Mechanisms of stimulation of SAGA-mediated nucleosome acetylation by a transcriptional activator

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1. Introduction

Transcriptional coactivators function directly downstream of sequence-specific DNA binding activator proteins to promote eukaryotic gene expression. In general, coactivators are large, multi-subunit complexes with multiple functions. Numerous categories of coactivators have been characterized, including coactivators that change the post-translational modification state of histones, those that alter nucleosome positioning and composition, and those that directly bridge activator proteins and the general transcriptional machinery [1–6].

The SAGA (Spt-Ada-Gcn5 acetyltransferase) complex from budding yeast was one of the first discovered histone modifying complexes [7,8], and has served as a prototype for understanding activator-coactivator function. SAGA is highly conserved across eukaryotes and regulates a wide range of inducible genes, including most stress response genes in higher eukaryotes [9,10]. SAGA is a 1.8 MDa complex and is composed of 19 subunits that are arranged into four functional modules [8,11]. These include the histone acetyltransferase (HAT), deubiquitination (DUB), Spt, and Taf modules [11].

The enzymatic modules can be isolated from the complex, and it has been shown that subunits within the modules modify the activity of the core catalytic subunits [12–15]. For example, the acetyltransferase enzyme Gcn5 alone can only acetylate a single lysine residue on free histone H3 [12,13]. When in a complex with Ada2 and Ada3, the specificity of Gcn5 expands to that of SAGA, acetylating four primary lysine residues on free and nucleosomal histone H3 [13], with even more sites of acetylation observed with the full complex [16,17]. Interestingly, the HAT module of SAGA is not limited to the SAGA complex. In addition to other complexes that are largely composed of the same components [18], the HAT module of SAGA is also found in complexes that differ significantly in their other subunits [19,20]. In budding yeast, this includes the ADA complex, which contains Ahc1 and Ahc2 proteins in association with the HAT module [11,21]. However, beyond its composition and the fact it too can acetylate nucleosomes [11,12], the ADA complex has not been characterized significantly.

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As a coactivator, SAGA stimulates gene expression through its acetyltransferase function and via interactions with activator proteins. Under stress or developmental conditions, SAGA complexes are localized to gene promoters and establish regions of hyperacetylation [22–24]. This localization was further shown to be dependent on SAGA interacting with DNA-binding activator proteins [25–28]. These results are complemented by numerous studies demonstrating that many subunits in SAGA interact with activator proteins [29–32]. Thus, these studies have led to a model in which activator proteins enhance SAGA-mediated nucleosome acetylation by stabilizing binding of SAGA to chromatin associated with activators.

Recently, our group has investigated the mechanism of Gal4-VP16 activator stimulation of SAGA-mediated nucleosome acetylation, and have observed a novel mechanism of activator action [33]. Stimulation of HAT activity is due to the increase in the turnover rate of the reaction, not, as is usually ascribed, to the apparent binding affinity of SAGA to chromatin or to a change in affinity for acetyl coenzyme A (CoA). Here, we further characterized the components required for the stimulation of the HAT activity of SAGA by Gal4-VP16 activator, including the binding requirements for activator stimulation and mapping of the SAGA subunit most responsible for stimulation. Results from these studies revealed novel properties of the HAT module in isolation and were then further examined within the context of the ADA complex.

2. Materials and methods

2.1. Nucleosome preparation

Biotinylated GBY and 147 bp DNA was prepared as previously described [33]. Briefly, recombinant Xenopus laevis histones were prepared [34] and assembled into octamers [35]. Nucleosomes were assembled by depositing the histone octamer onto DNA by the process of rapid dilution and then dialyzing into LDB buffer (2.5 mM NaCl, 10 mM Tris pH 7.4, 0.25 mM EDTA), as previously described [36]. The homogeneity and degree of saturation of nucleosome assemblies were assessed via 4% Native PAGE. Nucleosomes were immobilized on hydrophilic streptavidin beads from NEB as previously described [37].

2.2. ySAGA and yADA preparation

Endogenously expressed SAGA (TAP-tagged on Spt7) and ADA complex (TAP-tagged on Apc1) was expressed and purified as previously described [38]. Untagged SAGA complexes with wild type and mutant Tra1 subunits were expressed and purified as described [39]. After anion column purification, 2 μl of every other fraction was tested for HAT activity on 50 μM H3 peptide in 11 μl reaction volume. Reactions were incubated at 30 °C for 30 min before following the protocol described below. The purified enzymes were quantitated by Western blot (Santa Cruz Biotechnology sc-9078) in comparison to known amounts of recombinantly expressed and purified Gcn5.

2.3. Recombinant HAT module and Gal4-VP16 purification

Ada2/Ada3/Gcn5 complex was purified as previously described with some modifications [40]. In particular, after anion-exchange chromatography, pooled samples were concentrated to approximately 1 ml and applied to size-exclusion chromatography (GE Superdex 200, 25 ml) in SEC buffer (300 mM NaCl, 20 mM HEPES pH 8.0, 0.1 mM EDTA, 2 μg/ml Pepstatin A, 2 μg/ml Leupeptin, 1 mM PMSF, 1 mM Benzamidine, 10 mM BME). Peak fractions were analyzed by SDS-PAGE and fractions containing recombinant sub-complex were pooled and concentrated. Concentrated samples were then mixed with glycerol to a final concentration of 50%. The purified enzyme complex was quantitated by western blot as above. Analysis by SEC-MALS yielded a molecular weight of approximately 150 kDa. Addition of 15 μM NP-40 to HAT reactions was required to prevent non-specific binding of the HAT module to the magnetic streptavidin resin. Recombinant Gal4-VP16 was purified from E. coli X901 cells transformed with pJL2S plasmid (a generous gift from Dr. Steve Triezenberg) largely according to a previously published protocol [3].

2.4. Steady state kinetics assays

Bead-based assays were performed nearly the same as previously described [33]. Modifications to the assay include preincubating 18 μl of bead bound substrate at 2X the desired final concentration in LDB at 30 °C for 5 min with 18 μl of 2X HAT buffer (1X concentrations = 25 mM Tris pH 8, 150 mM NaCl, 5% glycerol, 1 mM PMSF, 1 mM DTT, 10 mM sodium butyrate, 4 μM AcCoA) with a specific activity of tritiated acetyl CoA of 13.9–22.6 Ci/mmol. Assays with the HAT module also included 15 μM of NP40. The reactions were initiated with addition of enzyme. Enzyme was always sub-stoichiometric relative to nucleosome (20 nM) and activator protein (40–800 nM) but varied in concentration for different assay. The enzyme concentrations used were 0.52–3.62 nM for the SAGA complex, 11 nM HAT complex, and 2.22 nM ADA complex. 8 μl aliquots were removed from the reaction and washed, counted, and initial rates calculated as previously described [33]. Assays on 20 μM H3 peptide (amino acids 1–20, Anaspec 62753) were performed similarly. 18 μl 20 μM final H3 peptide in LDB was mixed 1:1 with 18 μl 2X HAT buffer (1X concentrations = 25 mM Tris pH 8, 150 mM NaCl, 5% glycerol, 1 mM PMSF, 1 mM DTT, 10 mM sodium butyrate, 12 μM AcCoA) with a specific activity of tritiated acetyl CoA of 13.9–22.6 Ci/mmol. 8 μl aliquots were spotted onto P81 phosphocellulose discs, washed twice in 300 ml wash buffer (4 mM sodium carbonate and 46 mM sodium bicarbonate, pH 9.2) for 20 min and once in 200 ml acetone for 10 min. Discs were air-dried before being added to 3 ml liquid scintillation cocktail. Initial rates calculated as previously described and normalized for enzyme concentration [33].

2.5. EMSA and pull-down assays

EMSA assays of Gal4-VP16 (0, 20, 100, 200, 400 nM) activator and 5 nM GBY or 147 bp nucleosomes were performed as described previously [33]. Pull-down assays of 11 nM HAT module by 20 nM GBY nucleosome and increasing concentrations of Gal4-VP16 activator (0, 40, 100, 200 nM) were performed in buffers nearly identical to the steady state kinetics assays; 15 μl 20 μM final GBY nucleosomes in LDB mixed 1:1 with 2X HAT buffer (1X concentrations = 25 mM Tris pH 8, 150 mM NaCl, 5% glycerol, 1 mM PMSF, 1 mM DTT, 10 mM sodium butyrate, 4 μM CoA). Pull-down assays of the HAT module were performed with 200 μg of His-Tag Dynabead (Invitrogen), 20 nM GBY nucleosome, 11 nM HAT module, and 0–200 nM of Gal4-VP16. 36 μl reactions proceeded for 9 min at 30 °C under conditions used for the steady-state acetylation assays described above with cold AcCoA.

3. Results

3.1. Stimulation by activator occurs in cis

Gal4-VP16 activator was shown to stimulate nucleosome acetylation turnover by SAGA [33]. To characterize how activator interacted with the chromatin substrates to stimulate the HAT activity of SAGA, we used a bead-based, steady state nucleosome acetylation assay [33]. Briefly, endogenously expressed SAGA was purified from budding yeast and then incubated with nucleosomal substrates. One substrate, GBY (Gal4 binding site in the body of the DNA) nucleosome, possessed flanking DNA on both sides of the nucleosome, and a 17 bp consensus sequence for the yeast activator Gal4 was embedded in one side of the flanking DNA. Another nucleosome, 147 bp nucleosome, had no flanking DNA. To further characterize the minimal requirements for stimulation by activator, we also tested the HAT activity of SAGA on H3 tail peptides in a filter-binding assay [39].
To determine if nucleosomal flanking DNA was required for the stimulation by activator, we compared the rate of SAGA HAT activity on GBY nucleosomes to 147 bp nucleosome lacking flanking DNA. The addition of Gal4-VP16 activator to reactions of SAGA and GBY nucleosomes increased the HAT activity as previously seen (Fig. 1A, left graph, left columns; [33]). In contrast, when increasing concentrations of Gal4-VP16 activator were added to reactions of SAGA and 147 bp nucleosomes, acetylation was not significantly stimulated (Fig. 1A, left graph, right columns). A similar lack of stimulation was observed when Gal4-VP16 activator was added to HAT assays on H3 peptide (Fig. 1A, right graph). These results indicate that activator alone is not sufficient to stimulate HAT activity, and that DNA-binding is required.

DNA-binding might be necessary because such binding allows the activator protein to adopt an active conformation. In such a case, it should be possible to stimulate SAGA acetylation in trans. To test this idea, HAT assays were again performed on the 147 bp nucleosome and H3 peptide substrates, but this time with the addition of 17 bp Gal4 consensus DNA in addition to the Gal4-VP16 activator. Previously, we, and others, have observed that Gal4 DNA binding domain binds its consensus sequence with high affinity [33,41,42]. The inclusion of Gal4-VP16 activator to reactions of SAGA and 147 bp nucleosomes with increasing concentrations of Gal4 DNA did not stimulate the HAT activity of SAGA (Fig. 1B, left graph). Further, similar results were obtained when HAT activity was tested on the H3 peptide in the presence of Gal4-VP16 activator and Gal4 DNA (Fig. 1B, right graph). For each of the substrates, the acetylation activity of SAGA was not affected by the addition of the 17 bp Gal DNA alone (data not shown). Therefore, our results indicated that Gal4-VP16 activator does not stimulate the HAT activity of SAGA in trans, but rather in cis, where both the activator and activator-binding site are directly attached to the nucleosome.

### 3.2. SAGA HAT module activity inhibited by activator

Having determined substrate requirements for activator-mediated stimulation, we next sought to identify the factors required within the enzyme complex itself. Many subunits in SAGA have been shown to interact with activator proteins [29–32], including Ada2 and Gcn5, components of the HAT module [43,44]. The simplest way activators could influence the rate of acetylation by SAGA would be through direct interactions with components of the HAT module. We therefore recombinantly expressed and purified the Ada2/Ada3/Gcn5 HAT module with modifications to a previously described protocol in order to obtain more consistent HAT activities [40]. The complex of all three subunits was chosen because the inclusion of both Ada2 and Ada3 are activity of SAGA (Fig. 1B, left graph).
necessary and sufficient to obtain the same substrate specificity and HAT activity on H3 peptide substrates as observed for the SAGA complex [13].

To characterize the baseline kinetic properties of the HAT module, initial rate, steady state kinetic experiments were performed on increasing concentrations of GBY nucleosomes. The data was fit to the Michaelis-Menten equation (Fig. 2A), resulting in a $K_M$ of $50 \pm 15$ nM and a $k_{cat}$ of $0.24 \pm 0.04$ min$^{-1}$. In comparison to SAGA, the HAT module had 2.3-times weaker putative binding and a 4.2-times less efficient turnover rate. When increasing concentrations of Gal4-VP16 activator were added to reactions of the HAT module and GBY nucleosomes, in stark contrast to stimulation of SAGA HAT activity, the rate of nucleosome acetylation decreased (Fig. 2B, left). Inhibition was not simply due to non-specific disruption of the HAT module activity by protein, as increasing concentrations of a non-activator protein, BSA, to reactions of the HAT module and GBY nucleosomes did not drastically alter the rate of acetylation (Fig. 2B, right).

Another possible reason for the decrease in activity is that the Gal4-VP16 activator could cause the HAT module to dissociate in solution. Therefore, we confirmed that the HAT module was intact in the assays via a pull-down assay (Fig. 2C). In this assay, the HAT module was pulled down by magnetic beads specific for His-Ada3 in the presence of GBY nucleosomes and increasing concentrations of activator. Integrity of the complex was determined by the detection of Gcn5 by antibody, as
previous studies have shown that Ada3 and Gcn5 do not directly interact with each other, but are linked by Ada2 [45]. Our results showed that increasing concentrations of substrate and activator did not alter the amount of Gcn5 bound to the HAT module corroborating the integrity of the module under our experimental conditions. Additionally, Gcn5 alone has approximately 4-times lower rates of acetylation on core histones than the Gcn5-Ada2-Ada3 complex [13]. Thus, if the HAT module were being disrupted by Gal4-VP16, we would expect it to be reflected in its activity towards H3 peptide substrate. We did not observe such an effect with increasing concentrations of activator (Fig. 2D), further supporting our previous observation of the integrity of the HAT module.

Because inhibition of HAT activity was observed on the GBY nucleosomes but not on the H3 peptides, we next probed the influence of activator on HAT module acetylation of 147 bp nucleosomes to see if flanking DNA was a requirement for inhibition. Like the GBY nucleosome, the addition of increasing concentrations of Gal4-VP16 activator to reactions of the HAT module and 147 bp nucleosomes decreased HAT activity (Fig. 2E). This result suggested that activator inhibits the activity of the HAT module in a manner that does not require binding to its consensus sequence in the nucleosomal flanking DNA.

Taken together, our results led us to consider a hypothesis where Gal4-VP16 activator binds non-specifically to nucleosomes and that the HAT module is not able to overcome non-specifically bound Gal4-VP16 activator from nucleosomes. To test the first part of this hypothesis, we utilized an electrophoretic mobility shift (EMSA) assay as previously described [33]. In this assay, increasing concentrations of Gal4-VP16 activator were incubated with GBY or 147 bp nucleosomes in the presence of excess CoA (Fig. 3A). CoA was used in these assays to prevent enzymatic turnover that would occur with acetyl CoA. As expected, Gal4-VP16 activator could bind to and shift unbound GBY nucleosomes to a bound state (Fig. 3A, left lanes). We also observed that Gal4-VP16 activator could bind to 147 bp nucleosomes, albeit more weakly compared to the GBY nucleosomes (Fig. 3A, right lanes). That we observed shift on 147 bp nucleosomes supported our model that Gal4-VP16 activator binds non-specifically to nucleosome substrates. To determine if Gal4-VP16 activator also prevented the HAT module from binding its nucleosome substrates, we next performed a competition assay using our bead-bound GBY nucleosomes (Fig. 3B). With increasing amounts of Gal4-VP16 activator, the amount of HAT module associated with the bead-bound nucleosome decreased and a general increase in unbound HAT module. Thus, our results suggest that non-specific activator binding can prevent HAT-module binding. This is in contrast with the full SAGA complex where equivalent levels of activator are not inhibitory.

3.3. ADA complex can overcome non-specifically bound activators

The HAT module alone is neither stimulated by activator, nor capable of clearing non-specifically bound activator. However, the HAT module does not only exist in SAGA, but also comprises the bulk of the yeast ADA complex [11,21]. We wanted to determine if the Gal4-VP16 activator influenced the HAT activity of ADA, and if ADA could clear non-specifically bound activators from nucleosome substrates. To do so, we first purified endogenous ADA complex from budding yeast and characterized its kinetic parameters towards GBY nucleosomes. Fitting the data to a Michaelis-Menten equation resulted in a $K_m$ of 116 ± 25 nM and a $k_{cat}$ of 1.3 ± 0.2 min$^{-1}$ (Fig. 4A). In comparison to SAGA, ADA has a very similar turnover rate while having a 5.3-times weaker apparent binding affinity for GBY nucleosomes. When increasing concentrations of Gal4-VP16 activator were added to reactions of ADA and GBY nucleosome, neither stimulation nor significant inhibition of its activity was observed (Fig. 4B). This result suggests that HAT activity of ADA, unlike that of SAGA, is not stimulated by Gal4-VP16 activator, and therefore lacks the subunits to mediate activator-mediated stimulation. However, like SAGA, ADA can clear non-specifically bound activators from nucleosome substrates.

3.4. Activator stimulates SAGA HAT activity through interactions with Tra1

Because the recombinant HAT module did not show Gal4-VP16-mediated stimulation, we sought to determine which SAGA subunits might mediate this stimulation. While a number of SAGA subunits have been shown to interact with Gal4-VP16, Tra1, the largest subunit of SAGA, has been shown to interact with many activator proteins, including Gal4-VP16 [30,31,46]. Further, deletion of part of Tra1 resulted in decreased H3 acetylation at the promoters of genes in budding yeast while not influencing the localization of SAGA or its ability to interact with activators [47]. This suggests that in addition to interacting with activators, the Tra1 subunit also plays a role in the substrate specificity or HAT activity of SAGA.

Endogenously expressed untagged SAGA complexes were purified containing either a wild type or mutant Tra1 subunit (tra1-2) [31]. To ensure the innate HAT activity of SAGA was not affected by the Tra1 mutations in tra1-2, we performed HAT assays of the two SAGA complexes in the absence of Gal4-VP16 activator both on H3 peptide (Fig. 5A) and GBY nucleosomes (Fig. 5B). In both cases, wildtype and mutant enzyme activity using an H3 peptide did not vary significantly, demonstrating that the Tra1 mutations alone were not deleterious towards HAT activity. To test the ability of both strains to be stimulated by activator, increasing concentrations of Gal4-VP16 activator were added to the reactions of the SAGA complexes and GBY nucleosomes (Fig. 5B). Like the other TAP-tagged SAGA experiments described above and previously [33], Gal4-VP16 activator had a strong stimulatory effect on the HAT activity of the wild type Tra1 containing complex (Fig. 5B, left). In contrast, the addition of increasing concentrations of Gal4-VP16 activator resulted in a $K_m$ of 116 ± 25 nM and a $k_{cat}$ of 1.3 ± 0.2 min$^{-1}$ (Fig. 3A).
activator to tra1-2 containing SAGA complex and GBY nucleosomes resulted in a loss of nearly two thirds of the stimulation of HAT activity (Fig. 5B, right). Thus, our studies indicate that activator-dependent stimulation of SAGA HAT activity is predominantly mediated via interactions of the Tra1 subunit with activator bound to nucleosome substrates.

4. Discussion

Eukaryotic gene expression requires the coordinated efforts of many proteins and protein complexes to overcome the repressive nature of chromatin. In this study, we have probed how an activator protein stimulates the HAT activity of the SAGA complex.

Our results show that the HAT activity of SAGA is not significantly stimulated by activator when chromatin substrates lack flanking DNA. One possibility for this observation is that binding to the flanking DNA itself makes activator active by, for example, inducing a conformational change in the activator protein. Such a conformational change has been seen upon binding of the Gal4 activator to its consensus DNA; the structure of unbound Gal4(1–100) is more elongated compared to DNA-bound Gal4(1–100) [42,48]. However, our data seems to rule this out and instead supports a model where DNA-binding by activator occurs in cis. This requirement is likely due to a need for the activator to be held proximal to the nucleosome for it to affect the HAT activity of SAGA. Increasing the local concentrations of activators near nucleosomes could also be critical to increasing the binding affinity of or stabilizing weak affinity interactions between the activation domain of VP16 and the Tra1 subunit of SAGA. It should be noted that, in this model of weak binding affinity between activator and SAGA, it could still be possible to see stimulation by driving binding interactions at high concentrations of activator. Indeed, our studies show that high concentrations of Gal4-VP16 activator in reactions of SAGA and 147 bp nucleosome can increase the HAT activity up to two-fold. However, full stimulation is not observed at high concentrations of activator and, coupled with our results that Gal4-VP16 activator binds non-specifically to nucleosomes, may be due to competition of nucleosome binding with SAGA.

That the Gal4-VP16 activator can bind non-specifically to nucleosome substrates is not surprising. The DNA binding domains of many transcriptional activators have been structurally characterized, and in addition to unique recognition of specific bases [49], they also often make extensive interactions with the negatively charged phosphate backbone of DNA [50]. As this part of the DNA is shared by all sequences, this interaction generates non-specific binding. The effect of this non-specific binding can be especially pronounced under conditions of low ionic strength, where a lack of ions to shield the non-specific interactions between the phosphate backbone of DNA and activator
proteins strengthens this interaction. However, we should note, in our experiments, all kinetic and binding assays were performed at 150 mM monovalent ion – conditions with significant ionic strength and which are frequently used to approximate physiological conditions. Despite such non-specific nucleosome binding, activators can still potentially function in a specific manner. Activators may sample the chromatin by non-specifically binding nucleosomes until they find their consensus DNA sequences. At these sites, they then recruit and/or interact with coactivators to further stabilize their binding and influence their activities. Non-specific binding of activators may also inhibit untargeted enzymatic activities of chromatin modifying complexes, explaining why SAGA and ADA can compete for binding while the HAT module cannot.

We also note that in our previous studies of nucleosome acetylation by the SAGA complex, the presence of linker DNA beyond the nucleosome core increased acetylation activity [35]. For the HAT complex alone, such stimulation is not observed (Fig. 2E and B). In fact, nucleosome lacking linker DNA show higher activity. Because SAGA contains more than a dozen subunits outside of the HAT complex core [11]. We, we speculate that stimulation by linker DNA requires these additional subunits. Why the activity decreases with linker DNA is not clear. It is possible that the linker DNA directly inhibits the HAT module. However, it could be due to the accessibility of the histone tail with and without linker DNA. Others have previously shown that Gcn5-mediated acetylation in nucleosome-containing substrates is correlated with the accessibility of the histone tails [51], and linker DNA is known to interact with the H3 tail [52]. Thus, the HAT module could have reduced activity toward the H3 tail in the presence of linker due to competition for H3 tail binding between the HAT module and the linker DNA.

Our analyses of the effects of activator on the ADA complex and the minimal HAT module led us to conclude that stimulation of HAT activity occurred through interactions of activator with a subunit outside of the HAT module. Many subunits in SAGA have been shown to interact with activators [29–32]. We specifically focused on the Tra1 subunit as it has been shown to interact with multiple activators [30,31,46] and mutations have perturbed the HAT activity of SAGA [47]. When activator was added to a mutant Tra1 containing SAGA complex, we did indeed observe significantly diminished stimulation of HAT activity compared to wild type SAGA complex. Further work is required to determine if other subunits of SAGA interact with activators to stimulate its HAT activity.

Though the majority of the subunits that comprise the ADA complex are the same as the SAGA HAT module, little is known about its HAT activity. Like SAGA, it has been shown to acetylate both free and nucleosomal histones [11,12]. We too observe that ADA can acetylate nucleosomes substrates. However, unlike SAGA, the addition of activator to reactions of ADA on nucleosomes does not stimulate its HAT activity. Considering that stimulation of SAGA HAT activity occurs predominantly through interactions of activator with the Tra1 subunit, this result makes sense. Further, no interactions have been observed between ADA and transcriptional activators [25], even though the Ada2 and Gcn5 subunits has been shown to interact with the transcriptional activation domains [32,43,44,53]. Activator also does not inhibit the HAT activity of ADA. This could be due to subunits present in the ADA complex outside of the acetylation core interacting with DNA to outcompete or to clear nonspecifically bound activator. While interaction of the ADA complex with linker DNA was not specifically examined, we note that the Ahc1 subunit of the complex contains a putative zinc finger DNA binding domain. These results support a model where ADA can clear nonspecifically bound activators from nucleosome substrates, potentially enabling ADA to play a role in the maintenance of global histone acetylation.

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**CRediT authorship contribution statement**

**Sannie J. Culbertson:** Conceptualization, Investigation, Writing - original draft. **Michael A. Shogren-Knaak:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2020.100884.

**Abbreviations**

SAGA  Spt-Ada-Gcn5 acetyltransferase  
CoA  Coenzyme A  
GBY  DNA template with Gal4 binding site and 95 and 15 base pairs of DNA flanking a 147 bp nucleosome positioning sequence:147 nucleosome, DNA template with no DNA flanking a 147 bp nucleosome positioning sequence  
EMSA  electrophoretic mobility shift assays  
Gal4-VP16  activator fusion protein containing a Gal4 activator DNA binding domain and a VP16 activator activation region  
BSA  Bovine serum albumin protein; Tris, tris(hydroxymethyl)aminomethane  
EDTA  Ethylenediaminetetraacetic acid  
PAGE  polyacrylamide gel electrophoresis  
TAP  tandem affinity purification  
HEPES  4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid  
DTT  1,4-dithiothreitol  
PMSF  phenylmethylsulfonyl fluoride.  
Accession IDs.  
Tra1 P38811Spt3 P06844.  
Spt8 P38915Ub8 P50102.  
Sus1 Q6WNK7.  
Sgf11 Q03067.  
Sgf73 P5316SSpt7 P35177.  
Sp20 P50875.  
Ada1 Q12060.  
Taf5 P38129.  
Taf6 P53040.  
Taf9 Q05027.  
Taf12 Q03761.  
Sgf29 P25554Chd1 P32657.  
Gcn5 Q03330.  
Ada2 Q02336.  
Ada3 P32494.  
Ahc1 Q12433.  
Histone H4 P62799.  
Histone H3 Q92132.  
Histone H2A Q6AZJ8.  
Histone H2B Q92131.  
Gal4 P04386.  
VP16 P06492.
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