Functional analysis of *Drosophila melanogaster* histone H4 specific acetylase complex and its role in regulating chromatin structure

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Summary of PhD thesis

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Introduction

Numerous enzymes and protein complexes are known to bring about changes in the state of chromatin by different mechanisms with resultant effects on gene expression. One class of complexes, alter the DNA packaging in an ATP-dependent manner. Another class of chromatin structure regulating factors acts by covalently modifying histone proteins. The various modifications include phosphorylation, ubiquitination, ADP-ribosylation, methylation, sumoylation and frequently acetylation, catalyzed by histone acetyltransferases (HATs). In many cases HAT enzymes are components of complexes which also contain among others, ADA-type adaptors.

Recently our laboratory, in parallel with several others, has showed that contrary to the single ADA2 adaptor protein present in *Saccharomyces cerevisiae*, different GCN5-containing HAT complexes of *Drosophila melanogaster* cells contain two related ADA2 proteins encoded by genes referred to as *dAda2a* and *dAda2b*. In several other metazoan organisms, including mouse, human and Arabidopsis, there are also two ADA2-type coactivators. Biochemical separation of ADA2-containing *D. melanogaster* complexes indicated that dADA2a is present in a smaller (0.8 MDa) and dADA2b in a larger (2MDa) complex which corresponds to the Drosophila homologue of yeast SAGA complex. In a number of independent studies it was shown that in the absence of dADA2b or

dGCN5, in other words, in the absence of functional SAGA, the acetylation of histone H3K9 and K14 is greatly reduced, while the H4K8 acetylation is not affected.

In this work we provide evidence that the dADA2a protein is a specific component of the smaller Drosophila HAT complex which during the course of this work became identified as ATAC. We demonstrate the genetic interaction between *dAda2a* and *dGcn5* genes and show their role in H4 acetylation. Finally, we describe the functional interplay between components of the ATAC complex and the JIL-1 kinase, which plays a role in another type of modification that take place at the chromatin: histone phosphorylation. The role of acetylation and phosphorylation in maintaining the higher order chromatin structure is discussed.

Aims of study

1. In Drosophila and several other metazoan organisms, there are two genes that encode related but distinct homologues of ADA2-type transcriptional adaptors. By using mutant *Drosophila* lines, it was shown that Drosophila ADA2b protein is present in the SAGA complex and has a role in H3 acetylation. Since in Drosophila the existence of another ADA2-type adaptor was reported, but its role was unknown our aims were to characterize the Drosophila *Ada2a*

mutants and to investigate the *in vivo* functional role of ADA2a protein.

2. During this work it was found that dADA2a is a component of ATAC complex and we showed that it plays a role in H4 acetylation. In mutants of *dAda2a* and several ATAC subunits, (*dGcn5* and *dAda3)*, there is an alteration of chromosome structure similar to structural changes observed in the absense of JIL-1 kinase. Since chromosome structural defects are not seen in dADA2b containing-complex, we investigated the role of ATAC in maintaining chromatin structure and if the similar phenotypes of ATAC mutants and the *Drosophila* JIL-1 kinase reflect a functional interaction.

Materials and Methods

1. Maintaining and handling of Drosophila stocks

2. Genetic crosses and phenotype analysis

3. Immunohistochemistry, staining of polytene chromosomes, salivary glands and Drosophila tissues

4. Analysis of histone modifications and proteins by Western Blot

5. Obtaining of transgene contructs using the recombinant DNA technique.

6. Detection of mRNA levels of different genes by RT-PCR and Q-PCR.

7. Testing the ability of puff formation by *in vitro* and *in vivo*

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This work was carried out in the Institute of Biochemistry, Biological Research Center of the HAS and in the Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Szeged, Hungary.

5. EMBO Conferences of Chromatin and Epigenetics, EMBL, Heidelberg, Germany, May 3-6, 2007 – Evidence for the involvement of ATAC specific components Gcn5, Ada2a and Ada3 in common gene regulatory pathways
O. Komonyi, T. Pankotai, A. Ciurciu, C. Popescu, L. Tora, I. Boros

6. **EMBO Conferences of Chromatin and Epigenetics, EMBL, Heidelberg, Germany, May 3-6, 2007** - The two isoforms of SAGA-specific ada2b factors have different functions
T. Pankotai, N. Zsindely, Z. Ujfaludi, **A. Ciurciu**,L. Tora, I. Boros

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ecdysone treatment.

Results and Discussion

In this study we provide several lines of evidence for the functional linkage between dADA2a and dGCN5. We show their genetic interaction by analyzing the phenotype of specific single and double mutants. The loss of either *dGcn5* or *dAda2a* function results in similar chromosome structural and developmental defects. *dGcn5/dAda2a* double-null mutants or a combination of *dAda2a* and *dGcn5* hypomorph alleles result in a phenotype stronger than that of either of the two mutations alone. The overexpression of dGCN5 protein by the use of an act-GAL4 driver in *dAda2a* mutant background results in a partial rescue. Furthermore, the phenotypic features of *dAda2a* mutants indicate a developmental block at the time of larva-pupa transition similarly as it was shown by others for *dGcn5* mutants. In accord with this, by analyzing the puff formation at sites containing ecdysone induced genes and using RT-PCR and Q-PCR to measure specific mRNA levels we demonstrate that the expression of several ecdysone-induced genes such as BR-C, Eip74 and Eip75 are downregulated in the absence of dADA2a protein.

Immunostaining of Drosophila polytene chromosome and Western blot analysis revealed a significantly decreased level of K5 and K12 acetylated histone H4 in *dAda2a* and *dGcn5* mutants, while

the acetylation established by dADA2b-containing GCN5 complexes at H3K9 and K14 was unaffected.

We noticed that another important characteristic of ATAC subunit mutants (*dAda2a*, *dAda3* and *dGcn5)* is the altered structure of the polytene chromosomes observed as disturbed banding pattern and distortions shown most clearly by the male X chromosome. Similar alterations of polytene chromosome structure were observed by others in the absence of JIL-1, the kinase that phosphorylates H3 at serine 10. In order to determine if there is an interdependence between JIL-1 and ATAC functions we studied the levels of ATAC-deposited histone H4K12 acetylation and JIL-1-deposited histone H3S10 phosphorylation in *JIL-1* and ATAC subunit mutants and found that H3S10 phosphorylation in ATAC mutants was severely decreased, while no change was detectable in the level of H4K12-Ac in *JIL-1* mutants. Thus, phosphorylation by JIL-1 depends on ATAC function. Further experiments support this observation, the increase of the JIL-1 protein level by the use of JIL-1EGFP transgene in *dAda2a* and *dGcn5* mutants restored the H3S10 phosphorylation level and increased the survival of *dAda2a* and *dGcn5* null and hypomorph animals. Significantly, the effect of JIL-1 overproduction is also well observable in the change of chromosome structure of ATAC mutants as the bloated phenotype of male X chromosome characteristic for each ATAC mutant studied (*dAda2a, dGcn5* and *dAda3*) was significantly suppressed by JIl-1 overproduction.

Posters

1. 30th FEBS Congress - 9th IUBMB Conference Budapest, Hungary, July 2-7, 2005 – Structure and function of the Drosophila melanogaster Fcp1 phosphatase
I. Tombacz, I. Török, **A. Ciurciu,** I. Boros

2. 7th Transcription Meeting, EMBL Heidelberg, Germany, August 26-30, 2006 – The drosophila transcriptional adaptor Ada2a targets Gcn5 HAT complex to nucleosomal histone4 acetylation
A. Ciurciu, O. Komonyi, T. Pankotai, I. Boros

3. 7th Transcription Meeting, EMBL Heidelberg, Germany, August 26-30, 2006 -The role of Drosophila Ada2 proteins in histone acetylation
I. Boros, T. Pankotai, N. Zsindely**, A. Ciurciu,** Z. Ujfaludi, O. Komonyi

4. 12. Regional Drosophila Meeting (RDM12). Vienna, Austria, November 25-26, 2006- Two Ada2 proteins in Drsosophila plays different roles in histone acetylation
T. Pankotai, O. Komonyi, L. Bodai, Z. Ujfaludi, S. Muratlogu, **A. Ciurciu**,L. Tora, I. Boros

3. Schauer T., Tombacz I., **Ciurciu A.,** Komonyi O., Boros I - **Misregulated RNA Pol II C-terminal domain phosphorylation results in apoptosis.** Cell Mol Life Sci 2009

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Oral presentations

1. **3rd TAF-Chromatin meeting, Athens, Greece 28-29. September. 2006 –** The Drosophila HAT GCN5 and the transcriptional adaptor ADA2a are involved in nucleosomal histone H4 acetylation
   Anita Ciurciu, Orban Komonyi, Tibor Pankotai, Imre Boros

2. **4th TAF-Chromatin meeting, Dublin, Ireland 28-29. September. 2007 -** Chromatin remodeling complexes and their functional interaction
   Anita Ciurciu, Orban Komonyi, Tibor Pankotai, Imre Boros

3. **Straub Days, Szeged, Hungary 28-30.November.2007.** Step by Step Histone Acetylation, Phosphorylation, Methylation- who follows who?
   Anita Ciurciu, Orban Komonyi, Cristina Popescu, Imre Boros

4. **2nd Szeged Chromatin Minisymopsium, Szeged, Hungary, 1-3.May.2008 -** The effect of ATAC on JIL-1 function
   Anita Ciurciu, Orban Komonyi, Imre Boros

As phosphorylation of histone H3S10 by JIL-1 counteracts heterochromatin formation resulting from histone H3K9 dimethylation by Su(var)3-9 dimethyltransferase, we assumed that H4 acetylation at K5 and K12 also has an effect on heterochromatin spreading. In concert with this assumption, immunostaining of polytene nuclei or chromosome spreads with H3K9me2 specific antibodies revealed a spread of signal on *dAda2a* and *dGcn5* mutant chromosomes similarly to that observed on *JIL-1* chromosomes. For testing the genetic interaction between *Su(var)3-9* and ATAC subunit mutants, we studied the effect of *dAda2a* and *dGcn5* deficiency in *Su(var)3-9* heterozygous backgrounds and showed that a decreased level of SU(VAR)3-9 increased the survival and improved the chromosome structure of ATAC mutants.

In order to explain the mechanism of ATAC and JIL-1 functional interaction we analyzed the expression of JIL-1 in ATAC mutants by determining the JIL-1 mRNA and protein levels and showed that the JIL-1 was expressed in ATAC mutants to a similar level as in wild-type controls. However, the binding of the JIL-1 protein to the chromatin containing a low level of acetylated H4 was significantly reduced.

Summary of novel findings

In this study we showed for the first time:

1. The involvement of *D. melanogaster* ATAC histone acetylatransferase complex in histone H4 acetylation.

2. The existence of cross-talk between H4 acetylation and two other types of covalent modifications: H3S10 phosphorylation and H3K9 dimethylation.

Our results strongly suggest a functional interaction of nucleosome remodeling and histone acetyltransferase complexes and histone kinase and methyltransferase. Our data demonstrate that ATAC is required for acetylation of H4K12 and K5 residues and for subsequent binding of JIL-1 kinase to chromatin. A reduced level of histone H4K12 acetylation by ATAC attenuates the phosphorylation of histone H3S10 by JIL-1 that permits the spreading of H3K9 dimethylation by SU(VAR)3-9. These observations support and provide a mechanistic view for the interpretation of the so called histone code hypothesis in molecular terms.

List of publications

List of publications directly related to the subject of the thesis

1. Ciurciu A., Komonyi O., Pankotai T., Boros I. **– The Drosophila histone acetyltranferase Gcn5 and transcriptional adaptor Ada2a are involved in nucleosomal histone h4 acetylation.** Mol Cell Biol. 2006 26(24):9413-23.

IF: 7,093

2. Ciurciu A., Komonyi O., Boros I **- Loss of ATAC-specific histone H4 lysine 12 acetylation reduces JIL-1 binding to chromatin and phosphorylation of histone H3 at serine 10.** J Cell Sci. 2008 15;121:3366-72

IF: 6,543

List of publications indirectly related to the subject of the thesis

1. Pankotai, T., Komonyi,O., Bodai, L., Ujfaludi,Z., Selen Muratoglu, S., **Ciurciu, A.,** Tora, L., Szabad, J., Boros I. **– The Homologous *Drosophila* Transcriptional Adaptors ADA2a and ADA2b Are both Required for Normal Development but Have Different Functions,** Mol Cell Biol 2005 25(18):8215-8227

IF: 7,093

2. Carré C., **Ciurciu A.,** Komonyi O., Jacquier C., Fagegaltier D, Pidoux J., Tricoire H., Tora L., Boros I. & Antoniewski C. **- The *Drosophila* NURF remodeling and the ATAC histone acetylase complexes functionally interact and are required for global chromosome organization**. EMBO reports. 2008 9(2):184-92

IF: 7,663