

Ph.D. thesis summary

**The role of dSAGA specific histone acetylation in
regulation of gene expression**

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Introduction

Considerable and dynamic compaction of the genome is essential for normal function of eukaryotic cells. This is carried out by the chromatin structure, in which 147 base pairs of DNA is wound around a core of four basic histone proteins (H2A, H2B, H3, H4) to form the basic building blocks, the nucleosomes. Individual nucleosomes can pack against each other and adopt several higher-order chromatin structures which are thought to be the major obstacles to transcription, replication and repair. Therefore, compaction level and dynamic changing of chromatin packaging can possess a major regulatory role in gene expression regulation. Posttranslational modifications of histone proteins are one of the various known mechanisms altering the accessibility of the chromatin template. Among several described modifications acetylation of lysine residues is one of the most studied ones. Histone acetylation is a major determinant of chromatin compaction: hyperacetylation state is coupled to an open conformation, whereas hypoacetylated nucleosomes are characteristic for a compact, folded chromatin. Hyperacetylated, open chromatin has long been linked to active transcription; however, the exact details of its regulatory effect have not yet been established. Considerable knowledge provided from characterization of histone acetyltransferases (HAT) can contribute to better understanding the regulatory role of histone acetylation. One of the well characterized HAT enzymes is Gcn5, which functions as a subunit of large multiprotein complexes. These Gcn5-containing HAT complexes harbour ADA-type adaptor proteins, which regulate the template recognition and lysine specificity of Gcn5. dADA2b protein is a subunit of the acetylation modul of dSAGA, one of the two different *Drosophila* Gcn5-containing HAT complexes, that determines the histone H3 lysine 9 and 14 acetylation specificity of the complex. Diverse functions of ADA2 proteins are demonstrated by the fact *dAda2b* gene produces two mRNAs and protein forms. The N-terminal parts of dADA2b isoforms are identical; these shared parts harbor a zinc finger-like ZZ and a SANT domain, characteristic for all known ADA2 proteins. The isoforms differ in their C-terminal regions, which

contain ADA2 protein specific α -helical motifs so-called ADA boxes. The short isoform (dADA2bS) of dADA2b harbors two ADA boxes, while a third ADA box is present only in the longer isoform (dADA2bL). If these ADA boxes could contribute to the capability of ADA2 proteins to interact with other subunits of the complex, we hypothesized that the dADA2b isoforms varying in the number of the ADA boxes could function in different regulatory processes.

In my thesis I examined the presumed role of dSAGA specific histone acetylation in gene regulation by utilizing a *dAda2b*⁸⁴² deletion eliminating complete dADA2b function and dADA2bS isoform expressing transgenic strains.

Aims

1. Which genes are regulated by dSAGA specific acetylation in late L3 larval and early pupal stages?

- Transcriptome analysis of *dAda2b* mutant late L3 larvae and early pupae

2. How can the two isoforms of dSAGA specific dADA2b subunit contribute to the gene regulatory role of dSAGA?

- Investigation of the expression pattern of dADA2b isoforms and the function of the short dADA2b isoform

3. Whether does the dSAGA complex and dSAGA specific H3K9 acetylation directly regulate the expression of dSAGA-dependent genes?

- Determination of dADA2b binding and the level of dSAGA specific H3K9 acetylation on different regions of dSAGA-dependent and dSAGA-independent genes

4. What can be the function of dSAGA specific H3K9 acetylation in the process of gene activation?

- Determination of dSAGA specific H3K9 acetylation pattern and its effect on transcription during the activation of *Eip74EF* and *Eip75B* ecdysone-induced genes

Methods

- Transcriptome analysis of *dAda2b*⁸⁴² mutant and dADA2bS isoform expressing *Drosophila* larvae by DNA microarray
- Characterization of different dADA2b isoform expressing *Drosophila* strains
- Detection of H3K9ac level by immunohistochemistry on polytene chromosomes from salivary gland
- Detection of H3K9 acetylation level and expression of dADA2b proteins by Western blot
- Detection of apoptosis in *Drosophila* larval imaginal discs by acridine orange staining
- Determination of H3K9 acetylation level on several genomic regions by chromatin immunoprecipitation
- Measurement of gene expression by quantitative real-time PCR

RESULTS

1. Genes are regulated by the dSAGA acetyltransferase complex

We determined the genes regulated by dSAGA specific acetylation in well defined developmental stages. For this purpose, we performed transcriptome analysis on wild type and *dAda2b* mutant animals in two developmental stages (late L3 larvae and early pupa). By comparing the mRNA profiles we determined genes showing altered expression level in the absence of dADA2b. At both time points analyzed only a small subset of genes (5 %) were regulated by dSAGA complex. Despite the supposed role of histone acetylation in transcription activation, more than half of the dSAGA-dependent genes showed increased transcription levels in the absence of dSAGA specific acetylation. In both analyzed developmental stages the dSAGA-regulated genes showed similar distribution according to biological function. The significant enrichment of the upregulated genes in gene ontology categories involved immune response, might suggest the potential role of dADA2b protein in stress response.

2. Role of short isoform of dADA2b subunit in dSAGA functions

Previous studies revealed that *dAda2b* gene gave rise to two mRNAs and protein isoforms (dADA2bS and dADA2bL). Therefore, we examined whether these two dSAGA specific dADA2b subunits could contribute to the functional complexity of dSAGA in transcription regulation. By western blot analysis we demonstrated that the dADA2b protein isoforms corresponding to the above mentioned mRNA forms could be detected during *Drosophila* development in various amount in different stages. Therefore we studied the potential of the short isoform to occupy the *dAda2b* function. We concluded that the dADA2b short isoform alone can partially provide the *dAda2b* function both in development, in acetylation activity and in the regulation of apoptotic processes. Taken together, the

two dADA2b isoforms differ only in their C-terminus however functionally they are not equivalent. The partial functional rescue effect of dADA2bS can be explained by the fact the dADA2bS-containing dSAGA complex regulates the transcription of only the down- or only the upregulated dSAGA-dependent genes. In order to answer this question we performed transcriptome analysis on larvae expressing only the short dADA2b isoform in *dAda2b* mutant background. We concluded that the dADA2bS-containing dSAGA complex could contribute to the regulation of both the increased and the decreased ones of dSAGA-regulated genes but it was more effective in mediating of repressive function. Besides this, the presence of the short isoform alone was not sufficient for the proper regulation of more than the half of the dSAGA-dependent genes. Thus the normal expression of these genes might require the presence of either the long isoform or both dADA2b isoforms. Our data can not exclude the possibility that dSAGA could perform gene-selective transcription regulation by using distinct dADA2b isoforms however it is most probably not accomplished by the mechanism in which the dADA2bS-containing dSAGA induces or represses the expression of the regulated genes.

3. The role of dSAGA complex and dSAGA specific H3K9 acetylation in regulation of gene expression

The dADA2b protein is a component of the acetylation module of dSAGA complex and determinates substrate specificity of dGcn5. Therefore, we examined whether both the decreased and increased transcription in the absence of dADA2b can be the consequence of the function of dADA2b in histone acetylation. For this in *w¹¹¹⁸* and *dAda2b* mutant SpEv larvae we determined the presence of dADA2b protein and the H3K9 acetylation level on dSAGA-dependent (both up- and downregulated) and dSAGA-independent genes. According to the presence of dADA2b protein more dSAGA-dependent genes (*sug*, *enc*, *CycB*, *Hus1*) could be identified as direct dSAGA target genes. On both up- and downregulated genes we could detect dADA2b binding so the dSAGA function could be directly responsible for either expression changes. The presence of dADA2b could be detected not only on promoters but also on 3' end regions. Our data about dADA2b binding pattern

suggests the possible role of dSAGA in transcription initiation and elongation phases.

By examining the H3K9 acetylation level of dSAGA-dependent genes we detected the presence of dSAGA specific H3K9 acetylation on both up- (*Fst*, *Hus1*, *AttD*) and downregulated (*sug*, *cnc*, *CycB*) genes. Thus we concluded that the presence of the dSAGA specific acetylation on promoters is essential in the transcription regulation of several genes but it can contribute not only to gene activation but also to gene repression.

4. The function of dSAGA specific H3K9 acetylation in the process of gene activation

To understand whether the dSAGA specific H3K9 acetylation can contribute to the process of gene activation, we determined the level of histone acetylation during the progress of gene activation and its effect on the efficiency of transcription. According to immunostaining of polytene chromosomes of salivary gland in *Drosophila* we established that the presence of dSAGA specific acetylation is not an obligate prerequisite of activation of heat shock genes as the active RNA polymerase could bind to activated heat shock genes in the loss of dSAGA specific H3K9 acetylation.

To clarify in details the role of acetylation during gene activation *in vivo* we used two early ecdysone induced genes (*Eip74EF* and *Eip75B*) as models. First we defined the activation patterns of these genes; therefore three time points were selected (one before activation, two during activation) for the analysis of the acetylation pattern. At these time points we determined the level of H3K9 acetylation on the promoter, on the first intronic and on the 3' end exonic regions of these genes by chromatin immunoprecipitation. Our results demonstrated that the H3K9 acetylation occurred globally on the tested genes, its regional and temporal distribution could not be detected. Only moderate increase in the H3K9 acetylation level but not obligate appearance of it was observable nearby the promoter regions during active transcription. Chromatin immunoprecipitation analysis performed on *dAda2b* mutant wandering (wL3) larvae revealed that the dSAGA complex was

responsible for the establishment of a global H3K9 acetylation pattern on the *Eip74EF* and *Eip75B* genes.

To examine how this dSAGA specific global acetylation pattern could contribute to the transcription regulation of *Eip74EF* and *Eip75B* genes, we measured the level of nascent RNA produced from *Eip74EF* and *Eip75B* promoters in the absence of dADA2b proteins. We found that the induction of the moderately activated *Eip74EF-RB* and *Eip75B-RC* promoters did not change significantly, whereas the activation of the strongly induced *Eip74EF-RA* promoter failed to occur in the loss of dSAGA specific H3K9 acetylation. So the dSAGA specific global acetylation pattern on *Eip74EF* and *Eip75B* genes can play an important regulatory role in the activation of these genes.

SUMMARY

The supposed function of dSAGA specific histone acetylation in gene regulation

Previously detailed results in my thesis indicate the multiple role of dSAGA HAT complex in gene regulation. On one hand, interacting directly with transcription factors dSAGA functions in the generation of a site-specific acetylation, on the other hand it contributes to the establishment and maintenance of a global acetylation pattern. The two acetylation types do not occur independently, but on a dynamic global acetylation background a site-specific acetylation pattern can appear. Our results revealed that the H3K9 acetylation pattern of euchromatic regions changed in the loss of dSAGA activity. It is possible that the hypoacetylated chromatin structure weakens the binding affinity of regulatory factors – which can be either activators or repressors – and hence the transcription of genes under their control could be changed according to the loss of the regulatory function. Moreover, this dynamic global acetylation pattern may also contribute to the suitable kinetics of transcription regulation as our data indicating altered kinetics of the activation of the *in vivo* model genes (*Eip74EF* and *Eip75B*) by the loss of dSAGA specific H3K9 acetylation. Therefore, the dSAGA specific acetylation can also contribute to the proper gene regulation by an indirect way through the establishment of a chromatin structure that enables transcription.

LIST OF PUBLICATIONS

Publications related to the Ph.D. thesis:

Pankotai T*, **Zsindely N***, Vamos EE, Komonyi O, Bodai L, Boros IM.
Functional characterization and gene expression profiling of *Drosophila melanogaster* short dADA2b isoform-containing dSAGA complexes.
BMC Genomics 2013 14:(1) p. 44. (in press)
IF (2011): 4,073

Bodai L, **Zsindely N**, Gáspár R, Kristó I, Komonyi O, Boros IM.
Ecdysone induced gene expression is associated with acetylation of histone H3 lysine 23 in *Drosophila melanogaster*.
PLoS One. 2012; 7(7):e40565.
IF (2011): 4,092

Zsindely N*, Pankotai T*, Ujfaludi Z, Lakatos D, Komonyi O, Bodai L, Tora L, Boros IM.
The loss of histone H3 lysine 9 acetylation due to dSAGA-specific dAda2b mutation influences the expression of only a small subset of genes.
Nucleic Acids Res. 2009. 37(20):6665-80.
IF: 7,479

* Co-first authors

Other publication:

Pankotai T, Popescu C, Martín D, Grau B, **Zsindely N**, Bodai L, Tora L, Ferrús A, Boros IM
Genes of the ecdysone biosynthesis pathway are regulated by the ATAC histone acetyltransferase complex in *Drosophila*
Mol Cell Biol. 2010 30(17):4254-66.
IF: 6,188

Total impact factor: 21,832

Conference presentations:

9th EMBL Conference Transcription and Chromatin
2010. augusztus 28-31. Heidelberg, Németország
N Zsindely, T Pankotai, C Popescu, L Bodai, IM. Boros
The Drosophila GCN5 containing dSAGA and dATAC complexes have markedly different transcriptional effects
(Poster presentation)

Epigenetic World Congress

2009. IX. 17-18. Berlin, Németország

Zsindely N, Pankotai T, Bodai L, Tora L, Boros IM

Loss of the dSAGA subunit dADA2b influences the expression of a small subset of genes

(Poster presentation)

33rd FEBS Congress and 11th IUBMB Conference,

2008. június 28.-július 3. Athén, Görögország

N. Zsindely, T. Pankotai, Z. Ujfaludi, O. Komonyi, D. Lakatos, C. Thibault, L. Tora and I. Boros

The role of dADA2b proteins in dSAGA

(Poster presentation)

Spetses Summer School Chromatin & Transcription

2008. szeptember 3-8. Spetses, Görögország

N Zsindely, T Pankotai, Zs Ujfaludi, O Komonyi, D Lakatos, C Thibault, L Tora and I M Boros

The role of dADA2b proteins in dSAGA

(Poster presentation)

Genetikai Műhelyek Magyarországon minikonferencia

2008. szeptember 12. Szeged

Zsindely Nóra

Különböző adaptor izoformák a SAGA hiszton acetiláló komplexben

(Oral presentation)