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Insights into SAGA function during gene expression

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Histone modifications are a crucial source of epigenetic control. SAGA (Spt–Ada–Gcn5 acetyltransferase) is a chromatin-modifying complex that contains two distinct enzymatic activities, Gcn5 and Ubp8, through which it acetylates and deubiquitinates histone residues, respectively, thereby enforcing a pattern of modifications that is decisive in regulating gene expression. Here, I discuss the latest contributions to understanding the roles of the SAGA complex, highlighting the characterization of the SAGA-deubiquitination module, and emphasizing the functions newly ascribed to SAGA during transcription elongation and messenger-RNA export. These findings suggest that a crosstalk exists between chromatin remodelling, transcription and messenger-RNA export, which could constitute a checkpoint for accurate gene expression. I focus particularly on the new components of human SAGA, which was recently discovered and confirms the conservation of the SAGA complex throughout evolution.

Keywords: DUB; mRNA export; SAGA; SUS1; transcription

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See Glossary for abbreviations used in this article.

The SAGA complex

The transition from a compact and inactive chromatin state to open and active chromatin is a prerequisite for transcription. This is achieved through the post-translational modification of the amino-terminal tails of histones, which creates an appropriate epigenetic environment for gene expression (Suganuma & Workman, 2008). Histone acetylation is one of the best-studied post-translational modifications that has a role in this process. HATs have been isolated in organisms ranging from yeast to humans, several of which contain a Gcn5-related acetyltransferase (GNAT) catalytic subunit. In yeast, the GNAT family includes the Rtt109, SAGA, SLIK, Ada and HAT–A2 complexes. In humans, it comprises the STAGA, TFTC and PCAF complexes (Torok & Grant, 2004; reviewed in Nagy & Tora, 2007), which Pijnappel & Timmers (2008) renamed as human SAGA for simplicity. In addition, another complex known as ATAC, which also contains GCN5, has been recently identified in humans (Guelman *et al*, 2009; Wang *et al*, 2008).

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The yeast SAGA complex is composed of 21 widely conserved proteins that are organized into functional submodules (Table 1). Some subunits of the complex have been well established by both biochemical and genetic studies, such as Ada, Spt and a subset of TAFs, as reviewed by Daniel & Grant (2007). SAGA also comprises the product of the essential *tra1* gene, which has been proposed to interact with acidic activators—such as Gcn4—directly (Brown *et al*, 2001; Grant *et al*, 1998). In addition to these subunits, new yeast SAGA components have been discovered by biochemical approaches, including Ubp8 (Henry *et al*, 2003), Sgf11, Sgf29 and Sgf73 (Gavin *et al*, 2002; Helmlinger *et al*, 2004; Powell *et al*, 2004; Sanders *et al*, 2002) and Sus1 (Rodriguez-Navarro *et al*, 2004). Although our knowledge regarding the subunit composition of the yeast and human SAGA complexes has increased significantly, the process of understanding the contribution of each component to orchestrating gene expression in eukaryotes is ongoing. Here, I analyse new functions of SAGA during transcription, and the identification of new components of the human and *Drosophila* SAGA complexes.

The SAGA deubiquitination submodule

Several studies—reviewed by Weake & Workman (2008)—have shown that Lys123 of histone (H) 2B, which is located at its carboxy terminus, is ubiquitinated. This ubiquitination is essential for the *trans*-tail methylation of H3, and is also required for optimal gene activation. The first evidence that yeast SAGA could deubiquitinate H2B came from the Berger and Grant laboratories (Daniel *et al*, 2004; Henry *et al*, 2003), which showed that the SAGA-associated protein Ubp8 is required for this dubiquitination. Furthermore, Ubp8 is recruited to the *gal1* promoter and its deletion decreases the transcription of SAGA-responsive genes. Yeast Ubp8 is also a part of the SLIK complex and its activity is required to differentially regulate H3 methylation at some SAGA promoters. In 2005, the Berger and Workman laboratories identified another protein, Sgf11, as part of the SAGA deubiquitination module (DUBm; Ingvarsdottir *et al*, 2005; Lee *et al*, 2005). Sgf11 is an 11 kDa protein that contains a highly conserved zinc-finger domain, Zn-Sgf11, at its N-terminal end (Zhao *et al*, 2008). Berger and Workman reported that Sgf11 and Ubp8 are necessary to deubiquitinate H2B, and constitute a structural and/or functional submodule within SAGA. In addition, my laboratory reported that the small yeast SAGA subunit Sus1 forms a stable trimeric complex with Sgf11 and Ubp8 as part of this functional DUBm in SAGA, and is also required to deubiquitinate H2B and methylate H3 on Lys4 (Fig 1; Kohler *et al*, 2006). As Sus1 is important for mRNA export, my group proposed that the DUBm could link SAGA-dependent transcription and nuclear mRNA export (see below).

Glossary

Ada	adaptor
ARG1	arginine-requiring 1
ATAC	Ada Two-A-containing
ATXN	ataxin
BMI1	B-cell-specific Moloney murine leukaemia virus-integration site 1
Cdc31	cell division cycle 31
CTD	carboxy-terminal domain of Rpb1
Ctk1	carboxy-terminal domain kinase 1
DUBm	deubiquitination module
E(y)2	enhancer of yellow 2 *Drosophila*
ENY2	human homologue of enhancer of yellow 2
FACT	facilitates chromatin transcription
GAL1	galactose metabolism 1
GCN5	general control nonderepressible 5
GNAT	general control nonderepressible 5-related acetyltransferase
HAT	histone acetyltransferase
Mex67	mRNA export protein 67
mRNA	messenger RNA
mRNP	messenger ribonucleoprotein
PCAF	p300/CBP-associated factor
RING	really interesting new gene
Ris1/Uls1	role in silencing 1/ubiquitin ligase for SUMO conjugates protein 1
RNAPII	RNA polymerase II
RNF2	RING finger protein 2
Rpb1/RPO21	RNA polymerase II largest subunit B220
Rtt109	regulator of Ty1 transposition
Sac3	suppressor of actin 3
SAGA	Spt–Ada–Gcn5 acetyltransferase
SCA7	spinocerebellar ataxia type 7
Sgf	SAGA-associated factor
SLIK	SAGA like
Spt	suppressor of Ty element
STAGA	SPT3–TAF9–GCN5/PCAF
Su(Hw)	suppressor of Hairy wing
Sus1	Sl gene upstream of ySa1
TAF	TATA-binding protein-associated factor
TAP	tandem-affinity purification
TBP	TATA binding protein
TFTC	TAF-containing complex
TRA1	similar to human TRAAP
TREX2/THSC	Sac3–Thp1–Cdc31–Sus1 complex
Ubc	ubiquitin conjugating
UBP	ubiquitin protease
Ubp8	ubiquitin-specific processing protease 8
USP22	ubiquitin-specific peptidase 22
Yra1	yeast RNA annealing protein 1

Table 1 | SAGA subunits in yeast, human and *Drosophila*

SAGA	Yeast	Human	*Drosophila*
	Gcn5 (*ada4*)	GCN5/*PCAF*	Gcn5
Ada	Ada1	ADA1/*STAF42*	Ada1
	Ada2	ADA2b	Ada2b
	Ada3	ADA3	Ada3
Spt	Spt8	ND	ND
	Spt20 (*ada5*)	SPT20/*FAM48A*/p38IP	ND
	Spt7	SPT7/*STAF65γ*	CG6506
	Spt3	SPT3	Spt3
Taf	Taf5	TAF5L	Wda
	Taf6	TAF6L	ND
	Taf9	TAF9/*TAF9b*	Taf9
	Taf10	TAF10	Taf10
	Taf12	TAF12	Taf12
	Tra1	TRRAP	Tra1
	Sgf29	SGF29 (STAF36)	CG30390
DUBm	Ubp8	USP22	Nonstop
	Sgf11	ATXN7L3	Sgf11 (CG13379)
	Sus1	ENY2	e(y)2
	Sgf73	ATXN7	ND
	Chd1	ND	ND
	Rtg2	ND	ND

The names of homologous proteins encoded by paralogous genes, which can both be present in the complex, are separated by a forward slash. Alternative names for the same protein are shown in parenthesis. ND, not defined.

Work from several groups has recently identified higher eukaryote orthologues for yeast Ubp8, Sgf11 and Sus1 (Table 1); for example, *Drosophila* Nonstop and human USP22 have been identified as yeast Ubp8 orthologues. Weake and colleagues showed that Nonstop associates with *Drosophila* SAGA (dSAGA) and is required for accurate axon guidance in the optic lobe (Weake *et al*, 2008), thereby implicating dSAGA in the regulation of pathways essential for neural development. In addition, Nonstop and dSgf11 were proposed to function as an enhancer of position-effect variegation (Zhao *et al*, 2008). A recent study from the McMahon laboratory identified USP22 as a subunit of human SAGA (hSAGA) and, notably, as a component of an 11-gene signature associated with poor prognosis of diverse types of cancer (Zhang *et al*, 2008). Together with previous results (Kurabe *et al*, 2007; Liu *et al*, 2003), these findings support the idea that hSAGA is linked mechanistically to human cancer. Therefore, USP22 could regulate tumorigenesis by inducing histone modifications, as do the polycomb/cancer stem-cell signature proteins RNF2/RING1b and BMI1. Although USP22 is a component of hSAGA, the fact that it is the only SAGA subunit that has so far been found in an oncogenic signature suggests that this effect could be independent of SAGA (see below).

The *Drosophila* Sus1 orthologue, e(y)2, was initially cloned as a putative transcription factor and a component of a large multiprotein complex containing dTaf9 (Georgieva *et al*, 2001). As is the case for yeast Sus1, e(y)2 and ENY2 (which is the human homologue of yeast Sus1) are part of dSAGA and hSAGA, respectively (Kurshakova *et al*, 2007a; Zhao *et al*, 2008). Furthermore, ENY2 is required as a cofactor for the full transcriptional activity of nuclear receptors, and e(y)2 is essential for the barrier activity of Su(Hw)-dependent insulators (Kurshakova *et al*, 2007b; Zhao *et al*, 2008). In addition, there is an e(y)2 paralogue in *Drosophila* that has a testis-specific expression pattern (Krasnov *et al*, 2005), suggesting that it might have a gonad-specific function.

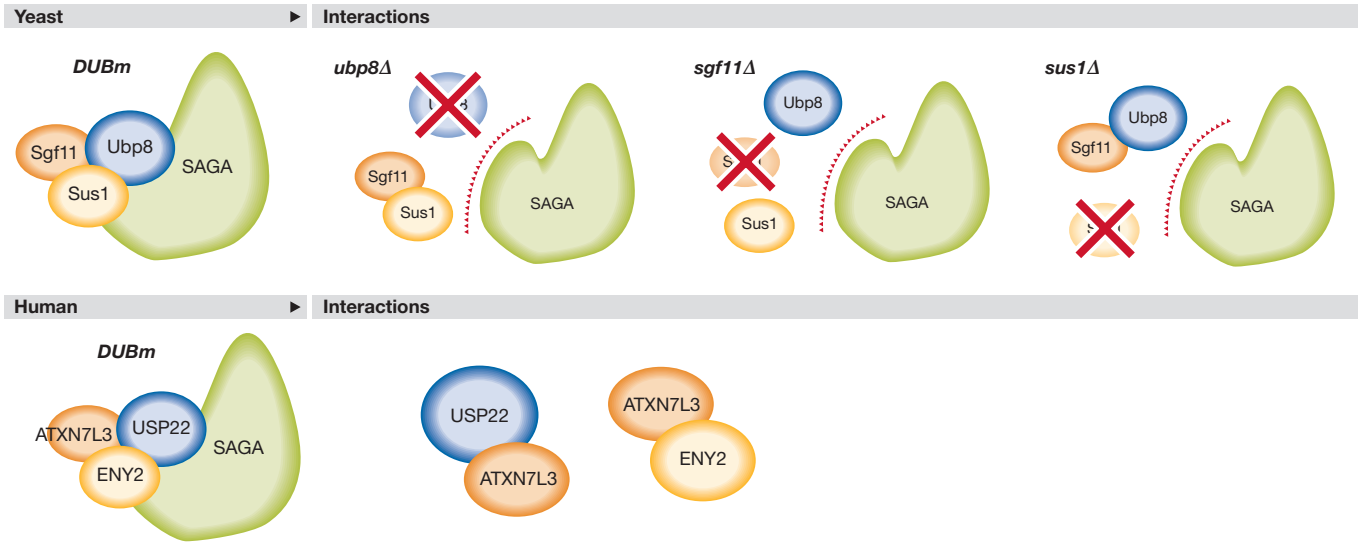

Fig 1 | The SAGA deubiquitination module is conserved from yeast to humans. (**A**) The yeast DUBm is composed of Ubp8, Sgf11 and Sus1. The elimination of *ubp8*, *sgf11* or *sus1* induces a pair-wise loss of the other members of the module and impairs SAGA association. (**B**) The human DUBm contains USP22, ATXN7L3 and ENY2. A direct interaction between ATXN7L3–USP22 and ATXN7L3–ENY2 has been shown in human cells. ATXN7L3, ataxin 7-like 3; DUBm, deubiquitination module; ENY2, human homologue of enhancer of yellow 2; SAGA, Spt–Ada–Gcn5 acetyltransferase; Sgf11, SAGA-associated factor 11; Sus1, Sl gene upstream of ySa1; Ubp8, ubiquitin-specific processing protease 8; USP22, ubiquitin-specific peptidase 22.

My group has shown that Sus1 and Sgf11 interact in the absence of Ubp8, whereas no association between Sus1 and Ubp8 was detected in the absence of Sgf11 (Fig 1; Kohler *et al*, 2006), and these results were subsequently confirmed (Lee *et al*, 2009). The Devys and Tora groups have recently shown the existence of the DUBm—composed of USP22, ATXN7L3 and ENY2—in human cells (Fig 1; Zhao *et al*, 2008). After immunoprecipitation of the different subunits, they observed interactions between ATXN7L3 and USP22, and between ATXN7L3 and ENY2; furthermore, the coexpression of these three proteins led to the formation of a stable complex. Recent work have also shown an association between ATXN7L3 and USP22 in the absence of human SPT20, although the authors did not assess the presence of ENY2 in this complex (Nagy *et al*, 2009). Altogether, these data point to a strong conservation throughout evolution not only of the SAGA protein composition but also of its modularity.

Despite these new findings, many questions remain unanswered and will undoubtedly be the focus of future studies (Sidebar A). The identification of the proteins that constitute the DUBm provides an opportunity to study the molecular environment in which this complex is active in more detail. Surprisingly, recombinant Ubp8 is inactive in isolation (Lee *et al*, 2005, 2009), and its ZnF-UBP domain—which is required for its binding to SAGA—is unable to bind to free ubiquitin (Ingvarsdottir *et al*, 2005). Therefore, the regulation of Ubp8 activity might involve conformational changes promoted by its binding to other partners, probably other DUBm subunits (Bonnet *et al*, 2008). Notably, by using multidimensional protein-identification technology analysis, my group found that a significant amount of ubiquitin is associated with Sus1 in a Sus1–TAP purification, suggesting a more direct role for Sus1 in this process (P. Pascual-Garcia & S.R.-N., unpublished data). H2B is subject to ubiquitination/deubiquitination cycles that are essential for optimal gene activation; however, most studies have analysed total histone modifications or particular modifications at some specific genes. Undoubtedly, a system-wide analysis, together with strong bioinformatic processing of the data, will lead to a better understanding of how these histone modifications are coordinated and how the chromatin environment has an impact on their function. How the chromatin context alters DUBm function remains to be seen.

Sgf73: the new piece of the DUBm puzzle

The identification of the DUBm raised many questions, such as how it is anchored to SAGA and which proteins mediate the DUBm–SAGA interaction. Sgf73 was initially identified as a yeast SAGA-interacting factor through a proteomic approach (Sanders *et al*, 2002), and was later shown to be the functional homologue of human ATXN7 (McMahon *et al*, 2005). Sgf73 interacts with upstream activiating sequences *in vivo*, thereby facilitating assembly of the transcription preinitiation complex (Shukla *et al*, 2006). In mammals, the Devys group showed that ATXN7 was also an integral component of hSAGA (Helmlinger *et al*, 2004), and Zhao and co-workers recently found that the hDUBm interacts with TAF5L and ATXN7, and proposed that the two proteins could mediate its association with the hSAGA complex (Fig 2; Zhao *et al*, 2008).

The ZnF-Sgf11 zinc-finger domain is poorly conserved between the ATXN7/Sgf73 and ATXN7L3/Sgf11 families. However, this low sequence identity suggested a link between Sgf73 and DUBm, which was confirmed by several studies showing that ySgf73 anchors the DUBm to the ySAGA complex and is further supported by the lack of copurification of Sus1–TAP or Ubp8–TAP with SAGA in *sgf73*-knockout cells (Fig 2; Kohler *et al*, 2008; Lee *et al*, 2009; Pascual-Garcia *et al*, 2008). Moreover, the Hurt and Workman groups showed that Sgf73 stimulates deubiquitinated H2B, and that both Sgf73 and the DUBm are necessary for full Upb8

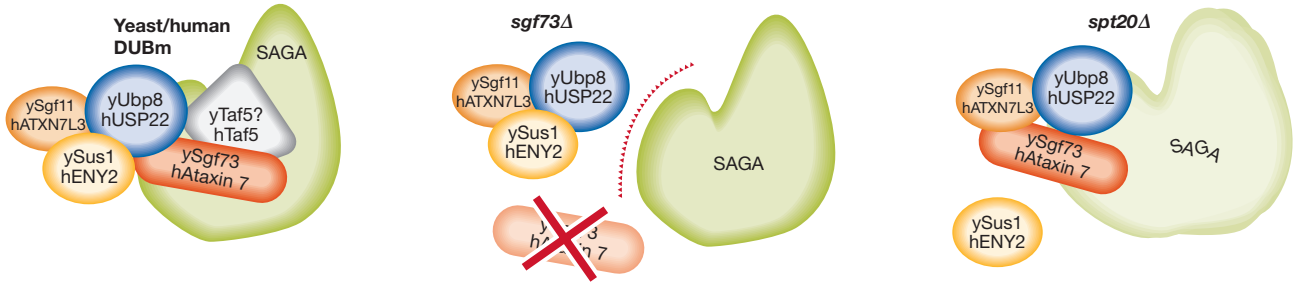

Fig 2 | ATXN7/Sgf73 anchors the SAGA deubiquitination module to SAGA. A loss of Sgf73 prevents DUBm–SAGA association. Ubp8, Sgf11 and Sgf73 were found to interact in DUBm purified from cells lacking *spt20*, which is required for SAGA stability and recruitment to promoters. In these cells, Sus1 is not associated with the submodule, indicating that SAGA has a role in DUBm assembly. ATXN7, ataxin 7; DUBm, deubiquitination module; ENY2, human homologue of enhancer of yellow 2; SAGA, Spt–Ada–Gcn5 acetyltransferase; Sgf, SAGA-associated factor; *spt*, suppressor of Ty element; Sus1, Sl gene upstream of ySa1; Taf5, TATA-binding protein-associated factor 5; Ubp8, ubiquitin-specific processing protease 8; USP22, ubiquitin-specific peptidase 22.

Sidebar A | In need of answers

(i) Is the SAGA protein puzzle solved?
(ii) Is SAGA the only way for the DUBm to contact chromatin?
(iii) Can the DUBm act independently of the SAGA complex?
(iv) Is the ySus1 that is involved in DUB independent of SAGA?
(v) How is Sgf73 linked to TREX2 and mRNA export?
(vi) Do histone modifications affect mRNA export?
(vii) Which proteins are required to activate the protease activity of Ubp8?

protease activity. In conclusion, mutations in Sgf73 result in the loss or release of the complete DUBm from SAGA, whereas the deletion of *ubp8*, *sgf11* or *sus1* partly affects the binding of the other members of the module to SAGA. Notably, the Sgf73–Ubp8–Sgf11 complex that can be purified from cells lacking *spt20*—which is required for SAGA stability—does not contain Sus1 and is unable to deubiquitinate H2B *in vitro* (Fig 2; Lee *et al*, 2009). At least two models could explain these results: Sus1 could be essential for the activity of the DUBm, although the other three subunits—Sgf73, Ubp8 and Sgf11—are able to interact physically in its absence; or, the presence of SAGA could be required to activate and/or assemble the DUBm *in vivo*, although it is dispensable for its activity *in vitro*. The fact that the reconstitution of the tetrameric DUBm complex *in vitro* required the coexpression of Sus1 and Sgf11 leaves this question unanswered. In any case, it seems that the incorporation of Sus1 into the DUBm requires an intact SAGA complex. Interestingly, an analysis of the evolutionary conservation patterns of DUBm components showed that Sgf11 and Sgf73 are restricted to the eukaryotic crown group, whereas Sus1 is found in kinetoplastids and parabasalids. This indicates that Sus1 was present in the last eukaryotic common ancestor and implies that it is the most conserved subunit of the DUBm (Venancio *et al*, 2009). Furthermore, Sus1 has significant functional links to two E2 ligases—Ubc11 and Ubc4—as well as to the E3 ligase Ris1/Uls1, which implies that, in addition to being a subunit of the DUBm, it might also function as a common adaptor for both chromatin protein ubiquitination and deubiquitination.

Remarkably, the fact that Ubp8, Sgf11 and Sus1 can interact *in vivo* in the absence of Sgf73 suggests that the DUBm could have an alternative role that is independent of SAGA; indeed, a trimeric Ubp8–Sgf11–Sus1 complex can be dissociated from SAGA under certain conditions, lending support to this idea (Kohler *et al*, 2006). Furthermore, yeast cells that lack SAGA—owing to the deletion of *spt20*—accumulate less ubiquitinated H2B than cells lacking Ubp8 (Henry *et al*, 2003), which suggests that residual Ubp8 DUB activity might exist in SAGA-deficient cells. Noticeably, Nagy and colleagues found significant differences between the recruitment kinetics of the DUBm and of other hSAGA subunits to the promoters of stress-responsive genes in human cells (Nagy *et al*, 2009), highlighting the possibility that the DUBm has an alternative, SAGA-independent function. Otherwise, different proteins could target the DUBm to specific promoters when SAGA is deleted. The fact that yeast UBPs are promiscuous enzymes increases the possibility of alternative functions of the DUBm in specific cellular environments; whether these possible alternative mechanisms act to regulate the levels of ubiquitinated H2B depending on the chromatin environment in eukaryotes is still unknown. Along these lines, two recent studies significantly advance our understanding of the context-dependent functions of ubiquitinated H2B in transcriptional regulation (Minsky *et al*, 2008; Shema *et al*, 2008). Shema and co-workers propose a role for the deregulation of H2B ubiquitination in cancer development, as ubiquitinated H2B increases the expression of growth-restricting genes while restraining the expression of growth-promoting genes. In addition, Minsky and colleagues suggest that H2B ubiquitination is intimately linked with global transcriptional elongation in mammalian cells. This raises several questions: Is the human DUBm involved in this process? Does it discriminate between these different sets of genes? Providing answers will require an in-depth study of the molecular function of the DUBm within SAGA and independently of SAGA.

SAGA acts during transcription elongation

Efficient elongation on chromatin involves the removal of the physical barrier imposed by nucleosomes to the transcribing RNAPII (Fuchs *et al*, 2009). Work performed in many laboratories supports the idea that the ubiquitination of H2B occurs cotranscriptionally and might

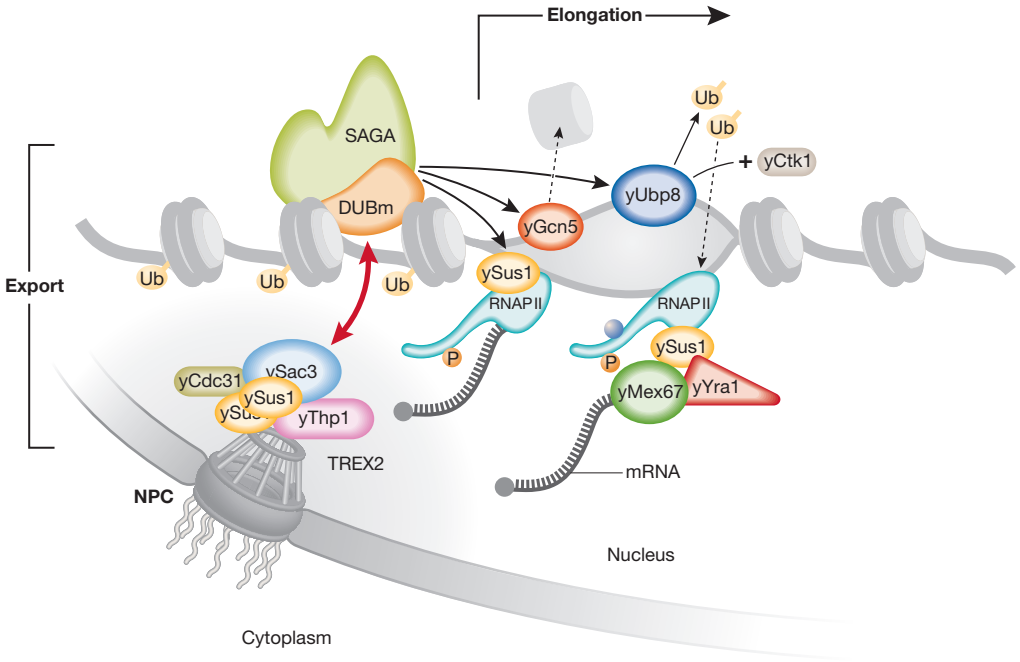

Fig 3 | Different SAGA subunits are involved in transcription elongation. The coordination of SAGA enzymatic activities helps transcription through coding regions. Among other SAGA components, Gcn5 and Ubp8 are recruited to coding regions to modify histones, which, in turn, facilitate transcription elongation. Gcn5 promotes nucleosome eviction and Ubp8 activity contributes to Ctk1 recruitment. Sus1 enters the coding regions and interacts physically with phosphorylated RNAPII and the mRNA-export factors Yra1 and Mex67 cotranscriptionally, ensuring an optimal coupling between transcription elongation and mRNA export. Cdc31, cell division cycle 31; Ctk1, carboxy-terminal domain kinase 1; DUBm, deubiquitination module; Gcn5, general control nonderepressible 5; Mex67, messenger RNA export protein 67; NPC, nuclear pore complex; RNAPII, RNA polymerase II; Sac3, suppressor of actin 3; SAGA, Spt–Ada–Gcn5 acetyltransferase; Sus1, Sl gene upstream of ySa1; Thp1, Tho2/Hpr1 phenotype; TREX2, transcription export 2; Ub, ubiquitin; Ubp8, ubiquitin-specific processing protease 8; Yra1, yeast RNA annealing protein 1.

directly stimulate transcription elongation (Laribee *et al*, 2007). The Osley group has recently shown that the stable association between FACT and transcribed chromatin depends on the ability to form ubiquitinated H2B, which has a role in nucleosome dynamics during elongation (Fleming *et al*, 2008). In addition, strong evidence from the Hinnebusch and Berger groups supports a role for the SAGA complex during transcription elongation. Yeast SAGA has been shown not only to be present at upstream activating sequences but also to localize to the coding sequences cotranscriptionally, and histone acetylation by Gcn5 promotes nucleosome eviction, thereby enhancing the processivity of RNAPII during elongation (Govind *et al*, 2007). The association of SAGA with coding sequences is dependent on phosphorylation of the CTD of Rpb1—the largest subunit of RNAPII—on Ser 5, indicating that SAGA or some of its subunits might interact with actively transcribing RNAPII during elongation. Furthermore, Wyce and colleagues showed that ubiquitination of H2B acts as a barrier for the association of Ctk1 with the coding regions of active genes, whereas subsequent deubiquitination by Ubp8 triggers Ctk1 recruitment and, therefore, productive elongation (Wyce *et al*, 2007). In addition, we have recently shown that Sus1—similar to other SAGA subunits—is strongly associated with the *ARG1* gene during transcription (Pascual-Garcia *et al*, 2008), and that it requires both the SAGA complex and the TREX2 complex (also known as THSC) to be efficiently targeted to open reading frames. Sus1 can be copurified with the elongating form of RNAPII, and also with the mRNA-export factors Yra1 and Mex67, which are recruited to the open reading frame cotranscriptionally. Therefore, Sus1 probably interacts with these factors on coding regions during transcription elongation and, consequently, could be required for gene transcription depending on the length of the mRNA.

Overall, these data suggest a mechanism by which the presence of SAGA in transcribing sequences facilitates the remodelling of the chromatin at these regions, increasing the accessibility for the elongation machinery (Fig 3). Sliding from the promoter, SAGA would be delivered to open reading frames in a transcription-dependent manner. At coding regions, several SAGA subunits can modulate the elongation process through acetylation (by Gcn5), deubiquitination (by Ubp8) and interaction with mRNA-export factors (through Sus1). Once within the coding region, Gcn5 activity promotes nucleosome eviction facilitating RNAPII displacement, and H2B Ubp8-dependent deubiquitination is necessary to recruit Ctk1, which, in turn, facilitates effective elongation. A pool of Sus1 also enters the gene with the aid of SAGA, where it contributes to Ubp8-mediated deubiquitination. Sus1 could also interact with downstream mRNA-export factors cotranscriptionally, thereby ensuring a perfect coupling between transcription elongation and

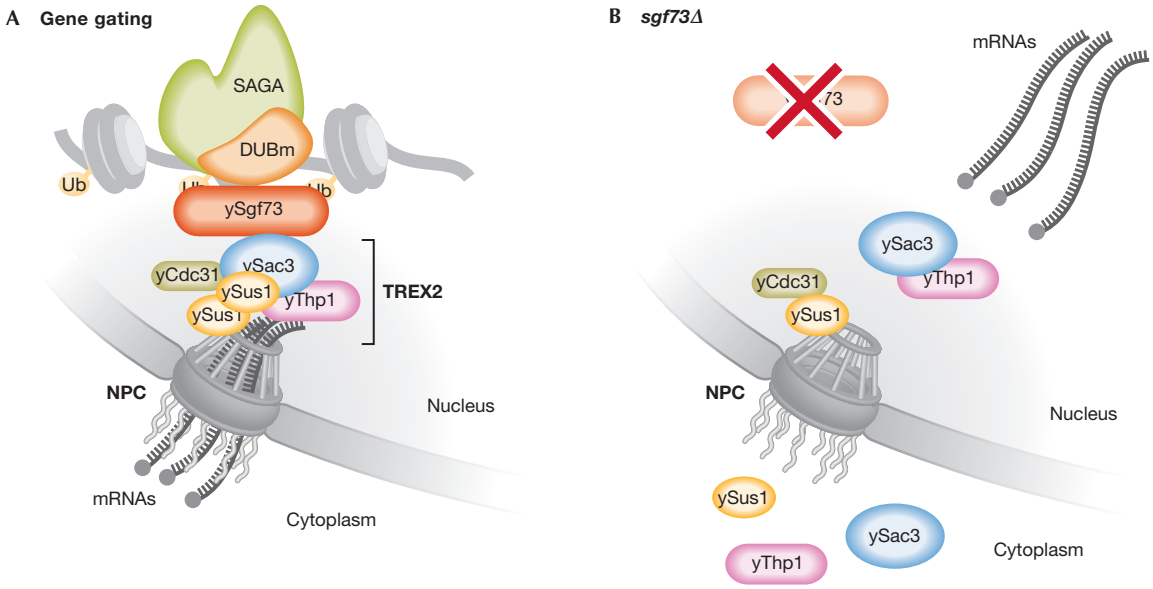

Fig 4 | SAGA is involved in messenger RNA export and gene gating. (**A**) Deletion studies have shown that the middle domain of Sgf73 is required for SAGA binding and to preserve the integrity of TREX2. Through its bifunctional protein design, Sgf73 could contribute to creating a link to the export of specific mRNA transcripts across the NPC. (**B**) Deletion of *sgf73* causes the partial disassembly of the TREX2 complex, and the mislocalization of Sus1, Sac3 and Thp1 away from the NPC and in the cytoplasm. In this mutant, *gal1* transcripts are unable to leave the nucleus. Cdc31, cell division cycle 31; DUBm, deubiquitination module; *GAL1*, galactose metabolism 1; NPC, nuclear-pore complex; Sac3, suppressor of actin 3; SAGA, Spt–Ada–Gcn5 acetyltransferase; Sgf, SAGA-associated factor; Sus1, Sl gene upstream of ySa1; Thp1, Tho2/Hpr1 phenotype; TREX2, transcription export 2; Ub, ubiquitin.

mRNA export. Interestingly, the DUBm subunit Sgf73 has a crucial role in loading Sus1 onto coding sequences and its absence also leads to elongation defects (Pascual-Garcia *et al*, 2008). What is the role of the DUBm at coding regions? Analysing the roles of the DUBm when it is located at promoters or coding regions constitutes a challenging task for future analysis.

Last step: SAGA links transcription to mRNA export

The transport of mRNA out of the nucleus is an essential step, which is necessary to accomplish gene expression. During their synthesis, mRNAs are packaged into mRNPs and exported through the nuclear pore complex (NPC; Iglesias & Stutz, 2008). Several studies have established links between the factors involved in transcription and mRNA export. Strong evidence that these processes are coupled was provided by the identification of yeast Sus1 as a subunit of both the SAGA complex and the TREX2 complex (Rodriguez-Navarro *et al*, 2004); the latter is associated with the NPC, and acts in mRNA export and transcription elongation (Pascual-Garcia & Rodriguez-Navarro, 2009). Notably, the function of TREX2 is conserved, as a homologous complex—known as AMEX—has been recently characterized in *Drosophila* (Kurshakova *et al*, 2007a). Sus1 participates in mRNA export and mediates the repositioning of active gene loci to the nuclear periphery (Cabal *et al*, 2006), and *Drosophila* e(y)2 interacts with the NPC and regulates mRNA transport by anchoring a subset of transcription sites to the nuclear periphery (Kurshakova *et al*, 2007a). The interactions of Sus1 with both SAGA and TREX2 have been recently characterized using a mutational approach (Klockner *et al*, 2009), and the uncoupling of the association of Sus1 with either complex was shown to result in a selective functional impairment of transcription-coupled mRNA export. These findings suggest that, although Sus1 might bind to SAGA and TREX2 differently, certain requirements are common to its association with both complexes. Moreover, the structure of Sus1 bound to the TREX2 subunits Sac3 and Cdc31 has been recently solved, reinforcing its role in TREX2 assembly (Jani *et al*, 2009). Sus1 and Cdc31 synergistically promote the association of TREX2 to the NPC and mRNA nuclear export. Sus1 has an articulated helical hairpin fold that facilitates its wrapping around Sac3. Whether Sus1 also adopts this structure when binding to SAGA is still unknown.

Several lines of evidence point to a role of SAGA in mRNA export. The removal of Sgf11 enhances the mRNA-export defect observed in *sus1*-knockout cells (Kohler *et al*, 2006), indicating that the function of SAGA in mRNA export is not limited to Sus1. Furthermore, new strong correlations between SAGA and mRNA export were established in 2008; unexpectedly, the loss of the SAGA subunit Sgf73 partly disrupted the association between Sus1 and TREX2, and elicited a partial mislocalization of Sus1 to the cytoplasm (Kohler *et al*, 2008; Pascual-Garcia *et al*, 2008). These results raised several questions in the field: How is Sgf73 linked to TREX2 and mRNA export? Does it have a direct role in this process? Sgf73 is necessary for the export of the *GAL1* transcript, and has been proposed to alter the structure of Sac3 to allow efficient TREX2 assembly (Fig 4A). However, the export defects associated with the lack of Sgf73 could be a mere consequence of Sus1 mislocalization, which would lead to a partial disassembly of TREX2 from the NPC, thereby leading to export defects

(Fig 4B). Whether other SAGA subunits—different from Sus1—have a direct role in mRNA export is unknown. Answering this question will require studies of the precise mechanism of mRNA export at the molecular level. Although Sgf73 is not believed to exist free, ATXN7 has been the only SAGA subunit reported so far to shuttle from the nucleus to the cytoplasm (Taylor *et al*, 2006). Is this shuttling important for ATXN7 function? Is it connected to mRNA export? A provocative speculation is that ATXN7, in complex with other proteins, is transported through the NPC together with the mRNAs transcribed from SAGA-dependent genes. Whether ENY2 and ATXN7 are necessary for the coupling of mRNA export to transcription and nuclear dynamics in human cells is still unknown. Therefore, further work should address their contribution to linking promoter-bound activators and the mRNA export machinery physically.

Concluding remarks

Here, I have highlighted the identification of new components of the human SAGA complex, showing that yeast, *Drosophila* and human SAGA are functionally and structurally equivalent entities. In addition, recently discovered roles of the complex have been described, emphasizing the broad contribution made by the SAGA complex to the entire process of gene expression. Whether an epigenetic mark—for example, H2B ubiquitination—controls mRNA export in eukaryotes is an exciting issue that should be explored in the future.

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