

The role of ADA2b adaptor protein containing histone
acetylation complexes in *Drosophila melanogaster*

Ph.D THESIS

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Introduction and aims

Chromatin modifying and chromatin remodeling complexes play central roles in eukaryotic transcription in overcoming the repressed state of genes resulting from the tight packaging of DNA and histone proteins in nucleosomes. Among chromatin modifying complexes those which carry histone acetyl transferase (HAT) activity deserve particular attention since acetylation of the N-terminal tails of nucleosomal histones is proven to have specific gene activation or in some cases repression effects. Histone acetylation has been linked to transcriptional activation for a long time, based on the observation that actively transcribed regions tend to be hyperacetylated, whereas transcriptionally silent regions generally are hypoacetylated. With the identification of the histone acetyltransferase H1 as the *Tetrahymena* homolog of the yeast transcriptional regulator GCN5 and the subsequent demonstration that other transcriptional regulators, such as the coactivators p300/CBP, steroid receptor coactivators, and the TFIID subunit TAF1, also possess intrinsic HAT activity, the link between acetylation and transcription has been firmly established.

GCN5 is an evolutionarily conserved protein which is present as the catalytic subunit in several multiprotein complexes, such as the SAGA and ADA complexes in yeast and the PCAF, STAGA, and TFIIIC complexes in mammals. Structural and functional differences of these complexes arise from the numerous factors present in them in addition to the common catalytic subunit. ADA (*alteration/deficiency in activation*) factors are also present in most of the known GCN5-containing complexes and are believed to play a role in determining the functions of the complexes in several ways, including the regulation of complex specificity and interaction with basal transcriptional factors and sequence specific activators.

The *Ada* genes were first identified in yeast, by the demonstration that their inactivation could relieve the toxicity caused by overexpression of strong transcriptional activators. ADA proteins were subsequently shown to exist in protein complexes containing the GCN5 histone acetyltransferase. The yeast ADA2 protein contains a zinc finger ZZ and a SANT domain. The SANT domain stimulates the HAT activity of GCN5

and is involved in substrate recognition. Recently, it was demonstrated in our laboratory that the *Drosophila melanogaster* genome contains two *Ada2* genes, designated as *dAda2a* and *dAda2b*. Biochemical characterization of the two dADA2 proteins demonstrated that both of them interact with the HAT GCN5 and participate in transcription activation. On the other hand, ADA2a and ADA2b exhibit marked differences, since they participate in distinct high-molecular-weight HAT-containing protein complexes, localize to different chromosomal loci, and have at least partly different partners of interaction. Two different ADA2 homologs encoded by two distinct genes have been found not only in *Drosophila* but also in *Arabidopsis* and several vertebrates. These observations raised several questions concerning the structural and functional differences of GCN5-containing HAT complexes of higher eukaryotes. Specifically, that do the two related dADA2 proteins have distinct or overlapping functions and what are their roles within the HAT complexes? The primary aim of the studies described in my thesis was to provide data for the answers to these questions by completing a detailed analysis one of the two identified *Ada2* genes of *Drosophila*. I used mostly genetic approaches to analyze the function of the *dAda2b* gene. Before these studies no previous genetic analysis on a component of a GCN5-containing HAT complex has been described in *Drosophila*. I found that *dAda2b* is required for viability and for maintaining normal histone H3 acetylation level. In the absence of *dAda2b* increased apoptosis can be observed in response to radiation-induced DNA damage. The main conclusions from my results are that ADA2b - as a component of the *Drosophila* SAGA HAT complex - is involved in histone H3 acetylation and in gene specific transcription regulation and it may also affect DNA damage recognition and repair.

Methods

Generation of *Drosophila* mutants by P-element remobilization and analysis of mutant phenotypes by genetic crosses. Complementation test analysis and lethal phase determination. Generation of transgene carrier *Drosophila* lines and analysis of these by genetic crosses. Generation of germ line and somatic mosaics and mosaic analysis. Immunostaining of larval tissue and polytene chromosomes to detect apoptosis and protein localization. Transfection of S2 cells in cell culture for gene expression determination. Determination of gene expression level by RT-PCR based RNA determination and microarray. Bioinformatic analysis of microarray data. Detection of *in vivo* protein expression by the use of epitope tag-containing transgene constructs and western blotting.

Results

In order to facilitate a detailed study of the *Ada2b* gene and to analyze the biological role of its product(s), first I generated *Ada2b* mutants by mobilizing a P-element insertion located close to the putative regulatory region of the gene. Following mobilization of the P-element I used genetic and molecular approaches to characterize the obtained mutants. Results of these experiments demonstrated the successful generation of a number of *Ada2b* alleles among them several null alleles.

Phenotype analysis of the generated mutants revealed that the lack of *Ada2b* function severely affected the development of animals. Homozygous *Ada2b* null mutants are pupa lethal. A direct proof that the lethality and other observed developmental defects of *Ada2b* null animals were the results of the loss of ADA2b function was provided by the use of transgenes corresponding to the genomic *Ada2b* region. Several independent transgene insertions were able to rescue the phenotypic defects and resulted in development of fertile adults. Furthermore, generation of germ line chimeras by pole cell transplantation demonstrated that the *Ada2b* function is also required for the start of normal embryonic development.

Northern-blot analysis of ADA2b specific RNA demonstrated that the transcription of *Ada2b* give rises to two messages. By the use of specific primers I PCR

amplified cDNA copies of both messages and constructed transgenes which direct the synthesis of EGFP fusion derivatives of both ADA2b proteins. Expression of these hybrid genes in S2 cells suggested that the two ADA2b protein isoforms (ADA2b¹ and ADA2b²) are localized in different cell compartments. The construction and analysis of transgenic *Drosophila* lines expressing either an ADA2b¹-EGFP or an ADA2b²-EGFP transgene under the control of UAS regulatory region also demonstrated the functional difference of the two ADA2b proteins. Both the *Ada2b*¹ and *Ada2b*² transgene show a rescue effect, but to a different extent and alone neither of them is capable for a complete rescue. The two transgenes, however, together are able to facilitate the development of animals to the extent close to that observed with a wild type transgene.

As ADA2 proteins are components of HAT complexes it was interesting to test the effect of *Ada2b* mutations on histone acetylation. I found that *Ada2b* null mutations have a strong effect on H3 acetylation at two specific lysine residues, K9 and K14. Interestingly, a difference between the two ADA2b proteins, ADA2b¹ and ADA2b² was also observed in H3 acetylation. With the use of transgenes directing only ADA2b¹ or ADA2b² production we could demonstrate that only one of the two ADA2b proteins is playing a role in histone H3 post-translational modification. I found that while the loss of both ADA2b isoforms interfere with both H3 K9 and H3 K14 acetylation, in the absence of ADA2b² H3 K14 and K9 acetylation is not affected. In contrast, in *Ada2b*¹ mutants a decreased H3 K14 acetylation was observed.

I detected even further functional differences between the two ADA2 factors of *Drosophila* by studying their role in eye pigment production, apoptosis and mitosis. A comparison of the pigment contents of *Ada2* heterozygotes and wild-type animals indicated that *Ada2b* mutation affected the pigment contents of the eyes of adult animals, while *Ada2a* mutation did not. In concert with this, the mRNA level of *rosy*, a gene encoding xanthine dehydrogenase, which is involved in the formation of red eye pigments, exhibited a significant reduction in *Ada2b* mutants as compared to wild-type animals, while the expression of *rosy* did not change in *Ada2a* null animals. This suggests that ADA2a and ADA2b are differently involved (directly or indirectly) in the transcriptional regulation of *rosy*. The difference between the two isoforms of ADA2b is

also observable in the different regulation of the *rosy* gene. The presence of ADA2b² is necessary and sufficient for normal expression level of *rosy* gene.

The physical interaction of ADA2b protein with the tumor suppressor p53 was demonstrated earlier by immunoprecipitation. This prompted me to test if a functional consequence of this interaction *in vivo* can be detected. To test the functional capability of Dmp53 preserving genome stability in *Ada2* mutant background I used loss of heterozygosity (LOH) assay based on the detection of appearance of cell clones displaying the recessive *mwh* (*multiple wing hair*) phenotype following low level X-ray irradiation. In *Ada2a* heterozygotes, the frequency of clones was similar to that seen in wild-type animals. In contrast, *Ada2b* heterozygotes exhibited substantially elevated numbers of *mwh* cells under conditions that had no effect on wild-type flies. This result clearly demonstrated that ADA2b, unlike ADA2a, is involved in the pathway induced by Dmp53 to preserve genome stability in response to low-level X-ray radiation induced DNA damage. The Dmp53 function is also essential for radiation-induced apoptosis. DNA damage leads to *Dmp53* activation, which through the transcriptional activation of proapoptotic factors brings about apoptosis. Radiation induced apoptosis in the discs of *Ada2b* mutants was significantly lower than that in wild type animals. In accord with that, in *Ada2b* mutant imaginal discs the number of cells stained with the vital dye acridine-orange was significantly decreased. The introduction of the *Ada2b* transgene into *Ada2b* null animals restored apoptosis to the level seen in the wild type, indicating that the decreased level of apoptosis was a result of the insufficiency of the *Ada2b* function. To test whether *Ada2* mediated *Dmp53* functions by altering the transcription of its proapoptotic target, *reaper*, I compared the *reaper* mRNA levels in wild-type and *Ada2* mutants. Interestingly, although high-dose X-ray irradiation resulted in a decrease in apoptosis in wing imaginal discs the induction of the *reaper* message was similar in wild-type and *Ada2b* animals. Surprisingly, I could not detect *reaper* mRNA induction under the same conditions in *Ada2a* mutants. Taken together, these data indicate that ADA2b exerts its effect on a Dmp53-related pathway(s) other than those including *reaper* induction.

The retarded development of organs containing mitotically dividing cells (gonads, imaginal discs) in *Ada2b* mutants as compared with the wild-type controls suggests a

delay in cell cycle progression. In the absence of *Ada2b*, the number of cells in mitosis is increased in imaginal discs. It is reasonable to assume that the primary cause of cell proliferation defects is a failure in transcription of genes required for proper cell cycle progression. I assume that in *Ada2b* animals the cell cycle is blocked in G2/M. In concert with that, I observed that the *Ada2b* mutation abolishes the expression of a mitotic regulator, *Map205*. For this function the ADA2² isoform of the protein is required since the presence of ADA2b² rescued the *Map205* mRNA level, but the ADA2b¹ did not.

Finally, I analyzed the effect of *Ada2b* mutation on the total cellular mRNA profile by microarray hybridization. The changes I observed in the level of specific mRNAs comparing wild type and *Ada2b* samples are in concert with the observations I made by studying the expression of individual genes. In *Ada2b* mutants the expression of genes playing role in eye pigment formation, in apoptosis and in mitosis can be detected by microarray hybridization. In addition, I also detected specific alterations in the expression of genes involved in cuticle formation and immunity. The determination whether these changes are primary or secondary effects of the loss of *Ada2b* requires further studies. A detailed analysis of the chip data and particularly a comparison of that with data obtained from mutants defective in other components of HAT complexes are in progress.

Main results

1. The construction of a genetic system for studying the *in vivo* role of a subunit of a HAT complex in a multicellular eukaryote by the isolation and characterization of *Drosophila* *Ada2b* mutants and *Ada2b* transgene carriers.
2. The determination that HAT complexes containing the dADA2b protein acetylate histone H3 at K9 and K14 residues.
3. The determination that the ADA2b-containing HAT complexes play a role in gene-specific transcription regulation effecting the expression of genes which are for example required for eye pigment formation, apoptosis and cell cycle regulation.

Publications

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