The role of SAGA in the transcription and export of mRNA

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SAGA/TFTC, which is a histone acetyltransferase complex, plays an important role in the regulation of transcription. We have identified that the metazoan TFTC/STAGA complexes had histone H2A and H2B deubiquitinase activity that is carried out by a DUBm (deubiquitination module). We studied the DUBm of SAGA in Drosophila melanogaster and identified Drosophila homologs of yeast DUBm components. Two subunits of DUBm (Sus1/ENY2 and Sgf11) were shown to have functions separate from DUBm function. Thus, Sus1/ENY2 was shown to be present in several different complexes. Sgf11 was found to be associated with the cap-binding module (CBC) and recruited onto growing messenger ribonucleic acid (mRNA). Also, we have shown that Sgf11 interacted with the TREX-2/AMEX mRNA export complex and was essential for mRNA export from the nucleus. Immunostaining of the polytene chromosomes of Drosophila larvae revealed that Sgf11 is present at the sites of localization of snRNA genes. It was also found in immunostaining experiments that dPbp45, the subunit of the PBP complex, the key player in the snRNA transcription process, is associated not only with the snRNA gene localization sites, but with other sites of active transcription by PolII. We also revealed that Sgf11 was present at many active transcription sites in interbands and puffs on polytene chromosomes. Sgf11 was localized at all Brf1 (the component of the RNA polymerase III basal transcription complex) sites. We concluded that SAGA coactivated transcription of both the PolII and PolIII-dependent snRNA genes. Key words: transcription; SAGA; DUB module; AMEX; Sgf11; ENY2; snRNA genes; PolII-dependent transcription; PolIII-dependent transcription.


Роль SAGA комплекса в транскрипции и экспорте мРНК

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Introduction

A large number of coactivator complexes are organized into transcription systems to provide accurate and precise functioning of the RNA polymerase II (RNAP II) machine. Coactivators are multisubunits complexes that are recruited to chromatin to promote transcription initiation by direct interaction with general transcription factors (GTFs) or RNAPII. They also may act indirectly through modification of chromatin structure (Li et al., 2007).

The yeast SAGA histone acetyltransferase (HAT) complex and its Drosophila and human homologs STAGA (the SPT3/TAF9/GCN5 acetyltransferase complex)/TFTC (the TBP-free...
TFTC/STAGA complex has a modular organization that links the deubiquitination function to HAT and DUBm

We analyzed a highly purified human TFTC fraction by MS-MS mass spectrometry and identified three additional subunits of TFTC/STAGA (USP22, ATXN7L3 and ENY2). Human USP22 is the homolog of yeast Ubp8; human ATXN7L3 is the homolog of the yeast Sgf11 protein; and ENY2, a 101 amino acid protein, is homologous to yeast Sus1. We showed that the ubiquitin protase USP22 together with ATXN7L3 and ENY2 forms a deubiquitination module (DUBm). Also, we revealed that two different TFTC/STAGA subunits, TAF5L and ATXN7, interact with this module and may mediate its association with TFTC/STAGA (Zhao et al., 2008).

Then, we studied the DUBm of SAGA in *Drosophila melanogaster*. *Drosophila* homologs of yeast DUBm components have been identified. Nonstop, Sgf11 and ENY2 are homologous to yeast Ubp8, Sgf11 and Sus1, respectively, and were shown to be components of SAGA (Weake et al., 2008). *Drosophila* Nonstop and Sgf11 have a role in H2B deubiquitination (Weake et al., 2008). A putative *Drosophila* ortholog of yeast Sgf73 was also identified (Weake et al., 2009). However, the existence of an integrated DUBm in *Drosophila* has not been shown. We demonstrated that endogenous *Drosophila* ENY2, Sgf11 and Nonstop form an integrated DUBm associated with SAGA.

First, we raised antibodies against Sgf11 and Nonstop (subunits of DUBm) in rabbits, antibodies against the ENY2 component were described previously (Georgieva et al., 2001) and performed immunoprecipitation experiments. Antibodies against Sgf11 co-precipitated Nonstop from the nuclear extract of *Drosophila* embryos. Moreover, antibodies against either Sgf11 or Nonstop co-immunoprecipitated not only ENY2, but also the Gcn5 subunit of SAGA (Fig. 1). Thus, we confirmed the existence of a SAGA-associated DUBm in *Drosophila melanogaster*. It should be noted that a certain amount of Sgf11 remained in the extract after treatment with antibodies against Nonstop or ENY2. Hence Sgf11 also may perform their function in a complex other than SAGA DUBm.

Our previous data showed that the SAGA complex participates in the transcription of the *Drosophila* heat-shock protein 70 (hs70) gene during heat shock (Lebedeva et al., 2005). So, we analyzed the function of Sgf11 in gene expression using the hs70 gene model. The antibodies against Sgf11 strongly stained hs70 puffs on *Drosophila* larval polytene chromosomes after heat shock, indicating that Sgf11 participates in the transcription of hs70. Then we used a chromatin immunoprecipitation (ChIP) assay to study the occupancy of the hs70 promoter by Sgf11 and other DUBm subunits before and after gene activation. Sgf11 was detected on the hs70 promoter, its association with the promoter turned out to be RNA-dependent unlike that of ENY2 or Nonstop.

Earlier we demonstrated an interaction between E(y)2/ENY2 and the nuclear pore complex (NPC) and showed that SAGA/TFTC also contacts the NPC at the nuclear periphery. E(y)2/ENY2 also forms a complex with the X-linked male sterile 2 (Xmas-2) protein to regulate mRNA export from nucleus to cytoplasm both in normal conditions and after heat shock. This complex was named AMEX/TREX2 (Fig. 2). Importantly, E(y)2/ENY2 and Xmas-2 knockdown decreased the contact between the hs70 gene loci and the nuclear envelope before and after activation and interfered with transcription.

Based on these data, we performed RNA-IP experiments and found that...
Sgf11 was associated with hsp70 mRNA and with mRNAs of two other genes (Ras2 and tubulin). Antibodies against Sgf11 immunoprecipitated mRNA even more efficiently than did antibodies against Xmas-2 or ENY2 (Fig. 3). We have also found that Sgf11 interacts with the AMEX/TREX2 complex and similarly to Xmas-2 and ENY2 co-localizes with NPC. We demonstrated that knockdown of Sgf11 disrupted mRNA export of both hsp70 mRNA and the total mRNA (Fig. 4). Therefore, Sgf11 interaction with AMEX/TREX-2 may play an important role in general mRNA export (Gurskiy et al., 2012).

**Fig. 3.** Sgf11 is associated with mRNAs of several genes.

*a* – RIP experiments with hsp70 mRNA after heat shock were performed using antibodies against Sgf11 or components of the mRNA-interacting AMEX complex (ENY2, Xmas-2); nonimmune IgG was used as control. The results are shown as a percentage of input; b – Sgf11 binds to mRNAs of ras and tubulin genes under normal conditions. The U1 snRNA was used as a control. Antibodies used in RIP experiments were the same as in Fig. 3, a. The results are shown as a percentage of input (adapted from Gurskiy et al., 2012).

**Fig. 4.** RNAi knockdown of Sgf11 interferes with general mRNA export.

*a* – RNAi knockdown of Sgf11, but not Nonstop, interferes with general mRNA export. Cells were treated with GFP dsRNA (control) or dsRNA corresponding to Sgf11 and Nonstop. Xmas-2 RNAi knockdown was performed as a positive control. Representative examples of the distribution of mRNA (green staining) and cell nuclei (red staining) and the corresponding merged images are shown for control cells and cells after Sgf11 or Nonstop knockdown (magnification, ×1000). RNA FISH was carried out using a Cy3-labeled oligo(dT) probe to identify poly(A)+ RNA. The nuclei were stained blue with DAPI. The images were recolored in Photoshop for better visualization.

*b* – quantitative presentation of the results of experiments shown in Fig. 4, a. Bars show the percentage of cells with disturbed hsp70 mRNA nuclear export (about 200 cells per RNAi experiment were examined) (adapted from Gurskiy et al., 2012).

**Fig. 5.** Sgf11 is associated with Cbp80 in a separate complex.

*a* – co-immunoprecipitation experiments with nuclear extract of *Drosophila* embryos reveal no interactions between Cbp80 and ENY2 or Nonstop DUBm components.

*b* – recombinant Sgf11 interact with Cbp80 but not with Cbp20. FLAG-tagged Sgf11 was co-expressed with HA-tagged Cbp80 or HA-tagged Cbp20 in transiently transfected S2 cells. Immunoprecipitation was performed with anti-FLAG or anti-HA antibodies or with IgG. The Western blot was stained with anti-FLAG or anti-HA antibodies. About 10 % of the input and 50 % of the precipitate were loaded onto the gel (adapted from Gurskiy et al., 2012).
SAGA is present at snRNA genes and interacts with Pbp45

Mapping the binding sites for Sgf11 component of the SAGA complex on the polytene chromosomes of Drosophila larvae by immunostaining revealed that Sgf11 is present at the sites of localization of snRNA genes (Gurskiy et al., 2012). To verify this result, we performed double immunostaining of polytene chromosomes from the salivary glands of Drosophila using antibodies against Sgf11 and Pbp45, the subunit of the PBP complex, the key player in the snRNA transcription process. It was found that Pbp45 can be found not only at the snRNA loci but also at other actively transcribed sites (in puffs and interbands). Similar data had previously been obtained by the whole genome sequencing of hSNAPC1, the human Pbp45 homolog (Baillat et al., 2012). Our findings are the first indication that similar to hSNAPC1, dPbp45 is associated in addition to the snRNA gene localization sites, with other sites of active transcription by PolIII.

Sgf11 and Pbp45 colocalized at many actively transcribed genes on polytene chromosomes, including those sites where U1–U6 snRNA genes are located. To confirm that the SAGA complex is indeed present at the promoters of the snRNA genes, we performed ChIP with antibodies against the components of SAGA (Sgf11, ENY2 and Gcn5) and Pbp45. All tested proteins were detected at the promoter regions of snRNA genes: at promoters of the genes transcribed by RNA polymerase II (snRNA U1 and snRNA U2) and at promoters of the U6 snRNA genes which are transcribed by RNA polymerase III.

To determine whether the subunits in the SAGA complex physically interact with the snRNA gene transcription apparatus, we co-immunoprecipitated the components of SAGA modules: the HAT module (Gcn5 and Ada2b factors), the DUB module (Sgf11, ENY2, and Nonstop) and TRRAP, with the antibodies against the Pbp45 protein from the nuclear extract of the S2 cells. Results obtained showed that protein components of both the HAT and DUB modules interact with Pbp45, and thus, with the PBP complex. Therefore, SAGA proteins are not only present at the promoter regions of snRNA genes, but they also interact with the snRNA transcription machinery. Moreover, SAGA participates in snRNA gene transcription guided by both PolIII and PolII.

To detect SAGA at the RNA polymerase III-transcribed genes, we analyzed colocalization of Sgf11 and Brf1 (the component of the RNA polymerase III basal transcription complex) on polytene chromosomes. In contrast to Sgf11, which is present at many active transcription sites in interbands and puffs, Brf1 was found in a relatively low number of sites of the PolIII-transcribed genes. However, the immunostaining experiments revealed that Sgf11 was present at all Brf1 sites. In particular, they colocalize at the loci corresponding to U6 and tRNA genes, as well as other RNA polymerase III target genes.

Recently, a ChIP-seq analysis of Drosophila embryos has shown that Ada2b, Nonstop, and Sgf11 occupied promoter regions of snRNA genes (Li et al., 2017). Our results obtained for Drosophila support these data and we have demonstrated that SAGA participates in the transcription of snRNAs. We have also shown that the SAGA complex participates in the transcription of other PolIII target genes: the U6 snRNA genes.

Chromatin immuno precipitation using antibodies against the components of the SAGA complex and Brf1 confirmed that SAGA is present not only at the promoters of individual U6 snRNA genes, but also at the promoters of other genes (RNase MRP and tRNA Lys) transcribed by RNA polymerase III (Fig. 6, a–c). Additionally, we have demonstrated that the protein components of both the HAT module and the DUB module interact with Brf1. All the tested SAGA subunits interacted with Brf1 in the nuclear extract. Therefore, the SAGA complex is present at the promoters of the RNA polymerase III-transcribed genes and interacts with the RNA polymerase III transcription factors.

The effect of mutations in the genes encoding SAGA complex subunits on snRNA transcription

Next, we checked whether mutations of subunits of the SAGA complex could change snRNA gene transcription in flies. The effect of mutations in genes encoding SAGA components on snRNA transcription in Drosophila showed that it is indeed the case. First, we showed that all studied factors were present on the promoters of snRNA genes in flies (chromatin was prepared from Drosophila...
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H2B monoubiquitylation. The antibodies against nonmodified H2B and against monoubiquitylated H2B (H2BK120Ub) were used. The data represent the ratio of H2BK120Ub to nonmodified H2B, verified by ChIP in the control and mutant strains. The results obtained demonstrated that H2B monoubiquitylation increased in all mutated flies comparatively to the control.

Conclusion

Recently, X. Li et al. (2017) investigating SAGA-independent properties of the DUB module, detected by a ChIP-seq assay an occupancy of snRNA promoter regions by Sgf11, Spt3, and Ada2b subunits. Our results are in agreement with the data that the DUB module has both SAGA-related and independent functions. In summary, it can be argued that the SAGA complex in metazoans is widely involved in the regulation of gene transcription (Popova et al., 2018).

Our model suggests that the SAGA complex is involved both in PolII and PolIII transcription, while some subunits of the complex participate in subsequent transcription steps (Fig. 7).

References


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