition could facilitate coregulation of spatially proximal genes by promoting cooperative nucleosome acetylation between genes.

The Spt-Ada-Gcn5-acetyltransferase (SAGA) complex in the budding yeast Saccharomyces cerevisiae, and highly homologous complexes in higher eukaryotes, plays a major role in regulating gene expression (1, 2). In yeast, this regulation occurs predominantly by enhancing transcription of inducible genes (3) and occurs in part through the SAGA-mediated acetylation of chromatin at the promoter and transcribed regions of genes (4, 5).

The chromatin targets of SAGA-mediated acetylation are the amino-terminal portions of histone proteins, also called histone tails, which extend past the DNA of nucleosomes. In vitro studies have shown that the histone H3 tail is the primary target of SAGA-mediated acetylation, although the histone H2B tail can also be weakly acetylated (6). Within the H3 tail, the side chain of lysine-14 is the major site of acetylation, with H3 lysine-9 and lysine-18 being secondary sites, and H3 lysine-23 being a tertiary site (7). Interestingly, the histone H3 tail not only interacts with the catalytic domain of the SAGA complex subunit Gcn5 (8) but can also bind to a number of other domains contained within the 20-protein subunits that comprise the complex. Some of the currently identified domains include the SANT domain of Ada2 (9) and the bromo domains of both Gcn5 and Spt7, which recognize acetylated H3 tails (10).

The ability of the H3 tail to serve as both substrate and binding partner of the SAGA complex potentially allows for complex regulation of the action of SAGA by chromatin. This regulation could occur at many levels of chromatin structure. In the most basic structural unit of chromatin, the nucleosome, all 4 histones, H2A, H2B, H3, and H4, are present in 2 copies, and these histones are associated with 147 bp of nucleosomal DNA wrapped 1.67 times around the histone octamer (11). Thus, the core nucleosome component and the presence of 2 copies of the H3 tails have the potential to influence SAGA activity. In addition, H3 tails are presented at relatively regular intervals because nucleosomes in the genome occur frequently and in relatively close proximity. For example, in yeast ≈69% of chromosome III is sequenced by nucleosomes, with an average distance between nucleosome centers of 212 bp (12). Although these nucleosomes are arrayed in a linear fashion in genomic sequence, in many cases they adopt more complex higher-order structures. Short-range interactions between nucleosomes within a strand of chromatin allow chromatin to adopt a more compact 30-nm fiber structure (13, 14), and long-range interactions between distant nucleosomes provide a means for chromatin to potentially adopt 100- to 400-nm fiber structures (15). Moreover, a number of recent studies suggest that different functional forms of chromatin can adopt complex looping structures, where regions of the genome that are separated by large distances in sequence can be brought together in space (16). These complex forms of higher-order chromatin structure further complicate the presentation of the H3 tails to the SAGA complex.

We hypothesized that the rich structural complexity of chromatin would be exploited by the SAGA complex to both regulate and adjust its acetyltransferase activity. To test this possibility, we measured rates of nucleosome acetylation by the SAGA complex and found 2 aspects of chromatin structure that direct SAGA acetylation activity. Within a nucleosome the enzyme complex interacts with both copies of the histone H3 tail, and this interaction stimulates SAGA to bind to different, noncontiguous nucleosomes, generating cooperative acetylation.

Results

SAGA-Mediated Nucleosome Acetylation Is Cooperative. To characterize the kinetics of chromatin acetylation by the SAGA complex, the enzyme complex was TAP-tagged on the carboxyl terminus of the Spt7 subunit and affinity-purified to a high degree of homogeneity, as described by Wu et al. (17). Nucleosomal arrays were assembled as a model of chromatin fibers by using a salt dialysis deposition strategy (18). In these nucleosomal arrays, 12 recombinant, wild-type, Xenopus laevis histone octamers are spaced approximately every 208 nt apart on a Lytechinus variegates 208-12 5S rDNA template. Initial rates of nucleosome acetylation were determined by measuring the time-dependent incorporation of the radiolabeled acetyl group transferred from acetyl-CoA to the histones (19). Initial velocities were determined over a large range of nucleosomal array concentrations and showed good linearity for early time points. In these time courses, the extent of acetyl incorporation per H3 histone was from 0.47% to 4.4%.

Originally, we sought to fit the extensive initial-velocity data with respect to nucleosome concentration according to the standard Michaelis–Menten model (Fig. 1A). However, the data were not well suited for such a model of hyperbolic saturation because few
data points were within error of the best theoretical fit, and systematic deviation above and below the fit was observed. Instead, a sigmoidal, cooperative saturation kinetics model provided a fit that closely tracked the data (Fig. 1A). Fitting these data to linear formulations of the noncooperative and cooperative models reinforces the superiority of this approach. By using the Lineweaver-Burk form of the Michaelis-Menten model, it is clear the data shows systematic deviation from linearity (Fig. 1B). However, a Hill plot of the data is highly linear (Fig. 1C). From both direct fitting of the cooperative saturation kinetics model and the Hill plot, it is possible to extract a cooperativity constant, a parameter that measures the extent of cooperativity of the system. Both methods of fitting are in good agreement, 1.97 ± 0.15 and 1.91 ± 0.08, respectively. This magnitude of cooperativity is at least comparable with most known cooperative enzymes and suggests that maximal histone acetylation activity of the SAGA complex is facilitated through the binding of multiple substrates.

Cooperativity Results from Interactions Within the Nucleosome. What is it about the nucleosomal substrate that creates such cooperativity of acetylation? Overall, the acetylation reaction consists of 2 substrates, nucleosomes and acetyl-CoA. Binding of nucleosome substrates to the SAGA complex could change subsequent kinetic activity by either changing enzyme activity toward additional nucleosome substrates or toward acetyl-CoA. The simplest model of the latter would be that binding of the nucleosome substrate could organize the acetyl-CoA binding site, increasing its affinity for the cofactor at higher nucleosome concentration. Previous kinetics studies with the catalytic core of Gcn5 and H3 peptide substrates argue against this possibility because substrate binding is ordered with acetyl-CoA binding preceding H3 tail binding (20). However, the more complex nature of both the nucleosome substrate and the SAGA enzyme complex in our reactions could change the overall reaction mechanism. To explore this possibility, initial-velocity kinetics assays were performed to determine whether the binding of acetyl-CoA was changed at different concentrations of nucleosomal array substrates (Fig. 2A). At both a low (15 nM) and high (150 nM) concentration of nucleosomal substrate, the $K_m$ for acetyl-CoA is nearly identical, being 4.94 μM and 4.16 μM, respectively. This result suggests that cooperativity of nucleosome acetylation does not come from changes in binding of acetyl-CoA, but instead from changes in activity toward nucleosomes.

What in the nucleosomal array is required to create cooperativity of acetylation? Nucleosomal arrays are very complicated substrates because they present many potential binding interactions, including 2 copies each of the histone tails, the histone globular domain, and the nucleosomal DNA. Moreover, the oligomeric nature of nucleosomal arrays potentially allows binding interactions to occur between nucleosomes in both a SAGA-dependent and -independent manner. To simplify the system, initial rate kinetic experiments were performed on mononucleosomes that constitute a single repeat unit of the studied nucleosomal arrays (Fig. 2B). Analysis of initial velocities reveals that acetylation of the mononucleosomes is also highly cooperative (cooperativity constant of 1.76 ± 0.17), similar to the complete nucleosomal arrays (Table 1). Thus, cooperative acetylation does not require contiguous strands of nucleosomes. Similarly, formation of higher-order chromatin structure, which cannot be adopted by mononucleosomes, is not necessary for substrate cooperativity.

Because mononucleosomes are sufficient to induce cooperativity of SAGA-mediated acetylation, we investigated whether an even simpler substrate could function equivalently. Initial-velocity kinetics assays were performed on a histone H3 peptide (Fig. 2 C and D). Unlike the mononucleosome substrate, the H3 tail peptide was noncooperative (cooperativity constant of 0.97), fitting a saturation curve resembling the hyperbolic Michaelis-Menten equation. This finding suggests that cooperative acetylation requires more than the H3 tail in isolation. Because the nucleosome presents a pair of H3 tails in the context of a nucleosome, we wondered whether a nucleosome with a single H3 tail would suffice to restore the cooperative behavior.

Asymmetric Histone Octamer Generation. To generate nucleosomes with a single H3 tail, we developed a general strategy for making asymmetric octamers (Fig. 3A). In this strategy, histone octamers are assembled from constituent histones similar to standard protocols (21). However, for the histone that is ultimately to be present in 2 different forms, in our case a full-length H3 histone and an amino-terminal truncated H3 histone (Fig. 2C), they are introduced as a mixture, with an untagged form present in vast excess of a tagged form. For our single H3 tail octamer preparation, statistically 3 forms of octamers are expected: a majority of octamers with 2 truncated H3 tails and no tags; a small population of octamers with a single full-length, tagged H3 histone and a single truncated H3; and a very small population of octamers with 2 full-length, tagged histones. Octamers containing no tag can then be removed by using affinity purification, to give a final mix of octamers where the majority consists of asymmetric octamers with a single, tagged H3 tail. In this final mixture, some symmetric octamer with 2 full-length tails will be present. However, varying the ratio of tagged and untagged histone initially can control the amount of this octamer. For example, by using a 9:1 ratio of untagged to tagged histone, a little more than 1 in 20 octamers that have been affinity-purified contain 2 tails, a level we deemed acceptable for our experiments. For the affinity purification, we opted to use a His$_6$ tag/nickel resin strategy because others had shown that octamers could be isolated under these conditions (22).

Employing this asymmetric octamer strategy resulted in the desired single H3 tail octamers (Fig. 3B). Before nickel affinity purification, assembly of histone octamers using a 9:1 ratio of
tailless H3 to His6-tagged full-length H3 resulted in a population with very little tagged histone. However, after nickel affinity purification, the level of tagged, full-length H3 was equal to that of the truncated histone, as would be expected for octamers containing a single full-length H3 tail. These asymmetric single H3 tail octamers formed nucleosomal arrays, saturating the 208-12 5S rDNA template in a manner similar to recombinant wild-type octamers (Fig. 3C).

**Substrate Cooperativity Requires Dual H3 Tail Interactions.** Initial-velocity kinetic analysis of single H3 tail nucleosomal arrays was performed (Fig. 4A), and this substrate was found to be noncooperative in SAGA-mediated acetylation (cooperativity constant of 0.97). This loss of cooperativity was caused by the truncation of the H3 tail and not by the presence of the His6 tag because arrays containing 2 full-length H3 histones with the His6 tag exhibit full cooperativity (Fig. 4B). These results indicate that both H3 tails within a nucleosome must be present to generate cooperative acetylation and that the presence of multiple full-length H3 tails in single copy across different nucleosomes is insufficient to generate cooperativity.

The simplest explanation for why both histone H3 tails are necessary for cooperative acetylation is that the SAGA complex must bind both tails during the course of the reaction. Presumably, this tail binding is made possible by having binding domains in the enzyme complex located in a specific spatial orientation. To test this idea explicitly, we investigated the effect of moving the histone H3 tails to a new position.

In the nucleosomes each pair of histone tails extends past the nucleosomal DNA at different locations (23). The H3 tails exit the nucleosome near the DNA entry/exit points and between gyres of the DNA (Fig. 4C). To a large extent, this puts the exit point of the H3 tails in the same plane defined by the trajectory of the nucleosomal DNA. In contrast, the histone H4 tails exit the nucleosome on opposite faces of the plane defined by the nucleosomal DNA. Thus, moving the H3 tail to the location of the H4 tail would present a different binding surface to the SAGA complex and potentially disrupt the cooperativity of acetylation. To prepare such a tail-swap nucleosome, recombinant histone containing the tetra-Ala H3 tail peptide (Fig. 2), was expressed and purified (plasmid no. 47). This histone H3 peptide and proteins used in this work. Initial-velocity kinetics were measured for the tail-swap nucleosome, recombinant histone containing the H3 tail peptide, in which [acetyl-CoA]0 is 10.0 μM, and reflects an initial concentration of acetyl-CoA of 4.0 μM. For the H3 tail peptide, in which [acetyl-CoA]0 is 10.0 μM, the apparent turnover rate constant has been normalized to be directly comparable with the other assay.
Acetylation has been lost (cooperativity constant 0.95) on mononucleosomes containing the tetra-Ala H3/WT H3 octamers (Fig. 5). H3 were produced in a manner similar to the single-tail octamers containing equal amounts of wild-type H3 and tetra-Ala H3 tail, which demonstrates enhanced octamer mobility, whereas full-length H3 histones and H3 histones with smaller tail truncations do not (24). To address both of these issues, we desired nucleosomes that acetylation of the target sites of the SAGA complex in 1 tail of the H3 histone, and histone H4 with the H3gH4 fusion histone. Data were fit to a cooperative saturation model to give a cooperativity constant of 1.86 ± 0.07. (C) Initial velocity of nucleosomal arrays per enzyme containing 2 copies of the amino-terminal His-tagged H3 histones as a function of nucleosome concentration. Data were fit to a cooperative saturation model to give a cooperativity constant of 1.86 ± 0.07. (D) Relative orientation of the H3 and H4 histone tails based on mononucleosome structure 1AOI.11 DNA is depicted in white, H3 and H3’ histones in blue, and H4 and H4’ histones in green. The points at which the H3 and H4 tails exit the nucleosome are labeled in yellow. The histone tails, histones H2A and histone H2B, are omitted for clarity. (Lower) The point at which the H4 tail exits the nucleosome occurs behind the DNA and is not labeled. (D) Initial velocity per enzyme of histone H3 tail-swap mononucleosome as a function of nucleosome concentration. These mononucleosomes replace the H3 histones with tailless H3 histone, and histone H4 with the H3gH4 fusion histone. Data were fit to a cooperative saturation model to give a cooperativity constant of 1.02 ± 0.07.

Fig. 4. Truncation of a single-histone H3 tail or repositioning of both H3 tails results in loss of cooperativity of substrate acetylation. (A) Initial velocity of nucleosomal arrays per enzyme containing octamers with a single H3 tail as a function of nucleosome concentration. Three independent trials with differing nucleosome concentration were performed. Shown is a representative trial. Each trial was fit to a cooperative saturation model to give an average cooperativity constant of 0.86 ± 0.13. (B) Initial velocity of nucleosomal arrays per enzyme containing 2 copies of the amino-terminal His-tagged H3 histones as a function of nucleosome concentration. Data were fit to a cooperative saturation model to give a cooperativity constant of 1.86 ± 0.07. (C) Relative orientation of the H3 and H4 histone tails based on mononucleosome structure 1AOI.11 DNA is depicted in white, H3 and H3’ histones in blue, and H4 and H4’ histones in green. The points at which the H3 and H4 tails exit the nucleosome are labeled in yellow. The histone tails, histones H2A and histone H2B, are omitted for clarity. (Lower) The point at which the H4 tail exits the nucleosome occurs behind the DNA and is not labeled. (D) Initial velocity per enzyme of histone H3 tail-swap mononucleosome as a function of nucleosome concentration. These mononucleosomes replace the H3 histones with tailless H3 histone, and histone H4 with the H3gH4 fusion histone. Data were fit to a cooperative saturation model to give a cooperativity constant of 1.02 ± 0.07.

Fig. 3. Generation of nucleosomes with asymmetric histone H3 composition. (A) Scheme for generation of histone octamers containing a single H3 tail. H3 histones are depicted as blue-gray wedges, whereas histones H2A, H2B, and H4 are a lighter orange. H3 histone with HHHHHH denotes full-length H3 histone with a His₆ amino-terminal tag. H3 histone with a short tail denotes amino-terminal truncated H3 histone. The first arrow indicates octamer assembly, whereas the second indicates affinity purification of the tagged octamers. (B) Histone composition of octamers prepared as described in A before and after affinity purification. Histones were resolved on an 18% SDS/polyacrylamide gel and stained with Coomassie blue. (C) Characterization of nucleosomal arrays prepared from single H3 tail octamers. 208-12 SS rDNA nucleosomal arrays prepared at varying molar ratios of single tail octamer to octamer positioning site were digested with EcoRI and then separated on a native 4% polyacrylamide gel and stained with ethidium bromide. Nuc and Naked indicate SS mononucleosomes and free DNA, respectively.

Discussion

In enzyme kinetics, substrate cooperativity can occur when the initial activity of an enzyme toward a substrate, be it binding or turnover, activates the system toward further reaction with substrate. This work shows that, in the case of SAGA-mediated histone acetylation, this activation requires that within a nucleosome both H3 tails exist in their proper orientation and that the target sites of the SAGA complex in 1 tail of a nucleosome complex disrupts cooperative acetylation and suggests that cooperativity is mediated by the recognition of free lysine side chains in the H3 histone tail.

Initially we considered cases where binding of a single unmodified or acetylated H3 tail facilitates acetylation of the second tail in the same nucleosomes. However, neither explicit rate equation solutions nor kinetic simulations based on our experimental results generated robust cooperativity (Figs. S2–S4). Instead, they con-
formed to Michaelis–Menten kinetics, where initial H3 tail binding could increase the overall apparent affinity for substrate. One reason that such a situation does not lead to cooperativity is because, although binding of the first H3 tail is directly related to the overall substrate concentration, increased binding of the second H3 tail is caused by an increase of effective concentration of the substrate and is not improved by increasing the actual substrate concentration. It is important to note that even though this model does not generate cooperative behavior, it could still be an important aspect of the overall kinetic mechanism.

An alternative kinetic model is that intramolecular binding of a nucleosome via both H3 tails facilitates intermolecular binding of a second nucleosome. In our experiments, this second nucleosome would be either another mononucleosome or a nucleosome located in a different nucleosomal array. Explicit rate equation solutions and kinetic simulations confirm that such models do readily generate substrate cooperativity consistent with our experimental results (Figs. S5 and S6). In our current working model (Fig. S5D), the binding of both unacetylated H3 tails within a nucleosome induces an allosteric change in the SAGA complex, facilitating intermolecular binding of another nucleosome and maximal acetylation. In this model, intramolecular binding of a second nucleosome within the same array could also occur. However, because this pathway is noncooperative it cannot occur exclusively, otherwise no positive cooperativity would be observed.

In the SAGA complex numerous protein domains are potential candidates for mediating binding to nucleosomes. Domains known to bind unmodified H3 tails include the active site of Gcn5 and the SANT domain of Ada2 (9). Furthermore, although binding of H3 tails is essential for substrate cooperativity, interactions with the whole-histone protein or other aspects of nucleosome structure, such as nucleosomal DNA binding by the SWIRM domain (26), could be required. Binding of multiple nucleosomes is consistent with either a monomeric or multimeric SAGA complex (Fig. S5A and B). Although isolated SAGA complex is monomeric (17), the complex might oligomerize in its biological context. Additionally, binding of the second nucleosome may only require interactions with a portion of it.

In vitro, nanomolar concentrations of nucleosomes are necessary to reveal kinetic cooperativity, whereas in vivo, average nucleosome concentrations are substantially higher. Within the nucleus, such modulation of presaturation binding of SAGA to nucleosomes may still be significant because nucleosome affinity could be significantly reduced by factors such as chromatin compaction and chromatin-associated proteins and because the effective concentration of nucleosomes could be reduced if cellular nucleosome are not freely mobile. More importantly, our kinetic analysis reveals a mechanistic aspect of SAGA activity that, although only observable at nanomolar concentrations, would occur over all concentrations. Specifically, it shows that the SAGA complex binds, and potentially acetylates, multiple noncontiguous nucleosomes. In our experiments, these binding interactions occurred intermolecularly. In the cell nuclei, this could occur either between different chromosomes or between nucleosomes that are separated by large distances in sequence, but that are organized to be close in 3-dimensional space. Such spatial colocalization of noncontiguous genes has been proposed as an important mechanism of coregulation of gene expression. A number of potential examples exist, including “transcriptional factories” of multiple copies of RNA polymerase II and associated transcriptional machinery (16) and localization of coregulated genes to nuclear pore complexes (27).

One of the best-characterized examples of spatial coregulation comes from chromatin that interacts with proteins associated with nuclear architecture. Kohwi-Shigematsu and coworkers (28) have shown that during thymocyte differentiation, the DNA-binding protein SATB1 is both necessary for organizing multiple cytokine genes into loop structures, where these gene promoters become colocalized to the hub of these loops, and is required for efficient coexpression of these genes (28). Further, SATB1 is essential for robust acetylation of coregulated promoters because loss of SATB1 reduces histone H3 K9 and K14 acetylation, presumably due in part to loss of chromosomal loop structure (29). These observations are consistent with the idea that SAGA cooperativity could promote colocalization of coregulated genes. Recruiting a coactivator complex like SAGA to the promoter of 1 of the coregulated genes would not only increase the effective enzyme concentration to promote acetylation of nucleosomes in the other spatially proximal coregulated promoters, but the dual H3–tail interaction at nucleosomes in the original promoter could effectively couple its acetylation to its functional interaction with other coregulated promoters. This high degree of cooperativity would not only help to couple the activation of coregulated genes, but might also help to protect these promoters from global, and potentially noncooperative, histone deacetylation.

The cooperativity of SAGA-mediated nucleosome acetylation demonstrates the functional utilization of the same pair of histone tails. It seems likely that other enzyme complexes that interact with nucleosomes might exploit such homotypic cross-tail interactions within the nucleosome. For example, the catalytic core of the SAGA complex, Gcn5, Ada2, and Ada3, is shared with a number of other histone acetyltransferase complexes in yeast as well as in higher eukaryotes (2). Additionally, many other chromatin-remodeling complexes contain multiple potential histone tail interaction domains, such as the SANT (30), chromo (31), bromo (32), and PHD finger domains (33). Further studies using tools such as asymmetric nucleosomes should help to address to what extent other systems use cross-talk between identical pairs of histone tails and how such cross-talk ultimately influences biological function.
Materials and Methods

DNA Template and Full-Recombinant Histone Preparation. These reagents were prepared by using standard techniques. Details are provided in the SI Text.

Tetra-Ala and Tetra-Ac H3 Preparation. Side-chain-protected, resin-bound peptides, HHHHHHARKQRTARSGAAPRQQATAA and HHHHHHARKQT-ARK(Ac)STGG K(Ac)APR K(Ac)QLAT K(Ac), were synthesized by Fmoc-based solid-phase peptide synthesis on Ctri-TTL support (Baylor Protein Chemistry Core Laboratory). Generation of fully deprotected thioester peptide was performed as described with modifications to the thioesterification step (25). Specifically, the peptide carboxyl terminus (5 mM) was activated with A/N-dicyclohexylcarbodi-imide (100 mM) and then reacted with benzyl mercaptan (100 mM) at 25 °C for 3 h, all in DMSO.

Pep tide ligation was performed as described in ref. 25. To remove unligated core histone, the crude reaction mixture was dissolved into 70% acetonitrile, 3 h, all in DMSO.

imidate (100 mM) and then reacted with benzyl mercaptan (100 mM) at 25 °C for 3 h, all in DMSO.

Histone Octamer Preparation. Octamers were prepared largely according to standard protocols (21). For asymmetric octamers, 0.02 μmol of His6-tagged H3 was combined with 0.18 μmol of untagged H3, 0.20 μmol of H4, and 0.22 μmol of H2A and H2B. Gel filtration-purified octamers were incubated with Ni-NTA resin (QIAGEN) for 40 min at room temperature. The resin was then washed twice with buffer C (buffer B at pH 6.3) and eluted with buffer D (buffer B at pH 4.5). The eluted fractions were then neutralized by adding 1 M Tris (pH 8.0) and ligated histone H3 was quantified by comparison with a known quantity of Xenopus recombinant His6-tagged H3 histone on an 18% SDS/polyacrylamide gel, stained by Coomassie blue. The identity of the ligated histones was confirmed by MALDI-TOF mass spectrometry.

Nucleosomal Arrays and Mononucleosomes Reconstitutions. Mononucleosomes and nucleosomal arrays were assembled with DNA template and purified histone octamers according to standard protocol with little modifications (18, 21). The level of saturation of the purified arrays was verified by EcorI digestion, followed by a 4% native polyacrylamide gel analysis with ethidium bromide staining. Mononucleosomes were analyzed by running and staining the 4% native polyacrylamide gel analysis with ethidium bromide staining.

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4. Robert F, et al (2006) Generation of fully deprotected thioester peptide was performed as described with modifications to the thioesterification step (25). Specifically, the peptide carboxyl terminus (5 mM) was activated with A/N-dicyclohexylcarbodi-imide (100 mM) and then reacted with benzyl mercaptan (100 mM) at 25 °C for 3 h, all in DMSO.
Supporting Information

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SI Text

DNA Template Preparation. Plasmid containing 208-12 5S rDNA sequence was digested with restriction enzyme HhaI and purified by gel filtration chromatography (1). The 196-1 5S rDNA was generated by digesting the 208-12 DNA plasmid with EcoRI for 24 h at 37 °C and purified by gel filtration chromatography.

Histone Purification. Expression and purification of recombinant *Xenopus* histones and their derivatives were performed according to standard methods (2). The plasmid encoding tail-swap histone H4 (T3G4) was a kind gift from Jeffrey C. Hansen, Colorado State University, Fort Collins, CO. His<sub>6</sub>-tagged H3 was amplified by PCR from the *Xenopus* histone H3 expression plasmid using the primers 5'-CC ACA CAT ATG CAT CAT CAT CAT CAT CAT CAT CGT GCC CGT ACC ACC ACC ACC GCC GCC-3' and 5'-GCA GCC AGA TCT CCG CCC TCT CTC AGC G-3'. The product was digested with NdeI and BglII and cloned into a pET3 expression vector digested with NdeI and BamHI.

Potential Acetylation Mechanisms. *Michaelis–Menten mechanism*. Under the standard Michaelis–Menten mechanism (Fig. S2A), the SAGA enzyme complex, *E*, binds a single mononucleosome substrate, SS (each S represent a separate H3 histone tail), and then generates and releases an acetylated product, PS, where a single H3 tail is acetylated. Under steady-state conditions, the initial rate of this product formation is:

\[
\frac{d[PS]}{dt} = \frac{k_3[E][SS]}{k_2 + k_3} - \frac{k_3[E][SS]}{k_2 + k_3} = \frac{k_{cat}[E][SS]}{K_m + [SS]}_o
\]  

(Note, acetyl-CoA is not explicitly included in the reaction mechanisms discussed. This is acceptable because our experimental results show that nucleosome binding does not change acetyl-CoA binding and because acetyl-CoA is present in vast excess of nucleosome and SAGA. Thus, the concentrations of acetyl-CoA and its bound intermediates effectively do not change during our assays and are implicitly included in the kinetic constants and reaction intermediates.)

In our experiments, such a mechanism is not observed because the initial rate of nucleosome acetylation as a function of substrate concentration shows a high degree of positive cooperativity (Fig. 1A). Positive cooperativity arises when binding of one substrate facilitates its activity toward another. The mononucleosome is the substrate but contains two H3 tails capable of acetylation. To determine whether the interaction of SAGA with the two tails of a single nucleosome can generate cooperativity, a number of mechanisms can be considered.

*Single-nucleosome mechanisms with multiple intermediates*. The simplest model of facilitated binding is one in which binding of one unacetylated H3 tail promotes the binding of the other unacetylated tail, and this bound intermediate can undergo acetylation (Fig. S2B). Because the intermediates only involve binding changes, it is reasonable to assume that they are formed rapidly and that under initial rate conditions they are at steady state. This assumption allows us to calculate their concentration as a function of free substrate and enzyme:

For the steady-state concentration of SAGA bound to both H3 tails, \([E^*SS]\):

\[
\frac{d[E^*SS]}{dt} = k_3[E][SS] - k_4[E^*SS] = 0
\]

\[
[E^*SS] = \left(\frac{k_3}{k_4 + k_5}\right) [E][SS]
\]  

To further express \([E^*SS]\) as the reaction concentration of free nucleosome, \([SS]\), and SAGA, \([E]\), we need to determine the reaction concentration of SAGA bound to a single H3 tail, \([ESS]\), which can be expressed in terms of \([E]\) and \([SS]\):

\[
\frac{d[ESS]}{dt} = k_1[E][SS] - k_2[ESS] - k_3[ESS] + k_4[E^*SS] = 0
\]

\[
[ESS] = \left(\frac{k_3}{k_2 + k_3 - \left(\frac{k_3 k_4}{k_4 + k_5}\right)}\right) [E][SS]
\]  

We can now go back to calculate the steady-state concentration of \([E^*SS]\):

\[
[E^*SS] = \left(\frac{k_3}{k_4 + k_5}\right) [ESS] = \left(\frac{k_3}{k_4 + k_5}\right) \left(\frac{k_1}{k_2 + k_3 - \left(\frac{k_3 k_4}{k_4 + k_5}\right)}\right) [E][SS]
\]  

Thus, the rate of product formation as a function of free substrate enzyme is:
In and of itself, this expression is not practically useful because the concentrations of free substrate and enzyme are not readily measured during the assay. However, these terms can be reexpressed in terms of a known quantities, \([SS]_o\) and \([E]_o\), initial substrate and enzyme concentration.

For \([SS]_o\), because in the experiment \([SS]_o \gg [E]_o\), and assays are performed during the initial course of the reaction where little total substrate is consumed:

\[
[SS] = [SS]_o \tag{S6}
\]

For \([E]_o\), during the reaction all species involving the enzyme must add up to the total initial concentration of enzyme, which allows the free enzyme concentration to be expressed as a function of the initial enzyme concentration:

\[
[E]_o = [E] + [ESS] + [E*SS]
\]

\[
[E] = \frac{[E]_o}{1 + k'[SS]_o} = \frac{[E]_o}{1 + k'[SS]_o} \tag{S7}
\]

Thus, the rate of product formation in terms of \([SS]_o\) and \([E]_o\) is:

\[
\frac{d[PS]}{dt} = k_5 \left( \frac{k_3}{k_4 + k_5} \right) \left( \frac{k_1}{k_2 + k_3 - \left( \frac{k_3k_4}{k_4 + k_5} \right)} \right) [E][SS]_o
\]

\[
= \frac{k_5 \left( \frac{k_3}{k_4 + k_5} \right) \left( \frac{k_1}{k_2 + k_3 - \left( \frac{k_3k_4}{k_4 + k_5} \right)} \right) [E]_o[SS]_o}{1 + k'[SS]_o} \tag{S8}
\]

\[
= \frac{k_5 \left( \frac{k_3}{k_4 + k_5} \right) \left( \frac{k_1}{k_2 + k_3 - \left( \frac{k_3k_4}{k_4 + k_5} \right)} \right) \left( \frac{1}{k' [E]_o[SS]_o} \right)}{1 + \left( \frac{k_1}{k' [E]_o[SS]_o} \right)
\]

\[
= \frac{k_5 \left( \frac{k_3}{k_4 + k_5} \right) \left( \frac{k_1}{k_2 + k_3 - \left( \frac{k_3k_4}{k_4 + k_5} \right)} \right) \left( \frac{1}{k' [E]_o[SS]_o} \right)}{1 + \left( \frac{k_1}{k' [E]_o[SS]_o} \right)
\]

Although this expression is complicated, it can be readily simplified by defining new kinetic constants, \(k_{cat}\) and \(K_m\), as composites of individual rate terms:

\[
= \frac{k_{cat}[E]_o[SS]_o}{K_m + [SS]_o} \tag{S9}
\]
This expression is simply the Michaelis–Menten equation. With the addition of all of the kinetics terms, the expression maintains the fact that the initial binding step facilitates the subsequent binding and acetylation steps, making the overall $K_m$ of the entire reaction different from the $K_m$ of the simpler mechanism. Nonetheless, this change in binding mechanism does not change the equation to become cooperative with respect to initial substrate concentration. One way to think about this is that in the first mechanistic step, the concentration of bound intermediate, $E_{SS}$, depends directly on the concentration of initial substrate. However, once formed, the conversion of any given molecule $E_{SS}$ to $E^{*}SS$ does not increase with an increase of SS. Thus, to summarize, this mechanism can change both the half-saturation concentration of the reaction ($K_m$) and maximum reaction rate (via $k_{cat}$), but it does not change the hyperbolic, noncooperative shape of the saturation curve.

A related mechanism is one in which either of the bound nucleosome intermediates, $ESS$ and $E^{*}SS$, can generate nucleosome product acetylated on a single tail (Fig. S2C). Like the previous mechanism, this mechanism is expected to be noncooperative for identical reasons. Specifically, once $ESS$ is formed, the amount of $E^{*}SS$ formed does not increase with increasing $SS$. Instead, it is only subject to the rate of conversion between $ESS$ and $E^{*}SS$ and the rate of turnover of these intermediates to product. Thus, if only product is detected, these parallel pathways combine to form a new effective rate of product formation according to the Michaelis–Menten equation. Even if the product for each pathway is not the same (Fig. S2D), the assay only counts the total amount of histone acetylation. Thus, the pathway that generates $PP$ kinetically just appears to be twice as fast as an identical pathway that generates $PS$.

In the mechanisms considered to this point, SAGA does not specifically recognize acetylated histone, $P$. In principle, this recognition could occur two different ways, either directly after its production without release, or through rebinding after release. A simple nondissociative mechanism is considered first. In this mechanism, a single H3 tail is acetylated, followed by enzyme rearrangement without release and then acetylation of the other histone tail (Fig. S2E). If all intermediates are under steady-state conditions, the rate of $PP$ formation can be derived in a manner nearly identical to that for mechanism 2B:

\[
\frac{d[PP]}{dt} = \frac{k_1 k_3}{k_2 + k_3}
\]

Again, this complicated rate expression essentially has the form of the Michaelis–Menten equation:

\[
\frac{k_{cat}[E][SS]}{K_m + [SS]} = \frac{k_{cat}[E][SS]}{K_m + [SS]}_0
\]

that is, this nondissociative mechanism is noncooperative in $PP$ formation with respect to changes in initial $SS$ concentration. An additional complication is that the current assay used likely also monitors $PS$ formation, requiring its inclusion. However, under steady-state conditions, the concentration of $EPS$ (and $E^{*}PS$) does not change over time. To be at steady state, $EPS$ formation must be fast relative to $PP$ formation and will be reflected in an initial burst of $PS$ counts, followed by a constant concentration of both it and $EPS$.

In our assays, no burst of acetylation is observed, so it seems unlikely that production of the $EPS$ intermediate reaches a steady state. In this case, both PS and $PP$ species with be changing with time and must be accounted for. However, in analogy to the mechanism in Fig. S2B, once $EPS$ is formed, its conversion to $E^{*}PS$ and $PP$ does not increase with increasing $SS$ concentration because no other pathways are available for either the enzyme or intermediate. Even if multiple pathways to product are available (Fig. S2F), because they both occur nondissociatively, they are not subject to changes in $[SS]$, similar to the mechanism of Fig. S2C.

A mechanism in which PS dissociates and can be bound and reacted with is shown in Fig. S2G. In this mechanism, a key difference from previous mechanisms is that once PS is formed, its binding with enzyme is in competition with SS, and so the rate of subsequent steps will potentially be affected by changes in the initial concentration of SS. To address this more complicated model directly, kinetic time course simulations were performed by using COPASI 4.2. In these simulations, the kinetic mechanism was defined, and then kinetic and initial reactant concentrations were set based on experimental results. Simulations were then run at various substrate concentrations for the time period of the experimental assays. These time courses were fit for initial rate as though they were experimental data, and then the initial rates were fit to the cooperative saturation kinetics model (Eq. 13) as a function of initial substrate concentration to determine the cooperativity constant of the mechanism. The validity of this approach can be seen in that simulation of conversion of mononucleosomes with a single-acetylated tail to double-acetylated mononucleosomes using Michaelis–Menten kinetics (second half of the mechanism depicted in Fig. S2G) agrees closely with kinetic constants that were experimentally derived (Fig. S3).

Because this conversion of PS to $PP$ constitutes half of the mechanism in Fig. S2G, it became the basis to simulate the full mechanism. For the first half of the mechanism, the conversion of SS to $PP$, we started with our experimental data for the acetylation
of mononucleosomes containing tetra-Ala H3/WT H3 asymmetric octamers. With this simulation we find that the generation of total acetylation as a function of time is not cooperative, with a cooperativity constant of 0.98 (Fig. S4A). In this case, it appears that during the course of the assay, the amount of PS generated cannot compete with SS, and so the amount of EPS and PP that is generated is relatively small (Fig. S4B), making the reaction largely reflect the first half of the mechanism. Furthermore, this lack of cooperativity appears robust. Individually changing $k_1/k_2$, $k_3$, $k_4/k_5$, or $k_6$ either up or down by 4- and 16-fold does not generate significant cooperativity (at most a cooperativity constant of 1.14), even when PP starts to be generated in significant quantities during the simulated assay (Fig. S4C). Thus, it seems unlikely that this dissociative mechanism provides the observed cooperativity constants (up to 1.97).

### Multiple nucleosome mechanisms.

In contrast to the mechanisms considered for the interaction of SAGA with single mononucleosomes, mechanisms in which SAGA interacts with multiple mononucleosomes can readily provide a high degree of positive cooperativity. The simplest of such mechanisms is one in which two substrates are bound simultaneously to one enzyme, resulting in the generation of a single product (Fig. S5A). Assuming initial velocity and steady-state conditions, the initial rate of product formation can readily be calculated in the same manner as used for the mechanism in Fig. S2B:

$$
\frac{d[PS]}{dt} = \frac{k_4[E]_0[SS]_0^2}{(k_2 + k_3)(k_1)} + [SS]_0^2
$$

[SI2]

Note, if SAGA exists as a dimer and generates two products simultaneously (mechanism in Fig. S2C), the equation for the initial rate of [PS] formation as a function of $[E]_0$ (not $[E]_0^2$) is exactly the same. Thus, our kinetic measurements cannot distinguish between these possibilities. Because an isolated SAGA complex exists as a monomer, further mechanisms considered use a monomeric SAGA complex.

In the mechanisms that generate Eq. 12, two substrates are bound simultaneously. However, when this mechanism is generalized to $n$ substrates, the rate expression becomes:

$$
\frac{d[PS]}{dt} = \frac{k_4[E]_0[SS]_0^n}{(k_2 + k_3)(k_1)} + [SS]_0^n
$$

[SI3]

This equation is in fact the cooperative saturation kinetics equation that we have used throughout to calculate the cooperativity constant, $n$, where when a single nucleosome is bound, the equation become the Michaelis–Menten equation. Because our wild-type nucleosomal array kinetic results fit this equation well with a cooperativity constant of 1.97 (Fig. 1A and Fig. S6A), a possible mechanism to explain this kinetic behavior is that the enzyme binds two nucleosomal arrays simultaneously to generate the product.

This requirement for simultaneous binding of two substrates makes this mechanism possible, but unlikely. An alternative formulation is one in which binding is sequential, where binding of a first substrate promotes binding of the second (Fig. S5C). Using previous assumptions and techniques, it can be shown that the initial rate of PS formation is as follows:

$$
\frac{d[PS]}{dt} = \frac{k_5k_4 + k_2k_3}{k_1k_3} + \left(\frac{k_5}{k_3} + \frac{k_4 + k_5}{k_5}\right)[SS]_0 + [SS]_0^2
$$

[SI4]

To what extent this mechanism would be well fit to the cooperative saturation kinetics equation (Eq. 13) with high positive cooperativity depends on the relationship between the binding affinities for the first and second substrates ($k_1/k_2$ and $k_3/k_4$, respectively) and the turnover rate ($k_5$). As an example, for simulations of SAGA acetylation of wild-type mononucleosomes, we could assume that the first $K_4$ is simply the $K_m$ of SAGA for the tetra-Ala H3/WT H3 mononucleosome, that the on rate for the first and second binding steps are near diffusion limited and identical, and that the turnover rate is defined by the $V_{max}$ for wild-type mononucleosome acetylation. This allows us simply to vary the off rate, $k_5$, for formation of the second intermediate. Plotting the initial rates as a function of nucleosome concentration (Eq. 14) and then fitting them to the cooperative saturation kinetics equation (Eq. 13) reveals several things (Fig. S6B). As $k_4$ decreases relative to $k_3$ [i.e., the dissociation constant for E(SS)$_2$ formation becomes tighter than that of ESS], the half-saturation concentration for the overall reaction becomes lower, and the cooperativity constant increases. This behavior is reasonable because the mechanism for simultaneous binding of two substrates can be thought of as binding of a first substrate, followed by extremely tight binding of the second substrate, effectively coupling the two binding events. Moreover, specific values for the simulated data ($K_{43} = (1/16) \times K_{40}$) are in relatively good agreement with the experimental data (cooperativity constants of 1.71 vs. 1.76 and half-saturation concentrations of 11.6 nM vs. 17.3 nM for simulated vs. experimental data, respectively).

Improving the fit of the data can be achieved a number of different ways. For example, in the generation of ESS, if the assumption that the $K_d$ for the process is not equal to the $K_m$ measured for the tetra-Ala H3/WT H3 mononucleosome, but instead is 2-fold greater [which is plausible if additional bound intermediates exist between ESS and E(SS)$_2$, then very good agreement between simulation and experimental data are observed (Fig. S6C; cooperativity constants of 1.80 vs. 1.76 and half-saturation concentrations of 15.7 nM vs. 17.3 nM for simulated vs. experimental data, respectively). Similar improvement could also potentially be achieved by varying other kinetic parameters, investigating mechanisms in which three or more substrates are bound sequentially, or considering concerted models of substrate binding. Thus, although this sequential model fits the data well, it is not the only possible model consistent with the data. However, unlike the mechanisms that consider only binding of a single nucleosome, this model does reproduce the high degree of positive cooperativity observed experimentally.

This sequential mechanism can be expanded in several ways to account for our other experimental observations (Fig. S5D). To account for the ability of single-tail nucleosome to be acetylated, an intermediate with single-tail binding, E*SS*, can be added. The intermediate can undergo noncooperative acetylation. From ESS* the second H3 tail within the nucleosome can be bound to form ESS. SAGA then undergoes an allosteric change to form the intermediate, E*SS, which exhibits a strong affinity for a second
nucleosome. For nucleosomal arrays, this second nucleosome can come from either a different nucleosomal array (which will show cooperative acetylation) or from the same strand to generate $E(SS)_2^*$ (which will not exhibit cooperative acetylation for the same reasons that the mechanism in Fig. S2B is not). Although the noncooperative acetylation pathways from $ESS^*$ and $E(SS)_2^*$ can occur, they cannot occur at the exclusion of the cooperative pathway, otherwise no cooperativity would be observed experimentally, suggesting that they are relatively minor compared with the cooperative pathway. Our model does not explicitly include the fact that the SAGA complex can acetylate multiple lysines on the H3 tail and instead considers them in aggregate. More complicated models that include these sites individually would not be expected to change the overall mathematics of the model, but could incorporate potential changes in the product specificity of the cooperative pathway relative to the noncooperative pathways.


Fig. S1. General scheme and analysis of the tetra-Ala H3 (Left) and tetra-Ac H3 (Right) histone ligation reaction. The crude reaction mixture was separated on an 18% SDS/polyacrylamide gel and stained with Coomassie blue.
I. Michaelis-Menten mechanism

A) \[ E + SS \xrightleftharpoons[k_1/k_2]{\text{ESS}} E - SS \xrightarrow[k_3]{\text{(ESS)}} E + PS \]

II. Multiple intermediates, no recognition of acetylation

B) \[ E + SS \xrightleftharpoons[k_1/k_2]{\text{ESS}} E - SS \xrightleftharpoons[k_3/k_4]{\text{ESS}} E + PS \]

C) \[ E + SS \xrightleftharpoons[k_1/k_2]{\text{ESS}} E - SS \xrightarrow[k_3/k_5]{\text{(ESS)}} E + PS \]

D) \[ E + SS \xrightarrow[k_3/k_4]{\text{ESS}} E - SS \xrightarrow[k_5/k_6]{\text{(ESS)}} E + PS \]

III. Multiple intermediates, recognition of acetylation

E) \[ E + SS \xrightarrow[k_1/k_2]{\text{ESS}} E - SS \xrightarrow[k_3]{\text{(ESS)}} E - PS \xrightarrow[k_4/k_5]{\text{(EPS)}} E + PP \]

F) \[ E + SS \xrightarrow[k_1/k_2]{\text{ESS}} E - SS \xrightarrow[k_3]{\text{(EPS)}} E - PS \xrightarrow[k_5/k_4]{\text{(EPS)}} E + PP \]

G) \[ E + SS \xrightarrow[k_1/k_2]{\text{ESS}} E - SS \xrightarrow[k_3]{\text{(EPS)}} E + PS \xrightarrow[k_4/k_5]{\text{(EPS)}} E + PP \]

Fig. S2. Potential SAGA-mediated acetylation mechanisms involving a single mononucleosome. Details are given in the SI Text.
Fig. S3. Kinetic simulation of acetylation of a mononucleosome preacetylated on a single H3 tail. (A) Simulated time course of acetylation. The model chosen is the second half of mechanism G in Fig. S2. A $V_{max}/[E]_0$ of 1.08 min$^{-1}$ and a $K_m$ of 11.1 nM were chosen based on the appropriate experimental data. Based on these values, rate constants of $k_4 = 6 \times 10^5$ M$^{-1}$ min$^{-1}$, $k_5 = 66.6$ min$^{-1}$, and $k_6 = 1.08$ min$^{-1}$, were chosen. The initial enzyme concentration was 1.0 nM. PS substrate was varied from 10 to 120 nM by steps of 10 nM. Simulated time points were plotted for every 0.2 min. Concentration of PP as a function of time was fit to a line to determine initial rates. Increasing PS concentration resulted in fits with increasing slope. (B) Plot of initial velocity of PP production per enzyme as a function of mononucleosome concentration. Open circles represent the initial rates from the simulated data in A. These data were fit to a cooperative saturation kinetics model (Eq. 13), resulting in a cooperativity constant of 1.06, a half-saturation concentration of 12.4 nM, and a $V_{max}/[E]_0$ of 1.07 min$^{-1}$. For comparison, the analogous experimental data are shown in black.
Fig. S4. Kinetic simulation of the sequential, dissociative, mononucleosome acetylation mechanism described in Fig. S2G. (A) Plot of initial velocity of total P production per enzyme as a function of mononucleosome concentration. Filled circles represent initial rates obtained for simulated time courses at that concentration of nucleosome. The mechanism used for these simulations is that shown in Fig. S2G. Simulation parameters used were the same as those described in Fig. S3, with the addition of $k_1/k_2$, $k_3$, $k_4/k_5$, and $k_6$, based on a $K_m$ of 38.9 nM and a $V_{max}/[E]_o$ of 1.08 min$^{-1}$ for tetra-Ala H3 WT H3 mononucleosomes. Fit (solid line) of the initial rates per enzyme to a cooperative saturation kinetics model (Eq. 13) resulted in a cooperativity constant of 0.98, a $V_{max}/[E]_o$ of 1.09 min$^{-1}$, and a $K_m$ of 39.2 nM. (B) Selected time courses for simulations described in A. Time courses are shown for initial mononucleosome concentrations of 10, 50, and 160 nM. Progress curves are shown for individual acetylated species (PS, EPS, PP) as well as for total acetylation (PS + EPS + 2PP). (C) Cooperativity constants obtained from simulation that result from the perturbation of kinetic parameters used in A. Individually, $k_1/k_2$, $k_3$, $k_4/k_5$, and $k_6$ were varied from 1/16th- to 16-fold of their values in A. Because it was assumed that the on rate for both SS and PS were constant and identical ($k_1$ and $k_4$, respectively), $k_1/k_2$ and $k_4/k_5$ were perturbed by changes in $k_2$ and $k_5$. 
I. Hill mechanisms

A) $E + 2(SS) \overset{k_1}{\underset{k_2}{\rightleftharpoons}} \frac{SS}{E} \overset{k_3}{\rightarrow} E + PS + SS$

B) $E_2 + 2(SS) \overset{k_1}{\underset{k_2}{\rightleftharpoons}} \frac{SS}{E_2} \overset{k_3}{\rightarrow} E_2 + 2(PS)$

II. Simple sequential mechanism

C) $E + SS \overset{k_1}{\underset{k_2}{\rightarrow}} \frac{SS}{E} \overset{k_3}{\rightleftharpoons} \frac{SS}{E} \overset{k_5}{\rightarrow} E + PS + SS$

II. Potential complete sequential mechanism

D) $E + SS -(SS)_{10} - SS \overset{k_1}{\underset{k_2}{\rightarrow}} E \overset{k_3}{\rightarrow} \frac{SS}{E} \overset{k_4}{\rightarrow} \frac{SS}{E} \overset{k_5}{\rightarrow} E + PS + SS$

Fig. S5. Potential SAGA-mediated acetylation mechanisms involving multiple nucleosomes. Details are given in the SI Text.
Fig. S6. Kinetic simulations of nucleosome acetylation mechanisms involving binding of multiple nucleosomes. (A) Plot of initial velocity of the total $P$ production per enzyme as a function of nucleosome concentration using the Hill mechanism (Fig. S5A and B). Open circles represent initial rates obtained for simulated time courses at that concentration of nucleosome. The mechanism used for these simulations is that shown in Fig. S5A. Simulation parameters used were based on the results from SAGA-mediated acetylation of nucleosomal arrays and include a half-saturation concentration of 33.6 nM, a $V_{\text{max}}/E_0$ of 1.42 min$^{-1}$, and cooperativity constant of 1.97. These data were fit to a cooperative saturation kinetics model (Eq. 13). Experimental data are shown with filled circles for comparison. (B) Plot of the initial velocity per enzyme of total $P$ production as a function of nucleosome concentration using a sequential binding mechanism (Fig. S5C). Open circles represent initial rates obtained using Eq. 14. For all plots the following kinetic parameters were used: $[E_0] = 1.0$ nM, $[S]_0$ from 0 to 300 nM by 5 nM steps, $k_1 = 6 \times 10^4$ M$^{-1}$ min$^{-1}$, $k_2 = 233.4$ min$^{-1}$, $k_3 = 6 \times 10^2$ M$^{-1}$ min$^{-1}$, and $k_5 = 1.09$ min$^{-1}$. The off rate, $k_4$, was varied and included values of 233.4, 58.35, 14.6, and 3.6 min$^{-1}$. $K_{D1}$ and $K_{D2}$ are defined as the ratios of $k_2/k_1$ and $k_4/k_3$, respectively. These initial rates per enzyme were fit to a cooperative saturation kinetics model (Eq. 13). From this fit, the following kinetic constants were obtained (from bottom to top fits): Cooperativity constants: 1.50, 1.61, 1.71, and 1.75; half-saturation concentrations: 59.0 nM, 25.0 nM, 11.6 nM, and 6.0 nM. For comparison, data for SAGA acetylation of wild-type mononucleosomes (filled circles), is included. For these data, a cooperativity constant of 1.76 and a half-saturation concentration of 17.3 nM was obtained. (C) As in B, but $k_2 = 466.8$ min$^{-1}$ and $k_4 = 14.6$ min$^{-1}$. From the fit of the simulated data, the cooperativity constant is 1.80 and the half-saturation concentration is 15.7 nM.