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Multiple faces of the SAGA complex

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Summary of Recent Advances

The SAGA complex provides a paradigm for multi-subunit histone modifying complexes. Although first characterized as a histone acetyltransferase, due to the Gcn5 subunit, SAGA is now known to contain a second activity, a histone deubiquitinase, as well as subunits important for interactions with transcriptional activators and the general transcription machinery. The functions of SAGA in transcriptional activation are well-established in *S. cerevisiae*. Recent studies in *S. pombe*, *Drosophila*, and mammalian systems reveal that SAGA also has important roles in transcript elongation, the regulation of protein stability, and telomere maintenance. These functions are essential for normal embryo development in flies and mice, and mutations or altered expression of SAGA subunits correlate with neurological disease and aggressive cancers in humans.

Introduction

Histone modifications, such as acetylation and ubiquitination, play a key role in facilitating a number of cellular events, including gene regulation. Acetylation of histones is largely associated with relaxing chromatin structure to support the entry of transcriptional machinery to genomic loci for activation, while histone ubiquitination has been linked to both gene activation and repression [1,2]. The histone modifying enzymes that catalyze these post-translational modifications are often integrated into large multi-subunit complexes to facilitate their enzymatic activity and substrate specificity. SAGA (Spt-Ada-Gcn5 acetyltransferase) is a 2MDa multi-protein chromatin modifying complex that is conserved between yeast and humans and harbors two known enzymatic modules that mediate the acetylation and deubiquitination of histones as well as non-histone substrates [3-5]. However, like many multi-subunit complexes, the functions of several components in the SAGA complex are still unknown. In addition, it is unclear exactly how the members of

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SAGA interact, coordinate and regulate the acetyltransferase and deubiquitinase activities of the complex, and whether the acetyltransferase and deubiquitinase (Dub) modules function in a cooperative or antagonistic manner to regulate cellular processes, such as gene regulation. Here we will discuss the most recent advances made toward deciphering how the catalytic activities of the SAGA complex function in development and disease.

The SAGA complex

The *Tetrahymena thermophila* protein, p55, was the first identified transcription related histone acetyltransferase (HAT) enzyme and is the ortholog of the yeast (y) transcriptional co-activator protein, Gcn5 (general control nonderepressible 5) [1]. Recombinant yGcn5 acetylates non-nucleosomal histone 3 (H3) lysine residue 14 (K14) [6,7]. However, the incorporation of yGcn5 into native multi-subunit complexes, such as ySAGA, expands its specificity to additional lysines in nucleosomal histones *in vitro*, including H3 K9, 14, 18, 23 and to a lesser degree histone H2B [7,8].

The ySAGA complex is modular in structure and has distinct functional units (Table 1) including a recruitment module (Tra1) [9,10]; acetylation module (Gcn5, Ada2, Ada3) [11-14]; TBP interaction unit (Spt3, Spt8) [13,15,16]; Dub module (Ubp8, Sus1, Sgf11 and Sgf73) [17-21]; and architecture unit (Spt7, Spt20, Ada1, TAF5, -6, -9 and -12) [8,13,22,23]. ySAGA is recruited to gene loci by the interaction of Tra1 with specific transcriptional activators [10], and the bromodomain of yGcn5 binds acetylated H3 and H4 N-terminal tails which potentiates cooperative nucleosome acetylation of histone H3 [24,25] (Figure 1A). The HAT module composed of yGcn5, yAda2 and yAda3 catalyzes this acetylation, opening up the chromatin landscape for binding of additional transcription factors and the pre-initiation complex (PIC) [14]. The ySpt3 subunit of ySAGA also recruits TBP to facilitate PIC formation and transcriptional activation (Figure 1B) [16]. In addition to promoting gene activation, a modified ySAGA complex, lacking ySpt8, accompanies Pol II during elongation and functions to acetylate and subsequently evict nucleosomes from gene coding regions (Figure 1 C, D) [26]. The Dub module of ySAGA, including Ubp8, Sus1 and Sgf11, also facilitates elongation through deubiquitination of H2B K123ub1 (Figure 1C), which allows for the recruitment of the Ctk1 kinase and subsequent Ser2 phosphorylation of the Pol II C-terminal domain (Figure 1D) [27]. ySAGA contributes to other aspects of gene expression as well. For example, SAGA-mediated histone acetylation functions in co-transcriptional spliceosome assembly and the recruitment of U2 snRNP to intron branchpoints [28], and SAGA-associated Sgf73 and Sus1 proteins link histone deubiquitination to the mRNA export machinery [29].

The components, modules and functions of the ySAGA are highly conserved across many species and act as a model for understanding the *Drosophila* (d) and human (h) SAGA complexes. The remainder of our discussion will focus on the enzymatic modules of these metazoan SAGA complexes and how their regulation is intimately linked to both development and disease.

The SAGA histone acetyltransferase module

Although the general compositions of dSAGA and hSAGA complexes are very similar to the ySAGA complex, some differences have been observed. The *GCN5* transcript is alternatively spliced in mouse and human cells, generating a short (GCN5S) isoform similar to yGcn5, and more predominantly expressed long (GCN5L) protein isoform that is incorporated into hSAGA and is highly analogous to the GCN5-related family member, PCAF [30,31]. This long GCN5 isoform is also conserved in *Drosophila* Gcn5 (dGcn5), suggesting an evolutionary gain of function in ancestral metazoans [32]. GCN5L (hereafter referred to as GCN5) contains both a HAT domain and a bromodomain similar to yGcn5,

but also has an extended N-terminal domain which confers the ability to acetylate mononucleosomal H3 as well as free H3 *in vitro* [30,33]. Metazoans also have two proteins similar to yAda2, ADA2A and ADA2B, which are found in distinct GCN5-containing complexes [33-36]. In particular, ADA2B is present in hSAGA and serves to bridge the interactions between GCN5 and ADA3 to facilitate acetylation of chromatin substrates [33]. Interestingly, both ADA2B and ADA3 may regulate distinct aspects of chromatin acetylation, as ADA2B-GCN5 dimeric complexes enhance the ability of GCN5 to acetylate mononucleosomes, whereas acetylation of polynucleosomes is strictly mediated by ADA2B-GCN5-ADA3 heterotrimeric complexes *in vitro* [33]. dAda2b and dAda3 also likely regulate some aspect of chromatin acetylation *in vivo*, as deletion of either dAda2b or dAda3 leads to decreased H3 K9, 14 acetylation levels on polytene chromosomes [37,38]. Furthermore, loss of Wda (a yTAF5 homolog) in *Drosophila* also reduces H3 K9 acetylation levels without compromising the integrity of the dSAGA complex, suggesting a role for Wda in chromatin acetylation [39]. Post-translational modifications identified on these and other subunits [40], as well as the acetylation of hADA3 by GCN5 [33], imply that PTMs may also play an important but unexplored role in regulating the functions of SAGA, including HAT activity.

As in yeast, hSAGA is recruited to specific target genes by activators, such as c-Myc, p53 and E2F, to stimulate the acetylation of histones and the activation of transcription [41-45].

While gene expression profiling for GCN5 has yet to be reported in mammalian cells, experiments in both budding (*S. cerevisiae*) and fission (*S. pombe*) yeast support the idea that GCN5 is a gene-specific co-activator, as yGcn5 only appears to regulate 4% and 1.1% of genes, respectively [46,47]. However, numerous genome-wide mapping studies of histone acetylation patterns demonstrate that yGcn5 globally acetylates H3 K9, 14 and that Gcn5 binds within the promoter and 5' coding regions of most highly transcribed genes in *S. cerevisiae* [48-52]. Additionally, genome-wide analysis of yGcn5 in *S. pombe* indicates that Gcn5 may have a general role in elongation, given that yGcn5 is found within the coding region of actively transcribed genes, and correlates with high H3 K14 acetylation levels [53]. Interestingly, hSAGA contains at least three subunits that have the ability to bind histone tails, including SGF29 which harbors a chromodomain, hSTAF65γ that displays a BTP domain (bromodomain transcription factors and PHD containing), and hADA2B that has a SANT domain (DNA binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIB). A role for these subunits in SAGA recruitment has not been reported; however, global Gcn5 mediated acetylation may be modulated by such domains. Collectively, these studies indicate that hSAGA functions both in gene specific and in global transcription events and that it can act at multiple steps in the transcription cycle.

The SAGA Deubiquitination module

Although first discovered and characterized as a HAT complex, SAGA was later discovered to harbor a second catalytic module centered on the Ubp8 (yeast) or USP22 (metazoan) deubiquitinases. As with GCN5, the Dub subunit may work most efficiently in the context of a module within SAGA that contains additional components [29]. In yeast, Sus1, Sgf11, and Ubp8 form a structural entity within the ySAGA holoenzyme, and the association of this Dub module with the rest of the complex is mediated by Sgf73 [29]. Sgf11 does not have a substantial role in maintaining the overall composition or integrity of SAGA but appears to mediate either recruitment or stable association of Ubp8 in ySAGA [20,54]. Sus1 is present in two functionally different protein complexes in both yeast and in flies, the SAGA complex where it regulates its deubiquitination activity through interactions with Ubp8 and Sgf11 and the TREX/AMEX complex, where it plays a role in mRNA export [55-57]. Such

dual functions of E(y) 2 (the *Drosophila* ortholog of Sus1) have implicated dSAGA in the positioning of specific gene loci (like hsp70) to the nuclear periphery, achieving a tightly regulated link between transcription and mRNA export under stress conditions [56]. hUSP22 deubiquitinates H2Bub, like the yeast ortholog, but can also deubiquitinate H2A *in vitro*, implicating its function in the regulation of Polycomb-mediated H2A ubiquitination [58,59].

Polyglutamine (polyQ) expansions in the N-terminal region of ATXN7 (the mammalian ortholog of Sgf73) are associated with spinocerebellar ataxia type 7 (SCA7), which is characterized both by motor coordination deficiencies (ataxia) and retinal defects. Mouse models of SCA7 indicate that both the wild type and mutant forms of ATXN7 integrate into the SAGA complex and do not affect SAGA composition. However the effects of polyQ ATXN7 on SAGA HAT activity or on SAGA-mediated gene activation are not clear. In one set of experiments, the acetyltransferase activity of the complex did not appear to be affected by transgenic polyQ ATXN7, even though chromatin immunoprecipitation experiments indicated aberrant SAGA recruitment and hyperacetylation of H3 K9, 14 on rod cell-specific gene promoters in the retinas of the transgenic mice. Surprisingly these hyperacetylation events correlated with decreased mRNA levels of the analyzed genes [60]. In contrast, two other studies which used either cultured cells or yeast expressing the polyQ forms of ATXN7/Sgf73 came to very different conclusions on the effects of the mutant protein on SAGA functions [61,62]. Reduced levels of acetylated H3 were observed on promoter-enhancer regions of photoreceptor genes, likely caused by impaired acetyltransferase activity of the complex in the presence of the polyQ expanded form of ATXN7 [62]. Neither of these studies addressed the effects of the polyQ allele on the Dub module. Clearly, much more work is needed to understand how the polyQ expansion affects SAGA recruitment and enzymatic activities.

The role of SAGA in development

Given that SAGA is conserved across many species and plays an essential role in regulating gene expression, it is perhaps not surprising that particular subunits of the SAGA HAT module control developmental processes. In *Drosophila*, loss of dGcn5 is lethal due to a lack of metamorphosis [63]. Furthermore, dAda2b and dAda3, which regulate H3 K9, 14 acetylation are also essential for *Drosophila* viability. In a similar manner, *Gcn5* is required for normal development in mice. *Gcn5* null (*Gcn5*^{-/-}) mice are embryonic lethal at 10.5 days post coitum (d.p.c.) and do not acquire normal mesoderm due to enhanced apoptosis in these tissues [64]. However, catalytically inactive *Gcn5* mice survive slightly longer (16.5 d.p.c.) and display both neural tube closure defects and exencephaly, implying that Gcn5 HAT activity is required for proper development and that SAGA also has HAT-independent functions [65]. Interestingly, *PCAF* null mice develop normally, but double homozygous null *Gcn5* and *PCAF* embryos die at 7.5 d.p.c. indicating a functional redundancy for these two HATs during embryonic development [64,66]. In cell culture, *Gcn5*^{-/-} mouse embryonic stem cells remain pluripotent and are capable of differentiation into all three germ layers [67]. The role of GCN5 in ES cells has yet to be defined, but is of considerable interest. H3 K56ac is linked to pluripotency of human embryonic stem cells and GCN5 acetylates H3 K56 on free histone *in vitro*, suggesting that GCN5 may play a role in maintaining stemness [68,69].

In addition to the HAT module regulating larval and embryonic development, the SAGA Dub module has been identified to control different aspects of neural development. This is observed by mutations in the *Drosophila* nonstop (USP22 ortholog) and dSgf11 proteins leading to defects in axonal targeting photoreceptors [70,71]. Furthermore, retinal defects

are also observed in the SCA7 neurodegenerative disease as discussed above, but the roles of USP22 or other Dub module subunits in development have not yet been defined.

The role of SAGA in cancer

The role of hSAGA in cancer is a good paradigm of its multi-functionality. For example, TRRAP and GCN5 regulate the oncogenic activity of the c-Myc oncoprotein and the p53 tumor suppressor [41]. Additional studies underscore that the whole SAGA complex may function in tumorigenesis. STAF65 γ (hSPT7) has a role in the transcription of several Myc-dependent genes [43]. Furthermore, SAGA recruitment may require SGF29, a poorly studied subunit of the complex, whose deregulated expression is implicated in malignant transformation [72]. In parallel, GCN5/PCAF acetylates non-histone proteins involved in transcriptional activation or repression, such as c-Myc [73].

The deubiquitination activity of the hSAGA complex has also been implicated in cancer, as USP22 has been grouped by transcriptional profiling into an 11-gene “signature” of poor prognosis in multiple types of cancer [59,74]. This signature also includes the PRC1 polycomb repressor complex subunit, Bmi1, a known regulator of self renewal in normal and malignant stem cells, suggesting USP22 may be a marker for cancer stem cells. Specifically, USP22 may be required for cell-cycle progression and could function with GCN5 to control certain cell cycle genes. GCN5 has been implicated in the transcriptional regulation of cyclin A, cyclin D3, PCNA and cdc25b, as well as CDK1 and cyclin B1 after DNA damage [75]. Furthermore, GCN5 acetylates the non-histone protein CDC6 to promote S-phase progression of the cell cycle. GCN5 and USP22 also protect telomeres from DNA damage response through the stabilization of a shelterin component called TRF1, and interestingly, this regulation is not transcriptional but involves USP22-mediated deubiquitination of TRF1 [76]. In addition, the Gcn5 related protein, PCAF, appears to regulate ubiquitination of Hdm2 through an intrinsic ubiquitination activity [77] and chk2 turnover [78], adding another level of regulation to an already complicated network.

Conclusions

SAGA is comprised of distinct modules that intimately collaborate to maintain structural integrity, genomic recruitment and interactions with the basal transcription machinery to localize the HAT and Dub activities of the complex to gene loci. Elegantly, SAGA mediates histone acetylation of gene promoters to enhance transcriptional activation and facilitates elongation by deubiquitinating histones downstream of Pol II and acetylating histones within the coding region to promote histone eviction (Figure 1). Functional HAT and Dub modules of the SAGA complex are central to normal mouse and *Drosophila* development. In addition, SAGA functions in cancer, as USP22 is part of an 11 gene “signature” associated with poor cancer prognosis and SAGA contributes to the activation of c-Myc responsive genes. However, many questions remain unanswered regarding the intricate regulation of this co-activator complex. Do SAGA components outside of the HAT and Dub modules either stimulate or suppress these enzymatic activities (Figure 1A)? What role do posttranslational modifications of the SAGA components play in complex regulation (Figure 1B)? Is SAGA-mediated histone acetylation required for Dub activity downstream of Pol II (Figure 1C), and do members of the HAT and Dub modules cross regulate each other (Figure 1C, D)? In addition to these questions, much remains to be elucidated about the biological function of SAGA. Little is known about the role of USP22 in mammalian development or the function of GCN5 and/or USP22 in embryonic stem cells. Furthermore, it is unclear how tightly the functions of GCN5 and USP22 are linked or whether the HAT and/or Dub module functions in SCA7 disease. Addressing these questions will undoubtedly lead to a clearer understanding for the roles of SAGA in development and disease.

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Glossary

Ada	adaptor
ATXN	ataxin
BrD	bromodomain
BTP	bromodomain transcription factors and PHD domain containing
CDK1	cyclin dependent kinase 1
Chk2	checkpoint kinase 2
ChrD	chromodomain
Ctk1	carboxy-terminal domain kinase 1
DEXDc	DEAD-like helicase
dE(y)2	<i>Drosophila</i> enhancer of yellow 2
Dub	deubiquitinase
EnY2	human ortholog of enhancer of yellow 2
FAT	FRAP/ATM/TRRAP domain
Gcn5	general control nonderepressible 5
HAT	histone acetyltransferase
Hdm2	human ortholog of mouse double minute 2 (mdm2)
LisH	Lis homology
nonstop	<i>Drosophila</i> analog of Ubp8
PCAF	p300/CBP-associated factor
PCNA	proliferating cell nuclear antigen
PI3Kc	phosphoinositide 3-kinase, catalytic domain
PIC	pre-initiation complex
Pol II	RNA polymerase II
PTM	post-translational modification
SAGA	Spt-Ada-Gcn5 acetyltransferase complex
SANT	Swi3/Ada2/N-CoR/TFIIIB domain
SCA7	spinocerebellar ataxia 7
Sgf	SAGA-associated factor
Spt	suppressor of Ty element
STAF	specificity protein transcription factor - associated factor
Sus1	S1 gene upstream of ySa1

SWIRM	Swi3/Rsc8/Moira domain
TAF	TATA binding protein-associated factor
TBP	TATA binding protein
TFIID	transcription factor II D
Tra1	yeast ortholog of human TRRAP
TREX/AMEX	transcription and export/mRNA and export
TRF1	telomeric-repeat binding factor 1
TRRAP	transformation/transcription domain-associated protein
Ubp8	ubiquitin specific processing protease
UBP-ZF	ubiquitin protease – zinc finger
UCH	ubiquitin carboxyl-terminal hydrolase
USP22	ubiquitin specific peptidase 22
WD40	40 amino acid protein domain often terminating in Trp-Asp
Wda	will decrease acetylation

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unique sites of protein acetylation and phosphorylation in ySAGA and TFIID were compared. Within the ySAGA complex, ySpt7 and ySgf73 were identified to be significantly acetylated, while ySpt7, yAda3, yTaf10, yTaf12 and ySgf73 were found to be phosphorylated.]

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evolutionary conservation of SAGA complex among species and implicating the *Drosophila* SAGA complex in the regulation of accurate axon guidance in the optic lobe of the fly.]

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Figure 1.

The enzymatic activities of SAGA facilitate transcription. Different modules of SAGA are required for various stages of gene activation. A. The recruitment module localizes the SAGA complex to target genes, via an activator protein, allowing the HAT module to acetylate neighboring histones. B. The TBP interaction module strengthens SAGA interactions with the transcriptional machinery and phosphorylation (P) of the Pol II CTD at Ser5 allows transcription to proceed. C. Upon initiation, SAGA is recruited to the Pol II machinery and assists in elongation, promoting further acetylation of histones within the coding region and the Dub module deubiquitinates histones H2B. D. Deubiquitinated histones allow the interaction of Ctk1 with H2B, which phosphorylates Ser2 of the Pol II

CTD to extend transcriptional elongation. Some of the questions remaining in this process include: A. Can other SAGA components (X) also contribute to HAT activity? B. Do PTMs (*) also regulate aspects of SAGA module interactions and activities? C. Does histone acetylation have any effect on SAGA Dub activity? C, D. Can the HAT module control Dub activity and vice versa?

Table 1

Components of the SAGA multi-subunit complexes in yeast, *Drosophila* and human. The protein structures of the SAGA orthologs are shown to scale. The legend denotes the domain names. The protein domains were determined with SMART (<http://smart.embl-heidelberg.de/>).

	Yeast (ySAGA)	<i>Drosophila</i> (dSAGA)	Human (hSAGA)
HAT	yGcn5	dGcn5	GCN5
BrD			
TFIID	ySpt3	dSpt3	SPT3
WD40			
ZnF	ySpt7	CG6506	STAF65 _y
SANT			
SWIRM			
Ada3			
LisH			
TAF			
SCOP	ySpt8		
FAT			
P13Kc			
Tudor-like	ySpt20		SPT20
UCH			
Sgf11			
SCA7	yAda1	dAda1	STAF42
EnY2			
ChrDc			
DEXDc			
Helicase	yAda2	dAda2B isoform-a dAda2B isoform-b	ADA2B
PCAF			
ZnF-UBP	yAda3		ADA3
BTP			
SUMO			
Ac			
Phos			
	yTaf5	dWda	TAF5L
	yTaf6		TAF6L
	yTaf9	dTaf9	TAF9
	yTaf10	dTaf10b	TAF10
	yTaf12	dTaf12	TAF12
	yTra1	dTra1	TRRAP
	ySgf29	CG30390	SGF29
	yUbp8	dNonstop	USP22
	ySgf11	dSgf11	ATXN7L3
	ySgf73		ATXN7
	ySus1	dE(y)2	ENY2
	yChd1		