Biological Research Centre of the Hungarian Academy of Sciences Institute of Biochemistry

Ecaterina Edith Vamoș

Analysis of the mechanism by which dADA2 factors contribute to the specificity of dSAGA and dATAC histone acetyltransferase complexes

PhD dissertation

Supervisor: Prof. Dr. Imre M. Boros

Szeged, 2013

Dedication

I dedicate this dissertation to the memory of my beloved parents, Ecaterina and Ioan, who always had confidence in me and offered me love, endless encouragement and support in all my endeavours.

Acknowledgments

I am most grateful to my supervisor, Dr. Imre Boros, for his guidance and support during my research. I appreciate all his contributions of time, ideas and funding to make my Ph.D. experience productive and stimulating.

I am thankful to Dr. László Bodai and Dr. Zoltán Villányi for their help with crosses and advices. Special thanks to our technician Katalin Gyuláné Ökrös for her technical assistance.

Also I would like to thank all my colleagues and coworkers in the laboratory for their cooperation, helping, and their entertaining and helpful discussions.

Many thanks to Ágota Tüzesi, Dr. Anita Ciurciu, Dr. Cristina Popescu and Dr. Manuela Jurca for all their help and moral support. Special thanks to my best friend Glăvan Alina for being there for me every time I needed it.

Last but not the least I would like to thank my late parents and my brother, Ionuţ, for all their love and encouragement in all my pursuits. Most of all I am grateful for my loving, encouraging, and patient future husband, Krzysztof, whose faithful support during the final stages of this Ph.D. is so appreciated. I am truly grateful to the Pawlak family for their love and moral support.

Table of Contents

Acknowledgments 3

List of abbreviations 6

Chapter 1 Introduction 9

1.1 Chromatin structure and dynamics 9

1.2 Histone modifications and chromatin regulation 12

1.3 Histone acetylation 15

1.3.1 Histone acetylation in transcription, DNA damage and repair 16

1.3.2 The variety of lysine acetyltransferases 18

1.3.3 The GNAT acetyltransferase family 19

1.4 The SAGA and ATAC acetyltransferase complexes 23

1.4.1 ADA2 coactivator proteins 26

1.5 Histone modifications and diseases 28

Chapter 2. Aims 30

Chapter 3. Materials and methods 31

3.1 Molecular cloning 31

3.1.1 Generation of chimeric DNA sequences 31

3.1.2 Constructs for co-immunoprecipitation 33

3.2 *Drosophila* S2 cell transient transfection 33

3.3 Co-immunoprecipitation 34

3.3.1 Purification of proteins expressed in S2 cells 34

3.3.2 Co-immunoprecipitation of proteins 34

3.4 Western blotting 34

3.5 *Drosophila* genetic analysis 35

3.5.1 Fly husbandry 35

3.5.2 *Drosophila* collection 35

3.5.3 Generation of transgenic *Drosophila* lines 36

3.5.4 Rescue experiments 37

3.6 Quantitative Reverse-Transcriptase PCR 37

3.7 Immunofluorescence of polytene chromosomes 38

Chapter 4. Results 40

4.1 Engineering the chimeric transgenes 40

4.2 Expression of dADA2 chimeras partially rescues the *ada2a*^{d189} or *ada2b*^{d842} mutants 44

4.3 The acetylation level of histones H3 or H4 at specific lysine residues is restored in *ada2* null mutants by the expression of dADA2 chimeric proteins 50

4.4 Chimeric dADA2 proteins did not improve the expression of selected genes affected by *ada2a*^{d189} or *ada2b*^{d842} mutation 55

4.5 *In vivo* interaction between dADA2b isoforms, dADA3 and dp53 57

Chapter 5. Discussion 61

Summary 69

Összefoglaló 72

List of publications 75

References 76

List of abbreviations

Abbreviation	*Description*
ADA	Alteration Deficiency in Activation
ATAC	Ada Two A containing Complex
CBP	CREB binding protein
CREB	cAMP response element-binding protein
CTK1	Carboxy-Terminal domain Kinase 1
dADA2bL	*Drosophila* ADA2b protein long isoform
dADA2b^{L}2a	Chimeric protein of N-terminal Ada2b long fragment fused to dAda2a C-terminal
dADA2b^{M}2a	Chimeric protein of N-terminal Ada2b medium fragment fused to dAda2a C-terminal
dADA2bS	*Drosophila* ADA2b protein short isoform
dADA2b^{S}2a	Chimeric protein of N-terminal Ada2b short fragment fused to dAda2a C-terminal
DHSs	DNase I-hypersensitive sites
DUB	Deubiquitination enzyme
ELP3	Elongator acetyltransferase complex subunit 3
FCS	Fetal calf serum
GCN5	General control nonderepressible-5′
GNAT	Gcn5-containing N-acetyltransferase complex
H2A and H2B	Histone 2A and 2B
H3 and H4	Histone 3 and 4
H3K14ac (K9)	Histone 3 acetylated at lysine 14 (lysine 9)
H3K4me3 (K27, K9)	Histone 3 tri-methylated at lysine 4 (lysine 27, 9)
H3S10ph	Histone 3 phosphorylated at serine 10
H4K5ac (K12)	Histone 4 acetylated at lysine 5 (lysine 12)
HAT	Histone Acetyltransferase

HDAC	Histone deacetylase
HPA2	Histone and other Protein Acetyltransferase
HP1	Heterochromatin protein1
INO80	INOsitol requiring 80
ISWI	Imitation switch
KAT	Lysine Acetyltransferase
MOF	Males absent on the first
MORF	Monocytic leukaemia zinc finger protein-related factor
MOZ	Monocytic leukaemia zinc finger protein
MSL	male-specific lethal
MYST	MOZ, Ybf2/Sas3, Sas2 and Tip60 acetyltransferase superfamily
NuA4	Nucleosome acetyltransferase of H4
NURF	Nucleosome remodelling factor
NUT1	Negative regulation of URS Two
p53	Tumour suppressor protein 53
PCAF	p300/CBP-associated factor
PHD	Plant Homeo Domain
PIC	Preinitiation complex
PP1	Protein phosphatase 1
PTM	post-translational modification
Rpb4	RNA polymerase 4
SAGA	Spt-Ada-Gcn-acetyltransferase
SANT	Swi3, Ada2, NCoR, TFIIIB
Sb	Stubble
SGF29	SaGa associated Factor 1
SLIK	SAGA like
SNF	Sucrose non-fermenting
SPT	SuPpressor of Ty's
STAGA	SPT3-TAFII 31-GCN5L acetylase

SU(VAR)3-9	Suppressor of position-effect variegation 3-9
SWI/SNF	Switching/sucrose non-fermenting
SWIRM	Swi3p, Rsc8p and Moira
Tb	Tubby
TBP	TATA binding protein
TFIID	Transcription Factor II D
TFTC	TATA-binding Protein-free TAF-containing Complex
TIP60	Tat-interactive protein 60
TSS	Transcriptional start site
UAS	Upstream Activation Sequences
Ubp8	Ubiquitin-specific processing Protease
w	white
ZZ	Zinc finger domain

Chapter 1.
Introduction

1.1 Chromatin structure and dynamics

In eukaryotic cells, DNA requires an extreme arrangement in order to be accommodated within the nucleus. The compaction of the DNA sequence into a dynamic chromatin fibre is achieved through association with strongly basic histone and non-histone proteins (*Fig. 1.1*). Cytologically two functional forms of the chromatin can be distinguished: heterochromatin, a condensed form during mitosis and meiosis that generally lacks DNA regulatory activity, and euchromatin described as open, gene rich regions found often (but not always) under active transcription (Jenuwein and Allis, 2001; Marmorstein, 2001). Within the chromatin, the fundamental repeating unit is represented by the nucleosome which consists of 147 bp DNA wrapped around a histone octamer of one H3-H4 tetramer and two H2A-H2B dimers (Roth et al., 2001; Li et al., 2012). The neighbouring nucleosomes are connected by a segment of linker DNA that varies from 10 to 80 bp in length depending on species and cell type. The presence of a fifth histone protein H1 stabilizes the assembly of the octameric core into a higher-order chromatin structure, the 30-nm fiber (Woodcock et al., 2006). The details of further chromatin folding are still uncertain (Bassett et al., 2009).

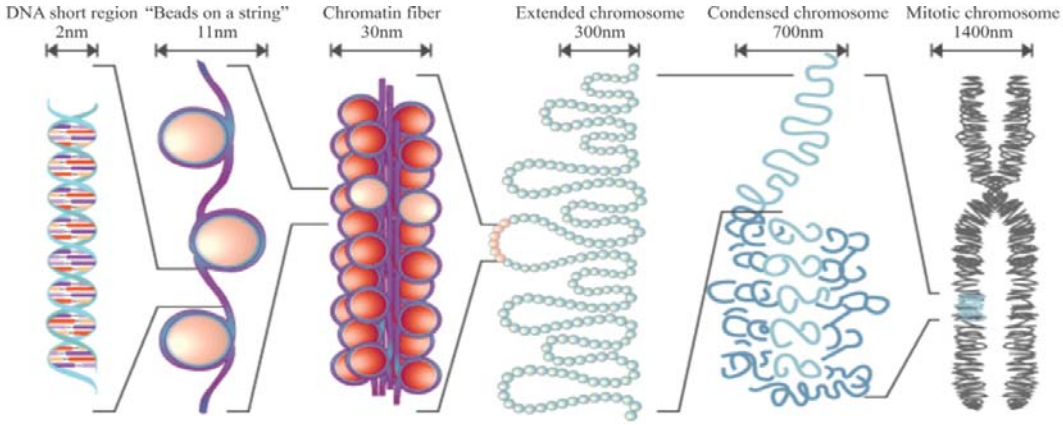

Fig. 1.1 *Different levels of DNA condensation in the nucleus (Felsenfeld and Groudine, 2003).*

Histones are small basic proteins consisting of a globular domain and a flexible, charged NH_2- and COOH-terminus (histone "tails") that pass over/between the gyres of the DNA superhelix (Luger et al., 1997). The histone tails are highly conserved across various species and are subjected to extensive post-translational modifications (PTMs), which are thought to contribute towards the formation of higher-order chromatin structure (Fletcher and Hansen, 1996; Dutnall and Ramakrishnan, 1997; Luger and Richmond, 1998). Besides covalent modifications of histone proteins, other types of mechanisms have been shown to contribute to the dynamics of chromatin structure. The activity of large ATP-dependent chromatin remodelling complexes and the incorporation of histone variants are two mechanisms linked to chromatin higher-order structure. While the first mechanism directly alters chromatin structure (Bouazoune et al., 2002), the subsequent process may act as an alternative to histone modifications for the generation of distinctive chromatin regions (Pusarla and Bhargava, 2005).

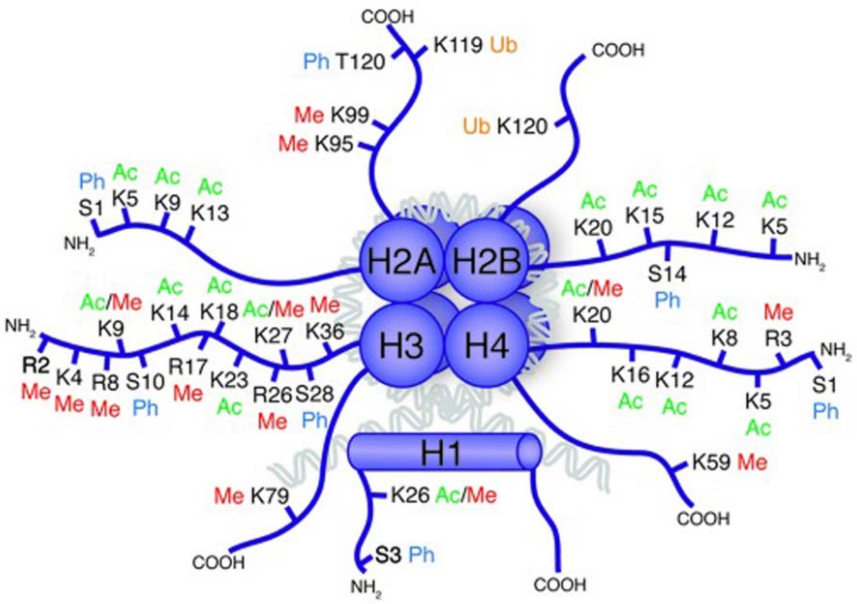

Fig. 1.2 *Schematic drawing of a nucleosome with post-translational modifications of the four canonical histones (H2A, H2B, H3 and H4) and the linker histone H1. The covalent PTMs [methylation (Me), acetylation (Ac), ubiquitination (Ub), and phosphorylation (Ph)] are highlighted on the N- and C-terminal tails of each histone (Tollervey and Lunyak, 2012).*

The amino terminal tail of histones has over 60 residues which have been shown to undergo enzymatic modifications in different cell types and during

developmental stages (Surani et al., 2007; Furdas et al., 2012). At least seven covalent modifications are known to date: acetylation (at lysine residues), methylation (lysine and arginine), phosphorylation (serine and threonine), ubiquitination (lysine), sumoylation (lysine), ADP ribosylation (glutamate and lysine), deamination (arginine) as well as proline isomerization (*Fig. 1.2*) (Kouzarides, 2007). Combinations of post-translational marks on single histones, single nucleosomes and nucleosome domains establish local and global patterns of chromatin modifications that may specify unique downstream functions (Strahl and Allis, 2000; Turner, 2000). Furthermore, combinations of interactions between complexes define substrate specificity and regulate the proper timing for covalent modifications on nucleosomes and chromatin (Suganuma and Workman, 2011). There are many studies which have shown that the site-specific combinations of covalent modifications collaborate to influence a multitude of cellular processes such as transcription, gene silencing, heterochromatization, DNA repair and replication (*Fig. 1.3*) (Munshi et al., 2009).

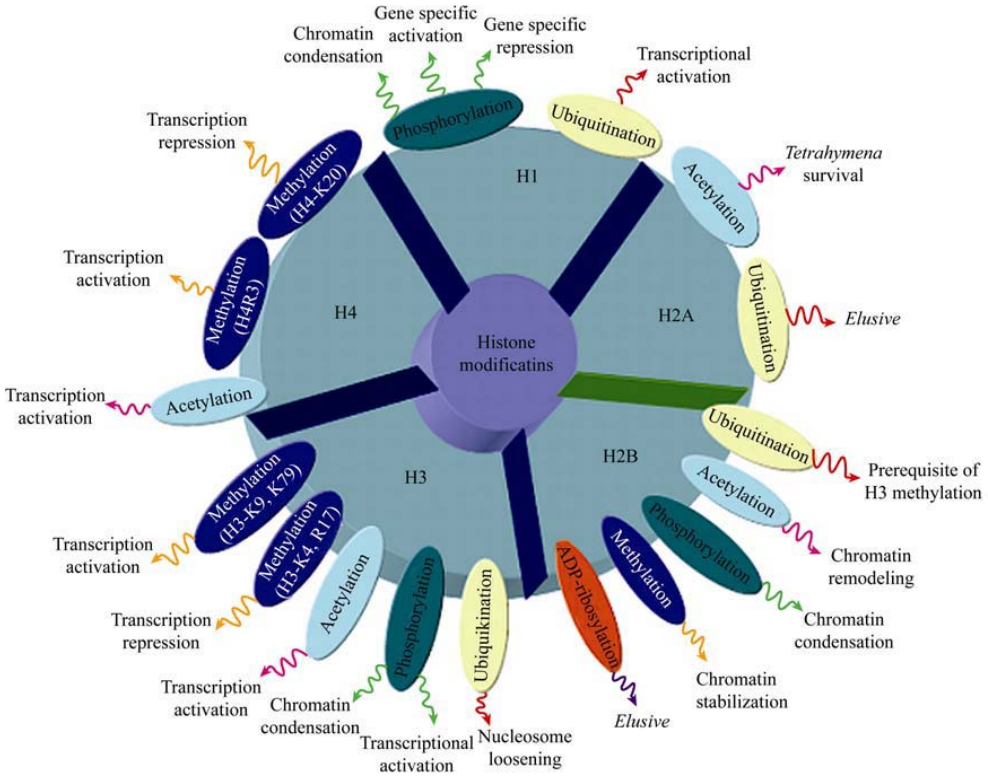

Fig. 1.3 *Graphical representation of histone modifications and their biological functions (Munshi et al., 2009**).***

It has been hypothesized that addition, interpretation and removal of the post-translational modification marks constitute the histone code, read by other proteins or protein modules and regulate chromatin states at the target genes (Strahl and Allis, 2000). The structure of chromatin provides a unique possibility to preserve the established arrangements of PTMs through cell divisions allowing the inheritance of cell type-specific gene expression patterns and thus to preserve cellular identity (Sawicka and Seiser, 2012).

1.2 Histone modifications and chromatin regulation

The highly dynamic structure of chromatin can respond to external stimuli and regulate all DNA-mediated processes within the nucleus. The major players in the chromatin regulation pathway are the histone proteins and their covalent modifications (Bannister and Kouzarides, 2011). It is proposed that histone modifications function as signalling platforms recognized by protein effectors. In turn, the effectors can ultimately determine the possible outcome of certain PTMs (Yun et al., 2011) which often depend on the chromatin and cellular context of such modifications (Berger, 2007). Proteins that are directly involved in post-translational modifications are often classified into three functional families: the enzymes which produce these modifications (the 'writers'), the proteins that recognize them (the 'readers'), and the enzymes that remove them (the 'erasers') (Yost et al., 2011). Given the importance of chromatin regulation in cell biology, the 'writers', 'readers', and 'erasers' are major targets for manipulation to further understand the histone code and the role it plays in the progression of human disease (Fletcher and Hansen, 1996; Luger and Richmond, 1998).

Post-translational modification of histones together with DNA methylation contribute to the epigenetic information within the chromatin (Munshi et al., 2009). The presence of different covalent modifications can alter the integrity and stability of a nucleosome. For example, lysine (K) acetylation (ac) has been shown to modify the secondary structure of the histone tail, weaken the interaction between the histones and DNA, and reduce internucleosomal interactions and chromatin folding (Fischle et al., 2003). Histone H4 acetylation at lysine 16 (H4K16ac) represents an essential

modification that can regulate the folding and chromatin alteration from heterochromatin to euchromatin (Shahbazian and Grunstein, 2007).

Phosphorylation of histone tails is controlled by the antagonistic action of protein kinases and phosphatases. The addition of a phosphate group from ATP to the target amino acid side chain and its removal regulates the chromatin structure (Oki et al., 2007). Histone H3 phosphorylation at serine (S) 10 and 28 are important covalent modifications for chromatin condensation and relaxation associated with mitosis, meiosis and transcription activation, respectively (Rossetto et al., 2012).

According to recent studies different histone methylations are recognized by 'readers' promoting the recruitment of other molecules that can regulate the chromatin and transcription states (Greer and Shi, 2012). In contrast to acetylation and phosphorylation, the methylation of lysines and arginines does not alter the charge of histone proteins (Bannister and Kouzarides, 2011). The degrees at which histone tails are methylated by methyltransferase enzymes (mono-, di-, tri-methylation of lysine; mono-, di-symmetric or asymmetric methylation of arginine) have been associated with distinct gene expression patterns. For instance, histone H3K4 tri-methylation (me3) is generally associated with active transcription or genes that are ready for activation. In contrast, repressed chromatin is marked by H3K27me3 as well as by H3K9me3, a binding platform for heterochromatin protein 1 (HP1) (Bernstein et al., 2002; Santos-Rosa et al., 2002).

ADP-ribosylation of histones by poly-ADP ribose polymerase family of enzymes has also been associated with an open chromatin state (Hassa et al., 2006). The relaxation of the chromatin might occur due to the negative charge of the ADP-ribose unit which repels the DNA (Messner and Hottiger, 2011). Little is known about the function of ADP-ribosylation, however increased levels of this modification were observed upon DNA damage (Hassa et al., 2006). The last 10 years of extensive research have linked ADP ribosylation with DNA replication and transcription that go far beyond the limited role in genotoxic stress response (Hottiger, 2011). These observations implicate ADP-ribosylation in different pathologies such as cancer, inflammation and neurodegenerative disorders (Hassa and Hottiger, 2008; Szabo et al., 2011; Yelamos et al., 2011).

A critical role in transcription, maintenance of chromatin structure and DNA repair has emerged for ubiquitination of histone proteins (Cao and Yan, 2012). The

mono-ubiquitynated histone H2A at lysine 119 correlates with gene silencing, whereas H2BK123 mono-ubiquitynation was shown to be associated with transcriptional initiation and elongation (Bannister and Kouzarides, 2011). Polyubiquitynation of K63 of histone H2A and H2AX, a variant of H2A, has been found to be required for DNA repair mechanism. Several studies demonstrated the occurrence of a cross-talk between histone ubiquitination and other covalent modifications. For example, mono-ubiquitination of H2B is a prerequisite mark for methylation of H3K4 and K27 and that H2A mono-ubiquitination is connected to H3K36me2 demethylation (Cao and Yan, 2012). Although the precise mechanism by which histone ubiquitination regulates different biological processes is poorly understood, aberrations within ubiquitination or deubiquitination of histones are clearly associated with multiple human diseases including cancer, making it a target for further investigations (Johnsen, 2012).

The language of histone modifications has an extra level of complexity. Certain covalent marks can influence the occurrence of one or more subsequent modifications either in *cis*, on the same histone molecule, or in *trans*, between histone molecules or across nucleosomes. The final biological read-out represents the crosstalk between distinct modifications with a particular impact on chromatin organization and structure (Fischle et al., 2003; Latham and Dent, 2007). There are several levels of "communication" between different histone modifications (Bannister and Kouzarides, 2011):

I. A particular residue can undergo multiple choices of post-translational modifications. For example the lysine residues on histone proteins can be acetylated, mono-, di-, tri-methylated or mono-ubiquitynated (Fischle et al., 2003; Kouzarides, 2007);

II. One covalent modification may depend upon a pre-existing mark. For instance, the methylation of H3K79 and H3K4 in yeast is dependent on the ubiquitynation of H2BK123 (Lee et al., 2007);

III. The affinity of a protein to a specific covalent mark can be disrupted by a neighbouring modification. During mitosis phosphorylation of H3S10 disrupts the binding of HP1 to methylated H3K9 and promotes acetylation on K14 in response to particular signals (Lee et al., 2007; Bannister and Kouzarides, 2011);

IV. A modification in an enzyme substrate can affect its enzymatic activity. For example, proline isomerization regulates methylation at H3K36 and the induction of transcription (Nelson et al., 2006);

V. The cross-talk between modifications can influence the recruitment of specific factors. For instance, the affinity binding of a PHD finger domain containing protein to H3K4me3 increases upon acetylation of H3K9 and H3K14 on the same tail of histone H3 (Vermeulen et al., 2010).

Taken together, histone modifications can act synergistically in a context-dependent manner to facilitate or repress chromatin-mediated processes. A major challenge is to decipher the many aspects that regulate changes in gene expression through histone and chromatin cross-talks (Fischle et al., 2003; Lee et al., 2010).

1.3 Histone acetylation

One of the most studied post-translational modifications is histone protein acetylation. The enzymatic reaction involves the covalent attachment of an acetyl group from acetyl-CoA to the ε-amino group of a lysine side chain. The positively charged amino group is neutralized and the interaction with the negative charges of the DNA phosphate backbone is blocked. It represents a highly dynamic process regulated by opposing action of two families of enzymes - histone acetyltransferases (HAT), recently designated as lysine acetyltransferase (KAT), and histone deacetylases (HDAC) (Allis et al., 2007; Munshi et al., 2009; Bannister and Kouzarides, 2011).

The level of histone acetylation is correlated with the level of transcription, operating as a hallmark of actively transcribed genes observed predominantly at promoters and 5′ ends of transcribed units (Choi and Howe, 2009). Lysine acetylation-dependent control of protein stability has also been reported. It has a direct regulatory potential that affects the turnover of different proteins such as transcription factors and proteins involved in nuclear functions (Sadoul et al., 2008). Considering its involvement in essential cellular processes such as gene expression, vascular remodelling, cell differentiation, neuronal plasticity, inflammation or metabolic events, lysine acetylation can be regarded as a master regulator in cellular biology (Furdas et al., 2012).

1.3.1 Histone acetylation in transcription, DNA damage and repair

Post-translational modifications that influence gene transcription can be classified in two groups: those that regulate gene activation and those that correlate with gene repression. As a general model it was suggested that acetylation, phosphorylation and ubiquitination are connected with activation while ubiquitination, sumoylation, deamination and proline isomerization have been implicated in repression. However, in the past decade the knowledge about these modifications has grown exponentially and it is more likely that any given PTM has the potential to play a role both in activation and repression of transcription under different conditions. For instance, histone methylation can have a positive effect when is observed in the coding region and a negative effect in the promoter region (Kouzarides, 2007).

Gene expression is highly regulated by the interplay between acetylation and deacetylation of nucleosomal histones through HAT and HDAC enzymes in the context of changing the tightly packed heterochromatin to a more relaxed euchromatin state (Munshi et al., 2009; Khan and Khan, 2010). The recruitment of HATs or HDACs to active transcriptional sites depends on different types of transcription factors. Their binding can enhance the addition of other factors to nucleosomal DNA, such as repressors or activators of transcription, thus preventing or triggering the formation of the RNA polymerase II preinitiation complex. For instance, the recruitment of the ATP-dependent SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodelling complex and the general transcription factor TFIID during transcription initiation requires histone H4K8 and H3K9/K14 acetylation (Agalioti et al., 2002).

The positioning of HATs and HDACs along the gene is highly regulated. At promoter regions the actively transcribed genes are marked by high levels of acetylation. In contrast, along the coding regions a low level of acetylation can be detected, that may help to inhibit initiation of transcription from cryptic intragenic promoters (Fukuda et al., 2006). This might suggest the presence of demarcation regions between hyper- and hypo-acetylated sites. Mutation of HAT and HDAC not only shifts the border between functional regions of chromosomes but can alter the segments of active regions within a gene (Fukuda et al., 2006).

In the presence of DNA damage the recognition and repair of the lesion must take place in the context of chromatin. In these conditions a more relaxed state is essential for the repair machinery to access the DNA breaks. Thus, ATP-dependent chromatin remodelling complexes and histone PTMs are important for this process and have been associated with DNA repair (Downs, 2008). Acetylation of histone H3 and H4 in their N-terminal tail have been found crucial for DNA repair in both mammalian and yeast cells (van Attikum and Gasser, 2005). Histone H3 acetylation occurs before and after double-strand break repair. The acetylation of H3K14 and H3K23 promotes H2AX ubiquitination, checkpoint activation and DNA repair, whereas H3K56 acetylation restores the chromatin structure and arrests checkpoint activation (Ikura et al., 2007; Chen et al., 2008).

The transient acetylation of histone proteins by HAT enzymes allows the repair machinery to access the lesion and bind to the chromatin. Indeed, a study performed on cells lacking histone acetylation demonstrated that the repair defect and impaired loading of repair factors was rescued by chromatin relaxation induced through chemical treatment (chloroquine, sodium butyrate or hypotonic conditions) (Murr et al., 2006). The TIP60 (Tat interactive protein 60) multiprotein complex in mammals, NuA4 (Nucleosome acetyltransferase of histone H4), SWR1 (SWI/SNF-related protein) and INO80 (Inositol-requiring protein 80) in yeast are responsible for the acetylation of several lysine residues (H3K14, K23, H4K5, K8, K12 and K14) following a double stranded DNA break (Kimura and Horikoshi, 1998). The mammalian TIP60 complex contains several subunits which provide acetylation and ATP-ase activities that contribute to histone exchange (Doyon and Cote, 2004). For instance, in *Drosophila melanogaster* in the presence of DNA damage the histone variant H2Av becomes rapidly phosphorylated on its C-terminus. The PTM is recognized by the TIP60 complex which can acetylate the histone variant and mediate its exchange with an unmodified H2Av (Kusch et al., 2004).

The tumour suppressor protein p53 functions as a transcription factor regulating gene expression in response to DNA damage. Following stress, p53 undergoes extensive PTMs to improve its function as a transcription factor and enable it to control the cellular decisions (cell cycle arrest, senescence or apoptosis). The tumour suppressor can bind to DNA and regulate the recruitment of general transcription factors thus promoting transcription of target genes (Patel et al., 2011).

The acetylation of the C-terminal end of p53 is crucial for its activation. More precisely, it enhances its DNA-binding ability, nuclear localization and its function in the recruitment of coactivators (Prives and Manley, 2001). To emphasize the importance of PTMs in the p53 regulatory mechanism, studies on mammalian cells demonstrated that in the presence of HDAC inhibitors the level of acetylated p53 was simultaneously increased and promoted apoptosis or senescence of cancerous and normal cells (Langley et al., 2002).

1.3.2 The variety of lysine acetyltransferases

The first lysine acetyltransferase was identified in 1995 when a polypeptide of 55 kDa (p55) was isolated from macronuclear preparation of *Tetrahymena thermophilia* and its HAT activity was demonstrated (Brownell et al., 1996a). The p55 enzyme proved to be surprisingly similar in amino acid sequence to the yeast transcriptional coactivator GCN5 (General Control Nonderepressible-5). These findings provided the first confirmation for a direct connection between histone acetylation and transcription regulation (Brownell et al., 1996b). In the past 15 years studies performed on different organisms have identified a significant number of coactivator proteins that possess HAT activity demonstrating their evolutionary conservation from yeast to humans (Fukuda et al., 2006).

According to their localization within the cell, HATs are classified in two different classes: type A-HATs and type B-HATs (*Table 1.1*). The members of type A class are found within the nucleus, often organized into large multiprotein complexes (Yang and Seto, 2007). They are responsible for the acetylation of histones and regulating the chromatin assembly and gene transcription (Munshi et al., 2009). Furthermore, the type A-HAT class can be divided into five families based on amino acid sequence homology as well as on the biochemical mechanism of acetyl transfer:

- **GNAT** (GCN5-related N-acetyltransferase) family members consist of HATs with sequence and structure similar to GCN5 (Dyda et al., 2000) and can promote the recruitment of transcription factors to the promoters (Vetting et al., 2005);
- **MYST** family, including the founding members MOZ, Ybf2/Sas3, Sas2 and Tip60 that are involved in the regulation of a variety of DNA-mediated reactions (Fukuda et al., 2006);

- **CBP/p300** family named after the two human paralogs p300 and CBP with a wide substrate specificity for histones and role in gene transcription regulation (Bannister and Kouzarides, 1996; Ogryzko et al., 1996);
- **Transcriptional Factors-related HATs** family (Mizzen et al., 1996);
- **Nuclear Receptor coactivator** families (Hodawadekar and Marmorstein, 2007; Furdas et al., 2012).

The type B-HAT class is represented by HAT1 (Histone Acetyltransferase 1), a primarily cytoplasmic enzyme involved in acetylating the newly synthesized free histones prior their incorporation into replicated chromatin (Munshi et al., 2009).

Most lysine acetyltransferase enzymes function as multisubunit complexes. These complexes are generally comprised of one protein, that serves as catalytic subunit, and accessory proteins which are required either for enzymatic activity or for targeting the HAT to specific locations within the genome (Choi and Howe, 2009). Lysine acetylation, in addition to its role in modulating the interaction between DNA and histones, can act as molecular tag particularly recognized by a structural motif called the bromodomain, named after the *Drosophila* protein BRAHMA (Yun et al., 2011). The evolutionarily conserved bromodomain was the first protein module to be shown to interact with acetylated lysines in the histone N-terminal tail (Jenuwein and Allis, 2001). The association of acetylated histones and bromodomains maintains the acetylation state and regulates the activities of bromodomain-containing factors (Fukuda et al., 2006). Bromodomains were identified in several HATs and chromatin remodelling complexes as well in general transcription factors with important functions in gene activation (Fischle et al., 2003; Suganuma and Workman, 2011).

1.3.3 The GNAT acetyltransferase family

Shortly after the yeast GCN5 protein was characterized as an acetyltransferase enzyme several other HAT proteins were identified and classified into distinct families (*Table 1.1*) (Roth et al., 2001). Each HAT family appears to have enzymatic preferences for distinct substrates and acting in different functional contexts (Marmorstein and Roth, 2001).

The GCN5-related N-acetyltransferase family is represented by class A-HAT histone acetyltransferase GCN5 and by class-B histone acetyltransferase HAT1, the Elongator complex subunit ELP3 (Elongator complex protein 3), the PCAF (p300/CBP associated factor), the mediator complex subunit NUT1 (Negative regulation of URS

Two) and HPA2 (Histone and other Protein Acetyltransferase) (*Table 1.1*) (Selvi et al., 2010). According to their amino acid sequences the members of GNAT family share several functional domains:

- N-terminal region of variable length;
- highly conserved acetyltransferase domain important for acetyl-CoA recognition and binding;
- domain that interacts with ADA2 (Alteration/Deficiency in Activation protein 2) coactivators,
- carboxy-terminal bromodomain that interacts with acetyl-lysine residues (Roth et al., 2001).

The ELP3 lysine acetyltransferase, member of the GNAT family, is the catalytic subunit of the Elongator complex. It was identified as a subunit of a multisubunit complex that co-purifies with the hyper-phosphorylated form of the RNA polymerase II holoenzyme in yeast and human cells (Singh et al., 2010). The ELP3 protein has been described to promote the acetylation of alpha-tubulin in microtubules of cortical neurons, hence modulating the migration and differentiation of projection neurons during corticogenesis (Creppe et al., 2009). ELP3 can promote histone H3 acetylation throughout the coding regions of certain neuronal cell motility genes in mammalian cells suggesting a role in neuronal gene regulation (Close et al., 2006). Disruption of the HAT activity of ELP3 has been identified in a number of human disorders that specifically affect neuronal functions (Singh et al., 2010).

Several functional studies have emphasized the role of GNAT members in regulating cell growth and development. Knockout of murine GCN5 resulted in embryonic lethality (Bu et al., 2007). In yeast, disruption of the gene encoding GCN5 impaired normal progression through the G2-M phase of the cell cycle, and mitotic gene expression (Trievel et al., 1999). PCAF, the homologue of GCN5 and another important member of the GNAT family plays essential role in transcriptional activation, cell cycle arrest and cell differentiation in mammalian cultured cells (Selvi et al., 2010). In young adult mice loss of PCAF expression resulted in neurological dysfunctions, including memory deficit and exaggerated responses to external stimuli (Maurice et al., 2008). Studies have also reported that PCAF possesses an intrinsic ubiquitination activity important for controlling the level of oncoprotein HDM2

(human double minute-2 protein) and, consequently, influencing the stability and activity of tumour suppressor p53 (Linares et al., 2007).

Both enzymes, GCN5 and PCAF, have been characterized as catalytic subunits of multiprotein SAGA-type (SPT-ADA-GCN5 acetyltransferase) complexes (Grant et al., 1997; Nagy and Tora, 2007). Furthermore, in humans, another multiprotein complex ATAC (Ada Two A containing) has been identified to contain GCN5 or PCAF as its catalytic subunit (Wang et al., 2008).

Table 1.1 *Lysine acetyltransferase families and their transcription related functions (Torok and Grant, 2004; Fukuda et al., 2006; Allis et al., 2007).*

Class	*Family*	*HAT*	*HAT complex*	*KAT*	*Histone substrate*	*Function*
A	GNAT	Gcn5	SAGA, SLIK, SALSA, ADA, HAT-A2, ATAC	KAT2	H3 and H2B	Transcription activation, DNA repair
		Gcn5L	STAGA, TFTC	KAT2A	H3 and H2B	Transcription activation
		PCAF	PCAF	KAT2B	H3 and H2B	Transcription activation
		Elp3	Elongator	KAT9	H3	Transcriptional elongation
		HPA2	HATB		H4	Transcriptional elongation
		Nut1	Mediator		H3, H4	RNA Pol II transcription
	MYST	Tip60	NuA4	KAT5	H4, H2A, dH2Av/yHtzl	Transcription activation, DNA repair
		Sas3	NuA3	KAT6	H3	Transcription activation, elongation, DNA replication
		MOF/MYST1	MSL	KAT8	H4	Chromatin boundaries, dosage compensation, DNA repair
		MOZ/MYST3		KAT6A	H3	Transcription activation
		MORF/MYST4		KAT6B	H3	Transcription activation
		HBOI/MYST2		KAT7	H4 and H3	Transcription, DNA replication
	p300/CBP	CBP	p300/CBP	KAT3A	H3, H4, H2A, H2B	Transcription activation
		p300	p300/CBP	KAT3B	H3, H4, H2A, H2B	Transcription activation
	TF-related HATs	TAF1/TBP	TFIID	KAT4	H3, H4	Transcription activation
		TFIIIC90	TFIIIC	KAT12	H3	RNA Pol III transcription
	Nuclear receptor co-activators	SRC-1		KAT13A	H3, H4	Transcription activation
		ACTR		KAT13B	H3, H4	Transcription activation
		P160		KAT13C	H3, H4	Transcription activation
		CLOCK		KAT13D	H3, H4	Transcription activation
B	GNAT related	Hat1	HAT1/2	KAT1	H4	Histone deposition, DNA repair

1.4 The SAGA and ATAC acetyltransferase complexes

Homologues of the GCN5 enzyme have been identified as the catalytic component of different multiprotein HAT complexes in nearly all eukaryotes (Brown et al., 2000; Sterner and Berger, 2000). In yeast, recombinant GCN5 could catalyse the acetylation of free histones on H3 at K9/K14 and H4 at K8/K16 (Kuo et al., 1996). However, in the context of the nucleosome core particle in which histones exist within chromatin, recombinant GCN5 was not able to acetylate histone proteins (Kuo et al., 1996). This implied that other factors are necessary to regulate the recognition of histone substrate or the acetyltransferase activity of GCN5 (McCullough and Grant, 2010). A combination of *in vitro* and *in vivo* genetic and biochemical studies have demonstrated that yeast GCN5 can functionally interact with two adaptor proteins, ADA2 and ADA3. Their association into a heterotrimeric complex could acetylate nucleosomal histones (Balasubramanian et al., 2002). Further studies led to the identification and characterization of two high-molecular-mass HAT complexes, SAGA and ADA, with apparent molecular masses of 1.8 and 0.8 mega Dalton (MDa). Both contain GCN5 as catalytic subunit and ADA2 as adaptor protein (Grant et al., 1997). The architecture and function of yeast acetyltransferase complexes have served as a foundation for the identification and characterization of similar complexes in higher eukaryotes (McCullough and Grant, 2010).

In contrast to yeast, vertebrates possess two GCN5 isoforms that are believed to originate from alternative splicing of a single gene product (Smith et al., 1998). The short isoform (GCN5S) is similar in size and function to the yeast GCN5 protein. The higher molecular weight isoform (GCN5L) is predominantly expressed and incorporated into human SAGA and shows high similarity in the amino-terminal domain to another GCN5-related family member, PCAF (Xu et al., 1998). The GCN5L isoform was also identified in *Drosophila melanogaster* (dGCN5) suggesting an evolutional conservation among metazoans (Smith et al., 1998). Moreover, during evolution the yeast *Ada2* gene diverged into two functionally distinct ADA2 paralogs in the animal kingdom, ADA2-alpha (ADA2a) and ADA2-beta (ADA2b). GCN5 was shown to interact with both ADA2 proteins in *Drosophila* (Muratoglu et al., 2003*)*, mice (Candau et al., 1996) and humans (Barlev et al., 2003). Biochemical purifications

have demonstrated that the two ADA2 proteins are present in distinct multiprotein complexes with different lysine target specificity. The ADA2b protein was found to be associated with SAGA-like complexes whereas ADA2a protein was identified as a subunit of ATAC and PCAF complexes (Kusch et al., 2003; Muratoglu et al., 2003; Guelman et al., 2006; Gamper et al., 2009). Characterization of the SAGA complex showed that GCN5, ADA2 and ADA3 were not required for the integrity of the complex, rather for its ability to recognize and acetylate nucleosomal substrates (Grant et al., 1997).

SAGA is a large complex composed of 18 to 20 protein subunits. It was initially characterized in yeast and is highly conserved throughout eukaryotes including *Drosophila melanogaster* and mammals (*Table 1.2*).

Table 1.2 *Subunit composition of SAGA and ATAC complexes in Saccharomyces cerevisiae, Drosophila melanogaster and Homo sapiens (Lee and Workman, 2007; Nagy and Tora, 2007; Wang et al., 2008; Nagy et al., 2010).*

Complex	**ySAGA**	**dSAGA**	**hSTAGA**	**dATAC**	**hATAC**
Organism	*S. cerevisiae*	*D. melanogaster*	*H. sapiens*	*D. melanogaster*	*H. sapiens*
Catalytic subunit	yGCN5	dGCN5	hGCN5	dGCN5	hGCN5
Complex subunits	yTRA1	dTRA1	hTRRAP		POLE4
	ySPT7	dSPT7	hSTAF65γ	D12	YEATS2
	ySPT8			CG30390	
	ySPT3	dSPT3	hSPT3	dATAC2 (CG10414)	hATAC2
	ySPT20			CG10238	MBIP
	yADA1	dADA1	hSTAF42	dATAC1 (CG9200)	ZZZ3
	yADA2	dADA2B	hADA2B	dADA2A	hADA2A
	yADA3	dADA3	hSTAF54	dADA3	hADA3
	ySGF29	dSGF29	hSTAF36	CG30390	hSGF29
	ySGF73		hATXN7		
	yUBP8		hSTAF60		
	ySGF11		hSTAF46		
	yTAF5	dTAF5	hTAF5L	WDS	WDR5
	yTAF6	dTAF6	hTAF6L		MAP3K7
	yTAF9	dTAF9	hTAF9	dATAC3	UBAP2L
	yTAF10	dTAF10B	hTAF10	dNC2β	hNC2β
	yTAF12	dTAF12	hTAF12	CHRAC14	POLE3
	yCHD1		hSTAF36		
	ySUS1	WDA			TBP
				dHCF	hHCF1

SAGA complex can be divided into four separate modules based on genetic, biochemical and structural studies from *Saccharomyces cerevisiae* (Weake and Workman, 2012).

- The HAT module contains GCN5, ADA2, ADA3 and SGF29 (SAGA-associated factors of 29) and catalyses the acetylation of multiple lysine residues in order to make the chromatin accessible for binding of transcription factors and the formation of the preinitiation complex (PIC) (Balasubramanian et al., 2002).
- The deubiquitinating (DUB) module consists of Ubp8, an ubiquitin specific protease which has been shown to associate with SGF11, SGF73 and SUS1 (Sl gene Upstream of ySa1) to facilitate elongation through deubiquitination of H2B which allows the recruitment of CTK1 (CTD kinase subunit alpha) kinase and subsequent phosphorylation of the Pol II C-terminal domain (Wyce et al., 2007).
- The TAF module includes several TATA-binding proteins (TBP)-associated factors that are shared with TFIID general transcription factor and might play an architectural role within SAGA (Lee et al., 2011).
- The TRA1 module that together with several SPT (SuPpressor of Ty) proteins function in the recruitment of Pol II and preinitiation complex formation (Bhaumik and Green, 2001).

The ATAC complex is exclusively present in multicellular eukaryotes and absent in yeast (*Table 1.2*) (Barlev et al., 2003; Muratoglu et al., 2003; Suganuma et al., 2008). Similarly to SAGA, the ATAC complex contains a HAT module composed of GCN5/PCAF enzymatic subunit and several adaptor proteins that modulate the catalytic activity of the enzymatic complex (*Table 1.2*) (Gamper et al., 2009). Moreover, ATAC harbours a secondary subunit, ATAC2 with putative HAT functions (Suganuma et al., 2008). *In vivo* and *in vitro Drosophila* studies demonstrated the existence of an interplay between dATAC and chromatin remodelling complexes. Thus, a mechanism might be proposed where binding of ATAC to the chromatin depends on chromatin remodelling, which is further regulated by the enzymatic activity of ATAC subunits (Carre et al., 2008; Suganuma et al., 2008; Spedale et al., 2012). Additionally, in pluripotent stem cells ATAC was found to form a stable

interaction with the Mediator complex, an important intermediary between activators and RNA Pol II (Krebs et al., 2010).

Genetic analysis in *Drosophila* established that both dGCN5-containing complexes are important during embryonic development (Pankotai et al., 2005; Carre et al., 2008), although, according to DNA microarray analysis, they regulate different sets of genes. The dATAC mutation affected the expression profile of 40% of all transcripts, mostly those involved in the ecdysone transcription pathway, while alteration of dSAGA function affected only 3% of the transcripts including those involved in antimicrobial defence mechanism (Zsindely et al., 2009). Recent studies on the binding profile of human ATAC and SAGA have indicated that while ATAC is able to bind to three distinct locations on the chromatin, known enhancers, transcription start sites (TSS) and at distal loci not marked by previously described enhancer features (H3K4me1, DHSs, Pol II), SAGA can only bind to known enhancers and TSSs (Krebs et al., 2011).

1.4.1 ADA2 coactivator proteins

Genetic studies in *Saccharomyces cerevisiae* have been essential for the discovery of several *Ada* genes as cofactor proteins involved in eukaryotic transcription. The ADA2 proteins have been reported to play an important role in transcriptional initiation site selection, they interact with basal transcription factors and potentiate the HAT activity of GCN5 (Berger et al., 1992; Barlev et al., 1995; Balasubramanian et al., 2002; Barlev et al., 2003). In contrast with the single *Ada2* gene characterized in yeast, in metazoan organisms (including mouse and human) and *Arabidopsis thaliana*, there are two genes encoding *Ada2*-type coactivators (Candau et al., 1996; Stockinger et al., 2001). In *Arabidopsis thaliana* the *Ada2b* gene is involved in abiotic stress response by controlling histone acetylation in the presence of salt stress or by regulation of the nucleosome occupancy in low temperature response (Vlachonasios et al., 2011).

Functional differences between the two human ADA2 proteins, ADA2-alpha and ADA2-beta, have been reported (Barlev et al., 2003). ADA2-alpha was found to be a stable component of the human PCAF complex (Ogryzko et al., 1998). In contrast, ADA2-beta appeared not to be a stable component of PCAF- or GCN5-containing macromolecular complexes, but interacted with subunits of the SWI/SNF chromatin-

remodelling complex (Barlev et al., 2003) and enhanced the acetylation of nucleosomes by GCN5L (Gamper et al., 2009).

The *Drosophila* genome also comprises of two *dAda2* genes encoding two adaptor proteins referred to as dADA2a and dADA2b. Studies have demonstrated their essential roles during development as subunits of two functionally and structurally distinct multiprotein complexes (Kusch et al., 2003; Muratoglu et al., 2003; Qi et al., 2004). dADA2b is found in the 2 MDa *Drosophila* dSAGA complex, while dADA2a is associated with dATAC, a 0.8 MDa complex (Kusch et al., 2003; Guelman et al., 2006). Gene characterization showed that through alternative splicing the *dAda2a* gene directs the synthesis of not only the dADA2a protein but that of the RNA Pol II subunit RPB4 as well. In the same manner, the *dAda2b* gene encodes two protein isoforms, dADA2bS and dADA2bL, identical in their N-terminal but distinct in their C-terminal regions (Muratoglu et al., 2003).

In *Drosophila* the *dAda2a* and *dAda2b* genes are located on the 3rd chromosome in the 90F10 and 84F5 cytological region. The two dADA2 proteins are similar in containing a ZZ domain in their N terminal region, followed by a SANT domain and two or three less conserved regions designated as ADA boxes (Kusch et al., 2003; Muratoglu et al., 2003). Similar structural organization for ADA2 is recognizable in most metazoans. The ZZ is a putative zinc-binding domain, the SANT is a DNA-binding domain and the function of ADA boxes is unknown. Recently, the last of the three ADA boxes of human ADA2-alpha has been identified as a SWIRM domain, a structural unit supposed to bear chromatin binding function (Qian et al., 2005; Yoneyama et al., 2007). Significantly, the four α-helices constituting the SWIRM domain in most ADA2 proteins can be identified in the C-terminal region of only one of the two dADA2 proteins (Yoneyama et al., 2007).

Although the similarity between the two dADA2 proteins in their N-terminal halves is over 50% (Kusch et al., 2003; Muratoglu et al., 2003), they participate strictly in distinct complexes and are not able to functionally substitute for each other (Pankotai et al., 2005). Null mutations of *dAda2b* (*ada2b*^{d842}) interfere with the function of dSAGA and result in decreased histone H3K9ac and H3K14ac levels *in vivo*, as clearly observable on antibody stained polytene chromosomes of mutant larvae (Qi et al., 2004; Pankotai et al., 2005). On the other hand, the *dAda2a* null

mutation (*ada2a*^{d189}) abolished the K5 and K12 acetylation of H4 while displaying no significant change in K9 and K14 acetylation of histone H3 (Ciurciu et al., 2006).

Overall, the ADA2 coactivator proteins together with other components of the histone acetyltransferase complexes are major determinants in specifying the substrate preference as well as the gene-specific targeting of these complexes.

1.5 Histone modifications and diseases

Histone modifications are known to regulate different physiological processes including transcription, replication, DNA repair and cell cycle progression (Ehrenhofer-Murray, 2004; Groth et al., 2007; Kouzarides, 2007). Thus, misregulation of the activity of the histone modifying enzymes can result in altered gene expression patterns that can lead to many human diseases, including cancer.

Although cancer is fundamentally a genetic disease, increasing evidence indicate that covalent histone modifications are globally and locally altered in cancer epigenomes (Jones and Baylin, 2007). The global reduction of H4K16ac and H4K20me3 has been associated with breast and liver cancer (Bhaumik et al., 2007). Decrease of H4K16 acetylation correlates with an increased genomic instability facilitating mutations and chromosomal rearrangements that might progress to cancer (Lennartsson and Ekwall, 2009). Mice deficient for the SUV39 (suppressor of variegation 3-9) H3K9 methyltransferase demonstrated reduced levels of heterochromatic H3K9me2/3 with severely impaired viability and chromosomal instabilities plus an increased risk of developing cancer (Peters et al., 2001). Mutations of certain HATs can also cause cancer as observed in mice (Kung et al., 2000) as well as in several cases of human leukaemia (Shigeno et al., 2004). At present, a promising therapeutic potential to reverse abnormal epigenetic changes associated with cancer is based on the administration of HDAC inhibitors. Studies in cell culture and animal models have shown that inhibition of histone deacetylases induces proliferation arrest, differentiation and apoptosis of cancer cells (Marks et al., 2000; Vigushin and Coombes, 2002).

In several neurodegenerative disorders such as Huntington's, Parkinson's, Alzheimer's diseases and amyotrophic lateral sclerosis altered levels of histone acetylation have been observed (Selvi et al., 2010). *In vitro* studies on cultured

cerebellar primary neurons have reported increased deacetylation of histone H3 and H4 that precede neuronal death (Rouaux et al., 2003). Numerous studies suggest the use of several HDAC inhibitors to restore histone acetylation and transcriptional activation in different neurodegenerative disease models (Selvi et al., 2010).

Histone covalent modifications are also important for the control of learning and memory functions. In mice, loss of PCAF function correlated with decreased learning competence and increased stress response (Maurice et al., 2008). The Rubinstein-Taybi syndrome is an autosomal dominant disorder associated in ~55% of cases with mutations in CREB binding protein (CBP) (Petrij et al., 1995).

Although little is known about the implication of covalent modifications in autoimmune diseases recent studies have described a role for histone modifications in rheumatoid arthritis. It is a chronic disease characterized by systemic inflammation, joint destruction, disability and pain (Klein and Gay, 2013). The transcription factor NF-κB - a key inflammatory regulator - binds very poorly to nucleosomal DNA. Different histone modifications like histone H3K9 and S10 phosphoacetylation, reduction in H3K9me and increase in H3/H4 acetylation, are needed to allow efficient NF-κB binding to its targets. Thus, in rheumatoid arthritis, the reduced activity of HDACs plays a key role in regulating NF-κB–mediated gene expression. Although treatments for rheumatoid arthritis with HDAC inhibitors in different animal models have shown promising results (Nishida et al., 2004; Lin et al., 2007; Joosten et al., 2011), the used inhibitors lack specificity for a single HDAC isoform and the mechanisms of action remains unknown (Klein and Gay, 2013).

Elucidating the role of HATs and HDACs in orchestrating proper cellular development and differentiation will enable the therapeutic usage of modulators aimed at preserving the physiological equilibrium of acetylation (Arrowsmith et al., 2012).

Chapter 2.

Aims

In *Drosophila melanogaster* dADA2a and dADA2b proteins are subunits of dATAC and dSAGA HAT complexes, respectively. Despite the fact that the two dADA2 proteins contain similar conserved domains and interact with similar partners they function in distinct HAT complexes. These observations made us interested in determining what particular domain within dADA2 proteins is responsible for their interaction with different transcriptional coactivators and the GCN5 acetyltransferase. The regions that specify the association of dADA2a and dADA2b proteins to one or the other HAT complexes were investigated by:

- Constructing transgenes that direct the expression of chimeric dADA2a/2b or dADA2b/2a proteins;
- Assessing their *in vivo* function by expressing the chimeric proteins in *D. melanogaster ada2a*^{d189} or *ada2b*^{d842} mutants;
- Determining the histone acetylation pattern on polytene chromosomes of both of the *ada2* null animals expressing the chimeric transgenes;
- Comparing the expression patterns of selected genes from *ada2a*^{d189} or *ada2b*^{d842} null mutants to those expressing the chimeric proteins.

Furthermore, two dADA2b protein isoforms have been reported in *Drosophila melanogaster* which might extend the functional complexity of dSAGA. The two dADA2b isoforms are identical at the N-terminal region but differ in their C-terminus. Both isoforms contain a Zinc finger and a SANT domain in their N-terminal region and two ADA boxes. Only the long dADA2b isoform contains a third ADA box in its C-terminal part. These observations made us interested to investigate whether the two isoforms are subunits of the same HAT complex or there are distinct HAT complexes with different dADA2b isoforms.

Chapter 3.
Materials and methods

3.1 Molecular cloning

3.1.1 Generation of chimeric DNA sequences

To generate plasmid constructs suitable for the expression of dADA2a, dADA2b (short isoform) and their chimeras in *Drosophila* S2 cells, and for the insertion of these transgenes in targeted landing sites in the *D. melanogaster* genome, we obtained the coding sequences of *dAda2b* and *dAda2a* from previously constructed pBTM-dADA2 plasmids (Le Douarin et al., 2001; Muratoglu et al., 2003). The coding regions were released by *EcoRI-SalI* digestions and reinserted into *EcoRI-XhoI* sites of pENTR3C vector (Invitrogen) generating pENTR3C-*dAda2*a and pENTR3C-*dAda2b*, which made possible the use of the Gateway cloning system (Gateway system, Invitrogen). This technology ensures fast, efficient directional cloning where the gene of interest is excised from the Entry clone and integrated into the Destination vector suitable for gene expression (*Fig. 3.1*).

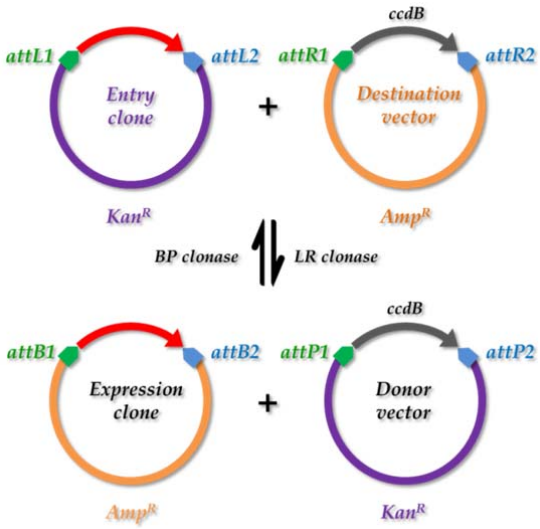

Fig. 3.1 *Overview of the Gateway technique. During LR reaction Entry clone with attL recombines with a Destination vector with attR to form a new expression clone with attB and a by-product with attP. The BP reaction is used to create an Entry Clone from Expression Clones or PCR products. Image adapted from http://www.invitrogen.com*

For generating dAda2b^{S}2a, dAda2b^{M}2a and dAda2b^{L}2a chimeric constructs, fragments corresponding to different sizes of the *dAda2b* N-terminal regions were amplified by PCR using pENTR3C-*dAda2b* as template with oligonucleotide primers which introduced a *BamHI* site. At the same time, the pENTR3C-*dAda2a* vector was digested with *BamHI* which removed a 791 bp fragment corresponding to the N-terminal region of *dAda2a*. The digestion fragments were separated, according to size, in agarose gel using electrophoresis. The fragments desired for ligation were then extracted using QIAquick Gel Extraction Kit (Qiagen). To ligate the dAda2b^{S}, dAda2b^{M} and dAda2b^{L} DNA fragments into the pENTR3C-*dAda2a* vector T4 DNA Ligase (Promega) was used according to the manufacturer's instructions.

A similar strategy was performed to generate dAda2a2b chimeric DNA by exchanging the C-terminus of pENTR3C-*dAda2a* (removed after *SpeI* and *XhoI* digestion) with amplified 903 bp sequence of *dAda2b*. The C-terminal region of *dAda2b* was amplified using pENTR3C-*dAda2b* as PCR template with dAda2bFw and dAda2bRv oligonucleotide primers (*Table 3.1*) which introduced *SpeI* and *XhoI* sites.

Table 3.1 *Primer sequences used in this study*

Construct	*Primer symbol*	*Primers* *Sequence 5′-3′***	*Restriction site*	*Amplicon size (bp)*	*Amplicon aa region*
dAda2b^{S}	pENTR3C Fw*	CTGATAGTGACCTGTT CGTTGCAA	-	486	1-144
	dAda2b^{S} Rv	GTCTGGATCCGTGCCA TTTACAAA	BamHI		
dAda2b^{M}	dAda2b^{M} Rv	ATCTGGATCCAAGGTA GAGAGAGC	BamHI	594	1-181
dAda2b^{L}	dAda2b^{L} Rv	GATTGGATCCAGCTTA AGCATCAC	BamHI	750	1-233
dAda2a2b	dAda2b Fw	GGACTAGTAAATGGCA CGATTGGAAGGGCC	SpeI	903	118-418
	dAda2b Rv	GACCTCGAGCTATCCA AGTAGTTTTTGCTG	XhoI		
dAda2bL isoform	AbNCO	CCATATGGCCATGGCA AG	NcoI	700	
	AbRIBH	CATGGATCCGAATTCA GTGGCTCAGCCAGC	BamHI		

*The pENTR3C Fw primer was used to generate dAda2b^{M} and dAda2b^{L} PCR fragments as well
**Restriction sites used for cloning steps are underlined
***aa represents amino acid

The chimeric constructs and the corresponding controls were transferred to pAFW or pAHW containing N-terminal FLAG or HA tag, by site-specific recombination according to the manufacturer protocol (Gateway system, Invitrogen).

3.1.2 Constructs for co-immunoprecipitation

The two *dAda2b* isoforms have the same 5′ and middle coding region. To generate *dAda2bL* (*dAda2b* long isoform) constructs suitable for transient transfection in S2 cells, the 3′ region was generated by PCR from a *Drosophila* pACT2 library using AbNCO-AbRIBH primer pairs (*Table 3.1*) (Pankotai et al., 2005). To generate pBTM-*dAda2bL* the corresponding PCR fragment was ligated into the *NcoI-BamHI* sites of the pBTM-*dAda2bS* vector (Muratoglu et al., 2003). The obtained full-length coding region of *dAda2bL* was further digested from pBTM with *EcoRI-SalI* and the fragment was ligated into *EcoRI-XhoI* sites of pENTR3C Gateway vector. Following an LR reaction as described in *3.1.1 dAda2bL* was introduced into pAFW vector containing an N-terminal FLAG tag.

For generation of N-terminal HA tagged dADA3 the corresponding coding sequence was subcloned from a previously described pBTM-*dAda3* plasmid (Muratoglu et al., 2003) into pENTR3C by digesting of both vectors with *EcoRI* followed by ligation of *dAda3* to pENTR3C. The obtained Entry clone was recombined into pAHW destination vector according to the Gateway Technology protocol. Cloning of the *Drosophila* p53 into pAHW vector suitable for transient S2 cell transfection was previously described (Pardi et al., 2011).

All cloned DNA vectors were sequenced to confirm fragments as being in the correct orientation and in-frame.

3.2 Drosophila S2 cell transient transfection

The S2 cell line derives from a primary culture of late stage (20-24 hours old) *Drosophila melanogaster* embryos (Schneider, 1972). Cells were cultured at 25°C in Schneider's *Drosophila* Medium (Sigma) supplemented with 10% FBS (fetal bovine serum, Sigma) and antibiotics (50 U of penicillin/ml, 100 U of streptomycin/ml). Every two days the cells were split when they had reached 80% confluence.

For transient transfection about 4×10^6 cells were seeded into a 60 mm culture dish containing 5 ml of culture medium. The next day cells were transfected using Effectene Transfection Reagent (Qiagen) according to the manufacturer's protocol.

3.3 Co-immunoprecipitation

3.3.1 Purification of proteins expressed in S2 cells

Transfection of S2 cells was performed as described above. After 48 h, cells were harvested by centrifugation (2000 rpm, 5 min), washed with 1 ml of ice-cold PBS and incubated in sonication buffer (50 mM TRIS-HCl pH 7.9, 2 mM EDTA, 50 mM NaCl, 0.1 mM PMSF, 0.1 mM aprotinin and 0.1 mM leupeptin) for 30 min on ice.

For co-immunoprecipitation the cells were lysed with 0.5 ml of RIPA buffer [10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.5% SDS, 0.1 mM PMSF, 0.1 mM aprotinin and 0.1 mM leupeptin] for 30 min on ice. The reaction tubes were centrifuged (13000 rpm, 10 min, 4°C), the pellet was discarded and the supernatant was frozen at -80°C for further use.

3.3.2 Co-immunoprecipitation of proteins

The clarified cell lysate was incubated with 20 µl anti-FLAG M2-agarose beads (Sigma) overnight at 4°C following the manufacturer's instructions. The immunoprecipitates were washed five times (4°C, 10 min) with TBST buffer [50 mM Tris-HCl (pH 7.5), 400 mM NaCl, 1% TWEEN 20] on gentle rotation and co-purified proteins were eluted from the beads in 2× sodium dodecyl sulphate (SDS) loading buffer for 5 min at 95°C.

3.4 Western blotting

For immunological detection of the hybrid proteins, transiently transfected cells were harvested and protein extraction was performed as described at *3.3.1*. The protein concentrations were measured using Bradford reagent and 20-25 µg protein was separated on 10% SDS-PAGE and transferred by electroblotting to 0.4 µm nitrocellulose membrane. The membranes were blocked for 1 h in 5% dry milk in TBST (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20) and incubated overnight with primary antibody diluted in TBST with 2% BSA. For the detection of

hybrid proteins M2 anti-FLAG antibody (Sigma) in 1:10000 dilution was used. The same membrane was washed 4× for 10 min in TBST and reprobed with anti-Tubulin monoclonal antibody (Sigma) at 1:5000 dilution.

To compare histone H3, H3K14ac and H4K12ac levels, total protein extracts were prepared from 10 third instar larvae of the $gcn5^{E333st}$ and w^{1118} genotype, crushed in sonication buffer containing 1mM DTT, and protease inhibitor mix (0.1 mM PMSF, 0.1 mM aprotinin and 0.1 mM leupeptin) and cleared by centrifugation (5 min 13000 rpm). Protein concentration was determined by Bradford assay and equal amounts of protein samples were loaded on 15% SDS-PAGE and transferred by electroblotting to 0.2 μm nitrocellulose membrane. For immunoblotting specific commercially available antibodies were used at the following dilutions: H3 (Abcam, ab1791) 1:5000, H3K14ac (Upstate, 06-911) 1:5000 and H4K12ac (Abcam, ab1761) 1:1000.

For the detection of co-immunoprecipitated proteins the following primary antibodies were used: Anti-HA tag antibody (Abcam, ab9110) in 1:5000 dilution, monoclonal Anti-FLAG M2 antibody (Sigma, F1804) in 1:10000 dilution. Membranes were washed in TBST, incubated with anti-rabbit-HRP (DACO, P0448) and anti-mouse-HRP (DACO, P0260) secondary antibodies, washed again extensively and developed using the ECL (Millipore) following the manufacturer's recommendations.

3.5 Drosophila genetic analysis

3.5.1 Fly husbandry

All flies were normally raised on standard *Drosophila* medium at 25°C (Ashburner, 1989). Flies were subsequently transferred to fresh vials approximately every two days.

3.5.2 Drosophila collection

The $ada2a^{d189}$ and $ada2b^{d842}$ null alleles have been previously described (Komonyi et al., 2005; Pankotai et al., 2005; Papai et al., 2005). The mutant alleles were kept over TM6C, Tb Sb balancer and homozygotes were selected on the basis of Tb+ phenotype. The phiX51D line with the following genotype M[vas-int.Dm]ZH-2A;M[3×P3-RFP.attP]ZH-51D, containing a *ϕC31* integrase source and an *attP* docking site, were kindly provided by Konrad Basler (University of Zurich, Switzerland)

(Bischof et al., 2007). The w^{1118} strain was used as control (Ryder et al., 2004). Other stocks used in this study were obtained from Szeged and Bloomington Stock Centres, unless otherwise indicated.

3.5.3 Generation of transgenic Drosophila lines

All crosses were performed at 25°C. The transgenic lines used in this study are described in *Table 3.2*.

Table 3.2 *Genotype and the number of analysed animals.*

Genotype	No. of analysed animals
w/w; P[dADA2b]/P[act-Gal4]; $ada2a^{d189}/ada2a^{d189}$ (control)	150
w/w; P[dADA2b]/P[act-Gal4]; $ada2b^{d842}/ada2b^{d842}$ (control)	155
w/w; P[dADA2a]/P[act-Gal4]; $ada2a^{d189}/ada2a^{d189}$ (control)	135
w/w; P[dADA2a]/P[act-Gal4]; $ada2b^{d842}/ada2b^{d842}$ (control)	87
w/w; P[dADA2b^{S}2a]/P[act-Gal4]; $ada2a^{d189}/ada2a^{d189}$	197
w/w; P[dADA2b^{M}2a]/P[act-Gal4]; $ada2a^{d189}/ada2a^{d189}$	241
w/w; P[dADA2b^{L}2a]/P[act-Gal4]; $ada2a^{d189}/ada2a^{d189}$	228
w/w; P[dADA2a2b]/P[act-Gal4]; $ada2a^{d189}/ada2a^{d189}$	175
w/w; P[dADA2b^{S}2a]/P[act-Gal4]; $ada2b^{d842}/ada2b^{d842}$	179
w/w; P[dADA2b^{M}2a]/P[act-Gal4]; $ada2b^{d842}/ada2b^{d842}$	167
w/w; P[dADA2b^{L}2a]/P[act-Gal4]; $ada2b^{d842}/ada2b^{d842}$	113
w/w; P[dADA2a2b]/P[act-Gal4]; $ada2b^{d842}/ada2b^{d842}$	85

For the generation of the transgenic lines expressing chimeric proteins, the amplified DNA fragments encoding chimeric proteins were first cloned into pENTR3C vector (Gateway Technology, Invitrogen) as described in *3.1.1*. Following LR reaction the chimeric genes were introduced into a modified pTWF plasmid contacting the 360 bp *attB* sequence (pTWF-*attB*). The Gateway vector containing chimeric transgenes were injected into *phiX51D* embryos and the site-specific insertion into *Drosophila* genome was mediated by *ϕC31* integrase. Since the pTWF-*attB* vector enables the fusion of a FLAG tag to the 3′ end of the expressed protein, the DNA sequences of the chimeric transgenes and the corresponding controls included a stop codon sequence at the C-terminal. Thus, untagged native proteins could be expressed.

The transgenic lines were kept as homozygotes on the second chromosome. To analyse the rescue capability of the hybrid transgenes in *ada2a*^{d189} or *ada2b*^{d842} mutant backgrounds, the following crosses were performed:

w;P[UAS-dAda2b^{S}2a];*ada2a*^{d189}/TM6C × w;P[act-Gal4];*ada2a*^{d189}/TM6C

or

w;P[UAS-dAda2b^{S}2a];*ada2b*^{d842}/TM6C × w;P[act-Gal4];*ada2b*^{d842}/TM6C

The same crosses were applied to all the transgenic lines in the rescue experiments. The genotypes analysed are presented in *Table 3.2.*

3.5.4 Rescue experiments

To determine the rescuing abilities of the hybrid transgenes in *ada2a*^{d189} or *ada2b*^{d842} mutant background, L3 animals identified as non-*Tubby* were gently transferred to new vials, allowed to develop at 25°C, analysed and scored for puparium formation or eclosion rate. The number of animals analysed is detailed in *Table 3.2*. The phenotypes of the progeny were inspected and pictures were taken under a Nikon SMZ-1000 stereo-microscope.

3.6 Quantitative Reverse-Transcriptase PCR

For quantitative determination of transcripts of specified genes total RNA were isolated from white pupae with RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. First-strand cDNA was synthesized from 1 µg RNA using TaqMan Reverse Transcription Reagent (ABI). Quantitative Real-Time PCR (qPCR) was performed in an ABI 7500 RT-PCR system using Maxima® SYBR Green/ROX qPCR Master Mix (Fermentas). mRNA levels were normalized to the expression level of the *Rp49* housekeeping gene and the $\Delta\Delta C_T$ method was used for the calculation of the relative abundances (Winer et al., 1999). The sequences of the primers are described in *Table 3.3* (Zsindely et al., 2009; Pankotai et al., 2010).

Table 3.3 *Oligonucleotides used for qPCR*

Oligonucleotides	*Sequence (5′-3′)*
Fst 3′ fw	ACTATCGATTCTTCAGCGGTCTA
Fst 3′ rv	GTTACTCGGAAACGCCAAAT
Cnc 3′ fw	TGGAATCAGTGAGCCAGGA
Cnc 3′ rv	TGTATAGTCGCCGGAAAAGG
Hus1-like 3′ fw	GGCCTTCTTTGGAGCACTT
Hus1-like 3′ rv	CCACATCCTGTCGTACATCG
phm fw	GGATTTCTTTCGGCGCGATGTG
phm rv	TGCCTCAGTATCGAAAAGCCGT
dib fw	TGCCCTCAATCCCTATCTGGTC
dib rv	ACAGGGTCTTCACACCCATCTC
sad fw	CCGCATTCAGCAGTCAGTGG
sad rv	ACCTGCCGTGTACAAGGAGAG
spo fw	TATCTCTTGGGCACACTCGCTG
spo rv	GCCGAGCTAAATTTCTCCGCTT
Rp49 fw	TGTCCTTCCAGCTTCAAGATGACCATC
Rp49 rev	CTTGGGCTTGCGCCATTTGTG

3.7 Immunofluorescence of polytene chromosomes

Polytene chromosome spreads were generated from the salivary glands of wandering L3 larvae of the genotypes indicated (*Table 3.2*). The larvae were dissected in 30 µl of Sol. I, transferred for 40 s to 30 µl of Sol. II, fixed for 40 s. in 3.7% formaldehyde Sol. III followed by incubation in Sol. IV for 1 min (the composition of the solutions are detailed in *Table 3.4*). The slides containing chromosome spreads were checked under a phase contrast microscope, immersed for a few seconds in liquid nitrogen and stored in PBST (1×PBS with 0.1% Tween 20). Samples were blocked in PBST plus 5% fetal calf serum for 1 hour at room temperature and incubated overnight at 4°C in a mixture of primary antibodies. Next day the slides were washed 3 times for 5 min in PBST followed by incubation with a mixture of secondary antibodies for 1 h at 25°C. The slides were washed in PBST 3× for 5 min and incubated with DAPI in PBST for 2 min at 25°C. Following the last 10 minutes

wash in PBST the slides were covered with Fluoromount aqueous based mounting medium (Sigma) and analysed under an epi-fluorescent compound microscope.

Specific polyclonal antibodies for histone H3 acetylated at lysine 9 (H3K9ac, ab4441), H3K14ac, H4K5ac, H4K12ac were from Abcam or UPSTATE and were used at 1:200 or 1:300 dilution. Mouse anti-Pol II (7G5) antibodies were kindly provided by Dr. László Tora and raised against specific peptides as previously reported (Georgieva et al., 2000). Secondary antibodies Alexa-Fluor-488-conjugated goat anti-mouse IgG (Invitrogen, A-11001) and Alexa-Fluor-555-conjugated goat anti-rabbit IgG (Invitrogen, A-21429) were used at 1:500 dilutions.

Table 3.4 *Solutions prepared for immunostaining of polytene chromosomes*

Components	Solution I (1000 µl)	Solution II (500 µl)	Solution III (500 µl)	Solution IV (1000 µl)
10% NP40	56 µl	100 µl	-	-
10x PBS	100 µl	50 µl	-	-
37% paraformaldehyde	-	50 µl	50 µl	-
96% acetic acid	-	-	225 µl	450 µl
H_2O	844 µl	300 µl	225 µl	550 µl

37% paraformaldehyde

0.185 g paraformaldehyde (SIGMA)

7 µl 1 M NaOH

Up to 500 µl distilled H2O

PBST (200 ml)

20 ml 10×PBS

200 µl Tween20

Blocking solution (1500 µl)

15 µl Tween 20

150 µl 10×PBS

150 µl FCS

Stained samples were examined with an OLYMPUS BX51 microscope and photos were taken with an Olympus DP70 camera using identical settings for mutant and control samples.

Chapter 4.
Results

4.1 Engineering the chimeric transgenes

Previous studies in *D. melanogaster* have reported the important role of the dSAGA and dATAC HAT complexes in modulating the chromatin structure by targeting specific lysine residues on histone H3 and H4 for acetylation (Pankotai et al., 2005; Ciurciu et al., 2006). GCN5 represents the catalytic subunit of SAGA and ATAC complexes, and together with ADA-type adaptor proteins modulates the activity and specificity of the HAT complexes (Wu et al., 2004; Nagy and Tora, 2007). Immunostaining of polytene chromosomes of $ada2b^{d842}$ null mutants indicated that the levels of histone H3K9 and K14 acetylation were severely decreased (Pankotai et al., 2005; Ciurciu et al., 2006). Independently obtained results corroborated these data and complex purification studies revealed that dADA2b was indeed a subunit of the SAGA-type *Drosophila* complex (Kusch et al., 2003). Similar experimental approaches demonstrated that, in contrast to the above described findings, the $ada2a^{d189}$ null mutation causes a decrease in the level of histone H4 acetylated on K5 and K12 (Ciurciu et al., 2006), and dADA2a was identified as a component of a smaller complex designated as dATAC (Guelman et al., 2006). Subsequently, detailed analysis of the dATAC complex revealed, in addition to dGCN5, the presence of another HAT catalytic subunit, dATAC2 (KAT14) with a pivotal role in H4K16 acetylation in embryos (Suganuma et al., 2008).

All the above observations led us to question whether the histone H4 acetyltransferase activity of dATAC can be attributed to dGCN5 or to another HAT of the complex. To find an answer to this question the effect of $gcn5^{E333st}$ mutation was tested on the acetylation of histones in L3 stage larvae. We performed western blot of protein extract of wild type (w^{1118}) and $gcn5^{E333st}$ late-third-instar larvae. As loading control the same membrane was blotted with H3 antibody. We observed that when the function of the dGCN5 catalytic subunit is altered a significantly reduced level of histone H3K14 and H4K12 acetylation is observed (*Fig. 4.1*). In light of the above and

earlier findings regarding the changes of acetylated histone levels in *ada2* null mutants, we wondered on the possible roles of dADA2 factors in dATAC and dSAGA complexes.

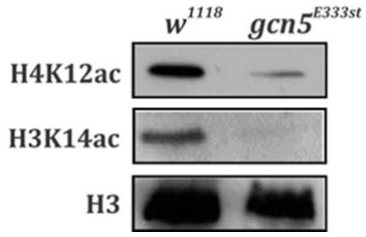

Fig. 4.1 *Loss of function of dGCN5 protein influences the acetylation level of H4K12 and H3K14. Immunoblots of total protein extracts of wild-type and dGcn5^{E333st} late third-instar larvae developed with antibodies specifically recognizing H4K12ac and H3K14ac.*

The high degree of sequence similarity between the two dADA2 proteins and the fact that they are specifically recruited to one or the other HAT complexes suggests the presence of a sequence unique for both dADA2 proteins that is essential for specific association to either dSAGA or dATAC complex. Both dADA2 proteins contain a Zinc finger domain and SANT domain within their highly conserved N-terminal region (>50%) (Kusch et al., 2003; Muratoglu et al., 2003). The homology between dADA2a and dADA2b at the C-terminus is much less pronounced (30-50% homology) (Kusch et al., 2003) where only dADA2a accommodates a SWIRM (Swi/RSC8/Moira) domain. Thus, we can hypothesize that their different recruitment to dSAGA or dATAC complexes can be attributed to the origin of the C-terminal region.

In order to gain more information regarding the hypothesis that the two dADA2 factors might play roles in modulating dGCN5 specificity in dATAC or dSAGA complexes, we constructed recombinant chimeric dADA2's where different regions of dADA2b protein was switched with domains of dADA2a protein. We believe that due to the sequence homology between dADA2 proteins the obtained chimeric proteins will maintain similar conformation to the wild-type proteins.

By joining PCR-generated DNA fragments corresponding to individual domains of dADA2a (light grey boxes – *Fig. 4.2*) and dADA2b (dark grey boxes – *Fig. 4.2*) we constructed four chimeric proteins (*Fig. 4.2*). A detailed description of their cloning is

presented in *Chapter 3-Materials and methods*. The four recombinant chimeras are as follows:

- dADA2b^{S}2a encodes amino acids 1-145 of dADA2b fused to amino acids 231-542 of dADA2a;
- dADA2b^{M}2a encodes amino acids 1-181 of dADA2b fused to amino acids 231-542 of dADA2a;
- dADA2b^{L}2a encodes amino acids 1-233 of dADA2b fused to amino acids 231-542 of dADA2a;
- dADA2a2b encodes amino acids 1-301 of dADA2a fused to amino acids 118-418 of dADA2b. Full length *dAda2a* and *dAda2b* genes were included as controls (*Fig. 4.2*). Restriction enzyme digestion and DNA sequencing confirmed the identity of each chimeric construct and controls.

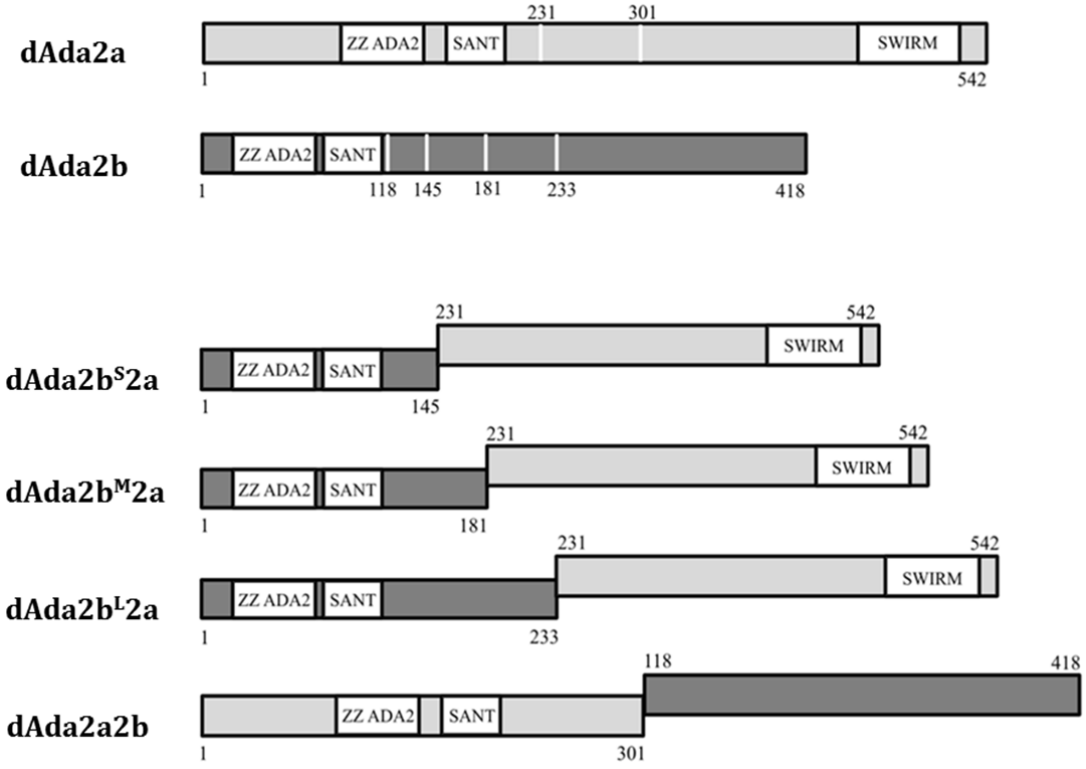

Fig. 4.2 *Domain structure of dADA2a, dADA2b proteins and overview of dADA2a/dADA2b chimeras. Numbers above and below the boxes indicate the relative amino acid sequence position within each protein. The vertical white bars indicate the positions where the two dADA2 proteins were assembled to generate chimeras.*

The chimeric constructs were first tested for their expression in *Drosophila* S2 cell line to confirm that improper folding of the chimeric proteins did not cause rapid degradation. Thus, the plasmids expressing FLAG epitope tagged chimeric constructs were transiently transfected into *Drosophila* S2 cells and verified by western blot that each plasmid directed the expression of a chimeric protein with the expected size. Immunoblotting with anti-FLAG antibody exhibits the same levels of expression for the wild-type proteins and chimeras. The same membrane was blotted with anti-Tubulin antibody as loading control (*Fig. 4.3*).

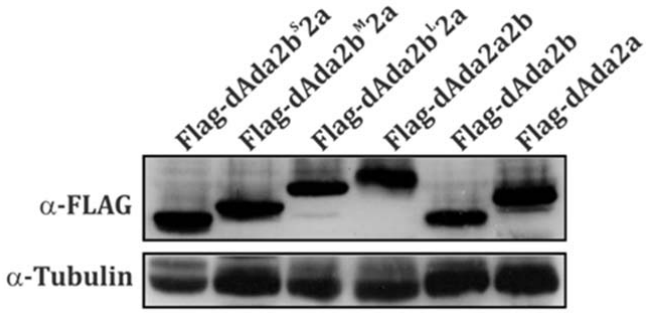

Fig. 4.3 *Transient expression of chimeric constructs in Drosophila S2 cell line. Cell lysate was prepared 24h following transfection and immunoblotted using FLAG antibody.*

The *in vivo* characterization of the function of the chimeric proteins was carried out by generating *Drosophila* transgenic lines aiming to assess the ability of chimeric genes to complement the loss of dADA2a and dADA2b functions. For the transgenic constructs we used coding regions of *dAda2* genes without any epitope tag to avoid possible interference with their function or the occurrence of unwanted interactions. We inserted the coding regions of the chimeric proteins into a transformation vector allowing site specific insertion into the *Drosophila* genome. This approach was chosen since although the classical method of P-element mediated transformation is quite efficient the transgenes can land almost anywhere in the genome, even in an essential gene. Moreover, depending on where the P-element lands, in some cases, it can be under the influence of nearby enhancers or silencers, which can and might affect a transgene's expression. Considering these factors and to bypass the randomness of P-element transformation which can directly influence the comparisons between the activities of two or more constructs, we generated transgenic lines using *ϕC31* integrase system (Groth et al., 2004).

The *ϕC31* integrase is a sequence-specific recombinase encoded within the genome of the bacteriophage and can mediate recombination between two sequences termed attachment sites (*att*), one found in the phage (*attP*) and the other in the bacterial host (*attB*) (Groth et al., 2004). *ϕC31* integrase can unidirectional integrate the transgene of interest into a target genome and requires no cofactors. The integrated transgenes are stably expressed and heritable. Since all constructs are integrated into the same locus within the genome the expression of the transgenes can be directly compared. By performing embryos injection with plasmids containing coding regions of the chimeras we generated transgenic lines and following genetic crosses carrier stocks were established. As control we generated transgenic lines with full-length coding regions of *dAda2a* and *dAda2b* (short isoform) genes. Subsequently, we tested the chimeric transgenes for their ability to rescue the *ada2a*^{d189} or *ada2b*^{d842} null mutations.

4.2 Expression of dADA2 chimeras partially rescues the ada2a^{d189} or ada2b^{d842} mutants

Functional characterization of *dAda2b* gene in *D. melanogaster* has been previously reported (Qi et al., 2004; Pankotai et al., 2005). *ada2b*^{d842} null mutants were generated by remobilizing P element localized close to the 5' ends of *dAda2b* gene and the biological consequences of this mutation was examined (Pankotai et al., 2005). The animals homozygous for the *ada2b*^{d842} null mutation have been described as pupal (P5 stage) lethal of which only a small percentage could reach the pharate adult (pA) stage (Pankotai et al., 2005). To learn more about the functional importance of dADA2b protein in *D. melanogaster* we generated transgenic lines expressing dADA2a/dADA2b chimeric proteins in *ada2b*^{d842} null animals and observed whether any phenotypical changes will occur.

The rescue experiments performed for the expression of the pUAS-*dAda2b* transgene as control under the regulation of the Actin-Gal4 driver partially rescues the P5 lethality phenotype of *ada2b*^{d842} null mutants and supports the development of 80% of the animals to pupal stage 14 (P14). The phenotypic features characteristic of the P5 and P14 stages (in the latter adult head, wings and legs are well differentiated and clearly visible, mature bristles are recognizable) made the scoring unbiased

(*Fig. 4.4*). Although the P14 stage animals are almost completely developed, they never emerge as adults. A full rescue with this transgene is not expected since the *dAda2b* cDNA that was used to generate the pUAS-*dAda2b* transgenic lines encodes only the short isoform of dADA2b thus explaining lack of full rescue. Recent studies in our group have also indicated that the short isoform of dADA2b alone is unable to complement the *ada2b*^{d842} null mutation (Pankotai et al., 2013b). The pUAS-*dAda2a* transgene expression in *ada2b*^{d842} mutant background resulted in 50% of the animals reaching P12 pharate adult stage (*Fig. 4.4*) with phenotype characteristics similar to those of escaper *ada2b*^{d842} mutants: the size of the head was reduced and the body dried out.

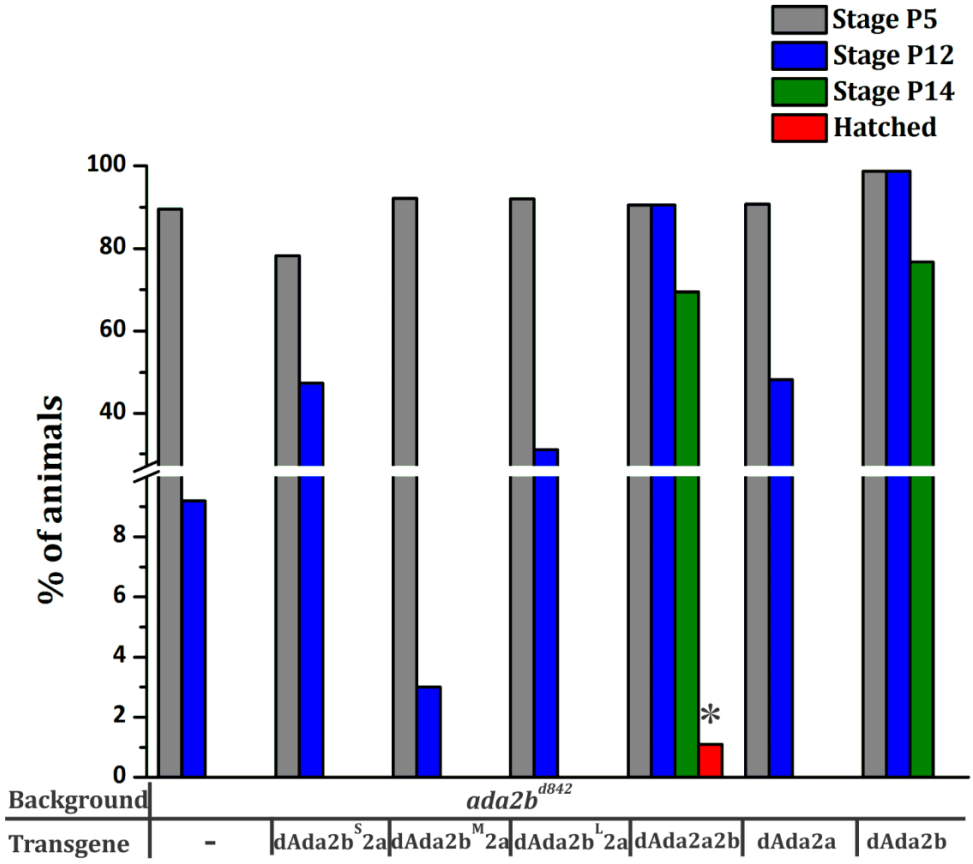

Fig. 4.4 *The dAda2a2b chimeric transgene partially rescues the phenotypic characteristics of ada2b*^{d842} *mutation. The percentages of animals that reach in the indicated developmental stage are shown. Each column represents specific developmental stages. The fifth stage pupal lethality (P5) associated with ada2b*^{d842} *null mutation is rescued to pharate adult stage by overexpression of pUAS-dAda2a2b chimeric transgene. A small percentage of transgenic animals complete the pharate adult stage and hatch (indicated by star).*

Out of the four of dADA2 chimeras tested in *ada2b*^{d842} mutant background, only the expression of chimeric transgene where N-terminal of *dAda2a* was fused to C-terminal of *dAda2b* resulted in partial but significant rescue of the null mutants phenotype (*Fig. 4.4*).

Notably, under the control of Actin-GAL4 driver more than 50% of the pUAS-*dAda2a2b* transgene-carrier animals could complete stage P5 and developed as far as P14 stage when an adult head, wings and legs are differentiated and clearly visible (*Fig. 4.4*). However, a two days delay was observed in their development as compared to pUAS-*dAda2a2b* transgene-carries *ada2b*^{d842} heterozygous siblings. Moreover, a small percentage of the *ada2b*^{d842} null mutant animals expressing the pUAS-*dAda2a2b* transgene could emerged as adults, although they died within 12 hours with their wings still folded (*Fig. 4.5-Hatched*).

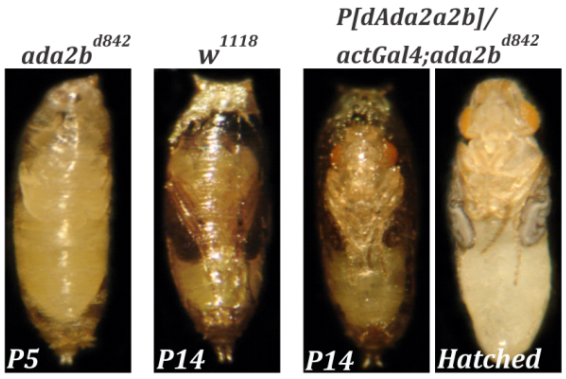

Fig. 4.5 *The dAda2a2b chimeric transgene partially rescues the phenotypic characteristics of ada2b*^{d842} *mutation. Images of ada2b*^{d842}*, wild type (w*^{1118}*) and ada2b*^{d842} *containing pUAS-dAda2a2b transgene at pupal stages (P) indicated on each separate picture. Compared to wild type, ada2b*^{d842} *mutants expressing dAda2a2b chimeric transgene exhibit a normal body development.*

The *ada2b*^{d842} animals expressing the dADA2b^{S}2a and dADA2b^{L}2a chimeric transgenes, where different lengths of dADA2b N-terminal was fused to the C-terminal half of dADA2a, reached P12 stage at a slightly higher percentage than compared with the null mutants. Although the data might suggest a partial rescue, we consider the results not significant since both dADA2b/2a transgene-carries *ada2b*^{d842} mutants had the same phenotypical characteristics as the null mutant animals. Moreover, the data obtained from *dAda2b*^{S}*2a* and *dAda2b*^{L}*2a* transgene carriers *ada2b*^{d842} mutants are less significant when compared with the results of the

rescue experiments for the *ada2b*^{d842} mutant animals expressing the *dAda2a2b* chimeric transgene.

The combined results of the rescue experiments have shown that chimeric protein dADA2a2b, where the N-terminal of dADA2a is fused to the C-terminal of dADA2b, can partially substitute the function of dADA2b protein in *ada2b*^{d842} null mutant animals.

The chimeric transgenes were next investigated for their potential to rescue the characteristic features of the *ada2a*^{d189} mutation. The *ada2a*^{d189} is a null allele mutation and homozygotes can survive for up to 2 weeks in L3 larval stage but fail to undergo prepupal contraction or form malformed brownish pupae with incompletely everted anterior spiracles (Ciurciu et al., 2006). In our rescue experiments Actin-Gal4 driven expression of the pUAS-*dAda2a* transgene resulted in complete rescue, while the pUAS-*dAda2b* transgene had no effect on the phenotype (*Fig. 4.6*).

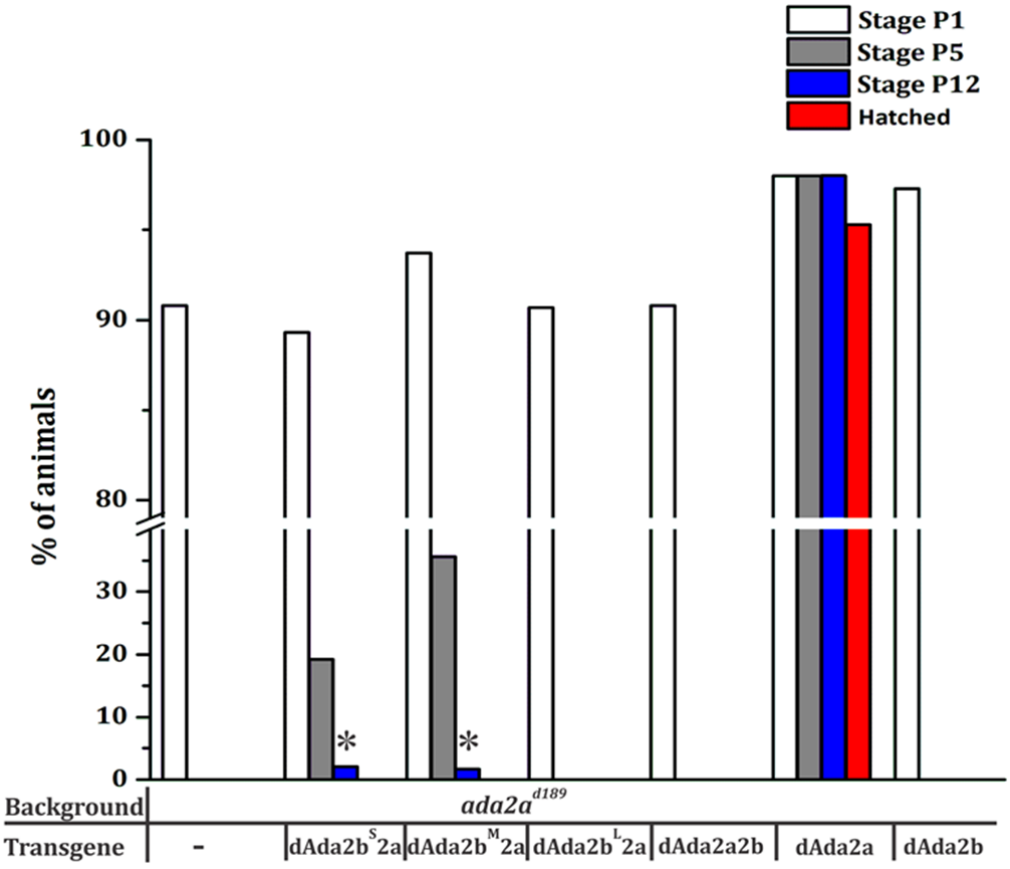

Fig. 4.6 *The L3 lethal phenotype of ada2a*^{d189} *mutation is rescued by expression of chimeric transgenes. The graphic depicts the quantification of the rescue experiments. Each column represents specific developmental stage. Animals expressing either of the two chimeras reached pharate adult stage P12 (indicated by star).*

Very similar results we could observe for *ada2a*^{d189} mutant animals that express dADA2b^{M}2a chimeric transgene. Out of 241 larvae expressing the transgene containing a medium fragment (1-181 aa) of *dAda2b* N-terminal region fused to the *dAda2a* C-terminal region, 226 larvae formed normal brownish P1 prepupae out of which 86 reached pupa stage (P5). Four animals out of 86 P5 stage pupae could develop further to pharate adult (P12) stage (*Fig. 4.6*). Compared to *ada2a*^{d189} null animals, the homozygous mutants expressing either dADA2b^{S}2a or dADA2b^{M}2a are more similar to wild type animals in shape, colour and texture (*Fig. 4.7*).

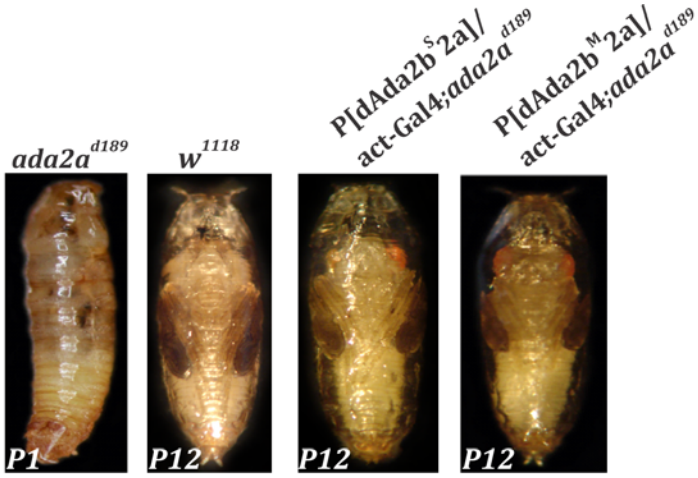

Fig. 4.7 *The L3 lethal phenotype of ada2a*^{d189} *mutation is rescued by expression of chimeric transgenes. Loss of dADA2a function results in malformed pupae-like structures while the expression of dAda2b*^{S}*2a or dAda2b*^{M}*2a transgene partly rescues the mutant phenotype. Compared to wild type the transgenic animals present the same features characteristic for pharate adult P12 stage when the abdominal bristle becomes visible and wings darken to black.*

In contrast to the *ada2a*^{d189} mutants that showed arrested development and persisted in the L3 larval stage for up to 2 weeks, the expression of *dAda2b*^{S}*2a* and *dAda2b*^{M}*2a* transgenes helped to rescue this developmental defect and the animals reached the P5 stage in 4 days. However, a two days delay in their development was recorded compared to that of wild type. Interestingly, the expression of dADA2b^{L}2a chimeric protein, where 1-233 amino acids of dADA2b N-terminus are fused in front of the dADA2a C-terminal region (231-542 amino acids), was not able to rescue the loss of dADA2a function (*Fig. 4.6*). A possible explanation for the failure of dADA2b^{L}2a chimeric transgene to show any phenotypical rescue for *ada2a*^{d189} mutant animals might be connected to its conformation. The chimeric ADA2b^{L}2a construct includes

not only the highly conserved N-terminal region but more than half of the *dAda2b* gene which comprises non-conserved regions as well. It is possible that the dADA2b^{L}2a chimeric protein is improperly folded and it is subjected to proteasomal degradation. Other possibility might be that the conformation of the dADA2b^{L}2a chimeric protein interferes with its proper function as substitute of dADA2a protein. As a result no significant effect can be observed on the rescue experiments for *ada2a*^{d189} null mutant animals which express the dADA2b^{L}2a chimeric transgene. Likewise, the pUAS-*dAda2a2b* transgene expression in *ada2a*^{d189} mutant background presented no significant rescue of phenotype relative to pUAS-*dAda2a* transgene (*Fig. 4.6*).

Taken together, the rescue experiments suggest that the C-terminal region of both proteins, dADA2a and dADA2b, has an essential role in determining the rescue ability of the chimeric transgenes in *ada2a*^{d189} and *ada2b*^{d842} mutant animals, respectively. The high homology between the N-terminal regions of the two dADA2 proteins is seemingly sufficient to maintain the proper folding of the chimeric proteins, while the C-terminal region might determine their incorporation into the dSAGA or dATAC complexes partially restoring one or the other dADA2 functions.

4.3 The acetylation level of histones H3 or H4 at specific lysine residues is restored in ada2 null mutants by the expression of dADA2 chimeric proteins

It is well established that GCN5 represents the catalytic subunit of SAGA and ATAC histone acetyltransferase complexes, although other ATAC subunits with catalytic activity have also been suggested (Grant et al., 1997; Guelman et al., 2006; Suganuma et al., 2008). Recent studies demonstrated that *gcn5*^{E333st} and *ada2a*^{d189} mutants display similar phenotypes in respect of both developmental failure and reduced acetylation on histone H4 at lysine 5 and 12 (Ciurciu et al., 2006). Moreover, mutation of the dADA2b protein reduces the level of acetylated H3 at lysine 9 and 14 as determined by polytene chromosome stainings (Qi et al., 2004; Pankotai et al., 2005; Ciurciu et al., 2006). In agreement with previous data we consistently found that *gcn5*^{E333st} null mutation affected the levels of histone H3 and H4 acetylation. Overall, these observations suggest that the presence of the dADA2a or dADA2b adaptor protein in a complex correlates with the histone specificity of that complex. To further support the role of dADA2 protein in histone acetylation and to emphasize the results of the rescue experiments we performed immunostaining of polytene chromosomes. We analysed whether the pUAS-*dAda2b*^{S}*2a*, pUAS-*dAda2b*^{M}*2a*, pUAS-*dAda2b*^{L}*2a* or pUAS-*dAda2a2b* transgenes are able to restore the decreased acetylation characteristic for *ada2a*^{d189} or *ada2b*^{d842} null mutation.

Polythene chromosome staining of *ada2b*^{d842} mutant L3 larvae that express pUAS-*dAda2a2b* chimeric transgene were prepared to analyse the chimera's effect on histone H3 acetylation on lysine 9 and 14. Chromosomes of the *ada2b*^{d842} mutants which express dADA2a2b chimeric protein where the N-terminal domain of the dADA2b protein had been replaced with the N-terminal 301 aa of dADA2a revealed increased acetylation on histone H3 at the lysines indicated when compared to the *ada2b*^{d842} null mutants (*Fig. 4.8*). In contrast, the pUAS-*dAda2b*^{S}*2a*, pUAS-*dAda2b*^{M}*2a* or pUAS-*dAda2b*^{L}*2a* chimeric transgenes had no detectable effect on either H3K9 or H3K14 acetylation being in agreement with their failure in rescue.

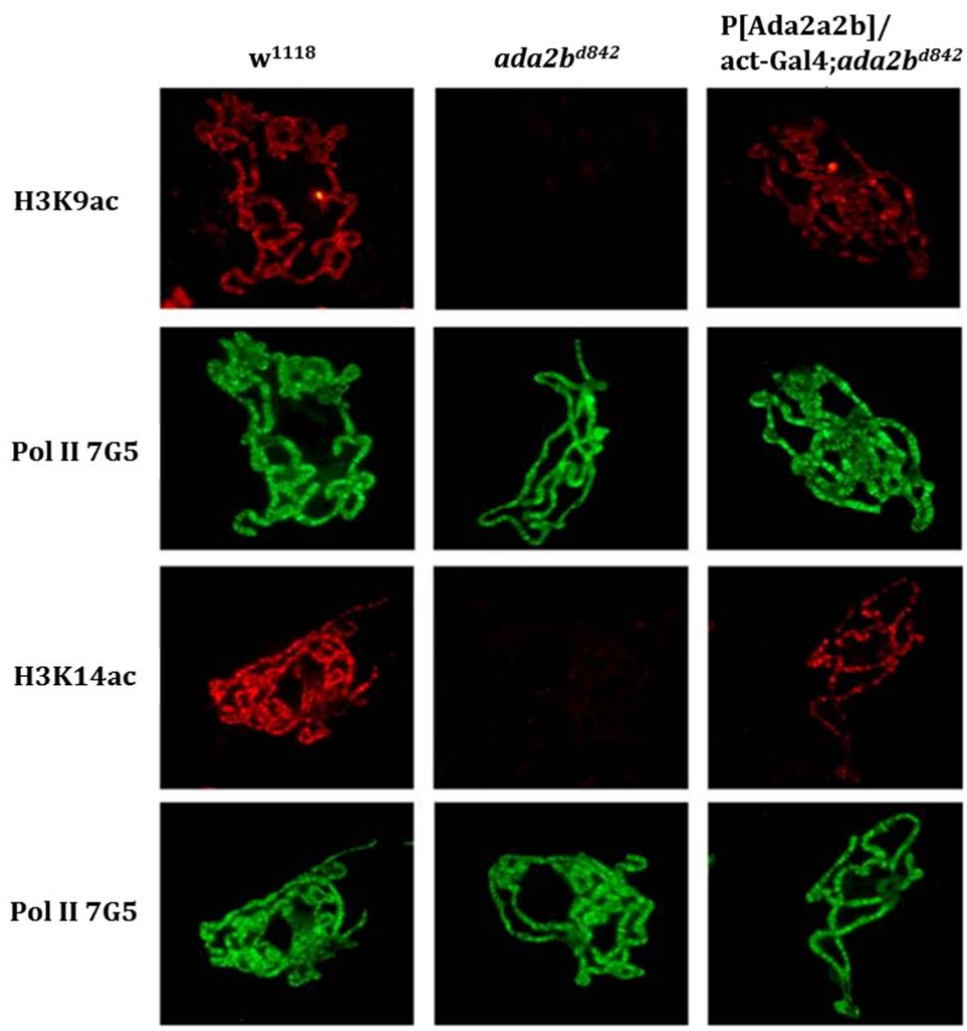

Fig. 4.8 *The effect of dAda2a2b chimeric transgene in ada2b^{d842} mutant on H3K9 and H3K14 acetylation. Chromosomes immunostained with polyclonal antibodies specific for individual acetylated lysine residues of H3 (red), as indicated on the left, and with a Pol II-specific monoclonal antibody (Pol II 7G5, green) are shown. Genotypes are indicated at the top. The images of the immunostaining in different mutants were obtained with identical data-recording settings.*

To validate our polytene chromosome staining we performed western blot of protein extracts from wild-type, *ada2b^{d842}* null mutants and *ada2b^{d842}* mutants expressing pUAS-*dAda2a2b* chimeric transgene. The immunoblots were developed with specific antibodies and we could observe significant increase in the acetylation levels for histone H3 at lysine 9 and 14 in *ada2b^{d842}* mutant animals expressing pUAS-*dAda2a2b* chimeric transgene compared to *ada2b^{d842}* null animals (*Fig. 4.9*).

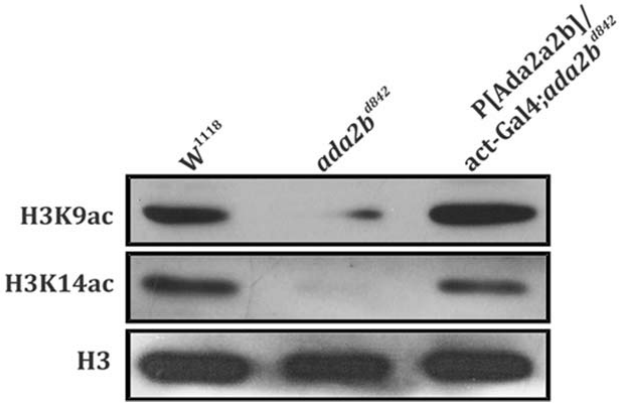

Fig. 4.9 *The effect of dAda2a2b chimeric transgene in ada2b^{d842} mutant on H3K9 and H3K14 acetylation. Western blot of protein extracts of wild-type, ada2b^{d842} and pUAS-dAda2a2b/act-Gal4; ada2b^{d842} L3 larvae developed with antibodies as indicated on the left. The blots were stripped and developed with H3-specific antibody to demonstrate equal loading.*

Previous immunostainings of polytene chromosomes have shown that the loss of dADA2a activity results in a dramatic decrease in the levels of H4K5ac and H4K12ac (Ciurciu et al., 2006). Our rescue experiments have demonstrated that the expression of dADA2b^{S}2a or dADA2b^{M}2a chimeric transgenes can partially restore the function of dADA2a protein in *ada2a^{d189}* mutant animals. We therefore found it interesting to determine whether these chimeric proteins can also restore the decreased histone acetylation pattern specific for *ada2a^{d189}* null mutation. For this, we studied the acetylation levels of H4K5 and K12 in *ada2a^{d189}* mutant animals in which dADA2a function was provided by the chimeric transgenes. Polytene chromosome staining of transgene carrier late-third-instar larvae with antibodies specific for acetylated H4K5 or H4K12 revealed that the two chimeric transgenes that contain the C-terminal region of *dAda2a* merged with N-terminal of *dAda2b* displayed similar staining intensity to wild type animals (*Fig. 4.10*). This might suggest that, in accord with the result of the rescue experiments, the pUAS-*dAda2b^{S}2a* or pUAS-*dAda2b^{M}2a* chimeric transgenes can partially substitute for the loss of dADA2a function and might produce acetylation competent HAT complexes in *ada2a^{d189}* mutant animals.

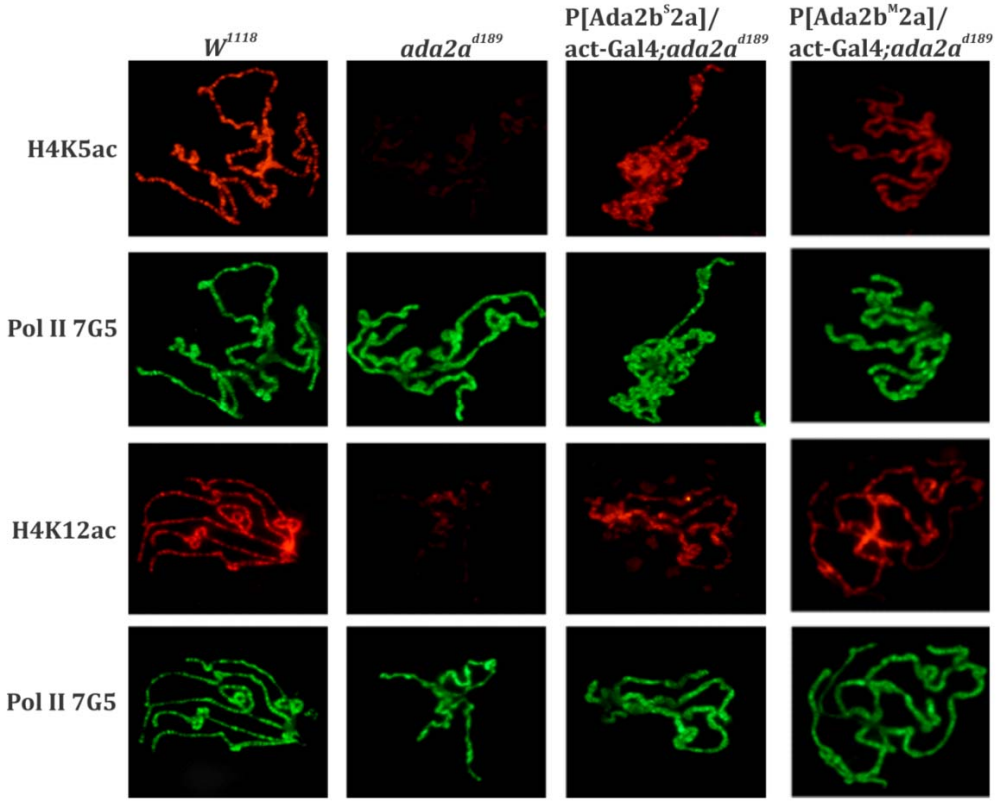

Fig. 4.10 *Immunostaining of polytene chromosomes obtained from w^{1118}, $ada2a^{d189}$ and pUAS-dAda2b^{S}2a, pUAS-dAda2b^{M}2a chimeric transgenes in $ada2a^{d189}$ mutant background. Antibodies specific for histone H4K5ac, H4K12ac (red) and Pol II 7G5 (green) were used as indicated on the left. Genotypes are indicated at the top.*

The immunoblotting of total protein extract from *$ada2a^{d189}$* larvae expressing pUAS-*dAda2b^{S}2a* or pUAS-*dAda2b^{M}2a* transgenes also indicated a restored level of H4K5 and K12 acetylation similar to wild type (*Fig. 4.11*).

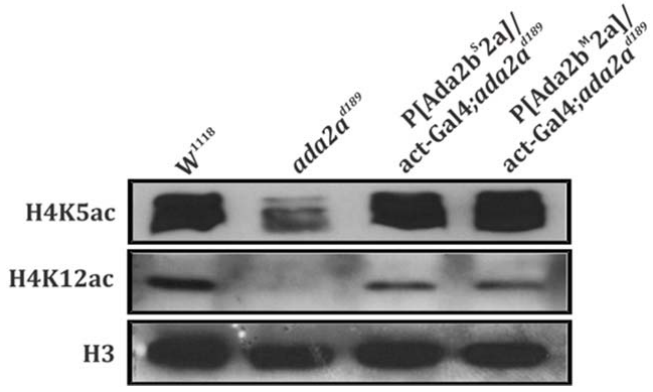

Fig. 4.11 *Western blots of protein extracts of w^{1118}, $ada2a^{d189}$ and $ada2a^{d189}$ mutants expressing pUAS-dAda2b^{S}2a or pUAS-dAda2b^{M}2a chimeric transgenes. The membranes were developed with the antibodies indicated on the left. The blots were stripped and developed with H3-specific antibody to demonstrate equal loading.*

In addition to the decrease acetylation for H4K5 and K12, the *ada2a*^{d189} null mutation has significant effect on the structure of the polythene chromosomes. They appear as fragile and display a distorted banding pattern (Pankotai et al., 2005; Ciurciu et al., 2006). It is not clear whether these structural characteristics of the polythene chromosomes are as a result of loss of *dAda2a* function or represents a more general effect due to the delayed development of these animals. In our experiments the ectopic expression of chimeric pUAS-*dAda2b*^{S}*2a* and pUAS-*dAda2b*^{M}*2a* transgenes in *ada2a*^{d189} mutant background substantially improved the distorted banding pattern of polytene chromosomes (*Fig. 4.12*).

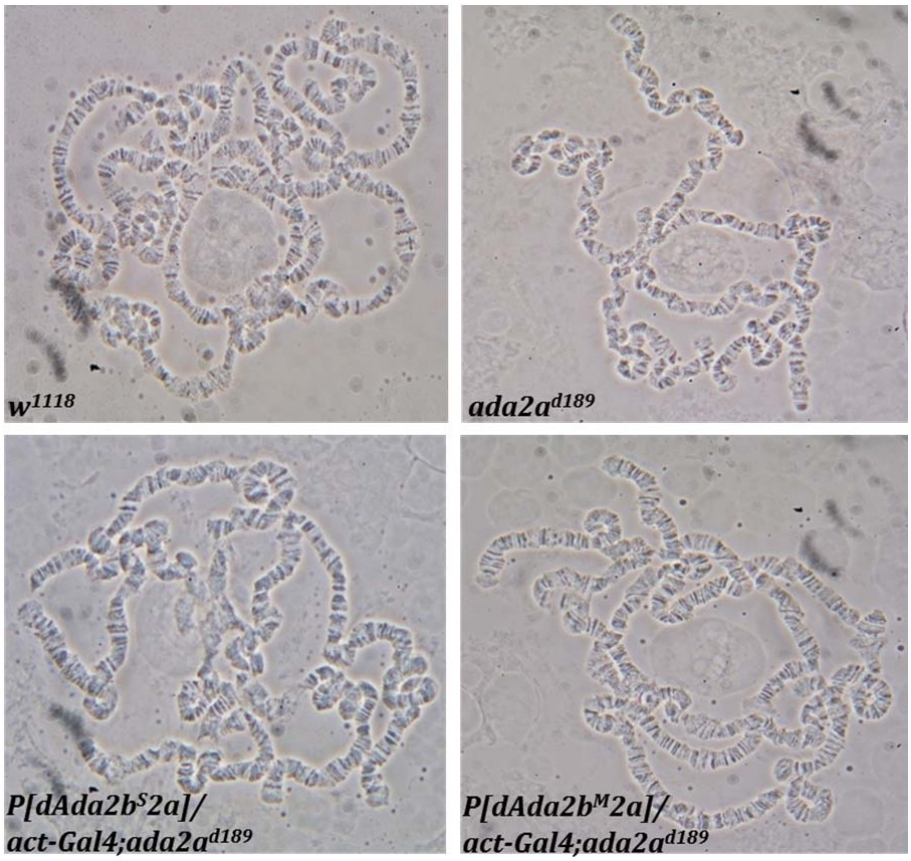

Fig. 4.12 *Phase-contrast images of polytene chromosomes prepared from wild type, ada2a*^{d189} *and pUAS-dAda2b*^{S}*2a or pUAS-dAda2b*^{M}*2a transgene-carriers ada2a*^{d189} *mutant animals. The abnormal morphology of ada2a*^{d189} *polytene chromosomes is rescued by pUAS-dAda2b*^{S}*2a and pUAS-dAda2b*^{M}*2a transgenes.*

The ectopic expression of Actin-Gal4-driven pUAS-*dAda2b*^{L}*2a* or pUAS-*dAda2a2b* chimeric transgenes in dATAC mutants had no significant effect on the

level of histone H4 acetylation at K5 or K12 being in agreement with the results of the rescue experiments.

According to the polytene chromosome immunostaining, we can hypothesized that the *in vivo* changes of the acetylation levels observed for histone H3 and H4 lysines in *ada2a*^{d189} or *ada2b*^{d842} mutants expressing chimeric transgenes, are influenced by the C-terminal regions of dADA2a or dADA2b proteins. These results might suggest that functionally distinct HAT complexes can be generated which contain as subunits the chimeric dADA2 proteins. These observations further validate our results of the rescue experiments.

4.4 Chimeric dADA2 proteins did not improve the expression of selected genes affected by ada2a^{d189} or ada2b^{d842} mutation

Recently it has been reported that the expression of a small set of genes are influenced by the *ada2b*^{d842} null mutation, whereas a considerable number of genes are affected by the loss of function of dATAC-specific subunit (Zsindely et al., 2009; Pankotai et al., 2010). Based on the results of the rescue experiments and the polytene chromosomes immunostainings we can hypothesize that the expression of the chimeric transgenes in *ada2* mutants might determine gene expression changes as well. To study the molecular consequences of the chimeric transgene expression in *ada2* mutants we compared the mRNA levels of selected genes from wild type and chimeric transgene expressers by Quantitative Real-Time PCR. mRNA samples were obtained from synchronized white pupae as described in *Chapter 3 – Material and Methods.*

From the genes affected by the *ada2b*^{d842} mutation we selected to analyse those which were found to be either down-regulated, such as *Sug (Sugarbabe*) and *Cnc* (*Cap 'n' collar*), or up-regulated, such as *Fst* (*Frost*) and *Hus 1-like* (Zsindely et al., 2009). *Sugarbabe* gene encodes a predicted zinc finger protein that regulates insulin gene expression in the *Drosophila* neurosecretory cells. *Sug* gene is highly induced in sugar-fed larvae in the fat body and parts of the midgut (Zinke et al., 2002). *Cnc* gene affects cell proliferation and development (Deng and Kerppola, 2013) while *Hus 1-like* gene is essential for the activation of the meiotic checkpoint (Abdu et al., 2007). The expression of *Frost* gene is essential for cold tolerance in *Drosophila melanogaster* and

may play an important role in thermal adaptation (Colinet et al., 2010). The analysis of mRNA in *ada2b*^{d842} null animals containing pUAS-*dAda2a2b* transgene revealed no significant changes in the expression levels of the selected genes compared to the wild type (*Fig. 4.13*).

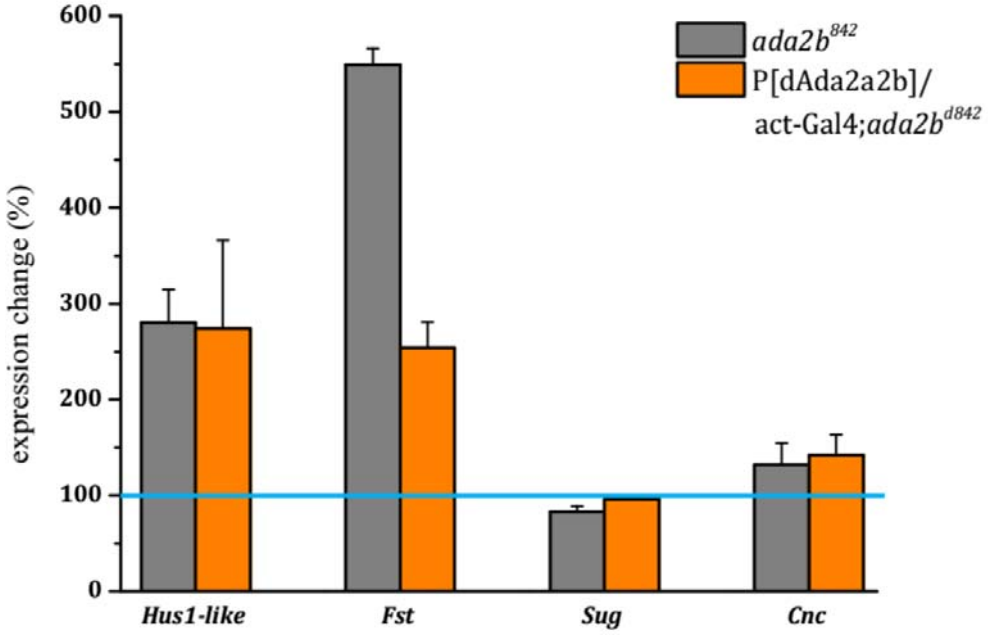

Fig. 4.13 *The mRNA levels of Hus1-like, Fst, Sug and Cnc in ada2b*^{d842} *mutant and dAda2a2b hybrid expressing transgenic white pupae. The changes in gene expression were determined by real-time PCR and compared to wild-type represented by the blue line. Error bars represent standard deviations calculated from three independent experiments.*

Previous microarray studies have indicated that dATAC is required for transcriptional activation of the *Halloween* genes which are involved in the synthesis of steroid hormone ecdysone critical for molting and metamorphosis of insects (Pankotai et al., 2010). In order to explore whether the altered mRNA levels of *Halloween* genes in *ada2a*^{d189} null mutants can be changed by expressing pUAS-*dAda2b*^{S}*2*a or pUAS-*dAda2b*^{M}*2a* chimeric transgenes, we carried out Quantitative Real Time PCR. Among the *Halloween* genes we chose to analyse *Phm (phantom)*, *Spok (spookier)* and *Sad (shadow)* which were found to be downregulated in *ada2a*^{d189} mutants (Pankotai et al., 2010). The *Phm* gene encodes a 25-hydroxylase, *Sad* gene encodes the 2-hydroxylase and *Spok* gene is believed to be important for a yet uncharacterized step (the Black Box) in the biosynthesis of ecdysone hormone (Ono et al., 2006; Rewitz et al., 2006). The Quantitative Real-Time PCR assay did not

indicated any significant change in the mRNA level corresponding to either of the above mentioned genes in the chimeric transgenes-carrier dATAC mutant animals compared to *ada2*^{d189} null mutants (*Fig. 4.14*). The failure to detect any change in mRNA level of the selected genes in either one or the other *ada2* mutants expressing chimeric transgenes might be the result of the partial rescue.

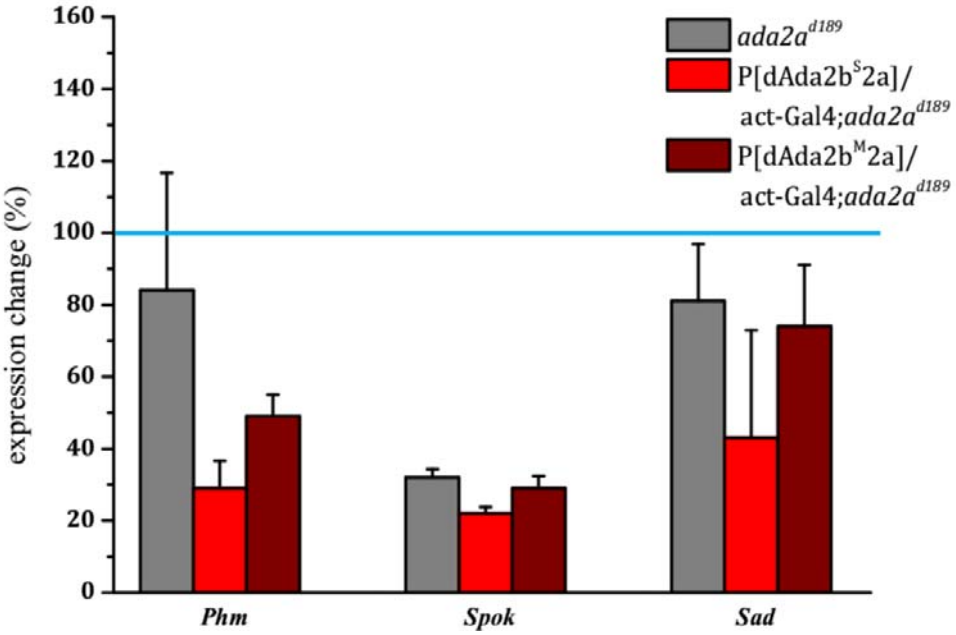

Fig. 4.14 *Expression of Halloween genes in ada2a*^{d189} *mutant and the effect of hybrid transgenes on mRNA levels. The changes in gene expression were determined by real-time PCR and compared to wild-type represented by the blue line. Error bars represent standard deviations calculated from three independent experiments.*

4.5 In vivo interaction between dADA2b isoforms, dADA3 and dp53

The *dAda2b* gene in *Drosophila melanogaster* gives rise to two protein isoforms designated as dADA2bS (short isoform) and dADA2bL (long isoform). Previous biochemical separation of GCN5-containing complexes have identified dADA2b proteins with different molecular masses in fractions corresponding to 1,8-2 MDa complexes (Muratoglu et al., 2003). The presence of two dADA2b isoforms and their co-fraction with subunits of dSAGA HAT complex might suggest that the short and the long dADA2b isoforms are subunits of either the same or similar multiprotein complexes.

The two dADA2b isoforms are identical in their N-terminal 330 aa region. These segments of the proteins harbour a ZZ zinc finger and a SANT domain, characteristic for all known ADA2 proteins, and two so-called ADA boxes (Muratoglu et al., 2003). The dADA2L protein has an extra ADA box within the C-terminal region. Although there is no similarity between dADA2bL and dADA2bS at their C-terminal regions (Qi et al., 2004), a sequence at the 3′ segment of dADA2bL (corresponding to 50 aa) is also present in two putative human hADA2b proteins. However, the SWIRM domain characteristic for the C-terminal region of human ADA2b proteins could not be recognized in the *Drosophila* counterparts.

It is well established that ADA2 proteins are components of multiprotein complexes and participate in protein-protein interactions. The occurrence of two dADA2b protein isoforms in *Drosophila* raised the intriguing possibility of different protein-protein interaction patterns. Thus, we performed experiments to analyse whether the two isoforms can interact with each other and whether both proteins, dADA2bS and dADA2bL, can interact with dADA3 protein, another component of ADA2b-containing HAT complexes.

To detect protein-protein interactions N-terminal FLAG or HA epitope tagged dADA2b isoforms were transiently expressed in S2 cells. Whole-cell lysates were obtained and incubated with M2 (anti-FLAG) agarose beads. Immunoprecipitates were analysed by immunoblotting using epitope-specific antibodies. Co-immunoprecipitation of dADA2bS with dADA2bL and vice versa indicated *in vivo* physical interaction between the two isoforms (*Fig. 4.15 A and B*).

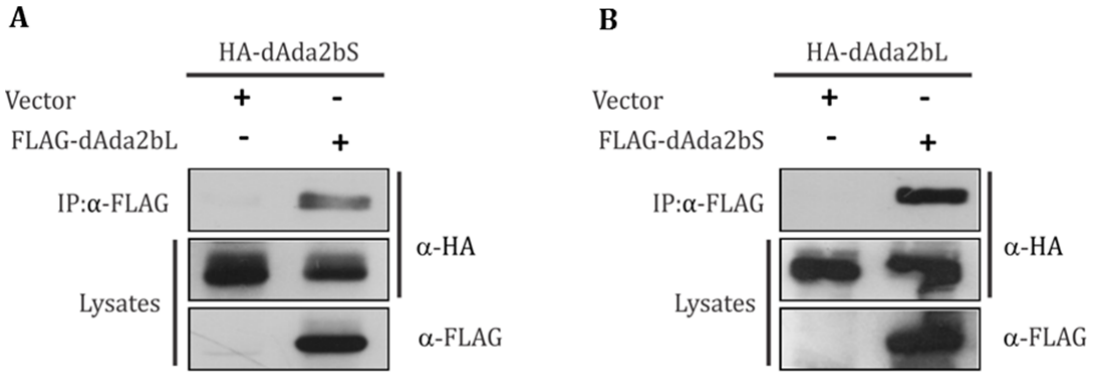

Fig. 4.15 *In vivo interaction between dAda2b isoforms. Cells were co-transfected with the indicated constructs for immunoprecipitation (on the left and upper part).* ***(A)*** *The FLAG-dAda2bL immunoprecipitated with HA-dAda2bS.* ***(B)*** *In a reciprocal experiment FLAG-dAda2bS co-immunoprecipitated with HA-dAda2bL.*

To further analyse the possibility that the two isoforms are components of the same or related multiprotein complexes we performed co-immunoprecipitation experiments to determine whether the two dADA2b isoforms can interact with dADA3, one of the dSAGA subunits. Using anti-FLAG M2 agarose beads we confirmed the association of the short and long dADA2b isoforms with dADA3 protein (*Fig. 4.16 A*). Together, the result from the co-immunoprecipitation experiments opens the possibility that both dADA2b isoforms are components of the same or similar HAT complexes.

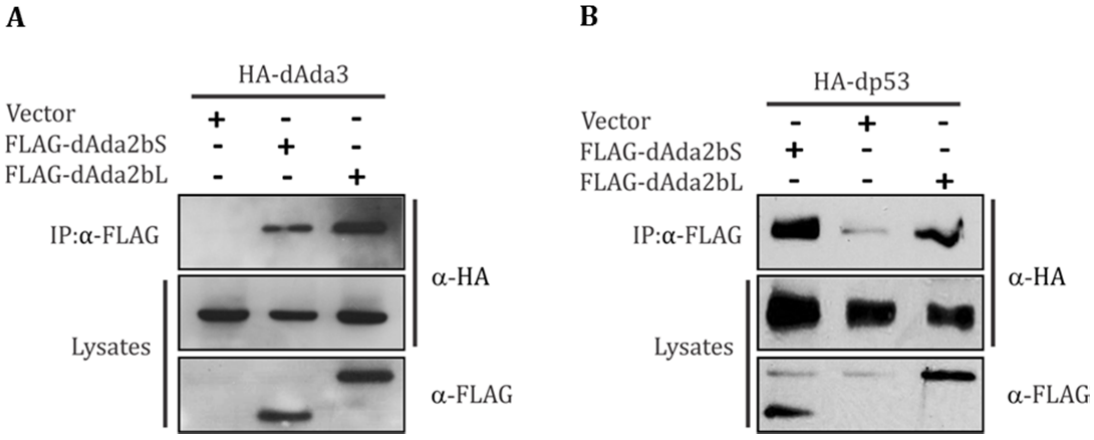

Fig. 4.16 *In vivo interaction between dAda2b isoforms, dAda3 and dp53.* ***(A)*** *Both dAda2b isoforms co-immunoprecipitated with HA-dAda3.* ***(B)*** *The FLAG-dAda2bL and FLAG-dAda2bS immunoprecipitated with HA-dp53. The antibodies used for developing the blots are shown on the right.*

Previous studies in human cells have demonstrated that the transcriptional activity of the tumour suppressor p53 requires the ADA2/ADA3/GCN5 HAT complex (Wang et al., 2001). Moreover, mutations of the *dAda2b* gene affected dp53 function (Pankotai et al., 2005), and a physical interaction between dADA2b and dp53 was also shown (Kusch et al., 2003). Based on these observations we found interesting to learn if dADA2b isoforms displayed differences in interaction with dp53. To determine whether dADA2b isoforms can associate with the tumour suppressor dp53 we performed anti-FLAG co-immunoprecipitation and probed the Western blots with antibodies against HA-dp53. We observed that both dADA2bS and dADA2bL isoforms co-immunoprecipitated with dp53 (*Fig. 4.16 B*). This suggests that the two dADA2b isoforms might participate in different dp53-mediated pathways.

The data obtained from the co-immunoprecipitation experiments did not reveal different abilities of the two dADA2b isoforms to participate in protein interactions, but demonstrated the possibility of dimer formation between dADA2b isoforms. Furthermore, the two dADA2b isoforms demonstrated the same ability to interact with the dADA3 adaptor protein and the tumour suppressor dp53.

Chapter 5.
Discussion

A considerable number of transcriptional regulatory proteins have been identified to interact with and alter chromatin. They display remarkable specificity regarding their histone selectivity and capacity to distinguish specific nucleosome substrates. How do proteins that alter chromatin structure accomplish preferential affinity to specific substrates represents an important mechanistic issue. One relevant aspect of ATP-dependent chromatin remodelling enzymes and histone modifying proteins is the fact that they function as large complexes and that their specificity is given in part by these assemblies. There are two important aspects to consider regarding the specificity of a complex. First, the domains within the enzymes themselves are necessary for targeting the complexes to nucleosomes, as exemplified by GCN5 bromodomain proven to be essential for nucleosome acetylation as a subunit of SAGA complex (Sterner et al., 1999). The second feature to consider is the presence of several interacting proteins which can influence the specificity of the complexes. For instance, TAFII68 is required for SAGA-dependent acetylation of nucleosomes.

In this dissertation, according to the results obtained from the rescue experiments and the chromosome immunostainings we propose that the C-terminal region within the dADA2 chimeric proteins is essential for selectively incorporating the chimeras into one or the other HAT complex. Although we did not perform HAT complex purifications the combined data strongly suggests that the chimeric proteins can partially substitute the function of dADA2a or dADA2b proteins as subunits of dATAC or dSAGA HAT complexes which in turn determines H3- or H4-specific targeting.

The *D. melanogaster* dATAC and dSAGA complexes share dGCN5 and dADA3 subunits, but differ in containing one of two complex-specific dADA2 type adaptors. Genetic analysis revealed that mutations removing dADA2b from dSAGA results in a decrease in histone H3K9ac and H3K14ac levels (Qi et al., 2004; Pankotai et al., 2005). Mutations affecting the dATAC subunit dADA2a result in decreased H4K5ac and

H4K12ac levels (Pankotai et al., 2005; Ciurciu et al., 2006; Guelman et al., 2006). Thus, the presence of dADA2a or dADA2b adaptor proteins in a complex correlates with the histone specificity of that complex. However, in addition to dGCN5 (KAT2), dATAC harbours at least one additional subunit with HAT activity. dATAC2 (KAT14) was identified to act as a second acetyltransferase enzyme of the dATAC complex with a preference for acetylating histone H4 (Suganuma et al., 2008). Mutations of *Atac2* resulted in a drop of H4K16ac levels in late embryos. The L2 lethality of *atac2* mutants prevented studying the effect of this HAT on the acetylation of polytene chromosomal histones. Both dSAGA and dATAC seem to be involved in global as well as promoter-specific histone modifications. Furthermore, in addition to its HAT activities SAGA plays a role in chromatin regulation via a deubiquitination module as well (Weake et al., 2008; Zhao et al., 2008). All the above illustrates a high level of interest in exploring molecular interactions determining SAGA and ATAC functions.

Previous studies have shown that distinct phenotypes arise from the loss of *Drosophila dAda2a* or *dAda2b* functions. Earlier results from our laboratory combined with data of others, established the complex-specific nature of dADA2a and dADA2b. As a further extension of those studies this dissertation work demonstrated that the C-terminal regions of both dADA2 proteins seem to play determining roles in complex-specific integration. Chimeric dADA2 proteins integrate into distinct HAT complexes depending on their C-terminal domains, which based on the *in vivo* rescue ability and in accordance with the histone acetylating activity, these complexes can be assumed of being in fact dSAGA and dATAC. We constructed chimeric dADA2 adaptors by switching the more conserved N-terminal and less preserved C-terminal regions between dADA2a and dADA2b. Three out of four tested constructs gave positive results in causing partial rescue of developmental defects *in vivo* and acetylating histones that were decreased due to $ada2a^{d189}$ or $ada2b^{d842}$ mutation. We assume that proper folding of these dADA2 proteins permitted the incorporation into dSAGA or dATAC complexes leading to partial recovery of functions.

Sequence comparisons and functional assays of ADA2 proteins identified three regions that are believed to mediate important functions. In yeast, *in vitro* experiments indicated that the N-terminal ZZ finger motif of ADA2 protein has an essential role in interacting with GCN5 (Candau and Berger, 1996). Deletion of the SANT domain attenuated the capacity of yeast ADA2 to support *in vivo* transcriptional

activity (Candau and Berger, 1996). The yADA2 SANT domain potentiated the catalytic function of yGCN5 and was important for acetylating nucleosomal histones (Balasubramanian et al., 2002; Sterner et al., 2002). The region following the SANT domain was shown to interact with yADA3 *in vitro* (Berger et al., 1992; Candau and Berger, 1996; Candau et al., 1996). A less conserved region among ADA2 proteins is the SWIRM domain. Studies on mammalian ADA2b protein revealed a conditional role of the SWIRM domain in chromatin acetylation by STAGA (Gamper et al., 2009). Our structure comparison revealed no SWIRM domain in dADA2b, while it is present in dADA2a *(Fig. 5.1)*. In this respect the two ADA2 factors of *D. melanogaster* differ more than ADA2 proteins of other metazoa.

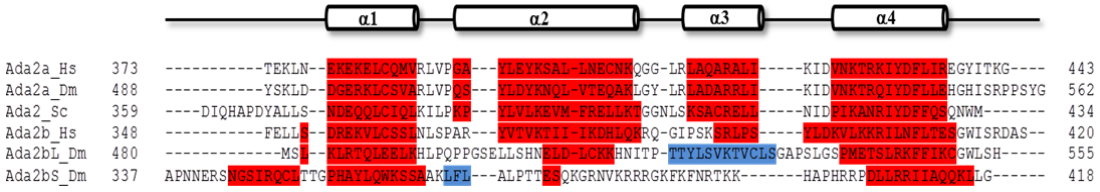

Fig. 5.1 *Sequence alignment of the SWIRM domains. The secondary structure prediction was performed using PRALINE Multiple Sequence Alignment (Heringa, 1999, 2000, 2002; Simossis and Heringa, 2003; Pirovano et al., 2008). The proteins are designated by their names followed by species abbreviations. Each sequence segment is flanked by the coordinates of its first and last amino acid. The proposed secondary structure elements in the predicted models are specified above the protein alignment (barrel shape figure representing the α-helix). The highlighted residues in the proteins represent the predicted secondary structure (red - alpha helix; blue - beta strand). The species listed are: Hs, Homo sapiens; Dm, Drosophila melanogaster; Sc, Saccharomyces cerevisiae. The NCBI Reference sequences are the following: NP_001479.3, NP_001014636.1, NP_010736.1, NP_689506.2, NP_001027151.1, NP_649773.1.*

In this dissertation it is demonstrated that the C-terminal region seems to determine the targeting of dADA2 proteins into GNAT complexes which differ in specificity to acetylate histone H3 or H4. Data on the physical and functional interaction of ADA2 proteins and other subunits within HAT complexes are mostly from studies performed on yeast and human SAGA. These complexes are thought to be built from structural and functional modules with poorly defined independence (Samara and Wolberger, 2011). The SAGA HAT module consists of GCN5, ADA3, ADA2 and SGF29. Detected physical interactions place ADA2 between GCN5 and ADA3. SGF29 makes contact with ADA3. Human ADA2 interacts with ADA3 through its N-terminal region following the SANT domain and its C-terminal SWIRM domain while

contacts with GCN5 are made through the SANT domain (Gamper et al., 2009). The homologous regions were found to be required for yeast ADA2 and ADA3/GCN5 interactions as well (Candau and Berger, 1996; Sterner et al., 2002). Yeast two-hybrid experiments detected physical interactions of dADA2 both with dADA3 and dGCN5 (Ciurciu et al., 2006).

At present, limited amount of data are available on the physical and functional relations between the HAT module and the rest of the SAGA (Suganuma et al., 2008; Lee et al., 2011). It is important to note here that physical interactions of ADA2b and ADA3 have also been detected with several non-SAGA subunit transcription regulators, for example the p53 tumour suppressor among others. Furthermore, it is also noteworthy that dADA2b give rise to two isoforms which differ in their C-terminal regions (Muratoglu et al., 2003). Biochemical purification of HAT complexes eluted two dADA2b isoforms in the same fraction (Muratoglu et al., 2003; Pankotai et al., 2013a). However, a considerable difference between *dAda2b* isoforms has been detected in the message and protein levels during fly development (Pankotai et al., 2013a).

All the above observations made us wonder whether different dSAGA complexes exist, in respect of their dADA2b content, or different dADA2b protein isoforms are present in the same complexes. Considering that SAGA possesses multiple activities in different biological processes (Weake and Workman, 2012) we can hypothesize that a slight change of subunit composition within the functionally independent modules can influence the activity of SAGA. In this respect we consider the isoforms of dADA2b protein as good candidates for having that particular role. According to co-immunoprecipitation experiments dADA2b isoforms can form dimers (*Fig. 4.15 A and B*). In addition, both dADA2bS and dADA2bL isoforms have been found to co-immunoprecipitate with dADA3 in *Drosophila* S2 cells (*Fig. 4.16 A*). Thus, the experimental data suggest that either of the two isoforms can participate as subunits of the HAT module within dSAGA complex. The partial phenotypic rescue observed during analysing the *in vivo* function of dADA2bS isoform transgene indicates that the shorter product alone can complement to some extend the dSAGA functions required for completing the fly development (Pankotai et al., 2013a).

In a mammalian system it was shown that the GCN5-containing acetyltransferase complex (STAGA) plays a role in p53-dependent gene activation.

ADA2b and GCN5 proteins are direct interacting partners of the p53 transcription factor (Gamper and Roeder, 2008). According to previous studies *Drosophila* p53 is a functional homologue of mammalian p53 (Ollmann et al., 2000). Physical interaction has been demonstrated between dADA2b and dp53 and loss of *dAda2b* function contributes to p53-dependent apoptosis in response to gamma radiation (Kusch et al., 2003; Qi et al., 2004). Even though only the animals carrying the short dADA2b isoform displayed reduced level of apoptosis in response to DNA damage caused by irradiation (Pankotai et al., 2013a), both dADA2b isoforms interacted with dp53 (*Fig. 4.16 B*). Thus, it appears that dADA2b may be necessary for efficient DNA repair or generation of a DNA damage signal and the interaction with dp53 might be required as an intermediary step along the complex apoptosis pathway.

Information on the structural organization of ATAC is more scarcely available. Since ATAC contains three of the subunits present in the HAT module of SAGA, and CG30390, a protein related to yeast SGF29 identified during dATAC subunit analysis (Suganuma et al., 2008), one would expect the existence of a dGCN5-containing HAT module in dATAC, built similarly to that of dSAGA. The detected interactions of dADA2a both with dGCN5 and dADA3 might give support to this assumption. This leads to the question whether the histone H4K5 and K12 acetyltransferase activity can be attributed to dGCN5 or to dATAC2. A third possibility could be that neither of these, but other HAT(s) which are affected by mutations of dATAC subunits are the underlying cause of the decrease in H4 acetylation. It is highly plausible that the loss of dADA2a disrupts the complex or distorts it in a way that inactivates dATAC2. Reduced acetylated histone H4 levels in $ada2a^{d189}$ mutants might result from knocking out the activity of the dGCN5 module.

Several observations can be put forward to argue for a direct role of dGCN5 in H4K5 and K12 modifications. First, dATAC2 was found to be specific for histone H4K16. Altered H4K16ac levels were never observed in $ada2a^{d189}$ mutants. Second, $gcn5^{E333st}$ mutants and $ada2a^{d189}$ mutants display practically identical phenotypes with respect to both developmental failures and reduced H4K5ac/K12ac levels. Actually, the loss of the third component of the dGCN5-centered HAT module, dADA3 caused similar changes (Grau et al., 2008). Importantly, *dGcn5* allele altered either in the HAT or the ADA2 interacting region of the protein (Carre et al., 2005) also showed identical phenotypes with $ada2a^{d189}$ null mutants, and genetic interactions between

ada2a^{d189} and *gcn5*^{E333st} mutants have been demonstrated in different settings (Ciurciu et al., 2006). Taken together, the possibility that dGCN5 acts on H4K5 and K12 side chains as a catalytic unit within the dATAC complex can hardly be excluded, though it can neither be proven by direct evidence. The observed loose specificity of dGCN5 and its reduced, but still existing affinity towards H4 gives support for this possibility.

Analyses of the functional consequences of yeast and human ADA2-ADA3-GCN5 interactions concluded that GCN5 activity, and significantly, its affinity towards histones depended upon the context the enzyme was positioned and also the context of its substrate (Grant et al., 1999; Balasubramanian et al., 2002; Sterner et al., 2002; Gamper et al., 2009). *Tetrahymena* GCN5 can accept histone H4 side chains as substrates (Poux and Marmorstein, 2003) and yGcn5 mutations cause reduced H4 acetylation (Zhang et al., 1998). Therefore, within ATAC, a GCN5 containing HAT module could be in suitable conformation for H4K5 and K12 acetylation Alternatively, ATAC2 enzymatic activity might be affected by ADA2a loss of function. However, in *Drosophila*, different lethal phases of *ada2a*^{d189} and *atac2* mutants do not support this assumption. *Atac2* mutants are L2 lethal, while *ada2a*^{d189} lethal phase is late L3. If indeed the product of *ada2a*^{d189} is required for dATAC2 function, than one would expect identical phenotypes. A counter argument to this can be based, however, on different amount of the maternal gene products. Interplay between units of dATAC could target the complex to chromatin via dGCN5 bromodomain interactions, while H4 acetylation is performed by dATAC2. In this respect the difference between the two dADA2 proteins in having or not having a SWIRM domain, which has chromatin binding affinity, might bear significance.

Regarding the question whether *ada2a*^{d189} mutations disable dGCN5 or dATAC2 (or both) to acetylate H4K5 and K12 residues, it was shown that the H4 specific acetylation is partially restored in *ada2a*^{d189} mutants by chimeric transgenes expressing dADA2a C-terminal region fused to N-terminal of dADA2b. The acetylation extends all over the polytene chromosomes. Similarly, the partial rescue of dSAGA-specific H3 acetylation by dADA2a/2b chimeric transgene expression seems to be more of a global than a gene specific modification change. The restored acetylation levels in neither case correlated with restored mRNA levels concerning the limited number of genes we tested by qPCR. This can be the result of the partial rescue, or it

could reflect a less direct link between these histone acetylation and gene expression changes.

Acetylation of H4K5 and K12 is linked to histone deposition into newly synthesized chromatin during S phase (Sobel et al., 1995). Among the identified *Drosophila* HATs (*Chameau, Tip60, Enok (enoki mushroom), CBP (nejire)*, *ELP3*), several have been shown to target these side chains of H4 (Allis et al., 2007). Our laboratory recently reported microarray data on the transcriptome of *ada2a*^{d189} mutants (Pankotai et al., 2010). In these mutants down-regulated mRNA levels were detected corresponding to HAT, HDAC and histone chaperone genes at late L3 stage, when a global decrease in K5 and K12 acetylated H4 on polytene chromosomes is clearly observable *(Table 5.1)*. The decreased mRNA levels are unlikely to be caused by an unspecific RNA degradation since other genes, among them several involved in acetyl-CoA metabolism, are represented by elevated mRNA levels as compared to wild type. Moreover, strong correlation exists between expression changes in *ada2a*^{d189} and *ada3*^{2} mutants. Strikingly, dGCN5 (KAT2) stands out being represented by an increased mRNA level in dATAC subunit mutants *(Table 5.1)*. Based on the microarray data one can hypothesize that a perturbed balance of HATs and HDACs leads to the global H4 acetylation changes in dATAC mutants. These indirect effects of dATAC mutations might cover gene specific alterations caused directly either by the dGCN5 or dATAC2 catalytic unit of dATAC complex.

The results reported within this dissertation are in accord with previously collected genetic data and underline the differences in histone specificity of the two *Drosophila* GCN5-containing HAT complexes. The demonstration of the important contribution of dADA2 C-terminal regions to complex formation pose questions and open ways to follow in the exploration of subunit interactions in order to elucidate the roles of these GCN5-containing complexes in chromatin regulation.

Table 5.1 *Expression changes of selected genes in ada2a^{d189} and ada3^{2} null mutants.*

Classification	*Gene symbol*	*Gene accession number*	*log$_2$ expression change*		*Biological function*
			ada2a^{d189}	*ada3^{2}*	
Histone acetyltransferases	Gcn5 (KAT2)	CG4107-RA	1.44	0.70	histone acetyltransferase activity
	Atac2 (KAT14)	CG10414-RA	-0.50	-0.33	N-acetyltransferase activity
	ELP3 (KAT9)	CG15433-RA	0.09	0.49	histone acetyltransferase activity
	HAT1 (KAT1)	CG2051-RC	-1.08	-0.99	histone acetyltransferase activity
	Chm (KAT7)	CG5229-RA	-2.14	-0.95	histone acetyltransferase activity
	MOF (KAT8)	CG3025-RA	-0.02	0.48	histone acetyltransferase activity
	Enok (KAT6A)	CG11290-RA	-1.15	-0.40	histone acetyltransferase activity
	Tip60 (KAT5)	CG6121-RA	-0.80	-0.78	histone acetyltransferase activity
	CG1894 (KAT6)	CG1894-RA	-1.26	-1.66	protein amino acid acetylation
	Nej (CBP)	CG15319-RB	-0.93	-0.79	histone acetyltransferase activity
Histone Deacetylases	HDAC3	CG2128-RA	-1.03	-0.88	histone deacetylase activity
	HDAC4	CG1770-RA	-0.77	-0.42	histone deacetylase activity
	Rpd3 HDAC	CG7471-RA	-2.00	-1.94	histone deacetylase activity;
	Sir2 HDAC	CG5216-RA	-0.93	-0.47	NAD-dependent histone deacetylase activity
	Sirt2 HDAC	CG5085-RA	-0.10	0.11	NAD-dependent histone deacetylase activity
Histone Chaperones/ nucleosome assembly	Ash1	CG8887-RA	-1.34	-0.85	histone chaperone
	Nap1	CG5330-RA	0.58	0.56	nucleosome assembly; histone binding
	Nlp	CG7917-RA	-0.17	-0.11	histone binding; ATPase activity
	Caf1	CG4236-RA	-0.93	-0.85	histone acetyltransferase binding
Acetyl-CoA metabolism	CG11198	CG11198-RA	2.65	2.87	acetyl-CoA carboxylase activity
	AcCoAS	CG9390-RA	1.26	1.61	acetate-CoA ligase activity
	CS	CG3861-RB	1.42	1.39	citrate (Si)-synthase activity

Summary

In eukaryotes, the association of the genomic DNA with histone and non-histone proteins into chromatin provides the means for compaction of the entire genome inside the nucleus. However, the compact structure of the chromatin restricts the access to the DNA for various regulatory proteins with essential biological functions in processes like transcription, replication and DNA repair. The repressive nature of the chromatin can be modulated by two mechanisms: covalent modifications of the histone tails and regulation of the chromatin structure by the ATP-dependent chromatin remodelling enzymes.

Histones are small basic proteins consisting of a globular domain and a flexible, charged amino- and carboxy-terminus (the histone "tails"). The histone tails are highly conserved across various species and are subjected to extensive post-translational modifications (PTMs) including acetylation and methylation of lysines and arginines, phosphorylation of serine and threonines, ubiquitination, sumoylation and biotinylation of lysines as well as ADP ribosylation (Dutnall and Ramakrishnan, 1997). One of the most studied PTMs is the histone protein acetylation. It is a highly dynamic process where the acetylation of the lysine residues is achieved by enzymes called histone acetyltransferases (HAT). However, the steady state balance of the histone acetylation is regulated by the opposing action of the HATs and histone deacetylases (HDAC) (Allis et al., 2007; Munshi et al., 2009; Bannister and Kouzarides, 2011).

Post-transcriptional modifications of histone tails serve as important signals for regulating chromatin-related processes. Recently it has been hypothesized that the histone tail modifications and/or specific combinations of the covalent modifications constitute a code, the "histone code", which regulates specific chromatin-related functions and processes (Jenuwein and Allis, 2001). Accumulating studies on the histone modifications demonstrated their important role during development and emphasize that misregulation of the epigenetic mechanisms can lead to human disease, including cancer (Furdas et al., 2012).

Dynamic and reversible acetylation of histone octamer N-terminal tails is strongly correlated with the transcriptional activity of a gene (Turner and O'Neill, 1995). Based on the sequence homology to the histone acetyltransferase p55 from Tetrahymena thermophilia, the yeast transcriptional adaptor GCN5 was identified as the first HAT enzyme linked to transcription activation (Brownell et al., 1996b; Kuo et al., 1996). Biochemical studies have identified that GCN5 represents the catalytic subunit of several transcriptional coactivator complexes raging from yeast to humans.

Most of the GCN5-containing histone acetyltransferase complexes also share ADA2 adaptor proteins. In *Drosophila melanogaster* there are two genes, referred to as *dAda2a* and *dAda2b*, encoding related dADA2 proteins (Kusch et al., 2003; Muratoglu et al., 2003). Biochemical separation of ADA2-containig *Drosophila* complexes indicated that dADA2a is present in a smaller complex (dATAC) responsible for acetylation of histone H4 at lysines K5 and K12 (Ciurciu et al., 2006; Guelman et al., 2006; Suganuma et al., 2008), whereas dADA2b is present in a larger complex (dSAGA) which targets H3 K9 and K14 for acetylation (Kusch et al., 2003; Qi et al., 2004; Pankotai et al., 2005). Additionally, the *Drosophila Ada2b* gene encodes two isoforms, dADA2bS (short) and dADA2bL (long), which are identical in their N-terminal, but differ in their C-terminal regions (Muratoglu et al., 2003; Qi et al., 2004). Glycerol gradient sedimentation analysis of the *Drosophila* nuclear extract have identified that the two dADA2b isoforms showed the same pattern of sedimentation in fractions corresponding to high molecular masses (Muratoglu et al., 2003). Thus, the composition and functional complexity of dSAGA might be influenced by the presence of two dADA2b isoforms.

In this dissertation several lines of evidence are provided for the role of the C-terminal domain of *Drosophila* dADA2 adaptor proteins in regulating the specificity of dSAGA or dATAC complexes *in vivo*. Using a series of domain swap chimeras, in which different fragments of dADA2b and dADA2a were mutually exchanged, we examined the importance of particular regions for the specificity of the HAT complexes. We tested the *in vivo* functions of the chimeric dADA2 proteins by determining the phenotypical rescue and histone modifying abilities of dADA2a/dADA2b chimeras in *ada2a*^{d189} and *ada2b*^{d842} null mutant background. We found that Actin-Gal4 driven expression of the chimeric dADA2 proteins had a partial phenotypical rescue effect for one or the other *ada2* mutants. Immunostaining of *Drosophila* polytene

chromosomes and western blot analysis revealed a significant restoration of the lost acetylation on H4K5 and K12 or H3K9 and K14 in *ada2* mutants carrying chimeric transgenes. In addition, the effect of the dADA2b/2a chimeric transgenes expressions was also noticeable on the chromosome structure of the dATAC mutants. The distorted phenotype of chromosomes characteristic for *ada2a*^{d189} null mutation was considerably improved in mutant animals expressing the pUAS-*dAda2b*^{S}*2a* or pUAS-*dAda2b*^{M}*2a* chimeric transgenes. These findings promote the hypothesis that the C-terminal domain of the dADA2 proteins plays an important role regarding the particular incorporation of dADA2 proteins into dSAGA or dATAC type complexes. Furthermore, the ability to use dADA2 chimeric proteins to rescue and examine developmental processes provides a strategy for associating specific structural domain of dADA2 proteins with functional specificity.

Biochemical and genetic evidences revealed the existence of two isoforms of dADA2b protein expressed in different quantities during fly development (Pankotai et al., 2013a). The experimental data presented in this thesis establish that both dADA2b proteins interact with each other and co-immunoprecipitated from S2 cells with dADA3, as well as with the tumour suppressor dp53. These findings show that both dADA2b proteins are functional and support the possibility that either both dADA2bL and dADA2bS can share the same HAT complex or they are subunits of different SAGA complexes. In either way their interaction with dp53 might suggest a role in the apoptotic pathway.

Összefoglaló

Eukariótákban a genomi DNS hiszton és nem-hiszton fehérjékkel való kapcsolata során kialakuló kromatin szerkezet biztosítja az egész genom kompaktálódását a sejtmagon belül. Ez a kompakt struktúra azonban megakadályozza számos, a transzkripcióban, a replikációban és a DNS-javításban részt vevő, esszenciális funkcióval rendelkező szabályozó fehérje DNS-hez való hozzáférését. A kromatin ezen gátló természete két mechanizmussal szabályozható: a hiszton végek kovalens módosításaival és a kromatin szerkezet ATP-függő kromatin átalakító enzimek segítségével történő szabályozásával.

A hisztonok kisméretű, bázikus fehérjék globuláris alegységgel és hajlékony, töltéssel rendelkező amino- és karboxi-terminálissal, melyeket "hiszton farki doménnek" is neveznek. A hiszton farki domének evolúcionálisan erősen konzerváltak és sokféle poszt-transzlációs módosításnak (PTM) vannak kitéve: a lizin és arginin oldalláncok acetilációja és metilációja, a szerin és treonin oldalláncok foszforilációja, a lizinek ubikvitinációja, SUMO-ilációja, biotinizációja és ADP ribozilációja egyaránt megfigyelhető rajtuk (Dutnall and Ramakrishnan, 1997). Az egyik legjobban tanulmányozott PTM a hiszton fehérjék acetilációja. Ez egy igen dinamikus folyamat, melynek során a lizin oldalláncok acetilációját speciális enzimek, az úgynevezett hiszton acetiltranszferázok (HAT) végzik. A hiszton acetiláció egyensúlyi állapotának fenntartását a HAT-ok és a hiszton deacetiláz (HDAC) enzimek ellentétes aktivitása biztosítja (Allis et al., 2007; Munshi et al., 2009; Bannister and Kouzarides, 2011).

A hiszton fehérjék poszt-transzkripcionális módosításai fontos jelként szolgálnak a kromatinszerkezettel kapcsolatos folyamatok szabályozásában. Feltételezik, hogy a hiszton fehérjék farki doménjeinek módosításai és/vagy a kovalens módosítások speciális kombinációi egy kódot („hiszton kód") alkotnak, ami specifikus kromatin-kapcsolt funkciókat és folyamatokat szabályoz (Jenuwein and Allis, 2001). Számos eredmény bizonyítja a hiszton módosítások egyedfejlődésben betöltött fontos szerepét, valamint azt, hogy az epigenetikai mechanizmusok szabályozatlansága különböző betegségek, mint például a rák kialakulásához vezethet (Furdas et al., 2012).

A hiszton oktamer N-terminális farki domének dinamikus és reverzibilis acetilációja erősen összefügg egy adott gén transzkripciós aktivitásával (Turner and O'Neill, 1995). Az élesztő transzkripcionális adaptor GCN5 fehérjéjét, mint az első HAT enzimet a *Tetrahymena thermophilia* p55 hiszton acetiltranszferáz enziméhez való szekvencia-homológia alapján azonosították (Brownell et al., 1996b; Kuo et al., 1996). Biokémiai vizsgálatok igazolták, hogy a GCN5 számos tarnszkripcionális ko-aktivátor komplex katalitikus alegysége. A legtöbb GCN5-tartalmú hiszton acetiltranszferáz komplex ADA2 adaptor fehérjéket is tartalmaz. *Drosophila melanogaster*-ben két, ADA2 fehérjét kódoló gént ismerünk: *dADA2a*-t és *dAda2b*-t (Kusch et al., 2003; Muratoglu et al., 2003). ADA2 fehérjéket tartalmazó *Drosophila* komplexek biokémiai vizsgálata során kimutatták, hogy a dADA2a fehérje egy kisebb komplex (dATAC) tagja, mely a hiszton H4 fehérjék lizin 5 és lizin 12 acetilációjáért felelős (Ciurciu et al., 2006; Guelman et al., 2006; Suganuma et al., 2008), míg a dADA2b fehérje egy másik, nagyobb komplexben (dSAGA) található, mely a hiszton H3 lizin 9 és lizin 14 acetilációjában játszik szerepet (Kusch et al., 2003; Qi et al., 2004; Pankotai et al., 2005). A *Drosophila Ada2b* génről két fehérje izoforma képződik: a rövidebb dADA2bS és a hosszabb dADA2bL, melyek N-terminális vége megegyezik, míg a C-terminális részükön különböznek (Muratoglu et al., 2003; Qi et al., 2004). *Drosophila* sejtmagi extrakt glycerol grádiens süllyedéses vizsgálata során megállapították, hogy a két dADA2b izoforma azonos süllyedési mintázatot mutatott a nagy molekulasúlyú frakciókban (Muratoglu et al., 2003). Ennélfogva a dSAGA komplex összetételét és funkcionális összetettségét a két dADA2b izoforma jelenléte befolyásolja.

Disszertációmban számos bizonyítékkal támasztom alá, hogy a *Drosophila* ADA2 adaptor fehérjék C-terninális doménje szerepet játszik a dSAGA vagy a dATAC komplex specifitásában *in vivo*. Számos domén-cserélt kiméra fehérje segítségével, melyekben a dADA2a és dADA2b különböző darabjait kölcsönösen kicseréltem, az egyes régiók HAT komplex specifitásának fontosságát vizsgáltam. A kiméra ADA2 fehérjék *in vivo* funkcióját a dADA2a/dADA2b fehérjék fenotipikus menekítésének és hiszton módosító képességének meghatározásával $ada2a^{d189}$ és $ada2b^{d842}$ null-mutáns háttéren teszteltem. Megállapítottam, hogy a kiméra dADA2 fehérjék Actin-Gal4 hajtotta túltermelése részleges fenotípus menekítést eredményez az egyik vagy a másik *ada2* mutánsban. A *Drosophila* polién kromoszóma immunfestése és western

blot analízis az elveszett H4K5 és K12 vagy H3K9 és K14 acetiláció visszaállítását mutatta a kiméria géneket hordozó *ada2* mutánsokban. Ezen túlmenően, a dADA2b/2a kiméra transzgének expressziójának hatása szintén jól megfigyelhető a dATAC mutánsok kromoszóma-szerkezetének megváltozásában. Az *ada2*^{d189} null mutációra jellemző deformálódott kromoszóma fenotípus jelentősen javult a pUAS-*dAda2b*^{S}*2a* vagy pUAS-*dAda2b*^{M}*2a* kiméra transzgént kifejező mutáns állatokban. Ezen megfigyelések bizonyítják, hogy az ADA2 fehérjék C-terminális doménje fontos szerepet játszik ezen fehérjék dSAGA vagy dATAC komplexekbe való beépülésében. Továbbá, a dADA2 kiméra fehérjék használata menekítésre és a fejlődési folyamatok vizsgálatára egy kiváló stratégiát biztosít a dADA2 fehérjék specifikus szerkezeti alegységeihez tartozó speciális funkciók hozzárendeléséhez.

Biokémiai és genetikai bizonyítékok támasztják alá, hogy a két dADA2b izoforma különböző mennyiségben termelődik a *Drosophila* egyedfejlődése alatt (Pankotai et al., 2013a). A kísérleti adatok alapján megállapítható, hogy a két dADA2b fehérje kölcsönhat egymással és ko-immunoprecipitálható dADA3-mal és a tumor szuppresszor dp53-mal S2 sejtekből. Ezen megfigyelések alapján elmondható, hogy mindkét dADA2b fehérje működőképes, és alátámasztja a lehetőséget, hogy a dADA2bL és a dADA2bS: 1. előfordulhat ugyanabban a HAT komplexben; 2. különböző SAGA komplexek alegységei. A p53-mal történő kölcsönhatásuk mindkét esetben az apoptotikus útvonalban betöltött szerepükre utal.

List of publications

Publications related to the thesis

- **Vamos E. E.**, Boros I. M. - *The C-terminal domains of ADA2 proteins determine selective incorporation into GCN5-containing complexes that target histone H3 or H4 for acetylation.*
FEBS Letters 2012 September 21; 586 (19):3279-86.

IF: 3.538

- Pankotai T., Zsindely N., **Vamos E. E**., Komonyi O., Bodai L., Boros I. M. - *Functional characterization and gene expression profiling of Drosophila melanogaster short dADA2b isoform-containing dSAGA complexes.*
BMC Genomics 2013 January 22; 14 (1):44.

IF: 4.07

Other publications

- Pankotai T., Ujfaludi Z., **Vamos E. E.**, Suri K., Boros I. M. - *The dissociable RPB4 subunit of RNA Pol II has vital functions in Drosophila.*
Mol. Genet. Genomics 2010 January 283 (1):89-97.

IF: 2.635

- Pardi N., **Vamos E. E**., Ujfaludi Z., Komonyi O., Bodai L., Boros I. M. - *In vivo effects of abolishing the single canonical sumoylation site in the C-terminal region of Drosophila p53.*
Acta Biol. Hung. 2011 December 62(4):397-412.

IF: 0.59

References

Abdu, U., Klovstad, M., Butin-Israeli, V., Bakhrat, A., and Schupbach, T. (2007). An essential role for Drosophila hus1 in somatic and meiotic DNA damage responses. Journal of cell science **120,** 1042-1049.

Agalioti, T., Chen, G., and Thanos, D. (2002). Deciphering the transcriptional histone acetylation code for a human gene. Cell **111,** 381-392.

Allis, C.D., Berger, S.L., Cote, J., Dent, S., Jenuwien, T., Kouzarides, T., Pillus, L., Reinberg, D., Shi, Y., Shiekhattar, R., Shilatifard, A., Workman, J., and Zhang, Y. (2007). New nomenclature for chromatin-modifying enzymes. Cell **131,** 633-636.

Arrowsmith, C.H., Bountra, C., Fish, P.V., Lee, K., and Schapira, M. (2012). Epigenetic protein families: a new frontier for drug discovery. Nature reviews. Drug discovery **11,** 384-400.

Ashburner, M. (1989). Drosophila: A laboratory handbook. (New York: Cold Spring Harbor Laboratory).

Balasubramanian, R., Pray-Grant, M.G., Selleck, W., Grant, P.A., and Tan, S. (2002). Role of the Ada2 and Ada3 transcriptional coactivators in histone acetylation. The Journal of biological chemistry **277,** 7989-7995.

Bannister, A.J., and Kouzarides, T. (1996). The CBP co-activator is a histone acetyltransferase. Nature **384,** 641-643.

Bannister, A.J., and Kouzarides, T. (2011). Regulation of chromatin by histone modifications. Cell research **21,** 381-395.

Barlev, N.A., Candau, R., Wang, L., Darpino, P., Silverman, N., and Berger, S.L. (1995). Characterization of physical interactions of the putative transcriptional adaptor, ADA2, with acidic activation domains and TATA-binding protein. The Journal of biological chemistry **270,** 19337-19344.

Barlev, N.A., Emelyanov, A.V., Castagnino, P., Zegerman, P., Bannister, A.J., Sepulveda, M.A., Robert, F., Tora, L., Kouzarides, T., Birshtein, B.K., and Berger, S.L. (2003). A novel human Ada2 homologue functions with Gcn5 or Brg1 to coactivate transcription. Molecular and cellular biology **23,** 6944-6957.

Bassett, A., Cooper, S., Wu, C., and Travers, A. (2009). The folding and unfolding of eukaryotic chromatin. Current opinion in genetics & development **19,** 159-165.

Berger, S.L. (2007). The complex language of chromatin regulation during transcription. Nature **447,** 407-412.

Berger, S.L., Pina, B., Silverman, N., Marcus, G.A., Agapite, J., Regier, J.L., Triezenberg, S.J., and Guarente, L. (1992). Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. Cell **70,** 251-265.

Bernstein, B.E., Humphrey, E.L., Erlich, R.L., Schneider, R., Bouman, P., Liu, J.S., Kouzarides, T., and Schreiber, S.L. (2002). Methylation of histone H3 Lys 4 in coding regions of active genes. Proceedings of the National Academy of Sciences of the United States of America **99,** 8695-8700.

Bhaumik, S.R., and Green, M.R. (2001). SAGA is an essential in vivo target of the yeast acidic activator Gal4p. Genes & development **15,** 1935-1945.

Bhaumik, S.R., Smith, E., and Shilatifard, A. (2007). Covalent modifications of histones during development and disease pathogenesis. Nature structural & molecular biology **14,** 1008-1016.

Bischof, J., Maeda, R.K., Hediger, M., Karch, F., and Basler, K. (2007). An optimized transgenesis system for Drosophila using germ-line-specific phiC31 integrases. Proceedings of the National Academy of Sciences of the United States of America **104,** 3312-3317.

Bouazoune, K., Mitterweger, A., Langst, G., Imhof, A., Akhtar, A., Becker, P.B., and Brehm, A. (2002). The dMi-2 chromodomains are DNA binding modules important for ATP-dependent nucleosome mobilization. The EMBO journal **21,** 2430-2440.

Brown, C.E., Lechner, T., Howe, L., and Workman, J.L. (2000). The many HATs of transcription coactivators. Trends in biochemical sciences **25,** 15-19.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996a). Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell **84,** 843-851.

Brownell, J.E., Zhou, J.X., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996b). Tetrahymena histone acetyltransferase A: A homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell **84,** 843-851.

Bu, P., Evrard, Y.A., Lozano, G., and Dent, S.Y. (2007). Loss of Gcn5 acetyltransferase activity leads to neural tube closure defects and exencephaly in mouse embryos. Molecular and cellular biology **27,** 3405-3416.

Candau, R., and Berger, S.L. (1996). Structural and functional analysis of yeast putative adaptors. Evidence for an adaptor complex in vivo. The Journal of biological chemistry **271,** 5237-5245.

Candau, R., Moore, P.A., Wang, L., Barlev, N., Ying, C.Y., Rosen, C.A., and Berger, S.L. (1996). Identification of human proteins functionally conserved with the yeast putative adaptors ADA2 and GCN5. Molecular and cellular biology **16,** 593-602.

Cao, J., and Yan, Q. (2012). Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. Frontiers in oncology **2,** 26.

Carre, C., Szymczak, D., Pidoux, J., and Antoniewski, C. (2005). The histone H3 acetylase dGcn5 is a key player in Drosophila melanogaster metamorphosis. Molecular and cellular biology **25,** 8228-8238.

Carre, C., Ciurciu, A., Komonyi, O., Jacquier, C., Fagegaltier, D., Pidoux, J., Tricoire, H., Tora, L., Boros, I.M., and Antoniewski, C. (2008). The Drosophila NURF remodelling and the ATAC histone acetylase complexes functionally interact and are required for global chromosome organization. EMBO reports **9,** 187-192.

Chen, C.C., Carson, J.J., Feser, J., Tamburini, B., Zabaronick, S., Linger, J., and Tyler, J.K. (2008). Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. Cell **134,** 231-243.

Choi, J.K., and Howe, L.J. (2009). Histone acetylation: truth of consequences? Biochemistry and cell biology = Biochimie et biologie cellulaire **87,** 139-150.

Ciurciu, A., Komonyi, O., Pankotai, T., and Boros, I.M. (2006). The Drosophila histone acetyltransferase Gcn5 and transcriptional adaptor Ada2a are involved in nucleosomal histone H4 acetylation. Molecular and cellular biology **26,** 9413-9423.

Close, P., Hawkes, N., Cornez, I., Creppe, C., Lambert, C.A., Rogister, B., Siebenlist, U., Merville, M.P., Slaugenhaupt, S.A., Bours, V., Svejstrup, J.Q., and Chariot, A. (2006). Transcription impairment and cell migration defects in

elongator-depleted cells: implication for familial dysautonomia. Molecular cell **22,** 521-531.

Colinet, H., Lee, S.F., and Hoffmann, A. (2010). Functional characterization of the Frost gene in Drosophila melanogaster: importance for recovery from chill coma. PloS one **5,** e10925.

Creppe, C., Malinouskaya, L., Volvert, M.L., Gillard, M., Close, P., Malaise, O., Laguesse, S., Cornez, I., Rahmouni, S., Ormenese, S., Belachew, S., Malgrange, B., Chapelle, J.P., Siebenlist, U., Moonen, G., Chariot, A., and Nguyen, L. (2009). Elongator controls the migration and differentiation of cortical neurons through acetylation of alpha-tubulin. Cell **136,** 551-564.

Deng, H., and Kerppola, T.K. (2013). Regulation of Drosophila metamorphosis by xenobiotic response regulators. PLoS genetics **9,** e1003263.

Downs, J.A. (2008). Histone H3 K56 acetylation, chromatin assembly, and the DNA damage checkpoint. DNA repair **7,** 2020-2024.

Doyon, Y., and Cote, J. (2004). The highly conserved and multifunctional NuA4 HAT complex. Current opinion in genetics & development **14,** 147-154.

Dutnall, R.N., and Ramakrishnan, V. (1997). Twists and turns of the nucleosome: tails without ends. Structure **5,** 1255-1259.

Dyda, F., Klein, D.C., and Hickman, A.B. (2000). GCN5-related N-acetyltransferases: a structural overview. Annual review of biophysics and biomolecular structure **29,** 81-103.

Ehrenhofer-Murray, A.E. (2004). Chromatin dynamics at DNA replication, transcription and repair. European journal of biochemistry / FEBS **271,** 2335-2349.

Felsenfeld, G., and Groudine, M. (2003). Controlling the double helix. Nature **421,** 448-453.

Fischle, W., Wang, Y., and Allis, C.D. (2003). Histone and chromatin cross-talk. Current opinion in cell biology **15,** 172-183.

Fletcher, T.M., and Hansen, J.C. (1996). The nucleosomal array: structure/function relationships. Critical reviews in eukaryotic gene expression **6,** 149-188.

Fukuda, H., Sano, N., Muto, S., and Horikoshi, M. (2006). Simple histone acetylation plays a complex role in the regulation of gene expression. Briefings in functional genomics & proteomics **5,** 190-208.

Furdas, S.D., Kannan, S., Sippl, W., and Jung, M. (2012). Small molecule inhibitors of histone acetyltransferases as epigenetic tools and drug candidates. Archiv der Pharmazie **345,** 7-21.

Gamper, A.M., and Roeder, R.G. (2008). Multivalent binding of p53 to the STAGA complex mediates coactivator recruitment after UV damage. Molecular and cellular biology **28,** 2517-2527.

Gamper, A.M., Kim, J., and Roeder, R.G. (2009). The STAGA subunit ADA2b is an important regulator of human GCN5 catalysis. Molecular and cellular biology **29,** 266-280.

Georgieva, S., Kirschner, D.B., Jagla, T., Nabirochkina, E., Hanke, S., Schenkel, H., de Lorenzo, C., Sinha, P., Jagla, K., Mechler, B., and Tora, L. (2000). Two novel Drosophila TAF(II)s have homology with human TAF(II)30 and are differentially regulated during development. Molecular and cellular biology **20,** 1639-1648.

Grant, P.A., Eberharter, A., John, S., Cook, R.G., Turner, B.M., and Workman, J.L. (1999). Expanded lysine acetylation specificity of Gcn5 in native complexes. The Journal of biological chemistry **274,** 5895-5900.

Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L., and Workman, J.L. (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes & development **11,** 1640-1650.

Grau, B., Popescu, C., Torroja, L., Ortuno-Sahagun, D., Boros, I., and Ferrus, A. (2008). Transcriptional adaptor ADA3 of Drosophila melanogaster is required for histone modification, position effect variegation, and transcription. Molecular and cellular biology **28,** 376-385.

Greer, E.L., and Shi, Y. (2012). Histone methylation: a dynamic mark in health, disease and inheritance. Nature reviews. Genetics **13,** 343-357.

Groth, A., Rocha, W., Verreault, A., and Almouzni, G. (2007). Chromatin challenges during DNA replication and repair. Cell **128,** 721-733.

Groth, A.C., Fish, M., Nusse, R., and Calos, M.P. (2004). Construction of transgenic Drosophila by using the site-specific integrase from phage phi C31. Genetics **166,** 1775-1782.

Guelman, S., Suganuma, T., Florens, L., Swanson, S.K., Kiesecker, C.L., Kusch, T., Anderson, S., Yates, J.R., 3rd, Washburn, M.P., Abmayr, S.M., and Workman, J.L. (2006). Host cell factor and an uncharacterized SANT domain protein are stable components of ATAC, a novel dAda2A/dGcn5-containing histone acetyltransferase complex in Drosophila. Molecular and cellular biology **26,** 871-882.

Hassa, P.O., and Hottiger, M.O. (2008). The diverse biological roles of mammalian PARPS, a small but powerful family of poly-ADP-ribose polymerases. Frontiers in bioscience : a journal and virtual library **13,** 3046-3082.

Hassa, P.O., Haenni, S.S., Elser, M., and Hottiger, M.O. (2006). Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? Microbiology and molecular biology reviews : MMBR **70,** 789-829.

Heringa, J. (1999). Two strategies for sequence comparison: profile-preprocessed and secondary structure-induced multiple alignment. Comput Chem **23,** 341-364.

Heringa, J. (2000). Computational Methods for Protein Secondary Structure Prediction Using Multiple Sequence Alignments. Curr Protein Pept Sc **1,** 273-301.

Heringa, J. (2002). Local weighting schemes for protein multiple sequence alignment. Comput Chem **26,** 459-477.

Hodawadekar, S.C., and Marmorstein, R. (2007). Chemistry of acetyl transfer by histone modifying enzymes: structure, mechanism and implications for effector design. Oncogene **26,** 5528-5540.

Hottiger, M.O. (2011). ADP-ribosylation of histones by ARTD1: an additional module of the histone code? FEBS letters **585,** 1595-1599.

Ikura, T., Tashiro, S., Kakino, A., Shima, H., Jacob, N., Amunugama, R., Yoder, K., Izumi, S., Kuraoka, I., Tanaka, K., Kimura, H., Ikura, M., Nishikubo, S., Ito, T., Muto, A., Miyagawa, K., Takeda, S., Fishel, R., Igarashi, K., and Kamiya, K. (2007). DNA damage-dependent acetylation and ubiquitination of H2AX enhances chromatin dynamics. Molecular and cellular biology **27,** 7028-7040.

Jenuwein, T., and Allis, C.D. (2001). Translating the histone code. Science **293,** 1074-1080.

Johnsen, S.A. (2012). The enigmatic role of H2Bub1 in cancer. FEBS letters **586,** 1592-1601.

Jones, P.A., and Baylin, S.B. (2007). The epigenomics of cancer. Cell **128,** 683-692.

Joosten, L.A., Leoni, F., Meghji, S., and Mascagni, P. (2011). Inhibition of HDAC activity by ITF2357 ameliorates joint inflammation and prevents cartilage and bone destruction in experimental arthritis. Molecular medicine **17,** 391-396.

Khan, S.N., and Khan, A.U. (2010). Role of histone acetylation in cell physiology and diseases: An update. Clinica chimica acta; international journal of clinical chemistry **411,** 1401-1411.

Kimura, A., and Horikoshi, M. (1998). Tip60 acetylates six lysines of a specific class in core histones in vitro. Genes to cells : devoted to molecular & cellular mechanisms **3,** 789-800.

Klein, K., and Gay, S. (2013). Epigenetic modifications in rheumatoid arthritis, a review. Current opinion in pharmacology.

Komonyi, O., Papai, G., Enunlu, I., Muratoglu, S., Pankotai, T., Kopitova, D., Maroy, P., Udvardy, A., and Boros, I. (2005). DTL, the Drosophila homolog of PIMT/Tgs1 nuclear receptor coactivator-interacting protein/RNA methyltransferase, has an essential role in development. The Journal of biological chemistry **280,** 12397-12404.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell **128,** 693-705.

Krebs, A.R., Karmodiya, K., Lindahl-Allen, M., Struhl, K., and Tora, L. (2011). SAGA and ATAC histone acetyl transferase complexes regulate distinct sets of genes and ATAC defines a class of p300-independent enhancers. Molecular cell **44,** 410-423.

Krebs, A.R., Demmers, J., Karmodiya, K., Chang, N.C., Chang, A.C., and Tora, L. (2010). ATAC and Mediator coactivators form a stable complex and regulate a set of non-coding RNA genes. EMBO reports **11,** 541-547.

Kung, A.L., Rebel, V.I., Bronson, R.T., Ch'ng, L.E., Sieff, C.A., Livingston, D.M., and Yao, T.P. (2000). Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. Genes & development **14,** 272-277.

Kuo, M.H., Brownell, J.E., Sobel, R.E., Ranalli, T.A., Cook, R.G., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996). Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. Nature **383,** 269-272.

Kusch, T., Guelman, S., Abmayr, S.M., and Workman, J.L. (2003). Two Drosophila Ada2 homologues function in different multiprotein complexes. Molecular and cellular biology **23,** 3305-3319.

Kusch, T., Florens, L., Macdonald, W.H., Swanson, S.K., Glaser, R.L., Yates, J.R., 3rd, Abmayr, S.M., Washburn, M.P., and Workman, J.L. (2004). Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. Science **306,** 2084-2087.

Langley, E., Pearson, M., Faretta, M., Bauer, U.M., Frye, R.A., Minucci, S., Pelicci, P.G., and Kouzarides, T. (2002). Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. The EMBO journal **21,** 2383-2396.

Latham, J.A., and Dent, S.Y. (2007). Cross-regulation of histone modifications. Nature structural & molecular biology **14,** 1017-1024.

Le Douarin, B., Heery, D.M., Gaudon, C., vom Baur, E., and Losson, R. (2001). Yeast two-hybrid screening for proteins that interact with nuclear hormone receptors. Methods Mol Biol **176,** 227-248.

Lee, J.S., Smith, E., and Shilatifard, A. (2010). The Language of Histone Crosstalk. Cell **142,** 682-685.

Lee, J.S., Shukla, A., Schneider, J., Swanson, S.K., Washburn, M.P., Florens, L., Bhaumik, S.R., and Shilatifard, A. (2007). Histone crosstalk between H2B

monoubiquitination and H3 methylation mediated by COMPASS. Cell **131,** 1084-1096.

Lee, K.K., and Workman, J.L. (2007). Histone acetyltransferase complexes: one size doesn't fit all. Nature reviews. Molecular cell biology **8,** 284-295.

Lee, K.K., Sardiu, M.E., Swanson, S.K., Gilmore, J.M., Torok, M., Grant, P.A., Florens, L., Workman, J.L., and Washburn, M.P. (2011). Combinatorial depletion analysis to assemble the network architecture of the SAGA and ADA chromatin remodeling complexes. Molecular systems biology **7,** 503.

Lennartsson, A., and Ekwall, K. (2009). Histone modification patterns and epigenetic codes. Biochimica et biophysica acta **1790,** 863-868.

Li, Q., Burgess, R., and Zhang, Z. (2012). All roads lead to chromatin: Multiple pathways for histone deposition. Biochimica et biophysica acta **1819,** 238-246.

Lin, H.S., Hu, C.Y., Chan, H.Y., Liew, Y.Y., Huang, H.P., Lepescheux, L., Bastianelli, E., Baron, R., Rawadi, G., and Clement-Lacroix, P. (2007). Anti-rheumatic activities of histone deacetylase (HDAC) inhibitors in vivo in collagen-induced arthritis in rodents. British journal of pharmacology **150,** 862-872.

Linares, L.K., Kiernan, R., Triboulet, R., Chable-Bessia, C., Latreille, D., Cuvier, O., Lacroix, M., Le Cam, L., Coux, O., and Benkirane, M. (2007). Intrinsic ubiquitination activity of PCAF controls the stability of the oncoprotein Hdm2. Nature cell biology **9,** 331-338.

Luger, K., and Richmond, T.J. (1998). The histone tails of the nucleosome. Current opinion in genetics & development **8,** 140-146.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature **389,** 251-260.

Marks, P.A., Richon, V.M., and Rifkind, R.A. (2000). Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. Journal of the National Cancer Institute **92,** 1210-1216.

Marmorstein, R. (2001). Protein modules that manipulate histone tails for chromatin regulation. Nature reviews. Molecular cell biology **2,** 422-432.

Marmorstein, R., and Roth, S.Y. (2001). Histone acetyltransferases: function, structure, and catalysis. Current opinion in genetics & development **11,** 155-161.

Maurice, T., Duclot, F., Meunier, J., Naert, G., Givalois, L., Meffre, J., Celerier, A., Jacquet, C., Copois, V., Mechti, N., Ozato, K., and Gongora, C. (2008). Altered memory capacities and response to stress in p300/CBP-associated factor (PCAF) histone acetylase knockout mice. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology **33,** 1584-1602.

McCullough, S.D., and Grant, P.A. (2010). Histone acetylation, acetyltransferases, and ataxia--alteration of histone acetylation and chromatin dynamics is implicated in the pathogenesis of polyglutamine-expansion disorders. Advances in protein chemistry and structural biology **79,** 165-203.

Messner, S., and Hottiger, M.O. (2011). Histone ADP-ribosylation in DNA repair, replication and transcription. Trends in cell biology **21,** 534-542.

Mizzen, C.A., Yang, X.J., Kokubo, T., Brownell, J.E., Bannister, A.J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S.L., Kouzarides, T., Nakatani, Y., and Allis, C.D. (1996). The TAF(II)250 subunit of TFIID has histone acetyltransferase activity. Cell **87,** 1261-1270.

Munshi, A., Shafi, G., Aliya, N., and Jyothy, A. (2009). Histone modifications dictate specific biological readouts. Journal of genetics and genomics = Yi chuan xue bao **36,** 75-88.

Muratoglu, S., Georgieva, S., Papai, G., Scheer, E., Enunlu, I., Komonyi, O., Cserpan, I., Lebedeva, L., Nabirochkina, E., Udvardy, A., Tora, L., and Boros, I. (2003). Two different Drosophila ADA2 homologues are present in distinct GCN5 histone acetyltransferase-containing complexes. Molecular and cellular biology **23,** 306-321.

Murr, R., Loizou, J.I., Yang, Y.G., Cuenin, C., Li, H., Wang, Z.Q., and Herceg, Z. (2006). Histone acetylation by Trrap-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks. Nature cell biology **8,** 91-99.

Nagy, Z., and Tora, L. (2007). Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. Oncogene **26,** 5341-5357.

Nagy, Z., Riss, A., Fujiyama, S., Krebs, A., Orpinell, M., Jansen, P., Cohen, A., Stunnenberg, H.G., Kato, S., and Tora, L. (2010). The metazoan ATAC and SAGA coactivator HAT complexes regulate different sets of inducible target genes. Cellular and molecular life sciences : CMLS **67,** 611-628.

Nelson, C.J., Santos-Rosa, H., and Kouzarides, T. (2006). Proline Isomerization of Histone H3 Regulates Lysine Methylation and Gene Expression. Cell **126,** 905-916.

Nishida, K., Komiyama, T., Miyazawa, S., Shen, Z.N., Furumatsu, T., Doi, H., Yoshida, A., Yamana, J., Yamamura, M., Ninomiya, Y., Inoue, H., and Asahara, H. (2004). Histone deacetylase inhibitor suppression of autoantibody-mediated arthritis in mice via regulation of p16INK4a and p21(WAF1/Cip1) expression. Arthritis and rheumatism **50,** 3365-3376.

Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H., and Nakatani, Y. (1996). The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell **87,** 953-959.

Ogryzko, V.V., Kotani, T., Zhang, X., Schiltz, R.L., Howard, T., Yang, X.J., Howard, B.H., Qin, J., and Nakatani, Y. (1998). Histone-like TAFs within the PCAF histone acetylase complex. Cell **94,** 35-44.

Oki, M., Aihara, H., and Ito, T. (2007). Role of histone phosphorylation in chromatin dynamics and its implications in diseases. Sub-cellular biochemistry **41,** 319-336.

Ollmann, M., Young, L.M., Di Como, C.J., Karim, F., Belvin, M., Robertson, S., Whittaker, K., Demsky, M., Fisher, W.W., Buchman, A., Duyk, G., Friedman, L., Prives, C., and Kopczynski, C. (2000). Drosophila p53 is a structural and functional homolog of the tumor suppressor p53. Cell **101,** 91-101.

Ono, H., Rewitz, K.F., Shinoda, T., Itoyama, K., Petryk, A., Rybczynski, R., Jarcho, M., Warren, J.T., Marques, G., Shimell, M.J., Gilbert, L.I., and O'Connor, M.B. (2006). Spook and Spookier code for stage-specific components of the ecdysone biosynthetic pathway in Diptera. Developmental biology **298,** 555-570.

Pankotai, T., Zsindely, N., Vamos, E.E., Komonyi, O., Bodai, L., and Boros, I.M. (2013a). Functional characterization and gene expression profiling of Drosophila melanogaster short dADA2b isoform-containing dSAGA complexes. BMC genomics **14,** 44.

Pankotai, T., Zsindely, N., Vamos, E.E., Komonyi, O., Bodai, L., and Boros, I.M. (2013b). Functional characterization and gene expression profiling of Drosophila melanogaster short dADA2b isoform-containing dSAGA complexes. BMC genomics **14,** 44.

Pankotai, T., Komonyi, O., Bodai, L., Ujfaludi, Z., Muratoglu, S., Ciurciu, A., Tora, L., Szabad, J., and Boros, I. (2005). The homologous Drosophila transcriptional

adaptors ADA2a and ADA2b are both required for normal development but have different functions. Molecular and cellular biology **25,** 8215-8227.

Pankotai, T., Popescu, C., Martin, D., Grau, B., Zsindely, N., Bodai, L., Tora, L., Ferrus, A., and Boros, I. (2010). Genes of the ecdysone biosynthesis pathway are regulated by the dATAC histone acetyltransferase complex in Drosophila. Molecular and cellular biology **30,** 4254-4266.

Papai, G., Komonyi, O., Toth, Z., Pankotai, T., Muratoglu, S., Udvardy, A., and Boros, I. (2005). Intimate relationship between the genes of two transcriptional coactivators, ADA2a and PIMT, of Drosophila. Gene **348,** 13-23.

Pardi, N., Vamos, E., Ujfaludi, Z., Komonyi, O., Bodai, L., and Boros, I.M. (2011). In vivo effects of abolishing the single canonical sumoylation site in the C-terminal region of Drosophila p53. Acta biologica Hungarica **62,** 397-412.

Patel, J., Pathak, R.R., and Mujtaba, S. (2011). The biology of lysine acetylation integrates transcriptional programming and metabolism. Nutrition & metabolism **8,** 12.

Peters, A.H., O'Carroll, D., Scherthan, H., Mechtler, K., Sauer, S., Schofer, C., Weipoltshammer, K., Pagani, M., Lachner, M., Kohlmaier, A., Opravil, S., Doyle, M., Sibilia, M., and Jenuwein, T. (2001). Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell **107,** 323-337.

Petrij, F., Giles, R.H., Dauwerse, H.G., Saris, J.J., Hennekam, R.C., Masuno, M., Tommerup, N., van Ommen, G.J., Goodman, R.H., Peters, D.J., and et al. (1995). Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature **376,** 348-351.

Pirovano, W., Feenstra, K.A., and Heringa, J. (2008). PRALINE (TM): a strategy for improved multiple alignment of transmembrane proteins. Bioinformatics **24,** 492-497.

Poux, A.N., and Marmorstein, R. (2003). Molecular basis for Gcn5/PCAF histone acetyltransferase selectivity for histone and nonhistone substrates. Biochemistry **42,** 14366-14374.

Prives, C., and Manley, J.L. (2001). Why is p53 acetylated? Cell **107,** 815-818.

Pusarla, R.H., and Bhargava, P. (2005). Histones in functional diversification. Core histone variants. The FEBS journal **272,** 5149-5168.

Qi, D., Larsson, J., and Mannervik, M. (2004). Drosophila Ada2b is required for viability and normal histone H3 acetylation. Molecular and cellular biology **24,** 8080-8089.

Qian, C., Zhang, Q., Li, S., Zeng, L., Walsh, M.J., and Zhou, M.M. (2005). Structure and chromosomal DNA binding of the SWIRM domain. Nature structural & molecular biology **12,** 1078-1085.

Rewitz, K.F., Rybczynski, R., Warren, J.T., and Gilbert, L.I. (2006). The Halloween genes code for cytochrome P450 enzymes mediating synthesis of the insect moulting hormone. Biochemical Society transactions **34,** 1256-1260.

Rossetto, D., Avvakumov, N., and Cote, J. (2012). Histone phosphorylation: A chromatin modification involved in diverse nuclear events. Epigenetics : official journal of the DNA Methylation Society **7**.

Roth, S.Y., Denu, J.M., and Allis, C.D. (2001). Histone acetyltransferases. Annual review of biochemistry **70,** 81-120.

Rouaux, C., Jokic, N., Mbebi, C., Boutillier, S., Loeffler, J.P., and Boutillier, A.L. (2003). Critical loss of CBP/p300 histone acetylase activity by caspase-6 during neurodegeneration. The EMBO journal **22,** 6537-6549.

Ryder, E., Blows, F., Ashburner, M., Bautista-Llacer, R., Coulson, D., Drummond, J., Webster, J., Gubb, D., Gunton, N., Johnson, G., O'Kane, C.J., Huen, D., Sharma, P., Asztalos, Z., Baisch, H., Schulze, J., Kube, M., Kittlaus, K., Reuter, G., Maroy, P., Szidonya, J., Rasmuson-Lestander, A., Ekstrom, K., Dickson, B., Hugentobler, C., Stocker, H., Hafen, E., Lepesant, J.A., Pflugfelder, G., Heisenberg, M., Mechler, B., Serras, F., Corominas, M., Schneuwly, S., Preat, T., Roote, J., and Russell, S. (2004). The DrosDel collection: a set of P-element insertions for generating custom chromosomal aberrations in Drosophila melanogaster. Genetics **167,** 797-813.

Sadoul, K., Boyault, C., Pabion, M., and Khochbin, S. (2008). Regulation of protein turnover by acetyltransferases and deacetylases. Biochimie **90,** 306-312.

Samara, N.L., and Wolberger, C. (2011). A new chapter in the transcription SAGA. Current opinion in structural biology **21,** 767-774.

Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J., and Kouzarides, T. (2002). Active genes are tri-methylated at K4 of histone H3. Nature **419,** 407-411.

Sawicka, A., and Seiser, C. (2012). Histone H3 phosphorylation - A versatile chromatin modification for different occasions. Biochimie.

Schneider, I. (1972). Cell lines derived from late embryonic stages of Drosophila melanogaster. Journal of embryology and experimental morphology **27,** 353-365.

Selvi, B.R., Cassel, J.C., Kundu, T.K., and Boutillier, A.L. (2010). Tuning acetylation levels with HAT activators: therapeutic strategy in neurodegenerative diseases. Biochimica et biophysica acta **1799,** 840-853.

Shahbazian, M.D., and Grunstein, M. (2007). Functions of site-specific histone acetylation and deacetylation. Annual review of biochemistry **76,** 75-100.

Shigeno, K., Yoshida, H., Pan, L., Luo, J.M., Fujisawa, S., Naito, K., Nakamura, S., Shinjo, K., Takeshita, A., Ohno, R., and Ohnishi, K. (2004). Disease-related potential of mutations in transcriptional cofactors CREB-binding protein and p300 in leukemias. Cancer letters **213,** 11-20.

Simossis, V.A., and Heringa, J. (2003). The PRALINE online server: optimising progressive multiple alignment on the web. Comput Biol Chem **27,** 511-519.

Singh, N., Lorbeck, M.T., Zervos, A., Zimmerman, J., and Elefant, F. (2010). The histone acetyltransferase Elp3 plays in active role in the control of synaptic bouton expansion and sleep in Drosophila. Journal of neurochemistry **115,** 493-504.

Smith, E.R., Belote, J.M., Schiltz, R.L., Yang, X.J., Moore, P.A., Berger, S.L., Nakatani, Y., and Allis, C.D. (1998). Cloning of Drosophila GCN5: conserved features among metazoan GCN5 family members. Nucleic acids research **26,** 2948-2954.

Sobel, R.E., Cook, R.G., Perry, C.A., Annunziato, A.T., and Allis, C.D. (1995). Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. Proceedings of the National Academy of Sciences of the United States of America **92,** 1237-1241.

Spedale, G., Timmers, H.T., and Pijnappel, W.W. (2012). ATAC-king the complexity of SAGA during evolution. Genes & development **26,** 527-541.

Sterner, D.E., and Berger, S.L. (2000). Acetylation of histones and transcription-related factors. Microbiology and molecular biology reviews : MMBR **64,** 435-459.

Sterner, D.E., Wang, X., Bloom, M.H., Simon, G.M., and Berger, S.L. (2002). The SANT domain of Ada2 is required for normal acetylation of histones by the yeast SAGA complex. The Journal of biological chemistry **277,** 8178-8186.

Sterner, D.E., Grant, P.A., Roberts, S.M., Duggan, L.J., Belotserkovskaya, R., Pacella, L.A., Winston, F., Workman, J.L., and Berger, S.L. (1999). Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Molecular and cellular biology **19,** 86-98.

Stockinger, E.J., Mao, Y., Regier, M.K., Triezenberg, S.J., and Thomashow, M.F. (2001). Transcriptional adaptor and histone acetyltransferase proteins in Arabidopsis and their interactions with CBF1, a transcriptional activator involved in cold-regulated gene expression. Nucleic acids research **29,** 1524-1533.

Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature **403,** 41-45.

Suganuma, T., and Workman, J.L. (2011). Signals and combinatorial functions of histone modifications. Annual review of biochemistry **80,** 473-499.

Suganuma, T., Gutierrez, J.L., Li, B., Florens, L., Swanson, S.K., Washburn, M.P., Abmayr, S.M., and Workman, J.L. (2008). ATAC is a double histone acetyltransferase complex that stimulates nucleosome sliding. Nature structural & molecular biology **15,** 364-372.

Surani, M.A., Hayashi, K., and Hajkova, P. (2007). Genetic and epigenetic regulators of pluripotency. Cell **128,** 747-762.

Szabo, E., Kovacs, I., Grune, T., Haczku, A., and Virag, L. (2011). PARP-1: a new player in the asthma field? Allergy **66,** 811-814.

Tollervey, J.R., and Lunyak, V.V. (2012). Epigenetics: judge, jury and executioner of stem cell fate. Epigenetics : official journal of the DNA Methylation Society **7,** 823-840.

Torok, M.S., and Grant, P.A. (2004). Histone acetyltransferase proteins contribute to transcriptional processes at multiple levels. Advances in protein chemistry **67,** 181-199.

Trievel, R.C., Rojas, J.R., Sterner, D.E., Venkataramani, R.N., Wang, L., Zhou, J., Allis, C.D., Berger, S.L., and Marmorstein, R. (1999). Crystal structure and mechanism of histone acetylation of the yeast GCN5 transcriptional coactivator. Proceedings of the National Academy of Sciences of the United States of America **96,** 8931-8936.

Turner, B.M. (2000). Histone acetylation and an epigenetic code. BioEssays : news and reviews in molecular, cellular and developmental biology **22,** 836-845.

Turner, B.M., and O'Neill, L.P. (1995). Histone acetylation in chromatin and chromosomes. Seminars in cell biology **6,** 229-236.

van Attikum, H., and Gasser, S.M. (2005). The histone code at DNA breaks: a guide to repair? Nature reviews. Molecular cell biology **6,** 757-765.

Vermeulen, M., Eberl, H.C., Matarese, F., Marks, H., Denissov, S., Butter, F., Lee, K.K., Olsen, J.V., Hyman, A.A., Stunnenberg, H.G., and Mann, M. (2010). Quantitative Interaction Proteomics and Genome-wide Profiling of Epigenetic Histone Marks and Their Readers. Cell **142,** 967-980.

Vetting, M.W., LP, S.d.C., Yu, M., Hegde, S.S., Magnet, S., Roderick, S.L., and Blanchard, J.S. (2005). Structure and functions of the GNAT superfamily of acetyltransferases. Archives of biochemistry and biophysics **433,** 212-226.

Vigushin, D.M., and Coombes, R.C. (2002). Histone deacetylase inhibitors in cancer treatment. Anti-cancer drugs **13,** 1-13.

Vlachonasios, K.E., Kaldis, A., Nikoloudi, A., and Tsementzi, D. (2011). The role of transcriptional coactivator ADA2b in Arabidopsis abiotic stress responses. Plant signaling & behavior **6,** 1475-1478.

Wang, T., Kobayashi, T., Takimoto, R., Denes, A.E., Snyder, E.L., el-Deiry, W.S., and Brachmann, R.K. (2001). hADA3 is required for p53 activity. Embo Journal **20,** 6404-6413.

Wang, Y.L., Faiola, F., Xu, M., Pan, S., and Martinez, E. (2008). Human ATAC Is a GCN5/PCAF-containing acetylase complex with a novel NC2-like histone fold module that interacts with the TATA-binding protein. The Journal of biological chemistry **283,** 33808-33815.

Weake, V.M., and Workman, J.L. (2012). SAGA function in tissue-specific gene expression. Trends in cell biology **22,** 177-184.

Weake, V.M., Lee, K.K., Guelman, S., Lin, C.H., Seidel, C., Abmayr, S.M., and Workman, J.L. (2008). SAGA-mediated H2B deubiquitination controls the development of neuronal connectivity in the Drosophila visual system. The EMBO journal **27,** 394-405.

Winer, J., Jung, C.K., Shackel, I., and Williams, P.M. (1999). Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. Analytical biochemistry **270,** 41-49.

Woodcock, C.L., Skoultchi, A.I., and Fan, Y. (2006). Role of linker histone in chromatin structure and function: H1 stoichiometry and nucleosome repeat length. Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology **14,** 17-25.

Wu, P.Y., Ruhlmann, C., Winston, F., and Schultz, P. (2004). Molecular architecture of the S. cerevisiae SAGA complex. Molecular cell **15,** 199-208.

Wyce, A., Xiao, T., Whelan, K.A., Kosman, C., Walter, W., Eick, D., Hughes, T.R., Krogan, N.J., Strahl, B.D., and Berger, S.L. (2007). H2B ubiquitylation acts as a barrier to Ctk1 nucleosomal recruitment prior to removal by Ubp8 within a SAGA-related complex. Molecular cell **27,** 275-288.

Xu, W., Edmondson, D.G., and Roth, S.Y. (1998). Mammalian GCN5 and P/CAF acetyltransferases have homologous amino-terminal domains important for recognition of nucleosomal substrates. Molecular and cellular biology **18,** 5659-5669.

Yang, X.J., and Seto, E. (2007). HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene **26,** 5310-5318.

Yelamos, J., Farres, J., Llacuna, L., Ampurdanes, C., and Martin-Caballero, J. (2011). PARP-1 and PARP-2: New players in tumour development. American journal of cancer research **1,** 328-346.

Yoneyama, M., Tochio, N., Umehara, T., Koshiba, S., Inoue, M., Yabuki, T., Aoki, M., Seki, E., Matsuda, T., Watanabe, S., Tomo, Y., Nishimura, Y., Harada, T., Terada, T., Shirouzu, M., Hayashizaki, Y., Ohara, O., Tanaka, A., Kigawa, T., and Yokoyama, S. (2007). Structural and functional differences of SWIRM domain subtypes. Journal of molecular biology **369,** 222-238.

Yost, J.M., Korboukh, I., Liu, F., Gao, C., and Jin, J. (2011). Targets in epigenetics: inhibiting the methyl writers of the histone code. Current chemical genomics **5,** 72-84.

Yun, M., Wu, J., Workman, J.L., and Li, B. (2011). Readers of histone modifications. Cell research **21,** 564-578.

Zhang, W., Bone, J.R., Edmondson, D.G., Turner, B.M., and Roth, S.Y. (1998). Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase. The EMBO journal **17,** 3155-3167.

Zhao, Y., Lang, G., Ito, S., Bonnet, J., Metzger, E., Sawatsubashi, S., Suzuki, E., Le Guezennec, X., Stunnenberg, H.G., Krasnov, A., Georgieva, S.G., Schule, R., Takeyama, K., Kato, S., Tora, L., and Devys, D. (2008). A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Molecular cell **29,** 92-101.

Zinke, I., Schutz, C.S., Katzenberger, J.D., Bauer, M., and Pankratz, M.J. (2002). Nutrient control of gene expression in Drosophila: microarray analysis of starvation and sugar-dependent response. The EMBO journal **21,** 6162-6173.

Zsindely, N., Pankotai, T., Ujfaludi, Z., Lakatos, D., Komonyi, O., Bodai, L., Tora, L., and Boros, I.M. (2009). 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 research **37,** 6665-6680.