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The SAGA continues: The rise of *cis*- and *trans*-histone crosstalk pathways

Brian D. Strahl[1,*], **Scott D. Briggs**[2,*]

[1]Department of Biochemistry and Biophysics, 120 Mason Farm Rd, University of North Carolina at Chapel Hill, NC, 27599; USA

[2]Department of Biochemistry and Purdue University Center for Cancer Research, Purdue University, Hansen Life Science Research Building, 201S. University Street, West Lafayette, IN 47907; USA

Abstract

Fueled by key technological innovations during the last several decades, chromatin-based research has greatly advanced our mechanistic understanding of how genes are regulated by epigenetic factors and their associated histone-modifying activities. Most notably, the landmark finding that linked histone acetylation by Gcn5 of the Spt–Ada–Gcn5–acetyltransferase (SAGA) complex to gene activation ushered in a new area of chromatin research and a realization that histone-modifying activities have integral genome functions. This review will discuss past and recent studies that have shaped our understanding of how the histone-modifying activities of SAGA are regulated by, and modulate the outcomes of, other histone modifications during gene transcription. Because much of our understanding of SAGA was established with budding yeast, we will focus on yeast as a model. We discuss the actions of *cis*- and *trans*-histone crosstalk pathways that involve the histone acetyltransferase, deubiquitylase, and reader domains of SAGA. We conclude by considering unanswered questions about SAGA and related complexes.

Keywords

SAGA; gene transcription; histone modifications; histone acetylation; histone methylation; histone deubiquitylation; Gcn5; Ubp8; crosstalk pathways; bromodomain; Tudor domains

[*]Corresponding Authors: sdbriggs@purdue.edu and brian_strahl@med.unc.edu.

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1. Introduction

1.1 A brief timeline leading to SAGA-associated histone-modifying activities

In 1951, Stedman and Stedman [1] proposed the notion that histones act as an inhibitory barrier for genes, and, in 1961, Allfrey confirmed that histones inhibit RNA synthesis [2] (Figure 1). Later, biochemical and cell biology approaches led Phillips [3] and Murray [4] to discover histone modifications such as acetylation and methylation, respectively. On the basis of these post-translational modifications (PTMs) and their association with RNA synthesis, Allfrey hypothesized that histone acetylation/deacetylation act as a switch to alter RNA synthesis [5]. Since Allfrey's 1964 hypothesis [5], the scientific community has been on a continuous "SAGA" that details how transcription is initiated, maintained, and regulated.

Allfrey's intriguing proposal notwithstanding, the importance of histone modifications acting as a switch wasn't realized until the mid-1990's when Allis and colleagues biochemically and genetically defined yeast Gcn5 (General control nonderepressible 5) as the first transcription-linked histone acetyltransferase (HAT) [6–8]. Similarly, Schrieber and colleagues also identified the first mammalian histone deacetylase, HDAC1, which was homologous to yeast Rpd3, a known transcriptional repressor [9]. These pivotal discoveries of enzymes that add or remove acetylation from histones completed our genetic understanding of a known activator (Gcn5) and repressor (Rpd3) in transcriptional regulation.

After Gcn5 was recognized as a specific HAT for histone H3 lysine 14 (H3K14) [10], Workman and colleagues showed that Gcn5 was part of a large multi-subunit complex coined SAGA that showed catalytic activity towards nucleosomes and an expanded set of lysine residues on histone H3 and other histones [11]. The significance of the SAGA complex in transcriptional regulation was initially established in classical genetic screens by various groups including the Thireos, Guarente, and Winston lab's, which provided an early view of how SAGA subunits contributed to gene regulation [12–14]. Interestingly, the HAT activity SAGA was not its sole activity. The discovery that SAGA contained histone H2B deubiquitylase activity expanded the toolkit by which this complex is able to contribute to chromatin organization and gene transcription [15]. From these two SAGA activities, we learned new twists and plots in how these activities are regulated by other histones and PTMs and how SAGA-associated activities modulate the outcomes of other histone PTMs.

In this review, we will focus on discoveries from mainly, but not limited to, investigations with the model organism *Saccharomyces cerevisiae* (*sc*). These discoveries have promoted the "rise" of *cis*- and *trans*-histone crosstalk pathways centered around the SAGA complex and its associated histone modifying activities. We will conclude by describing additional avenues of research and pertinent questions whose answers are necessary to fully understand how SAGA and chromatin merge to control gene expression and other DNA-templated processes.

2. SAGA (Spt-Ada-Gcn5 acetyltransferase)

SAGA is a multi-subunit protein complex that is conserved in many species such as *Saccharomyces cerevisiae* (*sc*SAGA), *Drosophila melanoga*ster (*dm*SAGA), *Homo sapiens* (*hs*SAGA) and *Arabidopsis thaliana* (*at*SAGA) [16–18]. Yeast SAGA (Spt-Ada-Gcn5 acetyltransferase) is a 2.0 MDa multi-subunit protein complex that is composed of 19 distinct proteins [16,19]. These proteins are separated into four distinct modules: the HAT module (Gcn5, Ada2, Ada3, and Sgf29), a deubiquitylase (DUB) module (Ubp8, Sgf11, Sgf73 (hSca7), and Sus1), the SPT (Suppressor of Ty) module (Tra1, Ada1, Spt3, Spt7, Spt8, and Spt20), and TAF (TATA-binding protein (TBP)-associated factors) module (Taf5, Taf6, Taf9, Taf10, and Taf12) (Figure 2) [17,19]. However, new Cryo-EM studies showed most of the proteins from the SPT and TAF module form a structural “core” complex involving Spt7, Spt3, Spt20, sgf73, Ada1, Taf5, Taf6, Taf9, Taf10, and Taf12 (Figures 2 and 3) [20,21].

SAGA from all species contains two distinct catalytic activities, HAT and DUB that are mediated by *sc*Gcn5 and *sc*Ubp8 (human ortholog *hs*Usp22), respectively. In contrast, there are also specific metazoan subunits which include two components of the SF3B spliceosomal complex, SF3B3 and SF3B5. These subunits have been found associated with *dm*SAGA and *hs*SAGA (STAGA) but not *sc*SAGA complexes suggesting a specialized function of these subunits in metazoan gene regulation [22,23]. In addition, there are key structural components such as Tra1, the largest subunit, that interacts with transcriptional activators; similarly, Spt3 and Spt8 interact with TBP for its promoter recruitment to TATA-containing promoters of stress responsive genes [24–26].

Though initially thought to only target stress responsive genes, SAGA appears to acts as a general coactivator, similar to Mediator, for pre-initiation complex (PIC) formation and all RNA Polymerase II (RNAPII) transcription [27,28]. In addition, several proteins in SAGA have distinct protein-binding or “reader” domains (Tudor or Bromodomains) that enable the SAGA complex to associate with chromatin or other proteins via PTMs. Moreover, several SAGA proteins are also associated with other protein complexes (reviewed in [16]). For example, Gcn5 is found in human ATAC and yeast SALSA/SILK complexes, Tra1 is part of the Nua4 acetyltransferase complex, and five TAF proteins (Taf5, Taf6, Taf9, Taf10, Taf12) are components in the TFIID complex [16,19,29]. Many of the aforesaid aspects of SAGA will be discussed further in other excellent reviews in this issue. In the following, we will specifically discuss how the HAT and DUB modules and their associated reader modules affect *cis*- and *trans*-histone crosstalk pathways.

2.1 HAT module and cis-histone crosstalk pathways

Gcn5 was originally discovered in a genetic screen for the inability of the yeast mutants to grow under amino acid-starved conditions [30]. However, Gcn5 was first cloned in 1992 by Georgakopoulos and Thireos, who also showed that Gcn5 was required for the transcriptional activator function of Gcn4 [12]. Once Gcn5 was identified to have enzymatic activity [7], Gcn5’s HAT activity was initially shown to have an *in vitro* preference for histone H3 at lysine 14 (H3K14) [10]. However, Gcn5 could also acetylate additional histone lysine residues, such as H3K9, H3K18, H3K23, H3K27, H3K36 and other histones

such as H4 and H2B [10,11,31,32]. Nonetheless, other proteins were needed for Gcn5 to acetylate nucleosomal substrates [11,33]. These additional proteins where found in the highly conserved HAT module of SAGA in which the association of Gcn5 with Ada2 and Ada3 subunits dictates Gcn5 specificity for nucleosomal substrates, whereas Sgf29 recognizes Set1-mediated H3K4 trimethylation [11,33–35]. Within the HAT module, there are two distinct reader domains - a single bromodomain within Gcn5 and a tandem Tudor domain located in the C-terminus of Sgf29 (Figures 2 and 3). Both reader domains have significant functions in *cis*-histone crosstalk pathways, as discussed below.

One of the earliest examples of a *cis*-histone crosstalk pathway involving Gcn5 was the connection between histone H3 phosphorylation and H3 acetylation in which Gcn5 prefers to acetylate histone H3K14 when H3 serine 10 (H3S10) is phosphorylated [36]. This type of crosstalk was further supported by *in vivo* analysis of the yeast *INO1* promoter where histone H3S10 phosphorylation by the Snf1 kinase is required for Gcn5 to acetylate H3K14 [37]. More recently, Shimada et al. reported that histone H3 threonine 11 (H3T11) phosphorylation by Chk1 facilitates Gcn5-mediated H3K14 acetylation and transcription of cell-cycle genes [38]. However, upon DNA damage, this *cis*-histone crosstalk pathway is reversed such that Chk1 is released from chromatin, thereby reducing H3T11 phosphorylation and H3K14 acetylation and resulting in transcriptional repression [38]. Intriguingly, histone H3 phosphorylation may also impact chromatin remodeling because H3S10 phosphorylation can antagonize binding of the Rsc4 bromodomain to H3K14 acetylation; thus, phosphorylation in this situation may act as a switch to inhibit and/or release chromatin remodeling factors from chromatin [39].

Gcn5 also contains a single bromodomain (BRD) that is adjacent to its catalytic HAT domain. The BRD of Gcn5 not only increases the retention of the SAGA complex on chromatin but it also facilitates *cis*-histone crosstalk [40,41]. For example, the BRD of Gcn5 binds to Gcn5-mediated H3K14 acetylated resides to facilitate acetylation at other histone lysine residues (H3K9, H3K18, K27, K36) neighboring H3K14, with H3K18 acetylation being the most dependent on the BRD [42]. Beyond facilitating acetylation sites on the same histone, the BRD can also enhance acetylation of the adjacent histone H3 within the same nucleosome and possibly within nearby nucleosomes [43]. In addition, the bromodomain of Gcn5 and H3K14 acetylation can promote chromatin remodeling by Switch/Sucrose non fermenting (SWI/SNF) and Remodels the Structure of Chromatin (RSC) complexes in which local hyperacetylation by SAGA marks promoters leading to subsequent recruitment of chromatin remodeling factors [39,44,45]. Interestingly, protein subunits that contain BRDs are common to most ATP-dependent chromatin remodeling complexes. SWI/SNF and RSC complexes contain subunits Swi2/Snf2 and Rsc4, respectively that harbor BRDs. Swi2/Snf2 has a single BRD and Rsc4 contains a tandem bromodomain (BRD1 and BRD2). The BRD of Swi2/Snf2 is needed for efficient remodeling of nucleosomes and displacement of SAGA from chromatin [46]. For Rsc4, Gcn5 HAT activity promotes its binding to H3K14 acetylated peptides via its BRD2 and inhibits Rsc4 binding to acetylated substrates via direct acetylation of Rsc4 at K25. Subsequently, BRD1 of Rsc4 binds to K25 acetylation, and this intramolecular interaction prevents BRD2 from binding acetylated histone substrates [39]. In a similar but distinct manner, Swi2/Snf2, the catalytic subunit of SWI/SNF complex, is also acetylated by Gcn5, which enables an intermolecular interaction between Swi2/Snf2's

bromodomain and its acetylated lysine residues that prevents or releases the bromodomain of Swi2/Snf2 from interacting with acetylated histone H3 [47]. Together, these regulatory intramolecular interactions would provide a temporal switch to release RSC and/or SWI/SNF complexes from the already remodeled chromatin so that chromatin remodeling complexes can be recycled for the next round of transcription.

Additional *cis*-histone crosstalk pathways were discovered using genome-wide chromatin immunoprecipitation (ChIP) and mass-spectrometry analyses. In these studies, histone H3K4 methylation and H3K14 acetylation were colocalized on the same genomic loci or on the same peptide when isolated from endogenous histones [48–50]. The importance and mechanism of the suggested *cis*-crosstalk between these two co-associated histone modifications was not realized until the discovery of histone H3K4 methyl readers that include chromodomain, plant homeodomain (PHD) fingers, and Tudor domains. In yeast, these readers include, Chd1 (chromodomain), Yng1 (PhD finger), and Sgf29 (tandem Tudor domain) [34,35,51–53]. Furthermore, each of these methyl-binding proteins is associated with a histone acetyltransferase complex where Yng1 is a subunit in the NuA3 complex, whereas Chd1 and Sgf29 are found associated with the SAGA complex [51,54]. Interestingly, both of these complexes utilize the same general mechanism in which the reader domains (PhD finger vs Tudor domain) recruit the HAT complexes to their histone methylated target genes to acetylate the histone H3 N-terminus [34,35,52,53]. In context of the HAT module, the tandem Tudor domains of Sgf29 also are required to stimulate processive multisite acetylation on H3K4 methylated nucleosomes [55]. Conversely, SAGA can also associate with the 5’ region of the ORF where histone acetylation appears to promote H3K4 methylation [56]. In this case, histone acetylation may directly stimulate Set1-mediated H3K4 methyltransferase activity, a function that has been observed for mammalian H3K4 methyltransferases MLL1 and MLL4 that prefer to methylate pre-acetylated substrates [57,58]. Alternatively, because Jhd2-mediated H3K4 demethylation is inhibited by histone H3 acetylation and prevents binding of Jhd2 to acetylated substrates ([59] and Briggs unpublished observations), acetylation by Gcn5 may also indirectly increase the steady state amount of H3K4 methylation on ORFs by preventing Jhd2 to recognize and demethylate its substrate. Overall, the HAT module is designed so that two distinct *cis*-histone crosstalk pathways enable local hyperacetylation of histone H3. In addition, these methyl-acetyl *cis*-histone crosstalk pathways appear to be bidirectional and may act as a feedback or feedforward loop to drive and control the histone PTM steps required for transcriptional initiation and elongation (see section 2.3).

2.2 DUB module and trans-histone crosstalk pathways

In 1980, West and Bonner first identified H2B ubiquitination in mouse histones. However, the function of H2B ubiquitination remained unknown until work performed by the Osley group in 2000 [60,61]. This ubiquitin-conjugating enzyme) in gene regulation and DNA repair to the mono-ubiquitylation of H2B at lysine 123 (H2Bub1). H2Bub1 is a highly conserved and dynamic modification that is localized to the promoters and bodies of actively transcribed genes. In addition to being required for proper tunable gene induction, H2Bub1 is necessary for the study critically linked the previously known functions of Rad6 (an E2

trans-histone crosstalk pathway of Set1/COMPASS-mediated H3K4 and Dot1-mediated H3K79 methylation [62–64].

Although the mechanistic basis of this histone crosstalk eluded discovery for nearly two decades, recent cryo-EM studies have defined the structural basis for how H2Bub1 directs the nucleosome binding and allosteric regulation of COMPASS and Dot1 methylation [65–68]. In the case of COMPASS, H2Bub1 prevents Set1 from making autoinhibitory contacts with deubiquitinated nucleosomes, thereby enabling Set1 to make key contacts with other COMPASS subunits so that histone H3K4 methylation can occur. In the case of Dot1, H2Bub1 binds and sequesters Dot1 in a more favorable orientation on the nucleosome for H3K79 methylation [65–68]. One striking difference between H2Bub1 and histone H3 methylation at K4 and K79 is their dynamics. H2Bub1 has a short half-life of ~1 min [69]. The H2B ubiquitin machinery (Rad6, Bre1 and Lge1) is associated with the Polymerase-Associated Factor 1 Complex (PAF1C) and the phosphorylated forms (serine 2 and 5) of the C-terminal domain (CTD) of RNAPII, which suggests that H2Bub1 is written and erased in a co-transcriptional manner during transcription elongation [70–75]. Unlike H2Bub1, however, H3K4 and H3K79 methylation are significantly more stable [69], and, in the case of H3K79me3, the modification is removed only passively via cell division [76]. These intriguing findings have helped to formulate the notion that, whereas H2Bub1 functions directly in transcription elongation, histone H3K4 and H3K79 methylation may function to generate transcriptional memory – perhaps to recruit or repel chromatin-associated proteins (e.g., ATP-dependent remodelers) that contain reader domains which maintain a chromatin state more amenable to further rounds of transcription [75,77].

Consistent with H2Bub1 functioning in transcription elongation, Chandrasekharan et al. and Batta et al. showed that deposition and removal of this chromatin mark correlated with the stability of nucleosomes genome-wide and at individual genes [78,79]. Although it was initially reasoned that the ubiquitin moiety on H2B serves as a “wedge” that “opens” chromatin domains during RNAPII elongation [80], high H2Bub1 levels are, in fact, associated with nucleosome assembly and/or stability, whereas low H2Bub1 levels lead to nucleosome loss. This finding is still poorly understood but may be due to the function of H2Bub1 in regulating the recruitment and/or activity of histone chaperones and/or ATP-dependent chromatin remodelers that contribute to the transient disruption of nucleosomes during transcription [81]. The precise mechanism for how H2Bub1 contributes to nucleosome stability is not well understood. However, the concept that H2Bub1 removal promotes nucleosome destabilization is consistent with the fact that SAGA, which stimulates transcriptional initiation and elongation, contains one of two major DUBs that remove this mark (i.e., Ubp8). The other major DUB involved in H2Bub1 removal is Ubp10, which functions independent of SAGA but in association with the FACT complex in gene bodies [82]. Although Ubp8 removes H2Bub1 more prominently at the 5’ ends of genes compared with 3’ ends, absence of Ubp8 greatly increases nucleosome assembly across the entire lengths of transcribed genes [79]. This finding is not only consistent with studies that showed SAGA within gene bodies, but it also implies that both DUBs contribute to the complete removal of H2Bub1 across genes undergoing transcription.

Within the SAGA complex, Ubp8, Sgf11, Sus1 and Sgf73 form a distinct DUB submodule responsible for H2Bub1 removal (Figure 2). Although the HAT and DUB modules are distinct structurally, they are in close proximity in cryo-EM analyses (Figure 3) [20,21]. Prior to binding the nucleosome, the DUB and HAT modules contact each another. However, the modules become displaced upon nucleosome binding, when the DUB module binds the nucleosomal H2A/H2B disk and the HAT module is more flexible and presumably free or accessible to acetylate histone H3. Detailed structural studies of the Ubp8 DUB module showed that an arginine cluster in the zinc finger of Sgf11 interacts directly with the H2A/H2B acidic patch, thereby stabilizing the DUB module on the nucleosomal disk [83]. In an earlier study, this zinc finger was proposed to also interact with DNA [84]. Additionally, Ubp8 makes contacts on H2B near the site of ubiquitin at K123 [83]. Although the cryo-EM structure of the Upb8 DUB module in isolation suggests that Ubp8 is bound to both sides of the nucleosomal disk, recent cryo-EM analyses of SAGA revealed that only one SAGA complex is likely to be bound to a single nucleosome [20].

An obvious expectation of having the HAT module in close proximity to the Ubp8-containing DUB module is that these two distinct activities are coupled events that enable transient passage of RNAPII during transcription. In this scenario, SAGA-dependent histone acetylation releases histone tails wrapped around DNA in the nucleosome to enable transient release of DNA and RNAPII passage. In parallel, Upb8 deubiquitylation may contribute to RNAPII passage by further facilitating nucleosome destabilization (likely in coordination with histone chaperones such as FACT). The combined activities would provide rapid and transient nucleosome destabilization needed for efficient RNAPII elongation through the nucleosome (Figure 4). Although it is widely assumed that nucleosomes are disassembled during transcription elongation, more recent cryo-EM studies that examined the passage of RNAPII through the nucleosome suggest that nucleosomes may be held together throughout elongation [85]. Additionally, in promoting transcription elongation, it may be that the key functions for histone chaperones and elongation factors (e.g., FACT, Spt6 and PAF) are to employ their histone-interacting acidic regions to maintain nucleosome integrity and decrease pausing during elongation [86]. In this model (see Figure 4), Ubp8-directed removal of H2Bub1, along with histone H3 acetylation by the HAT module in SAGA, may couple histone-DNA tail release activity with nucleosome destabilization to transiently facilitate RNAPII passage through the nucleosome. Following RNAPII passage, the PAF complex, Rad6/Bre1, Spt6 and Set2 (note that many of these factors are biochemically and genetically in the same pathway) may create nucleosome stabilization and re-association of histone tails with DNA and perhaps neighboring nucleosomes [77,87]. Although H2Bub1 is transient and may be removed rapidly, the deposition of histone H3K36 methylation by Set2 and the deacetylation “memory” that this pathway has would ensure long-term nucleosome integrity during cell growth.

Although the function of SAGA-mediated Ubp8 deubiquitylation during RNAPII elongation is still in need of investigation, there is a more defined activity of Ubp8 deubiquitylation at the 5’ ends of genes. Specifically, and during the transition from serine 5 phosphorylation to serine 2 phosphorylation of the CTD of RNAPII, H2Bub1 removal by Ubp8 is required for proper recruitment and function of the major serine 2 CTD kinase, Ctk1 [88]. This work not only defined a key activity of SAGA and deubiquitylation of H2Bub1 in the transition step

from transcription initiation to elongation but revealed how deubiquitylation at 5' ends is essential for establishing downstream activities in chromatin and transcription (Figure 4). It is likely that the function of Ubp8 at the 5' end to control Ctk1 activity is different from its nucleosome destabilizing activity that is performed across the transcribed regions of genes in coordination with RNAPII.

2.3. SAGA reader modules facilitate stable feedback loops that promote and create transcriptional memory

In addition to the function of SAGA-associated HAT and DUB modules in the control of nucleosome stability and histone PTMs during RNAPII elongation and nucleosome passage, multiple subunits of these two SAGA catalytic modules also "read" histones or specific histone PTMs (see Figure 3). This so called "read-write" capacity of chromatin-associated enzyme complexes like SAGA to read their own products and/or use embedded reader domains to engage the nucleosome in the correct context has become a common theme in chromatin biology. Below, we describe how SAGA histone reading, and writing, regulates its cellular functions and histone crosstalk abilities.

The BRD of Gcn5 binds to histone H3K14ac, and this association is critical for SAGA recruitment, chromatin retention, and HAT activity [40]. Additionally, and in the central SAGA module, Spt7 contains a BRD domain that, at least *in vitro*, can interact with histone H3K9ac [89]. These findings have reinforced the concept that SAGA reads its own product, thereby stabilizing itself on the nucleosome in a feed-forward loop that maintains robust HAT activity on that nucleosome or perhaps neighboring nucleosomes. This feed-forward circuit may be a critical rheostat in gene expression control, whereby high activator recruitment of SAGA promotes greater histone acetylation at the promoters to reinforce promoter escape and enhance transcription elongation by RNAPII.

Similar to histone H3 N-terminal acetylation that stabilizes or recruits SAGA by way of its BRDs, Sgf29 binding of histone H3K4 trimethylation (H3K4me3) via its tandem Tudor domain may further enhance and stabilize SAGA binding and activity on nucleosomes, specifically the +1 nucleosomes at gene promoters that are largely marked with H3K4me3 [34,55] (Figure 4). Perhaps, because Gcn5 exists at both promoters and in the transcribed regions of genes, H3K4me3 marked +1 nucleosomes may have a unique function on SAGA HAT activity at gene promoters that is not necessarily carried over to the gene bodies that lack high levels of H3K4me3. Thus, H3K4me3 may provide a robust feed-forward loop at promoters to ensure significant SAGA recruitment and histone acetylation that enables either RNAPII initiation or release into gene bodies. Finally, it is intriguing to consider how the crosstalk between H3K4me3 and H3 N-terminal acetylation may contribute to SAGA's feed-forward circuit and chromatin association. Specifically, the BRDs within SAGA promote further levels of histone H3 tail acetylation that should repel the H3K4me3-specific demethylase Jhd2 ([59], Briggs unpublished observation), thereby increasing H3K4me3 levels that increase still more SAGA binding by Sgf29. Thus, SAGA's read-write (and repel) capabilities likely have a critical function in SAGA's association with chromatin and likely help to direct histone modifications that control gene transcription. In addition, it appears that a feedback loop may exist wherein H3K4 methylation is stimulated by Gcn5-mediated

H3 acetylation [56]. This feedback loop may be a conserved function because histone acetylation appears to stimulate H3K4 methylation via *sc*Set1, MLL1, and MLL4 H3K4 methyltransferases as mentioned in section 2.1 [56–58].

Another potential feed-forward loop may exist to control histone H3 methylation by SAGA/Ubp8-mediated removal of H2Bub1. Because H2Bub1 regulates the *trans*-histone crosstalk methylation pathway, one could envision that SAGA-directed removal of H2Bub1 also acts to decrease the levels of H3K4 and H3K79 methylation. Although the potential for such regulation is possible, even low levels of H2Bub1 are sufficient to promote at least some or normal levels of H3K4 and H3K79 methylation [90]. Thus, although some H2Bub1 is needed for *trans*-histone methylation, the normal cycle of its removal by SAGA does not appear to contribute to reducing these histone methylation events.

Lastly, SAGA may have yet another putative histone interacting domain. Within the HAT module, the ADA2 subunit contains a conserved ZZ-SANT domain (Figure 3) that, in several human proteins was shown to interact with unmodified histone H3 tails [91]. Thus, this domain may read histone tails to further enhance SAGA recruitment or function on chromatin. The importance of this domain in SAGA biology awaits confirmation.

3. Future perspectives

Although we have learned about how SAGA contributes to transcription, there remain many unanswered questions and unsolved mysteries concerning how SAGA functions. Some of these issues include the functions of the different SAGA-like complexes, how histone acidic and basic patches contribute to SAGA activity, and how SAGA modification of non-histone targets contributes to transcriptional regulation.

The discovery and purification of multiple Gcn5-containing SAGA-like complexes has prompted many unanswered questions about their functions. In most cases, these noncanonical SAGA-like complexes share similarities to SAGA, but they also differ in subunit composition and function. For example, SALSA/SLIK possesses two major differences compared with SAGA. First, SALSA/SLIK contains an N-terminal truncated version of Spt7 [92,93]. Second, the Spt8 subunit in the SAGA complex is replaced by Rtg2 in the SALSA/SLIK complex, thereby linking SALSA/SLIK to the retrograde pathway [94]. Clarification is needed as to whether this altered complex is associated with gene bodies and is governed by the same crosstalk and regulation described for SAGA. The Berger group showed that SAGA and SALSA/SLIK differentially regulate gene transcription [95]; thus, the mechanisms by which SAGA and SALSA/SLIK contribute to this differential regulation may be due to the differences these complexes have in regulating the multiple crosstalk mechanisms discussed above. Additionally, Gcn5 was observed in yet even smaller subcomplexes called ADA and HAT-A2 [16]. These subcomplexes still possess the histone-reading subunit Sgf29; thus, it is likely that their functions and localizations across genes are also influenced by histone crosstalk and the histone PTM landscape.

Acidic and basic (charged) patches on histones appear to exert distinct but important activities in SAGA regulation. For example, we recently revealed the ability of the basic

patch in the H4 tail to restrict SAGA histone acetylation and H2B deubiquitylation [96]. Yet, how the basic patch contributes to this regulation is unknown, but it appears to be independent of the ability of the basic patch to control ATP-dependent remodelers or Dot1 [96,97]. An attractive hypothesis is that the basic patch contributes to chromatin accessibility by its interaction with the H4 tail, thereby limiting the DUB and HAT modules from interacting with nucleosomes. Another charged patch, the acidic patch of H2A/H2B, is critical for the interaction and function of the Upb8-containing DUB module of SAGA. An obvious question is how other chromatin regulators that require this same acidic patch (e.g., RSC) and that presumably work in coordination with SAGA can simultaneously use this same region of the nucleosome. Furthermore, it is notable that SAGA, and many of the other acidic-patch utilizing complexes [98], operates at the +1 nucleosome that is defined as having H2A.Z-containing nucleosomes that create a larger acidic patch [99,100]. The interesting question is how this unique +1 acidic patch regulates the activities of SAGA and other complexes.

An important regulatory mechanism for gene expression involves SAGA-mediated HAT activity via direct acetylation of non-histone subunits of RSC and SWI/SNF ATP-dependent chromatin remodeling complexes. As mentioned in section 2.1, Rsc4 and Snf2 are acetylated by Gcn5, which results in an intramolecular interaction with their respective bromodomains [39,47]. Could the BRD of the SAGA-associated subunits Spt7 or Gcn5 interact with a yet to be identified PTM on SAGA? Furthermore, this type of intramolecular interaction may exist for other chromatin-associated factors that have reader domains. For example, several proteins contain tandem Tudor domains, such as Sgf29, JMJD2A, FMR1, SND1, Rad9, and 53BP1 [34]. Although each of these proteins contains tandem Tudor domains classified in distinct subfamilies, it is possible that non-histone methylation provides mechanism distinct from, but analogous to the mechanism of non-histone acetylation-BRD intramolecular interactions. Interestingly, many readers also have single or double PhD domains that may be regulated not only by non-histone methylation but by non-histone acetylation because PhD fingers also bind to acetylated histone substrates [101]. Additionally, histone deacetylases and demethylases may target non-histone substrates to reverse the above-mentioned intramolecular interactions to recycle the “active” state for recognizing their chromatin substrates. The discovery of other key intramolecular interactions will explain how chromatin-associated factors are released or prevented from interacting with their substrates. These types of intramolecular switches could also contribute to feedforward and/or feedback loops that control gene expression and transcriptional memory. Overall, the questions raised here will likely be addressed in the coming years, thereby ensuring that the “SAGA” of understanding how gene expression is fundamentally regulated will continue to unfold and yield new insights.

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function. Finally, we dedicate our review in memory of Dr. Susan Abmayr and Dr. Craig Mizzen for their seminal contributions to the field.

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Highlights:

- Discusses advances in understanding SAGA regulation of transcription via histone post-translational modifications (PTMs).
- Discusses the functions of the HAT and DUB modules of SAGA in establishing, and regulating, histone PTMs.
- Discusses the activities of *cis*- and *trans*-histone crosstalk pathways and how reader domains promote feedforward and feedback loops.

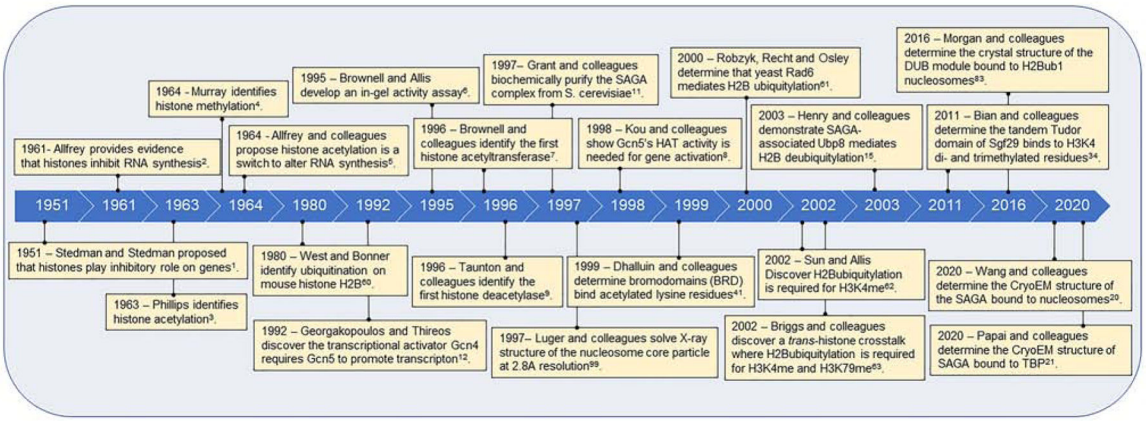


Figure 1. Histone acetylation and SAGA timeline.

Notable discoveries contributing to the current understanding of SAGA's function.

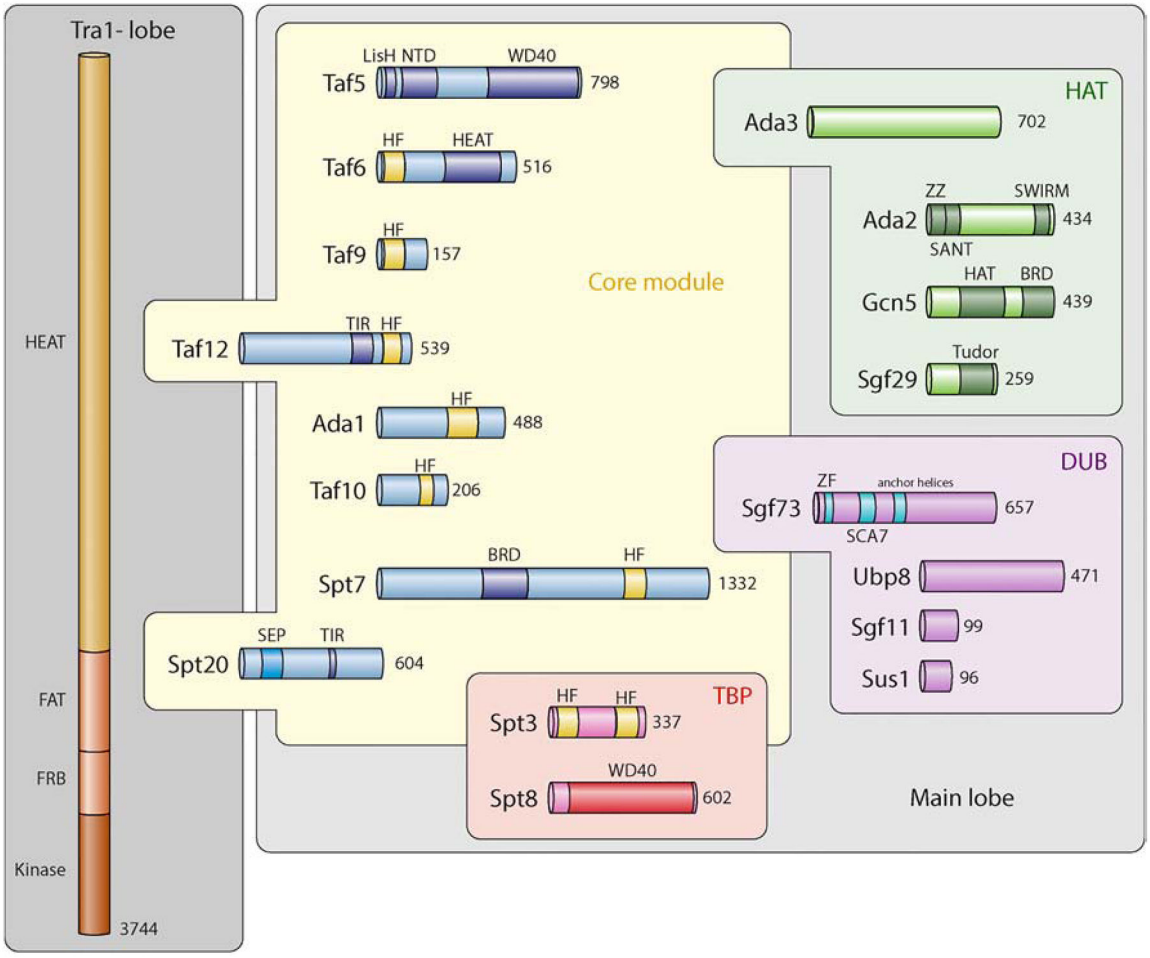
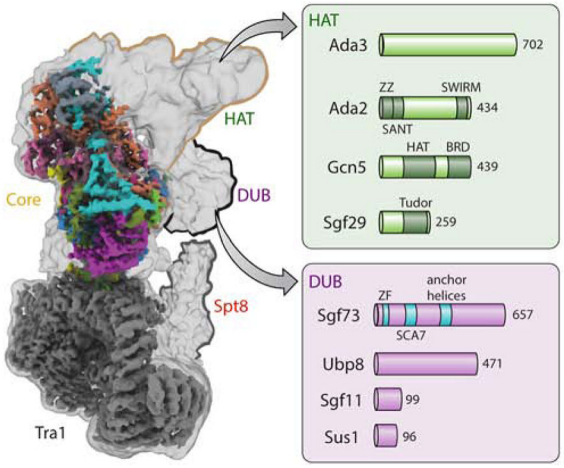


Figure 2. SAGA subunits and their domains.

Cartoon description showing the individual subunits of the SAGA complex with their functional domains highlighted. The distinct lobes containing various SAGA subunits are grouped together with subunits that connect the various lobes together intersecting. HEAT = Huntington, Elongation Factor 3, PR65/A, TOR; FAT = FRAP, ATM and TRRAP; FRB = FKBP12-rapamycin binding domain; TIR = Tra1-interacting; SEP = SEP domain; HF = histone fold domain; LisH = lis homology domain; BRD = bromodomain; WD40 = WD domain, G-beta repeat; ZF = Zinc Finger; SWIRM = SWIRM domain; HAT = histone acetyltransferase; Tudor = Tudor domain; ZZ = Zinc finger, ZZ type. Figure design is adapted from Papai et al. [21].



Modules and subunits	Chromatin reader and writer domains	Histone recognition function
Ada3	None	?
Ada2	ZZ, SANT	Histone binding?
Gcn5	HAT, BRD	Histone acetylation, H3K14ac binding
Sgf29	Tudor	H3K4me2/3 binding
Sgf73	ZF	H2A/H2B acid patch binding
Ubp8	H2B deubiquitination	H2Bub1 binding/removal
Sgf11	None	None
Sus1	?	Phospho-CTD interaction

Figure 3. HAT and DUB subunits within SAGA that contribute to chromatin engagement.

Left, Cryo-EM structure of the SAGA complex with HAT and DUB modules represented (from Wang et al. [20]. Arrows extending from the SAGA structure show the individual subunits of the HAT and DUB modules with their functional domains shown (see Figure 2). Right, Table highlighting the functional domains found in each DUB or HAT subunit with the documented histone or RNA polymerase II interactions made.

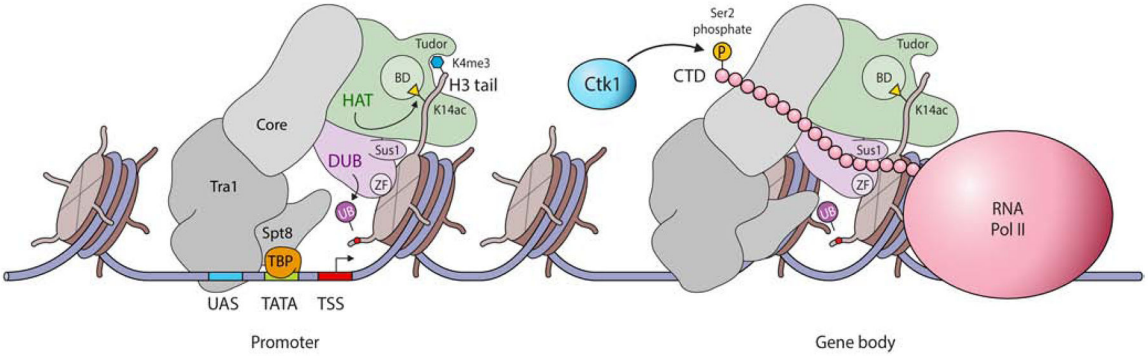


Figure 4. Transcription model for SAGA.

A cartoon model of the function of SAGA and its DUB and HAT modules in gene transcription. The left side shows the role of various subunits in mediating histone-binding and crosstalk at promoters, while the right-side highlights various activities of the SAGA complex during transcription elongation.