The function of dTAF10 proteins in *Drosophila melanogaster*

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Ph.D. thesis summary

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1. Introduction

As a first step of eukaryotic transcription initiation, the general transcription factor TFIID binds to the promoter region of transcribed genes. TFIID is a multiprotein complex consisting of TBP, which binds to the TATA box region, and several TAF proteins, which associate with TBP. TFIID is evolutionary well-conserved in human and *Drosophila* cells and it consists of double copies of five TAFs (TAF4, TAF5, TAF6, TAF9 and TAF12), which together create a two-fold symmetric structure. Then TAF8-TAF10 heterodimer joins to this complex and induces conformational changes by abolishing the two-fold symmetry. Further peripheral TAF proteins and TBP bind to the 7 TAF containing TFIID core, forming the functional complex. TAF proteins, however are parts not only the TBP, but the histone acetyl transferase (HAT) SAGA complex, as well. *Drosophila* dSAGA acetylates histone proteins by transferring acetyl groups from acetyl-coenzyme A to lysine side chains. The HAT enzyme of dSAGA is dGCN5, which on the other hand is also present in another histone acetyltransferase complex as well, that is designated as dATAC in *Drosophila*. Curiously, while dSAGA and dATAC complexes have very similar HAT modules, in which they share common subunits, such as dGCN5, dSGF29 and dADA3, they regulate different

biological processes. The HAT complexes dATAC and dSAGA have different substrate specificity: dSAGA acetylates lysines at the 9th and 14th positions of H3 histone, while dATAC acetylates the lysines at the 5th and 8th positions of H4 histone.

In *Drosophila melanogaster* two TAF proteins (dTAF10 and dTAF10b) have been identified, which show homology with the human TAF10. dTAF10 is part of the dSAGA complex, while dTAF10b is subunit of dTFIID. Both proteins have a conserved C-terminal domain, which is characteristic for the TAF10 family, but they are divergent in their N-terminal parts. In respect of their length, the dTAF10 and dTAF10b proteins show 48% identity to each other. Compared to the human TAF10, dTAF10b shows 54%, while dTAF10 shows 48% identity with it. There is limited information available on the function of genes encoding dTAF10/dTAF10b proteins. It is unclear how these proteins influence the histone acetyltransferase activity of the dSAGA complex or if they take part in the assembly of the dTFIID.

In my thesis work I studied *Drosophila* dTAF10 and dTAF10b containing complexes. During my PhD work, I investigated mutant animals from which the coding regions of dTAF10 and dTAF10b were eliminated.

2. Aims

2.1. Determining how the dTAF10/dTAF10b proteins influence the development of *Drosophila melanogaster*

2.2. Studying how the dTAF10/dTAF10b proteins influence the function of dSAGA histone acetyl transferase complex

2.3. Investigating how the dTAF10/dTAF10b proteins influence the assembly of dTFIID complex

3. Methods

- Phenotype analysis of *dTaf10* mutants, determination of the lethal phase resulting from the loss of dTAF10 function.
- Transcriptom analysis of *dAda2b*^{d842}, *dTaf10*^{d25}and *dAda2a*^{d189}mutant *Drosophila* larvae
- Detection of the changes in H3K9-, H3K14-, H4K8- and H4K12- acetylation levels by immunohistochemistry on polytene chromosomes of *dTaf10* mutant larvae
- Detection of specific proteins and histone acetylation levels by Western blot
- Determination of protein localization by immunohistochemistry on polytene chromosomes
- Investigation of protein-protein interaction by co-immunoprecipitation
- Determination the effects of ecdysone and cholesterol feeding of *dTaf10*^{d25} mutant larvae
- Usage of RNA interference to silence *dTaf5, dTaf8* and *dTaf10* expression
- Determination of gene expression level by RT-PCR

4. Results

1. Previously in our lab we generated *Drosophila* mutant animals in which P-element remobilization caused deletions eliminated the coding regions of dTAF10 and dTAF10b. One of the isolated *dTaf10* allele (*dTaf10*^{d25}) is a result of a 900 base pair deletion that removed the dTAF10 and dTAF10b coding regions but did not affect the neighboring genes (Aph-1, Colt). The phenotype analysis showed that the lack of dTAF10 and dTAF10b proteins caused late L3 and pupal lethality. We found that 60% of *dTaf10*^{d25} mutants were unable to form normal pupa and approximately 40% of animals died at L3 larval stages.

2. Although the dTAF10b is part of dSAGA complex, the microarray analysis showed that mutations either in gene encoding a dATAC subunit (*dAda2a*^{d189}) or in *dTaf10* genes resulted similar changes in the steady state mRNA levels. This observation suggests that dTAF10/dTAF10b containing complexes and dATAC take part in similar biological pathways.

3. We investigated by immunohistochemistry whether the ablation of dTAF10 and dTAF10b has any effects on the HAT activity of dATAC and dSAGA complexes. For this we examined the global histone acetylation level in *dTaf10*^{d25} mutant animals. We found that dSAGA specific H3K14 acetylation level was reduced by the ablation of

dTAF10/dTAF10b but the dATAC dependent H4K12 and GCN5 specific H4K8 acetylation levels remained comparable to the controls in *dTaf10*^{d25} mutants. Furthermore, the level of dSAGA specific H3K9 acetylation did not show any differences between the control and the *dTaf10*^{d25} mutants. These results indicate that the dTAF10b protein is a stable component of dSAGA and the ablation of dTAF10/dTAF10b does not affect the HAT activity of the dATAC complex.

4. In another set of experiments we investigated whether the ablation of dTAF10 and dTAF10b has any effects on the binding of dTFIID and dSAGA subunits to *Drosophila* polytene chromosomes. To test this, we performed immunostaining experiments on polytene chromosomes of *dTaf10*^{d25} mutant larvae and we examined the localization of dTBP, several dTAF proteins and dADA2b. The localization of dTAF5 was reduced on the polytene chromosomes in *dTaf10*^{d25} mutants, while the level of dTAF1 and dTBP on the polytene chromosomes were comparable with that of the control animals. Furthermore, we observed that dADA2b localization was not altered in the absence of dTAF10/dTAF10b compared to the control. The decreased level of dTAF5, which is a subunit of the core dTFIID, indicates that the ablation of dTAF10 and dTAF10b results in the degradation of dTFIID complex. Therefore, we

analysed the protein levels of several dTAFs and dTBP in *dTaf10*^{d25} mutant larvae by Western blot. We did not detect alterations in the protein level of dTAF1 and dTAF5 in the absence of dTAF10/dTAF10b compared to the control samples. On the contrary, the levels of dTAF6 and dTBP were decreased in *dTaf10*^{d25} mutant larvae. These results indicate that while the lack of dTAF10/dTAF10b proteins do not affect the dTAF5 protein level, the absence of dTAF10 proteins disturbs the binding of dTAF5 containing complexes on polytene chromosomes. dTAF1 and dTBP containing dTFIID is able to bind to chromosomes in the absence of dTAF10 proteins.

5. The interaction of dTAF4 and dTAF5 proteins plays an important role in maintaining the integrity of the dTFIID complex. Therefore, we used co-immunoprecipitation to reveal whether dTAF4-dTAF5 subcomplex was formed in *dTaf10*^{d25} mutants. We found that dTAF4 and dTAF5 can interact with each other even in absence of dTAF10 and dTAF10b. This indicates that the dTAF4-dTAF5 containing dTFIID can be formed even in the absence of dTAF10/dTAF10b proteins.

6. We found that the *dTaf10*^{d25} mutants were unable to form normal pupa and approximately 50% of the animals died at L3 larval stages. Earlier experiment showed that the absence of dADA2a proteins caused similar phenotype. In dATAC

mutants the absence of molting hormone ecdysone resulted in animal lethality. We assumed that the *dTaf10*^{d25} mutants also died in L3 larval stage due to the lower level of ecdysone. To verify this, we fed *dTaf10*^{d25} L3 larvae with 20-hidroxyecdysone. We observed that as a result of ecdysone feeding, more than 80% of L3 larvae reached the pupal stage. Ecdysone is synthesized from cholesterol in the ring gland and the failure of its production might result from defects in cholesterol uptake or in cholesterol conversion. Therefore we checked whether a failure in cholesterol uptake resulted in pupariation defects. We fed L3 larvae with cholesterol containing medium but this did not rescue the pupariation defects. This result indicated that the lack of the molecular precursor was not responsible for the absence of ecdysone.

7. Next, we investigated whether the gene expression changes and the morphogenesis delay caused by the lack of dTAF10 were due to defects in the synthesis of ecdysone hormone. Since ecdysone is synthesized in the prothoracic gland, we eliminated d*Taf10* messengers specifically in the prothoracic gland cells by producing siRNA against *dTaf10* with the help of a *phantom*-GAL4 driver. We found that the decreased level of *dTaf10* mRNA in the ring gland caused similar phenotype to that we detected in the absence of dTAF10 proteins. Since ecdysone

feeding restored the ability of puparium formation, this result indicated that the gene expression changes and the failure in morphogenesis of dTAF10 mutant animals were due to the failure in the synthesis of the molting hormone ecdysone. The targeted silencing of *dTaf5* and *dTaf8* resulted in similar loss of molting phenotype, as we detected in *dTaf10*-silenced animals. Similar to *dTaf10*-silenced animals, *dTaf5*-, and *dTaf8*-silenced ones could reach early pupal stages by ecdysone feeding. Together, these results suggest that the dTAF5, dTAF8 and dTAF10 containing dTFIID has an essential regulatory function in early stages of ecdysone hormone biosynthesis in the prothoracic gland cells.

8. The similar changes in the transcriptomes of dADA2a and dTAF10/dTAF10b mutants prompted us to check whether dTAF10 proteins are also necessary for the expression of genes involved in the ecdysone biosynthesis pathway. Microarray data showed that the *Halloween* genes (*spookier, phantom, disembodied, shadow, neverland),* which are expressed in the ring gland and are involved in the ecdysone biosynthesis, were down-regulated in $dTaf10^{d25}$ mutant animals. In contrast to that, the expression of *shade*, which is expressed in peripheral tissues, such as midgut and larval fat body, increased in the absence of dTAF10/dTAF10b.

We found similar changes in the expression levels of ecdysone biosynthetic genes in dATAC mutants when we compared the microarray data of *dAda2a*^{d189} with the microarray results of *dTaf10*^{d25} mutants. These results and the similar phenotypes, which were detected in the absence of both dTAF10 proteins and dADA2a, indicate that dTAF10/dTAF10b containing complexes and the dATAC complex together regulate the ecdysone biosynthesis.

5. Publications

MTMT number: 10048807

Article related to this Ph.D.thesis:

Pahi Z, Kiss Z, Komonyi O, Borsos BN, Tora L, Boros IM, Pankotai T.
dTAF10-and dTAF10b-Containing Complexes Are Required for Ecdysone-Driven Larval-Pupal Morphogenesis in *Drosophila melanogaster*
PLOS ONE 10:(11) Paper e0142226. (2015)
IF: (2015) 3,06

Other publications:

Borsos BN, Pankotai T, Kovacs D, Popescu C, Pahi Z, Boros IM.
Acetylations of Ftz-F1 and histone H4K5 are required for the fine-tuning of ecdysone biosynthesis during *Drosophila* metamorphosis.
DEVELOPMENTAL BIOLOGY 404**:**(1)pp. 80-87.(2015)
IF: (2015) 3,16

Villanyi Z, Ribaud V, Kassem S, Panasenko OO, Pahi Z, Gupta I, Steinmetz L, Boros I, Collart MA.
The Not5 subunit of the ccr4-not complex connects transcription and translation.
PLOS GENETICS 10:(10) Paper e1004569. 15 p. (2014)
IF: (2014) 7,15

Total impact factor: 13,37

Conference posters:

Annual Meeting of the Hungarian Biochemical Society Szeged 2016
Zoltan Gabor Pahi, Zsuzsanna Kiss, Orbán Komonyi, Barbara N. Borsos, Laszlo Tora, Imre M. Boros, Tibor Pankotai.
The role of TAF10/TAF10b containing complexes in *Drosophila melanogaster.*

FEBS3+ Meeting Molecules of Life. Portoroz, Slovenia 2015
Zoltan Gabor Pahi, Zsuzsanna Kiss, Orbán Komonyi, Barbara Nikolett Borsos, Laszlo Tora, Imre Miklos Boros, Tibor Pankotai.
The TAF10-containing TFIID is necessary for the ecdysone induced larval-pupal transition of *Drosophila melanogaster*.

Hungarian Molecular Life Sciences Conference, Eger 2015
Zoltán Gábor Páhi, Zsuzsanna Kiss, Orbán Komonyi, Barbara Nikolett Borsos, László Tora, Imre Miklós Boros, Tibor Pankotai.
The TAF10-containing TFIID is necessary for the ecdysone inducing larval-pupal transition in *Drosophila melanogaster*.

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Barbara N. Borsos, Tibor Pankotai, Dávid Kovács, Christina Popescu, Zoltán Páhi, Imre M. Boros.
Acetylations of Ftz-F1 and histone H4K5 are required for the fine-tuning of ecdysone biosynthesis during Drosophila metamorphosis.

The 18Th Annual Meeting of The RNA Society; Davos, Switzerland 2013
Zoltan Villanyi, Sujatha Subbana, Olesya Panasenko, Zoltan Pahi, Imre Boros, Martine Collart.
A New Role for NOT5 of the CCR4-NOT Complex in Connecting Transcription with Translation

Hungarian Molecular Life Sciences Conference, Siófok 2013
Zoltán Gábor Páhi, Tibor Pankotai, Barbara Nikolett Borsos, Imre Miklós Boros.
TAF10 proteins indicate structural and functional links between histone acetyltransferase and basal transcription factor complexes.

Magyar Biokémiai Egyesület 2011. évi Vándorgyűlése
Páhi Z. G., Pankotai T, Boros I. M.
The role of TAF10 in *Drosophila melanogaster*.

Magyar Biokémiai Egyesület 2010. évi vándorgyűlése
Páhi Z. G., Schauer T., Boros I. M. Investigation of mutant DTL proteins in *Drosophila melanogaster*.

Other articles:

Páhi Zoltán Gábor
Mérföldkövek a rákkutatásban
ÉLET ÉS TUDOMÁNY 70:(6) pp. 174-175. (2015)

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