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The Gcn5 complexes in *Drosophila* as a model for metazoa☆

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ABSTRACT

The histone acetyltransferase Gcn5 is conserved throughout eukaryotes where it functions as part of large multi-subunit transcriptional coactivator complexes that stimulate gene expression. Here, we describe how studies in the model insect *Drosophila melanogaster* have provided insight into the essential roles played by Gcn5 in the development of multicellular organisms. We outline the composition and activity of the four different Gcn5 complexes in *Drosophila*: the Spt-Ada-Gcn5 Acetyltransferase (SAGA), Ada2a-containing (ATAC), Ada2/Gcn5/Ada3 transcription activator (ADA), and Chiffon Histone Acetyltransferase (CHAT) complexes. Whereas the SAGA and ADA complexes are also present in the yeast *Saccharomyces cerevisiae*, ATAC has only been identified in other metazoa such as humans, and the CHAT complex appears to be unique to insects. Each of these Gcn5 complexes is nucleated by unique Ada2 homologs or splice isoforms that share conserved N-terminal domains, and differ only in their C-terminal domains. We describe the common and specialized developmental functions of each Gcn5 complex based on phenotypic analysis of mutant flies. In addition, we outline how gene expression studies in mutant flies have shed light on the different biological roles of each complex. Together, these studies highlight the key role that *Drosophila* has played in understanding the expanded biological function of Gcn5 in multicellular eukaryotes.

1. Introduction

Chromatin provides a barrier to processes that require access to the underlying DNA such as transcription and replication [1,2]. The nucleosome is the repeating unit of chromatin, and is composed of a hetero-tetramer of histones H3 and H4 flanked by two histone H2A/H2B heterodimers [1,2]. Histones can be post-translationally modified, predominantly on the N-terminal tails of the histone proteins [1,2]. These histone marks provide binding sites for other proteins that "read" these post-translational modifications, and can also potentially alter the nucleosome structure [1,3]. One of the first and most well studied histone modifications is acetylation, whereby an acetyl group is added to lysine residues often on the N-terminal tails of histones H3 and H4 [1]. Histone acetylation is generally associated with increased DNA accessibility because it stimulates chromatin remodeling [4]. Thus, histone acetylation usually correlates with, and contributes to active transcription [1,5]. Gcn5 was the first nuclear histone acetyltransferase (HAT) identified, first in *Tetrahymena thermophila* as described in this Special Issue by Brownell and Allis [141], and subsequently in the yeast *Saccharomyces cerevisiae* as outlined by [6]. Gcn5 has since been characterized in a wide range of eukaryotes [142,143], and here we focus on how studies in the model insect species *Drosophila melanogaster* have provided insight into the expanded biological roles for Gcn5 in multicellular eukaryotes.

The fruit fly *Drosophila* has been used as a model organism extensively for genetic studies and developmental biology [7]. The *Drosophila* genome shares 60% homology with humans, and about 75% of the genes responsible for human diseases have homologs in flies [8,9]. Moreover, *Drosophila* possess homologs of nearly all of the key factors involved in chromatin modification and transcription, making the fruit fly a powerful model organism for studying chromatin biology [10]. In contrast to mammals, which often possess multiple paralogs of histone modifying enzymes, *Drosophila* usually encodes only a single gene for different histone modifying enzymes, providing a simpler genetic system in which to dissect biological functions of various chromatin-based processes [11]. The *Drosophila* life cycle takes place within 10 days under standard laboratory conditions, beginning with the hatching of an egg into a larval stage, followed by several larval molts, formation of a

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pupa, metamorphosis (transformation from an immature, larval form to the adult fly), and finally eclosion (emergence from the pupal case) into an adult fly [7]. In this review, we provide a historical perspective on the identification of the *Drosophila* Gcn5 complexes. First, we describe the composition of the Gcn5 complexes in *Drosophila* in comparison to the orthologous complexes in *S. cerevisiae* and human cells. Next, we outline the essential subunits for fly development based on studies in mutant flies, and describe a subset of representative mutant phenotypes that highlight specialized functions for each Gcn5 complex in development. Finally, we describe the genome-wide localization patterns and biochemical roles for each Gcn5 complex in gene expression and other chromatin-based processes, where this has been defined. Last, we briefly discuss Gcn5 complexes in other insect species, and provide an overview of outstanding questions for future studies.

2. Gcn5 and its associated protein partners are conserved in *Drosophila*

Immediately after the discovery that the *Tetrahymena* p55 HAT corresponded to *S. cerevisiae* Gcn5, it became clear that Gcn5 was conserved throughout eukaryotes: "*it seems likely that the yeast 55-kDa polypeptide is conserved across a wide range of eukaryotes*" [12,13]. Indeed, only three years later, the Allis group identified Gcn5 in the model insect species, *Drosophila* [14]. In contrast to humans, who possess two Gcn5 paralogs (PCAF and Gcn5) [15,16], there is only one Gcn5 homolog in *Drosophila:* Gcn5 (FBgn0020388, CG4107). The gene encoding Gcn5 was historically named *pcaf* in flies [17], but it has since been renamed *gcn5* on FlyBase and in much of the recent literature; we refer to this gene as *gcn5* throughout this review. Gcn5 shares the domains that are common to all Gcn5 homologs including its HAT catalytic domain (469 - 634aa), Gcn5-N-Acetyltransferase (GNAT) domain (514 - 598aa), and bromodomain (717 - 795aa) [14], which binds acetylated lysine (Fig. 1) [18]. However, *Drosophila* Gcn5 shares higher similarity with both of the human Gcn5 paralogs than with *S. cerevisiae* Gcn5 (yGcn5). Moreover, Gcn5 contains a conserved N-terminal domain that is only found in metazoan Gcn5 homologs like human Gcn5 and PCAF [14]. It has been suggested that this N-terminal PCAF domain in human PCAF has E3 ubiquitin ligase activity [19], but this activity has not been demonstrated for human or *Drosophila* Gcn5.

In all organisms, Gcn5 associates with other proteins that are critical for both its activity and targeting. Although both *S. cerevisiae* and *Drosophila* Gcn5 can acetylate free histone H3 *in vitro*, they are unable to acetylate nucleosomal substrates on their own [14,20]. This lack of nucleosomal acetyltransferase activity is in contrast to human PCAF, which has been shown to acetylate nucleosomal substrates *in vitro* [16], and shares substantial homology with *Drosophila* Gcn5 (Fig. 1). In *S. cerevisiae*, Gcn5 associates tightly with two other proteins, Ada2 and Ada3, forming an heterotrimeric complex *in vitro* [21,22]. A third Gcn5-interacting protein, Sgf29, was later identified in *S. cerevisiae* [23,24]. Together, Gcn5, Ada2, Ada3, and Sgf29 constitute the core Gcn5 HAT module that is sufficient for nucleosomal histone acetylation [20] (see X in this Special Issue for further discussion on the core Gcn5 HAT module). *Drosophila*, like *S. cerevisiae*, has single homologs of Ada3 (FBgn0030891, CG7098) and Sgf29 (FBgn0050390, CG30390), which were readily identified by sequence comparisons with the *S. cerevisiae* proteins (Table 1) [25–27]. In contrast, there are two paralogs of Ada2 in *Drosophila*: Ada2a (FBgn0263738, CG43663) and Ada2b (FBgn0037555, CG9638) [26–28]. Both Ada2a and Ada2b share a similar domain structure to *S. cerevisiae* Ada2, possessing conserved ZZ and SANT domains (Fig. 2) [26–28]. Ada2a also contains a C-terminal SWIRM domain that is present in *S. cerevisiae* Ada2 and human Ada2a and Ada2b [29]. In addition, there are two splice isoforms of Ada2b resulting from alternative usage of splice acceptor sites in the third exon: Ada2b-PA encoding a 418-aa protein (FBppp0081303, also referred to as

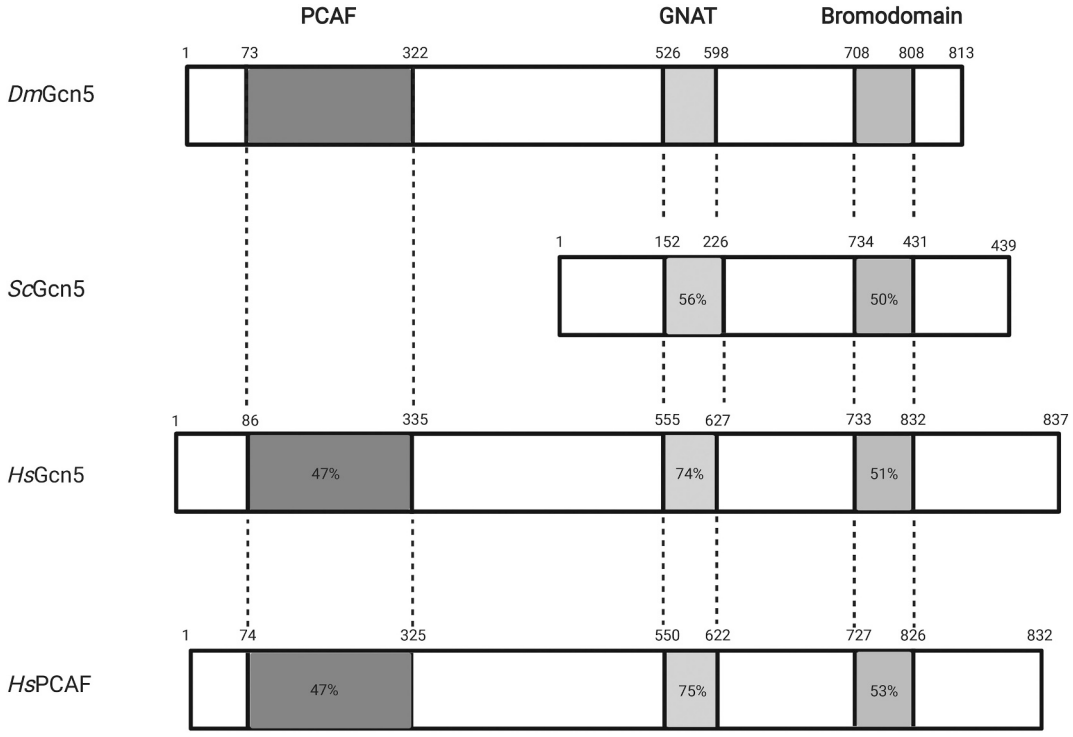

Fig. 1. Schematic comparison of *Drosophila* Gcn5 orthologs. Gcn5 amino acid sequences were aligned using Clustal Omega, and a schematic comparison of Gcn5 orthologs in *D. melanogaster*, *S. cerevisiae*, and *H. sapiens* was constructed. Accession numbers are as follow: *D. melanogaster* Gcn5, NP_648586.2; *S. cerevisiae* Gcn5, NP_011768.1; *H. sapiens* Gcn5, XP_006721880.1; *H. sapiens* PCAF, NP_003875.3. The highly conserved GNAT and Bromodomain, and the metazoan conserved PCAF domains are boxed in gray, and aligned in each ortholog as indicated by dotted lines. The amino acid positions for each domain are indicated by the numbers on top of each box. The percentage identity within the conserved domains in each Gcn5 ortholog relative to the corresponding domains in *Dm*Gcn5 is indicated by the % within each boxed domain.

Table 1
Drosophila SAGA subunits. The 20 *Drosophila* SAGA subunits can be organized into HAT, DUB, Core Structural, TBP binding, TF-binding, and splicing modules. The FlyBase ID, Annotation symbol (CG ID number), full gene name, and abbreviated gene symbol are shown for each *Drosophila* subunit, together with the orthologs from *S. cerevisiae* and *H. sapiens* (if present). Paralogous subunits are separated with a "/" sign. Alternative gene names are listed in parentheses. The protein domain and enzymatic activity (E.C. number) are based on FlyBase definitions for each *Drosophila* subunit. Note that Spt7 contains a bromodomain only in *S. cerevisiae*, but not in the metazoan orthologs. In addition, Spt8 is only present in the *S. cerevisiae* SAGA complex and is not listed here.

	FlyBase ID	Annotation symbol	Gene name	Gene symbol	*S. cerevisiae ortholog*	*H. sapiens* ortholog	DNA/histone domain/enzymatic activity
HAT module	FB gn0030891	CG7098	*Transcriptional Adaptor 3 (diskette)*	*Ada3*	*ADA3*	*TADA3*	
	FB gn0020388	CG4107	*Gcn5 acetyltransferase (Pcaf)*	*Gcn5*	*GCN5*	*GCN5/PCAF (KAT2A/KAT2B)*	PCAF, GNAT domain, Bromodomain, acetyltransferase (EC 2.3.1.48)
	FB gn0050390	CG30390	*SAGA-associated factor 29 kDa*	*Sgf29*	*SGF29*	*SGF29*	Tudor-like domain
	FB gn0037555	CG9638	*Transcriptional Adaptor 2b*	*Ada2b (PB isoform)*	*ADA2*	*TADA2B*	Zinc finger ZZ-type, SANT Myb domain
DUB module	FB gn0013717	CG4166	*Nonstop*	*Not*	*UBP8*	*USP22 (UBP22)*	Zinc finger-UBP-type, Ubiquitin protease
	FB gn0036804	CG13379	*SAGA associated factor 11 kDa*	*Sgf11*	*SGF11*	*ATXN7L3*	
	FB gn0031420	CG9866	*Ataxin 7*	*Atxn7*	*SGF73*	*ATXN7/ ATXN7L1/ ATXN7L2*	SCA7 domain
	FB gn0000618	CG15191	*Enhancer of yellow 2*	*e(y)2*	*SUS1*	*ENY2*	
Core structural module	FB gn0039067	CG4448	*Will decrease acetylation*	*Wda*	*TAF5*	*TAF5L*	WD40 domain
	FB gn0030874	CG6506	*Spt7*	*Spt7*	*SPT7*	*SUPT7L (STAF65G)*	Histone-fold domain
	FB gn0036374	CG17689	*Spt20*	*Spt20*	*SPT20*	*SUPT20H*	
	FB gn0051865	CG31865	*transcriptional Adaptor 1*	*Ada1*	*ADA1*	*TADA1*	Histone-fold domain
	FB gn0031281	CG3883	*SAGA factor-like TAF6*	*Saf6*	*TAF6*	*TAF6L*	Histone-fold domain
	FB gn0000617	CG6474	*Enhancer of yellow 1*	*e(y)1*	*TAF9*	*TAF9/TAF9b*	Histone-fold domain
	FB gn0026324	CG3069	*TBP-associated factor 10b*	*Taf10b*	*TAF10*	*TAF10*	Histone-fold domain
	FB gn0011290	CG17358	*TBP-associated factor 12*	*Taf12*	*TAF12*	*TAF12*	Histone-fold domain
TBP binding	FB gn0037981	CG3169	*Spt3*	*Spt3*	*SPT3*	*SUPT3H*	Histone-fold domain
TF-binding module	FB gn0053554	CG33554	*Nipped-A*	*Nipped-A*	*TRA1*	*TRRAP*	PIK-related pseudokinase
Splicing module	FB gn0035162	CG13900	*Splicing factor 3b subunit 3*	*Sf3b3*	–	*SF3B3*	Cleavage/polyadenylation specificity factor
	FB gn0040534	CG11985	*Splicing factor 3b subunit 5*	*Sf3b5*	–	*SF3B5*	

Ada2bS) and Ada2b-PB encoding a 555-aa protein (FBppp0099776, also referred to as Ada2bL) [28]. These Ada2b splice isoforms are expressed at equivalent levels during different developmental stages in flies, and share both the ZZ and SANT domains, differing only in their C-terminal regions (Fig. 2) [28]. The longer Ada2b-PB isoform contains the SWIRM domain in its unique C-terminal region, while the Ada2b-PA isoform lacks this domain (Fig. 2). In flies, like in *S. cerevisiae*, the nucleosomal HAT activity of Gcn5 requires interactions with either Ada2a or Ada2b, and Ada3 [14,20,26]. Both Ada2 paralogs, Ada2a and Ada2b, are conserved in other multicellular eukaryotes including *Arabidopsis* and humans [27], providing an early hint that Gcn5's association with other proteins might expand its biological role in multicellular organisms.

3. *Drosophila* Ada2 proteins nucleate formation of distinct Gcn5 complexes

Gcn5 resides within three different multi-subunit complexes in *S. cerevisiae*: the large, highly similar Spt-Ada-Gcn5 Acetyltransferase (SAGA) and SAGA-like (SLIK/SALSA) complexes, and the small Ada2/Gcn5/Ada3 transcription activator (ADA) complex [20,30,31,X et al.]. The presence of multiple versions of Ada2 in flies suggested that Gcn5 might reside within additional complexes in *Drosophila*, and raised the question as to which version of Ada2 was present in each complex. During the first decade of the twenty first century, a series of studies led by the Boros and Workman groups revealed the existence of two large multi-subunit Gcn5 complexes in flies. In *Drosophila*, the Ada2b paralog (specifically the Ada2b-PB isoform) is present in the SAGA complex, similar to that found in *S. cerevisiae* [26,27,32]. In contrast, Ada2a resides within a Gcn5 complex that is not present in *S. cerevisiae*, the Ada2a-containing (ATAC) complex [25]. ATAC was first identified in *Drosophila*, and it has since been characterized in mammalian cells and appears to be widely conserved in multicellular eukaryotes [33]. More recently, an ADA-like complex was also identified in *Drosophila* [34], together with an insect-specific Gcn5 complex that contains the shorter Ada2b-PA splice isoform [35]. With the exception of the small ADA complex, which contains only the core HAT subunits, the *Drosophila* Gcn5 complexes each possess additional protein subunits that contribute to their unique biological activities.

3.1. SAGA

The first of the *Drosophila* Gcn5 complexes to be identified, SAGA, is

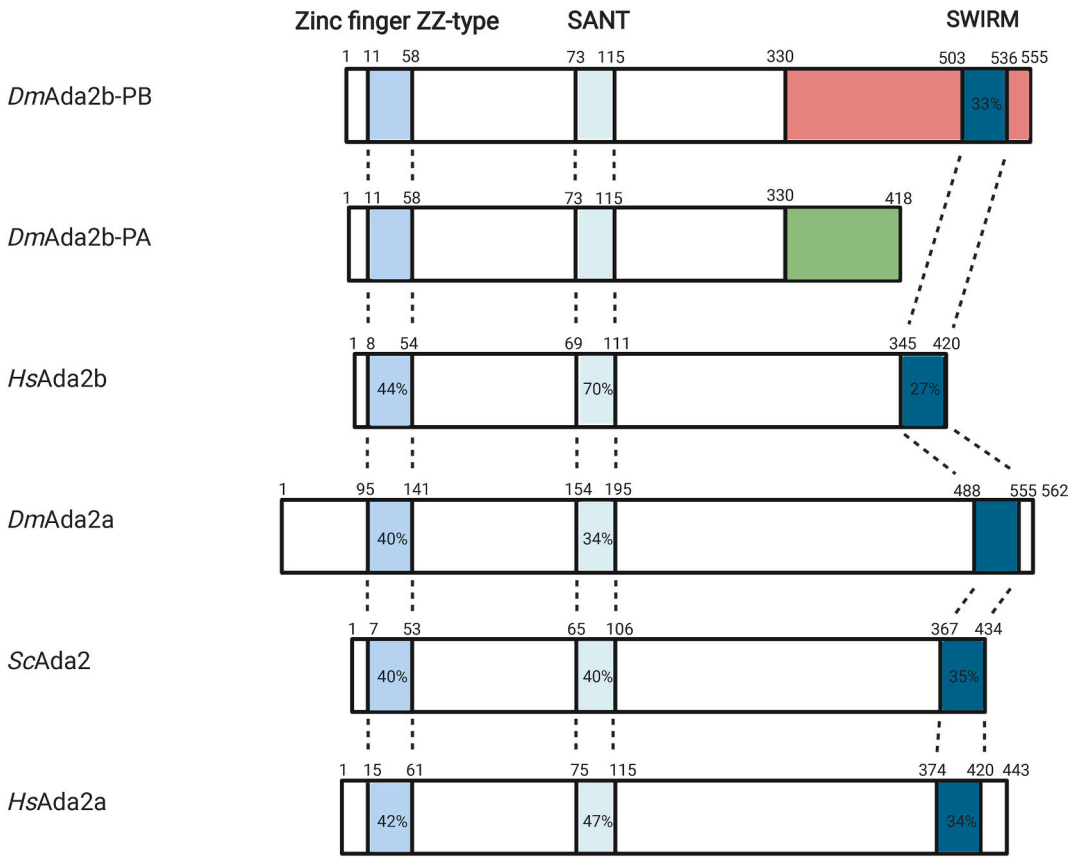

Fig. 2. Schematic comparison of *Drosophila* Ada2a and Ada2b orthologs. Ada2a and Ada2b amino acid sequences were aligned using Clustal Omega and a schematic comparison of *D. melanogaster* Ada2a and Ada2b (PA and PB isoforms) with *S. cerevisiae* Ada2 and *H. sapiens* Ada2a and Ada2b was constructed. Accession numbers are as follow: *D. melanogaster* Ada2a NP_001014636.1, Ada2b-PA NP_649773.1, Ada2b-PB NP_001027151.1; *H. sapiens* Ada2a NP_001159577.2, Ada2b NP_689506.2; *S. cerevisiae* Ada2 NP_010736.3. The conserved Zinc finger ZZ-type and SANT domains, and the SWIRM domains are boxed and aligned between the orthologs as indicated by dotted lines. The C-terminal specific domains for Ada2b-PA and Ada2b-PB are colored in green or orange, respectively. The amino acid positions for each domain are indicated by the numbers on top of each box. The percentage identity within the conserved domains in each Ada2a or Ada2b ortholog relative to the corresponding domains in *Dm*Ada2b or *Dm*Ada2a respectively is indicated by the % within each boxed domain. The % identity within the SWIRM domain is compared to *Dm*Ada2a. *Sc*Ada2 was aligned with *Dm*Ada2a.

a large 2 MDa complex that contains 20 different protein subunits [26,27]. SAGA has been well characterized in *S. cerevisiae* where its subunits were historically first organized into four major modules: the HAT module (Gcn5, Ada2, Ada3, Sgf29), a deubiquitination module (DUB; Ubp8, Sus1, Sgf11, and Sgf73), the TATA binding protein-Associated Factor (TAF) module (Taf5, Taf6, Taf9, Taf10, and Taf12), and the Suppressor of Ty's (SPT) module (Ada1, Spt3, Spt7, Spt8, and Spt20), with Tra1 originally being classified as a Spt protein, although it was not identified in the original genetic screen [36]. More recent structural studies have resulted in a re-organization of the subunits in the TAF and SPT modules into a structural core (Taf5, Taf6, Taf9, Taf10, Taf12, Spt7, Ada1, and Spt20), a TATA binding protein (TBP) binding module (Spt3 and Spt8), and a transcription factor (TF) binding module consisting only of Tra1 [24,37] (see Helmlinger et al. [144] in Special Issue for further discussion about the structural organization of SAGA). The composition of the HAT and DUB modules remains unchanged from the original modular organization. Ubp8 within the DUB module provides SAGA with a second histone modifying activity, catalyzing deubiquitination of monoubiquitinated histone H2B (H2Bub1) [38,40] (see [39] in Special Issue for more details regarding the DUB activity of SAGA). *Drosophila* SAGA (dSAGA) contains orthologs of all *S. cerevisiae* SAGA subunits with the exception of Spt8. In fact, no ortholog of the *Spt8* gene is present in the genome of any metazoan organism [41]. A variant of SAGA, termed SLIK/SALSA, has been purified in *S. cerevisiae*, and contains a C-terminal truncated version of Spt7 and lacks Spt8 [31]. Although dSAGA, like SLIK/SALSA, lacks Spt8, the *in vivo* existence of SLIK/SALSA remains controversial because Spt7 can be cleaved at its C-terminus by the Pep4 protease *in vitro*, resulting in removal of the Spt8-binding domain and subsequent loss of Spt8 [42]. Moreover, the C-terminal domain that is absent from the SLIK/SALSA Spt7 variant is conserved in metazoan Spt7 [43,44], while the N-terminal bromodomain appears to be unique to *S. cerevisiae* Spt7 [45]. Below, we outline the composition of each module of dSAGA, and describe the subunits that differ from their *S. cerevisiae* counterparts.

Although some dSAGA subunits could be identified by sequence similarity with their *S. cerevisiae* SAGA counterparts, mass spectrometry of affinity-purified SAGA complexes revealed incorporation of novel subunits that were not predicted by sequence comparisons with the *S. cerevisiae* SAGA components. The HAT module in dSAGA contains Gcn5, Ada3, and Sgf29, which are shared between all of the *Drosophila* Gcn5 complexes (Fig. 3) [25–26,34,35]. Although initially both splice isoforms of Ada2b were presumed to be part of the SAGA complex, mass spectrometry of affinity-purified SAGA complexes demonstrated that only the longer Ada2b-PB splice isoform is part of the dSAGA HAT module [32,35]. Orthologs of all four DUB module subunits are also present in flies. The histone deubiquitinase Ubp8 in *S. cerevisiae* corresponds to Nonstop in flies (FBgn0013717, CG4166), which was originally named for the axon targeting defect observed in *nonstop* mutants during neuronal development [46]. Both Nonstop and Sgf11 (FBgn0036804, CG13379) are necessary for deubiquitination of H2Bub1 in flies [47]. The last two DUB module subunits in flies are Ataxin 7 (FBgn0031420, CG9866, the ySgf73 ortholog) and E(y)2 (FBgn0000617,

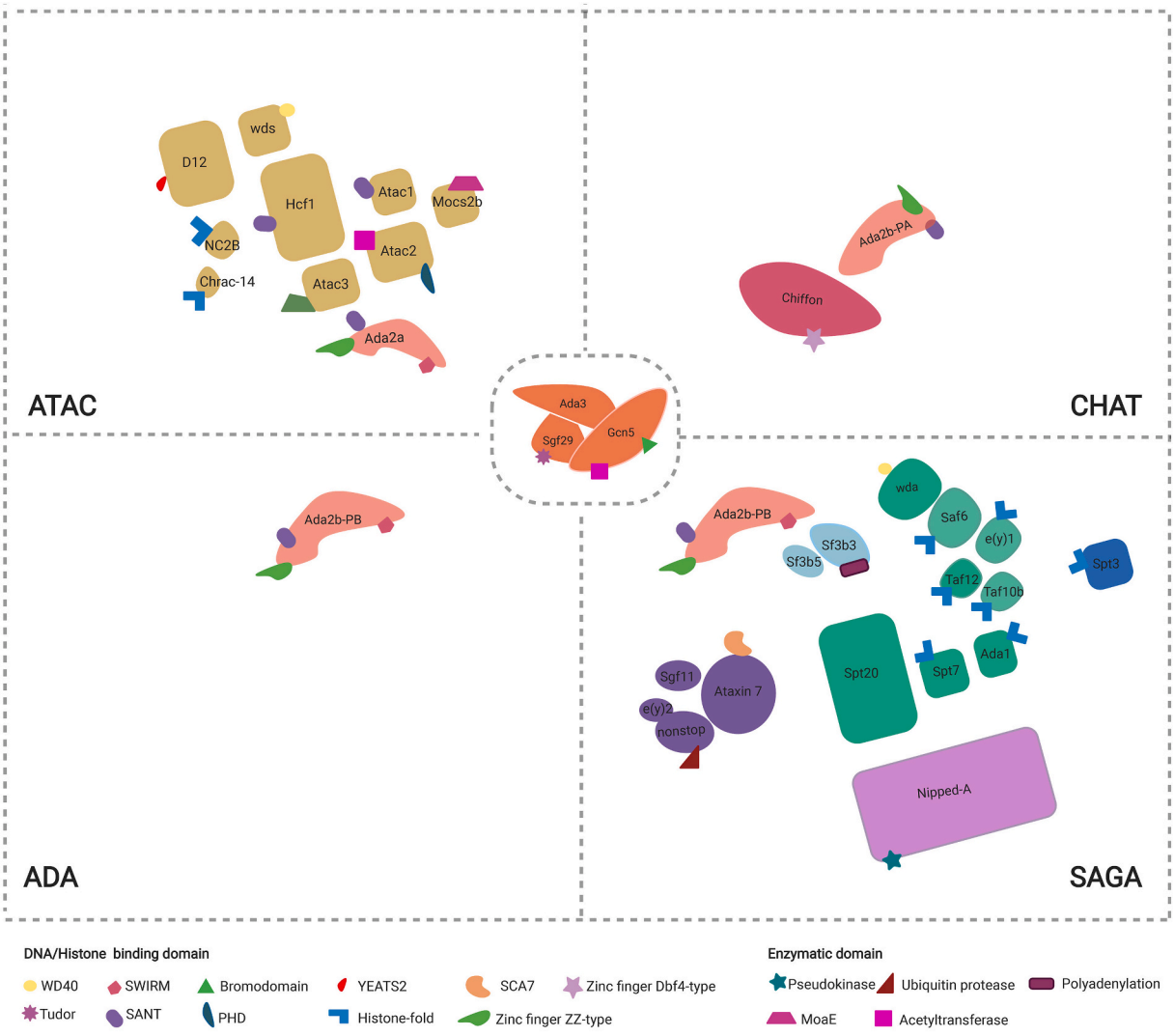

Fig. 3. Schematic illustration of the subunit composition of SAGA, ATAC, ADA, and CHAT. The subunits in the four Gcn5 complexes are shown in the four sections, with shared subunits of the core Gcn5 HAT module indicated in the central box. The area of each subunit is proportional to its relative molecular mass. Subunits are colored by complex, or by modules for SAGA, and the yeast Ada2 orthologs that nucleate formation of each complex shown in orange. Domains present in individual subunits are shown in the key below the figure.

CG6474, the ySus1 ortholog) (Table 1) [48–50]. In flies, like *S. cerevisiae*, several of the structural core subunits are shared between the transcription coactivator complex Transcription Factor II D (TFIID) and SAGA, namely E(y)1 (Taf9, FBgn0000617, CG6474), Taf10b (FBgn0026324, CG3069), and Taf12 (FBgn0011290, CG17358) [32,41]. However, other structural core subunits are unique to dSAGA and are not present in *Drosophila* TFIID. For example, the TAF5-like Wda (will decrease acetylation, FBgn0039067, CG4448), and TAF6-like Saf6 (SAGA factor-like TAF6, FBgn0031281, CG3883), are specialized TAF paralogs that are present in SAGA but not in TFIID [32,51]. Similar specialization of TAF proteins has occurred in other metazoan organisms with incorporation of Taf5-like and Taf6-like subunits in mammalian SAGA [41] (See Timmers [145] in this Special Issue for further discussion on the TAF subunits in SAGA). Other SAGA subunits in flies are much more conserved, although with considerable variation in some dSAGA subunits at the sequence level compared to their *S. cerevisiae* counterparts. For example, although Tra1 (Nipped-A, FBgn0053554, CG33554), Ada1 (FBgn0051866, CG31866), Spt3 (FBgn0037981, CG3169), and Spt7 (FBgn0030874, CG6506) were readily identified by sequence comparison with *S. cerevisiae* [51], Spt20 (FBgn0036374, CG17689) was not identified in flies until mass spectrometry of purified SAGA revealed the presence of this subunit [32]. Tra1/Nipped-A is the largest SAGA subunit (411 kDa in flies) and is shared with another transcriptional coactivator complex, the Tat interactive complex 60 kDa (TIP60; also known as the Nucleosome Acetyltransferase of H4 (NuA4) complex), which also possesses HAT activity [41,52]. In contrast to the SAGA HAT module, which preferentially acetylates histone H3, TIP60/NuA4 acetylates histone H4 and H2A.Z [53,54]. Last, SAGA contains two spliceosomal proteins, Sf3b3 (FBgn0035162, CG13900) and Sf3b5 (FBgn0040534, CG11985), that are not present in *S. cerevisiae* SAGA despite the existence of *S. cerevisiae* homologs corresponding to these proteins (Rse1 and Ysf3) [55]. Sf3b3 and Sf3b5 are shared with the Sf3b complex, a component of the U2 small nuclear ribonucleoprotein (snRNP), which recognizes the branch point sequence to facilitate spliceosome assembly [56,57]. Both of these spliceosomal proteins are also present in hSAGA (Table 1) [41,43,58]. Overall, *Drosophila* SAGA resembles the human SAGA complex more closely than either of the *S. cerevisiae* SAGA or SLIK/SALSA complexes, possessing similar specialized Taf-like proteins and containing the two additional spliceosomal proteins. The presence of these additional subunits in the metazoan SAGA complex suggests that SAGA may have gained more specialized roles in gene expression in animals compared to *S. cerevisiae*.

Several recent studies have investigated the structure of SAGA, and have provided insight into how each SAGA subunit integrates into the complex as a whole. These studies are described in more depth in another article in this Special Issue by Helmlinger et al., but are briefly described here to provide context for understanding the organization of *Drosophila* SAGA [144]. In *S. cerevisiae*, Cryogenic Electron Microscopy (cryoEM) data revealed a existence of a central module containing the structural core and the TBP binding module subunits that forms flexible connections to the HAT and DUB modules, while the large Tra1 subunit exists as a separate module that can bind the activation domain of transcription factors [59–62]. In *S. cerevisiae*, the HAT module is anchored to SAGA by Ada3 binding to Taf6, and HAT module subunits are lost from SAGA when it was purified from Ada3 or Ada2 mutant *S. cerevisiae* [24,60,63]. Similarly, Sgf73 (Ataxin 7) anchors the DUB module to the *S. cerevisiae* SAGA complex, and DUB subunits are lost from SAGA purified from Sgf73 mutant *S. cerevisiae* [64,65]. The DUB module requires Sgf73 for activity in *S. cerevisiae* [66], but in flies and plants, an enzymatically active DUB module can exist in the absence of the Sgf73 ortholog Ataxin 7 [49,67]. Notably, there is no ortholog of Sgf73/Ataxin 7 in *Arabidopsis*, suggesting that the DUB module may function independent of SAGA as the major H2Bub1 deubiquitinase [67,143]. In human cells, the protease Caspase 7 has been shown to cleave ATXN7, which could potentially release a free DUB module from SAGA [68]. This mechanism may also exist in *Drosophila*, although it has not yet been demonstrated. Thus, an open question remains as to whether the biological functions attributed to the DUB module subunit in flies (see Section 6) are due to its role in SAGA or represent its independent activity. These questions are discussed further by Mohan and colleagues in this Special Issue [39].

3.2. ADA

Recently, the Workman group has also identified an ADA-like complex in flies [34]. In *S. cerevisiae*, ADA contains the HAT module and two additional proteins, ADA HAT component 1 and 2 (Ahc1 and Ahc2) [24,30]. Early biochemical studies suggested that an ADA complex might also exist in flies because a small Ada2b-containing complex was detected by glycerol gradients of Ada2b-containing complexes [27]. Indeed, recently Soffers et al. showed that there is an ADA complex in flies, which like SAGA contains the Ada2b-PB splice isoform [34]. In contrast to *S. cerevisiae* ADA, the *Drosophila* ADA complex does not contain subunits corresponding to *S. cerevisiae* Ahc1/2, which do not have sequence homologs in flies or humans (Table 2) [34]. Thus, the ADA complex in flies does not possess any unique subunits that can be used to genetically distinguish it from SAGA.

3.3. ATAC

In addition to SAGA and ADA, flies also have an additional 820 kDa multi-subunit Gcn5 complex that is nucleated by the Ada2 paralog Ada2a: ATAC. Size-exclusion chromatography of the *Drosophila* Gcn5 complexes provided an early hint that Ada2a and Ada2b resided in distinct complexes [26,27]. Indeed, three years after the identification of the Ada2a paralog, the 13 subunit ATAC complex was first characterized in flies, providing the foundation for studies on this Gcn5 complex in other organisms [25]. ATAC shares the core HAT module subunits (Gcn5, Ada3, and Sgf29) with SAGA. In addition to the HAT module subunits, nine ATAC-specific subunits exist in flies. Six of these ATAC subunits are also present in the mammalian ATAC complex: Atac1 (FBgn0031876, CG9200, human ZZZ3 ortholog), Atac2 (FBgn0032691, CG10414, the human CRBP2 ortholog), D12 (FBgn0027490, CG13400, the human YEATS2 ortholog), Mocs2B (FBgn0039280, CG10238, equivalent to both the human hMoaE and hMBIP proteins), NC2β (FBgn0028926, CG4185, the human NC2B ortholog), and Wds (FBgn0040066, CG17437, the human WDR5 ortholog) (Table 3) [69,70]. The human ortholog of Chrac-14 (FBgn0043002, CG13399) has been detected in some human ATAC purifications [71], but was absent from others [72]. In contrast, two of the *Drosophila* ATAC subunits, Atac3 (FBgn0052343, CG32343) and Hcf (FBgn0039904, CG1710), appear to be specific to the fly ATAC complex and have not been detected in human ATAC [25,71,73]. Like SAGA, *Drosophila* ATAC contains a second histone modifying activity. The Atac2 subunit of ATAC contains a HAT domain, and *Drosophila* Atac2 possesses HAT activity toward histone H4 and H2A *in vitro* and *in vivo* [70]. However, the human counterpart for Atac2 (CRBP2) does not possess detectable HAT activity toward histone H4, suggesting that Gcn5 is the only active HAT within the human ATAC complex [72,73]. Thus, *Drosophila* ATAC contains two distinct acetyltransferase enzymes: Gcn5 and Atac2 [70]. Less is known about the modular organization and structure of the ATAC complex compared with SAGA. However, ATAC contains several histone-fold domain proteins, NC2β, D12 and Chrac-14, which may play a structural role in ATAC similar to that involving the structural core subunits in SAGA. While Chrac-14 and NC2β fail to form heterodimers, human YEATS2 (the *Drosophila* D12 ortholog) and NC2β interact *via* their histone-fold domains [70,71]. In addition, both *Drosophila* Chrac-14 and NC2β have the ability to form homodimers [70]. Wds also contains seven WD repeats, and this motif is often involved in protein–protein interactions (Fig. 3). In humans, YEATS2 (the *Drosophila* D12 ortholog) and Atac2 play a role in the integrity of the ATAC complex [71,72], suggesting that these subunits, together with Wds, Chrac-14 and NC2β, may play a central role in structural organization within the ATAC complex. Like SAGA, several ATAC subunits are shared with other chromatin modifying complexes. For example Chrac-14, Hcf, and Wds are also subunits of the COMPASS-like methyltransferase complexes, which are responsible for the bulk of di- and tri-methylation at histone H3K4 in *Drosophila* [74].

3.4. CHAT

Last, *Drosophila* possess a unique Gcn5 complex that appears to be

Table 2
Drosophila ADA subunits. The FlyBase ID, Annotation symbol (CG ID number), full gene name, and abbreviated gene symbol are shown for each *Drosophila* ADA subunit, together with the ortholog from *S. cerevisiae*. Alternative gene names are listed in parentheses. The ADA complex has not been yet characterized in human cells. The protein domain and enzymatic activity (E.C. number) are based on FlyBase definitions for each *Drosophila* subunit. Note that the *S. cerevisiae* ADA complex contains two additional subunits AHC1 and AHC2 that are not present in the *Drosophila* ADA complex.

FlyBase ID	Annotation symbol	Gene name	Gene symbol	*S. cerevisiae ortholog*	DNA/histone domain/enzymatic activity
FBgn0030891	CG7098	*Transcriptional Adaptor 3 (diskette)*	*Ada3*	*ADA3*	
FBgn0020388	CG4107	*Gcn5 acetyltransferase (Pcaf)*	*Gcn5*	*GCN5*	PCAF, GNAT domain, Bromodomain, acetyltransferase (EC 2.3.1.48)
FBgn0050390	CG30390	*SAGA-associated factor 29 kDa*	*Sgf29*	*SGF29*	Tudor-like domain
FBgn0037555	CG9638	*Transcriptional Adaptor 2b*	*Ada2b (PB isoform)*	*ADA2*	Zinc finger ZZ-type, SANT domain

Table 3
Drosophila ATAC subunits. The FlyBase ID, Annotation symbol (CG ID number), full gene name, and abbreviated gene symbol are shown for each *Drosophila* ATAC subunit, together with the ortholog from *H. sapiens*. Alternative gene names are listed in parentheses. The ATAC complex is not present in *S. cerevisiae*. The protein domain and enzymatic activity (E.C. number) are based on FlyBase definitions for each *Drosophila* subunit.

FlyBase ID	Annotation symbol	Gene name	Gene symbol	*H. sapiens* ortholog	DNA/histone domain/enzymatic activity
FBgn0030891	CG7098	*Transcriptional Adaptor 3 (diskette)*	*Ada3*	*TADA3*	
FBgn0020388	CG4107	*Gcn5 acetyltransferase (Pcaf)*	*Gcn5*	*GCN5*	PCAF, GNAT domain, Bromodomain, acetyltransferase (EC 2.3.1.48)
FBgn0050390	CG30390	*SAGA-associated factor 29 kDa*	*Sgf29*	*SGF29*	Tudor-like domain
FBgn0263738	CG43663	*Transcriptional Adaptor 2a*	*Ada2a*	*TADA2A*	Zinc finger ZZ-type, SANT domain, SWIRM domain
FBgn0039904	CG1710	*Host cell factor*	*Hcf*	–	
FBgn0040066	CG17437	*Will die slowly*	*Wds*	*WDR5*	WD40 domain
FBgn0027490	CG13400	*D12*	*D12*	*YEATS2*	YEATS
FBgn0043002	CG13399	*Chromatin accessibility complex 14 kD-protein*	*Chrac-14*	–	Histone-fold domain
FBgn0052343	CG32343	*Ada2a-containing complex component 3*	*Atac3*	–	
FBgn0028926	CG4185	*Negative Cofactor 2β*	*NC2β*	*NC2β*	Histone-fold domain
FBgn0031876	CG9200	*Ada2a-containing complex component 1*	*Atac1*	*ZZZ3*	SANT domain
FBgn0032691	CG10414	*Ada2a-containing complex component 2*	*Atac2*	*CRBP2*	GNAT domain/ acetyltransferase (EC 2.3.1.48)
FBgn0039280	CG10238	*Molybdenum cofactor synthesis 2B*	*Mocs2B (dModE, Mocs2)*	*MBIP*	Molybdopterin biosynthesis MoaE

specific to insects: the Chiffon Histone Acetyltransferase (CHAT) complex. Whereas the Ada2b-PB splice isoform is present in SAGA, the shorter Ada2b-PA splice isoform is not part of the SAGA, ADA or ATAC complexes [32]. Instead, Ada2b-PA nucleates formation of a fourth Gcn5 complex in flies that contains the shared HAT module subunits (Gcn5, Ada3, and Sgf29) together with a fifth protein, Chiffon (FBgn0000307, CG5813) (Table 4, Fig. 3) [35]. Chiffon is the *Drosophila* homolog of Dbf4, which binds and activates the Cdc7 kinase, forming the Dbf4-dependent kinase (DDK) complex [75]. DDK phosphorylates the Mcm2–7 helicase, activating the initial step in DNA replication [76–78]. In contrast to SAGA and ATAC, the CHAT complex is unlikely to exist in *S. cerevisiae* or humans, because Dbf4 does not co-immunoprecipitate with Gcn5 in either of these organisms [35]. Moreover, Chiffon interacts with directly with Gcn5 *via* its C-terminal domain, and this region of the protein is not conserved outside of insects [35].

4. Substrate specificity of the Gcn5 complexes

In general, the *Drosophila* Gcn5 complexes preferentially acetylate histone H3 *in vitro* and *in vivo* exhibiting the highest activity on K9 and K14 of both recombinant histone H3 peptides and nucleosomal substrates [25,34,35,79]. Although SAGA, ADA, and CHAT show this characteristic HAT activity toward histone H3, the presence of the second HAT in *Drosophila* ATAC expands its activity toward both histones H3 and H4 [25,70]. In fact, *Drosophila* ATAC shows strong specificity for histone H4 in nucleosomal substrates *in vitro* [70]. Moreover, mutations in Atac2 result in reduced global levels of acetylated H4K16 in fly embryos, and *ada2a* mutations decrease levels of acetylated H4K5, H4K12, and H4K16 in polytene chromosomes [70,80,81]. Depletion of Atac2 or Gcn5 from *Drosophila* cells by RNAi revealed that Gcn5 selectively acetylates histone H3, whereas Atac2 has a narrow but not absolute substrate preference for lysines on both H3 and H4 [82]. Other HATs have been shown to work together to deposit particular combinations of acetyl marks on chromatin; for example, CBP, MGEA5, and NAA10 act together to acetylate H4 on both K5 and K8 [82]. Similarly, Atac2's preference for different lysine residues on histones H3 and H4 was modulated by the pre-existing acetylation pattern on those histones [82].These data suggest that both Gcn5 and Atac2 contribute to the expanded HAT activity of the ATAC complex, which is likely influenced *in vivo* by the activity of other HATs.

Gcn5 specificity may be altered by its interaction with each Ada2 paralog because rescue experiments with hybrid Ada2 proteins showed that combining the unique C-terminal domain of Ada2a and Ada2b with the N-terminal domain of the other Ada2 paralog was sufficient to rescue the respective mutants and restore histone acetylation patterns [83]. Notably, the two Ada2b splice isoforms also only differ in their C-terminal domains (Fig. 2). Thus, the divergent C-terminal domains of the different Ada2 paralogs and splice isoforms in *Drosophila* likely contribute to both the formation of the different Gcn5 complexes and to the differences in HAT specificity of each complex.

In addition to histones, Gcn5 acetylates a number of non-histone targets in flies, which expand the biological functions of the Gcn5 complexes. For example, *Drosophila* Gcn5 acetylates the chromatin remodeling ATPase subunit Imitation SWI (FBgn0011604, CG8625, Iswi) at K753 both *in vivo* and *in vitro* [84]. This region in Iswi (747 – 756aa) is similar to the N-terminal domain of histone H3, suggesting that Gcn5 may recognize Iswi in a similar fashion to histone H3 [84]. Iswi is part of two nucleosome remodeling complexes in *Drosophila*: Nucleosome remodeling factor (NURF), and the Chromatin accessibility (CHRAC) complex [85]. However, the acetylated form of Iswi is only found in NURF, and is not present in the CHRAC complex [84]. Notably, as discussed in more detail in Section 7, mutations in the NURF subunit *iswi* or the ATAC subunit *ada2a* show similar phenotypes, and there is a genetic interaction between Ada2a and Iswi in flies [86]. These data suggest that in *Drosophila*, ATAC might target Iswi as a substrate for acetylation by Gcn5, although this has not been tested. In addition to

Table 4
Drosophila CHAT subunits. The FlyBase ID, Annotation symbol (CG ID number), full gene name, and abbreviated gene symbol are shown for each *Drosophila* CHAT subunit. Alternative gene names are listed in parentheses. The CHAT complex is not present in *S. cerevisiae* or human cells. The protein domain and enzymatic activity (E.C. number) are based on FlyBase definitions for each *Drosophila* subunit.

FlyBase ID	Annotation symbol	Gene name	Gene symbol	DNA/histone domain/enzymatic activity
FBgn0030891	CG7098	*Transcriptional Adaptor 3 (diskette)*	*Ada3*	
FBgn0020388	CG4107	*Gcn5 acetyltransferase (Pcaf)*	*Gcn5*	PCAF, GNAT domain, Bromodomain, acetyltransferase (EC 2.3.1.48)
FBgn0050390	CG30390	*SAGA-associated factor 29 kDa*	*Sgf29*	Tudor-like domain
FBgn0037555	CG9638	*Transcriptional Adaptor 2b*	*Ada2b (PA isoform)*	Zinc finger ZZ-type, SANT domain
FBgn0000307	CG5813	*Chiffon*	*Chif*	Zinc finger DBF-type

Iswi, *Drosophila* Gcn5 has been shown to acetylate Transcription factor EB (TFEB; FBgn0263112, CG43369), the ortholog of Mtif in flies [87]. Gcn5 acetylates K445 and K450 in Mtif, inhibiting autophagy and lysosomal biogenesis [87]. *Drosophila* Gcn5 also acetylates the Cyclin A associated protein Adenomatous polyposis coli 2, Apc2 (FBgn0026598, CG6193) [88]. Acetylation of Apc2 promotes ubiquitination and degradation of Cyclin A, resulting in its turnover, which regulates the maintenance (both self-renewal and differentiation) of *Drosophila* germline stem cells [88]. More details about acetylation of non-histone substrates by Gcn5 across a variety of organisms including *Drosophila* are described in this Special Issue by Michael Downey [148].

5. Gcn5 is essential for development in flies

Although Gcn5 is not essential in for proliferation in *S. cerevisiae*, loss of one of the human Gcn5 paralogs, Gcn5 (KAT2A), results in embryonic lethality [89,90]. Thus, the Gcn5 complexes appear to have an essential role in development in multicellular eukaryotes. To characterize the function of Gcn5 in *Drosophila*, Antoniewski and colleagues generated several different null *gcn5* alleles (Table 5). Loss of *gcn5* blocks two critical stages in *Drosophila* development: oogenesis (egg development) and metamorphosis. In flies lacking Gcn5, oogenesis is arrested at stage 5 and 6, and zygotic *gcn5* mutants die during the late third instar larval stage (Fig. 4) [17]. Moreover, adults with hypomorphic *gcn5* alleles show malformation of appendages such as abnormal elongated metathoracic twisted legs, and also exhibit a reduction in wing size and defects in wing-vein patterning, together with defects in cuticle formation [17]. In addition, null *gcn5* mutants fail to form a puparium, one of the initial steps in metamorphosis, potentially due to defects in expression of genes that respond to the insect hormone ecdysone [17]. Notably, *gcn5* mutants also exhibit severely reduced imaginal discs, suggesting that Gcn5 is required for cell proliferation in flies. Consistent with a potential role in cell proliferation, *gcn5* mutant imaginal discs showed a higher number of cells in S-phase, significantly more cells undergoing mitosis, and higher levels of apoptosis [17]. Mutations in another shared HAT module subunit, *ada3*, result in similar phenotypes to those observed in *gcn5* mutants, with reduced size of imaginal discs and defects in oogenesis [91]. The small imaginal discs in the *ada3* mutant led to the original name *diskette* [91], although this gene has since been renamed *Ada3* on FlyBase. *ada3* mutants also exhibit abnormal structure of polytene chromosomes; in particular showing changes in the banding pattern of the male X chromosome [91].

The severe developmental defects in *gcn5* mutants are likely to result from the combinatorial loss of all four *Drosophila* Gcn5 complexes. However, the identification and analysis of mutants that specifically disrupt each of the four Gcn5 complexes in flies suggests that at least three of these Gcn5 complexes are essential for development in flies. For example, mutations in *ada2a* or *ada2b* both result in developmental lethality and oogenesis arrest (Fig. 4) [28,79]. Further, mutations that disrupt the SAGA-specific subunits *nonstop*, *sgf11*, *wda*, *taf10b*, and *saf6,* or the CHAT-specific *chiffon* and *ada2b-PA* subunits, also result in larval lethality (Table 5, Fig. 4) [32,35,47,51,92]. Thus, SAGA, ATAC, and CHAT are essential for fly development. Unfortunately, ADA function cannot be separated genetically from SAGA in flies because both complexes share the Ada2b-PB isoform, and ADA contains no unique subunits in flies [34]. It should be noted that it remains unclear as to why mutations that disrupt different subunits of Gcn5 complexes result in lethality at different developmental stages (Fig. 4). Some mutants may exhibit more severe defects and earlier lethality due to their function in complexes outside the Gcn5 complexes, such as *sf3b5*, which is present in both SAGA and the U2 snRNP [57]. In addition, there may be a different amount of maternally supplied gene product that allows some Gcn5 complex mutants to survive to a later developmental stage. Germline mutants in several SAGA-specific mutants either fail to complete oogenesis, or cannot progress through embryogenesis (Fig. 4), supporting the idea that maternally supplied gene product is required for these zygotic mutants to progress to a later stage in development. However, the level or stability of maternally supplied gene product for different Gcn5 complex subunits has not been examined in flies. Overall, the characterization of mutants that specifically disrupt SAGA, ATAC, or CHAT provides some insight into the different roles of these complexes, and we outline specific biological functions of each complex in the following sections beginning with SAGA.

6. SAGA is critical for developmental processes defined by its modules

SAGA promotes transcription through both its catalytic activities and *via* interactions with the transcription machinery [93]. In *Drosophila*, SAGA colocalizes extensively with RNA polymerase II (Pol II) and is present at the both the promoter-proximal pause site of lowly expressed or highly regulated genes, and on the gene body of actively transcribed genes [94,95]. Although SAGA colocalizes with Pol II at most actively transcribed genes, gene expression profiling studies of SAGA mutants originally suggested that different SAGA modules might be required for transcription of particular subsets of genes [96]. For example, only a subset of the genes bound by SAGA in embryonic muscle were downregulated in *sgf11* mutants, and these genes showed enriched expression in muscle and functions related to muscle development, suggesting a potential role for the SAGA DUB module in expression of tissue-specific genes [94–96]. However, in human cells SAGA acetylates histone H3K9 and deubiquitinates H2Bub1 on all expressed genes [97], suggesting a much broader role in regulating transcription. This broader role in transcription is consistent with the extensive colocalization of SAGA with Pol II in flies and in human cells [95,97]. Since many of the early gene expression studies on *Drosophila* mutants used microarray analysis approaches that may not have been able to detect global changes in transcription (Table 6), it is possible that a much larger group of genes requires SAGA for proper expression in flies. In addition, studies in *S. cerevisiae* suggest that global changes in transcription can be buffered by changes in mRNA stability [98–100], and most gene expression studies in flies have examined steady-state mRNA levels. Thus, the genes identified in the expression profiling experiments in SAGA mutants may represent those subsets of genes that are most sensitive to loss of particular SAGA activities.

Despite the caveat that the gene expression profiling of SAGA mutants in flies may underestimate the number of genes regulated by SAGA, these studies have provided important insight into key developmental

Table 5
Phenotypes associated with mutant alleles that disrupt subunits of Gcn5 complexes in *Drosophila.* Mutant alleles or RNAi knockdown that disrupt subunits that are shared or specific to the SAGA, ADA, ATAC and CHAT complexes result in the described lethality and phenotypes, as outlined in the listed references. Only mutant alleles/RNAi knockdown that have been described in the literature are listed in this table. *, designated amino acid is altered to a stop codon.

	Gene	Mutant allele	Nature of allele	Viable/lethal?	Phenotype	Reference
SAGA/ADA/ ATAC/CHAT	*Ada3*	*ada3*^{3}	Nonsense: 371*	Lethal - early pupa	Decreased acetylation at H3K9, K14, K12; failure metamorphosis; reduced imaginal disc size.	[91]
	Gcn5	*gcn5*^{E333st} *gcn5*^{C137T} *gcn5*^{Q186st}	Nonsense: E333* Missense: C137Y Nonsense: Q186*	Lethal - late pupa	Decreased acetylation at H3K9, H3K14; Defects cell proliferation; failure to form puparium; photoreceptor axon mistargeting during eye development; oogenesis arrested in stage 5 and 6. Reduced imaginal disc size.	[17,47]
SAGA/ADA/ CHAT	*Ada2b (PA- and PB isoforms)*	*ada2b*^{1} *ada2b*^{2} *ada2b*^{842}	Null: 1077 bp deletion Null: 2.77 kb deletion Null: 800 bp deletion	Lethal - early pupa	Decreased acetylation at H3K9 in embryos and polytene chromosomes and H3K14 in ovary follicle cells and imaginal discs; defects in oogenesis; photoreceptor axon mistargeting during eye development.	[28,35,47,79]
SAGA	*Nonstop*	*not*^{2}	Null: 538 bp deletion	Lethal - pupa	Increased H2Bub1; photoreceptor axon mistargeting during eye development.	[46,47]
	Sgf11	*sgf11*^{e01308}	Null: 5.97 kb del etion	Lethal - late larva/early pupa	Increased H2Bub1; photoreceptor axon mistargeting during eye development.	[47,95]
	Ataxin 7	*ataxin 7*^{KG02020}	Null:	Lethal - late larva	Neural and retinal degeneration; reduced locomotion; cellularization defects.	[49,94]
	e(y)2	*e(y)2*^{1}	Null: 167 bp deletion	Viable	Short stocky body and separated wings; eyes with altered facets; low fertility.	[48,112]
	Nipped-A	*nipped-A*^{NC186}	Missense: V885D	Lethal - early pupa	Defects in Notch signaling.	[136]
	wda	*wda*^{11} *wda*^{4} *wda*^{8}	Null: 1510 bp deletion Null: 857 bp deletion Null: 864 bp deletion	Lethal - second instar larva	Decreased acetylation at H3K9.	[51]
	Saf6	*saf6*^{303}	Null: 303 bp deletion	Lethal - second instar larvae		[32]
	e(y)1	*e(y)1*^{17} *e(y)1*^{190}	Null: 79 bp deletion Null: 339 bp deletion	Lethal - larva	Dysregulation of ovary follicle cell development.	[137]
	Taf10b	*taf10*^{d25}	Null: 900 bp deletion	Lethal - pupae	Decreased acetylation at H3K14; defects in DNA repair efficiency.	[92,138]
	Sf3b5	*sf3b5* ^{EY12579}	Transposable element insertion.	Lethal - second instar larva	Reduced cell viability in eyes.	[55]
ATAC	*Ada2a*	*ada2a*^{189}	Null: 720 bp deletion	Lethal - pupa	Oogenesis arrested; altered structure of the polytene chromosomes; banding pattern is distorted.	[79]
	hcf	*hcf*^{HR1}	Null: 4348 bp deletion	Lethal - pupa	Heterozygous females are Sterile; oogenesis arrested at stage 8; decreased pupae size.	[107]
	wds	*wds*^{G0251} *wds*^{j25}	Not specified	Lethal - larva	Defects in wristles and wing veins; heterozygous male and female are unfertile.	[106]
	Chrac-14	*chrac-14*^{KG01051}	Not specified	Viable	Eclosion defective; flight defective; radiation sensitive.	[139]
	Atac3	*atac3* ^{GD4326}	RNAi	Lethal - pupa		[108]
	Atac2	*atac2* ^{e03046}	Transposable element insertion.	Lethal - second instar larva	Decreased acetylation at H4K16.	[70]
CHAT	*Chiffon*	*chif*^{Dsred} *chif*^{ETEB3} *chif*^{WF24}	Null:5.3 kb deletion Null: 6 kb deletion Missense: T521C	Lethal - third instar larva Viable	Decreased acetylation at H3K9, H3K14, and H3K18 in ovary follicle cells and imaginal discs; gene amplification disrupted; thin embryo chorion and rough eyes for *chif*^{WF24}.	[35,78,124]

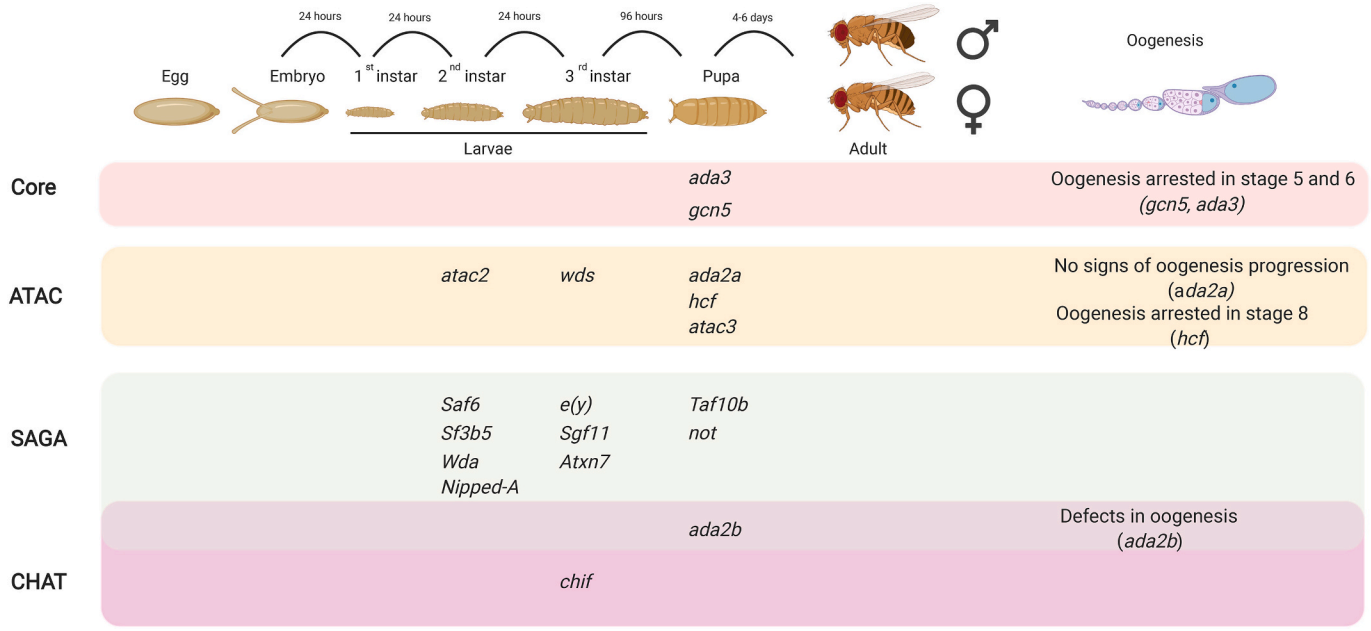

Fig. 4. The *Drosophila* Gcn5 complexes are essential for fly development. The life cycle of *Drosophila* comprises four successive stages, namely, egg, larva, pupa, and adult. Twenty-four hours after a female fly lays her eggs, larvae hatch. Larvae then undergo molting stages known as instars (three instar stages), during which the head, mouth, cuticle, spiracles, and hooks are shed. After ninety-six hours, the third instar larva encapsulates itself, forming a pupa. Metamorphosis takes place during the pupal stage, giving rise to all the structures in the adult fly. Oogenesis takes place within the ovary of female flies, and consists of 14 stages prior to deposition of the fertilized egg. The mutants shown disrupt subunits in the SAGA, ADA, ATAC or CHAT complexes, and result in lethality at the indicated developmental stage of the *Drosophila* life cycle. Mutations that have been shown to impact oogenesis are also indicated, but this has not been tested for all the mutant alleles shown. The *ada2b* mutant allele disrupts all three of the SAGA, ADA and CHAT complexes. The mutant alleles shown in this figure correspond to those listed in Table 5.

processes that require SAGA. Importantly, mutants that disrupt different modules of SAGA show different effects on gene expression, and exhibit specific developmental phenotypes. For example, mutations in *ada2b* disrupt oogenesis, whereas oogenesis progresses normally in *ataxin 7* or *nonstop* mutants [94]. Moreover, genes involved in DNA replication, eggshell formation, and chromosome organization were significantly downregulated in *ada2b* oocytes, but did not change in *ataxin 7* or *nonstop* mutants (Table 6) [94]. While the early zygotic genes are expressed properly in embryos that lack the maternal contribution for *ataxin 7* and *nonstop*, these embryos show later defects in cellularization and nuclear anchoring [94], suggesting that maternally contributed SAGA is required for proper development during embryogenesis. Interpreting these phenotypes is complicated by the recent finding that *ada2b* encodes two splice isoforms, only one of which is in the SAGA complex

Table 6
Gene expression analysis for Gcn5 complexes in *Drosophila*. Gene expression studies have been performed on homozygous mutants that disrupt subunits of the Gcn5 complexes SAGA, ADA, ATAC and CHAT. The number of differentially expressed genes identified using microarray or RNA-seq analysis by each study is listed, together with the major gene ontology processes and/or signaling pathways identified in the associated reference.

Complex	Gene	Approach	# genes identified	Differentially expressed genes-pathways/processes	Reference
SAGA/ATAC/ ADA/CHAT	*Gcn5*	Microarray; third instar larvae	~284 genes	Morphogenesis.	[86]
	Ada3	Microarray; third instar larvae	~5565 genes	Cuticle formation and ecdysone response.	[110]
SAGA/ADA/ CHAT	*Ada2b (PA & PB isoforms)*	RNA-seq; ovaries	>1000 genes	DNA replication, eggshell formation, chromosome organization, and DNA repair.	[94]
		Microarray; third instar larvae	~344 genes	Early ecdysone response genes: glue proteins.	[47]
		Microarray; third instar larvae	~580 genes	Ecdysone-induced genes, cuticle formation, and defense mechanisms.	[110,140]
SAGA	*Nonstop*	RNA-seq; embryos (stage 5)	>6000 genes	Cellularization, embryonic development, and tissue morphogenesis.	[94]
		Microarray; third instar larvae	~987 genes	Early ecdysone-response genes, puparial adhesion, eclosion, signal transduction, and central nervous system remodeling	[47]
		RNA-seq; third instar larvae glia	~1802 genes	Axon guidance, protein folding, cell morphogenesis, axon guidance, synaptic transmission.	[102]
	Sgf11	Microarray; embryonic muscle or neurons	~443 genes (muscle); ~390 genes (neuron)	Protein folding, nervous system development, mesoderm development, muscle development, and anatomical structure development.	[95]
		Microarray; third instar larvae	~618 genes	Early ecdysone response genes, puparial adhesion, eclosion, signal transduction, and central nervous system remodeling	[47]
		RNA-seq; third instar larvae glia	~1644 genes	Axon guidance, protein folding, cell morphogenesis, axon guidance synaptic transmission.	[102]
	Ataxin 7	RNA-seq; embryos (stage 5)	>6000 genes	Cellularization, embryonic development, and tissue morphogenesis.	[94]
ATAC	*Ada2a*	Microarrays; third instar larvae	~7306 genes	Cuticle formation and ecdysone pathway response.	[78]

[32,35]; thus, *ada2b* mutants disrupt all three of the SAGA, ADA and CHAT complexes, making it difficult to distinguish as to which complex is required for oogenesis in flies (Fig. 4).

The disruption in eye development caused by mutations in SAGA's DUB module provides a second example of how different activities of SAGA control development in flies. Although mutations that disrupt the DUB module such as *sgf11* and *nonstop* are lethal during the late larval/early pupal stage of development (Fig. 4), these mutants show characteristic defects in eye development in the late larval stage just prior to their death [46,47,101]. During the third larval instar, photoreceptor neurons in the developing eye imaginal disc project their axons to specific regions of the developing brain [101]. The SAGA subunit *nonstop* was first identified in a screen for genes involved in this photoreceptor axon targeting process [101]. Mutations in *nonstop* result in a failure of photoreceptor axons to project to their correct target layer in the developing brain, the lamina, instead mistargeting into the deeper medulla region [46]. This axon targeting defect is caused by loss of *nonstop* or *sgf11* within the glial cells that mark the target layer in the lamina [46]. Transcriptome profiling of these glial cells from *nonstop* and *sgf11* larval brains identified genes involved in axon guidance (Table 6) [102]. Moreover, RNAi knockdown or loss of function mutants in one of these DUB-regulated genes in glia, *multiplexin* (FBgn0260660, CG42543, Mp), resulted in axon targeting defects that were similar to those observed in *sgf11* mutants, arguing that at least some of these DUB-regulated genes in glia control axon targeting [102]. Since *ada2b* mutants also show axon mistargeting phenotypes, albeit substantially weaker than those observed in *nonstop* or *sgf11*, the DUB module likely controls expression of these genes as part of the SAGA complex [47,102]. However, in flies the DUB module can bind to chromatin independently of SAGA's HAT or structural core subunits [94], and loss of *ataxin 7* results in decreased H2Bub1 levels due to promiscuous binding of the DUB module [49]. Genes involved in locomotion, organ morphogenesis, and eye and neuronal development were highly regulated by the DUB module [94], suggesting that it remains possible that the DUB module could control some aspects of eye development independent of SAGA.

Third, analysis of mutations that disrupt the structural core and spliceosomal modules of SAGA suggests that like in *S. cerevisiae*, *Drosophila* SAGA can act as a transcriptional coactivator independent of its HAT or DUB activities [93]. In *S. cerevisiae*, Tra1 recruits SAGA to promoters through interactions with transcription factors [103], allowing Spt3 and Spt8 to interact directly with component of the transcription machinery such as TBP [104]. In flies, mutations in the structural core subunit *Saf6* result in defective expression of SAGA-regulated genes without altering global levels of acetylated histone H3 or H2Bub1 [32]. Likewise, mutations in the *sf3b5* spliceosomal SAGA subunit result in decreased expression of SAGA-regulated genes independent of changes in histone acetylation [55]. Analysis of the relative levels of spliced and unspliced transcripts for genes that are down-regulated in *sf3b5* mutants shows that the decreased mRNA levels in *sf3b5* mutants are not necessarily due to changes in splicing efficiency [55]. However, unlike other SAGA mutants, *sf3b5* is required for cell viability in flies, most likely due to its role as part of the U2 snRNP [55,57]. It is unclear how Sf3b5 regulates gene expression as part of SAGA, although it is possible that it may mediate transient interactions between the transcriptional and splicing machinery, which share a common spatial and temporal distribution during the coupled processes of transcription and splicing [95,105] (See Nuño-Cabanes and Rodríguez-Navarro [146] in this Special Issue for more discussion of SAGA subunits that are shared with other complexes). Together, these studies suggest that SAGA plays a fundamental role in fly development because it regulates the expression of genes that are required for processes such as oogenesis, metamorphosis, and neuronal development. However, fundamental questions remain as to whether the distinct roles of SAGA in particular developmental processes result from independent activity of particular modules or subunits. In addition, it is unclear as to whether SAGA has overlapping or distinct roles with the ADA and CHAT complexes that are also disrupted in *ada2b* mutants. *Drosophila* SAGA may also regulate a broader set of genes than indicated by past gene expression studies that have profiled steady-state mRNA levels and have not been able to detect global changes in active transcription. In *S. cerevisiae*, data suggests that SAGA regulates expression of all genes [97,100], while in human cells, SAGA deubiquitinates H2Bub1 on the transcribed region of all expressed genes, suggesting a widespread role in transcription regulation [97].

7. ATAC is a double HAT complex required for development

The ATAC complex is exclusive to multicellular eukaryotes, suggesting a potential function unique to development in multicellular organisms. Mutations that disrupt subunits of ATAC show developmental lethality during the larval or pupal stages (Table 5, Fig. 4). For example, *ada2a* mutants die during the pupal stage, and Ada2a is also essential for oogenesis [79]. In addition, mutant flies that lack *hcf*, *wds*, *atac3*, and *atac2* die during either the larval or pupal stage of development (Table 5) [70,106–108]. The developmental lethality of ATAC mutants may be due to defects in response to the insect hormone ecdysone, which triggers molting during the larval instars, and is also required for the larval–pupal transition at the onset of metamorphosis [109]. Both ecdysone levels and binding of its receptor to polytene chromosomes are reduced in *ada2a* and *ada3* mutants [110]. Moreover, genes required for ecdysone biosynthesis are misregulated in third instar larvae lacking Ada2a and Ada3 (Table 6) [110]. Thus, ATAC may be essential for viability in flies in part because it controls levels of hormones that trigger formation of the adult fly.

Histone acetyltransferases often act synergistically with nucleosome remodeling complexes to regulate chromatin structure and gene expression [111]. In flies, ATAC interacts genetically and biochemically with the chromatin remodeling complex, NURF [86]. Mutations in the NURF subunit *iswi* or the ATAC subunit *ada2a* show similar defects in eye development, with both mutants exhibiting small and rough eyes [86]. In addition, ATAC and NURF coregulate expression of a subset of genes including *Ultrabithorax* (*Ubx*), *engrailed* (*en*), and *heat-shock protein 70* (*hsp70*) [86]. Moreover, ATAC and NURF are both necessary to maintain proper chromatin structure, particularly on the X chromosome in male flies [86]. In flies, expression of genes on the single male X chromosome is doubled to equal that from the two female X chromosomes in a process termed dosage compensation [112]. During this process, the Males absent on the first (Mof) HAT within the Male Specific Lethal (MSL) complex acetylates H4K16 on the male X chromosome [112]. Mutations in ATAC and NURF subunits such as *ada2a, gcn5*, and *nurf301* show increased frequency of bloated X chromosomes in male flies [86], suggesting that ATAC and NURF maintain proper chromosomal structure of the dosage compensated male X chromosome. Although ATAC acetylates H4K16 [70], the bloated X chromosomes observed in *ada2a* and *gcn5* mutant males show similar levels of acetylated H4K16 compared to their wild-type counterparts [86]. Moreover, X-linked genes are not preferentially misregulated in *ada2a* or *gcn5* mutants [86]. Thus, ATAC and NURF may work together to maintain the chromosomal structure of the dosage compensated male X chromosome, rather than playing a specific role in expression of X-linked genes [86]. Notably, H4K16 acetylation by Mof antagonizes activity of another related chromatin remodeler, Iswi, in flies [113], and negatively regulates interactions between Iswi and its nucleosomal substrate *in vitro* [114]. Thus, the MSL and ATAC complexes may function synergistically with the related NURF and ISWI chromatin remodelers to maintain the structure, acetylation, and expression levels of dosage compensated genes in *Drosophila*. It is possible that the cooperative activity between ATAC and NURF could involve the direct acetylation of one of the NURF subunits, Iswi, by Gcn5 (see Section 4) [84], although this remains to be tested.

Drosophila ATAC contains three histone-fold domain proteins, D12, Chrac-14 and NC2β, leading to the question as to whether ATAC itself

possessed nucleosome remodeling activity because histone-fold domains can bind DNA [115], and Chrac-14, as part of the CHRAC complex, facilitates nucleosome sliding [116]. In addition, the human ortholog of Chrac-14, Chrac-17, enhances nucleosome sliding by the Iswi complex [117]. Purified ATAC does not show remodeling activity by itself on nucleosomal substrates *in vitro* [70]. However, ATAC can stimulate nucleosome sliding by the chromatin remodelers Iswi or SWItch/Sucrose Non-Fermentable (SWI-SNF) *in vitro* [70]. Similarly, recombinant Chrac-14 or NC2β also stimulated nucleosome remodeling by SWI/SNF [70], suggesting that the histone-fold domain proteins in ATAC contributes to its impact on chromatin remodeling. Notably, the inclusion of acetyl-CoA in these *in vitro* nucleosome sliding assays enhanced the effect of ATAC, suggesting that the HAT activity of ATAC also contributes to stimulation of chromatin remodeling by complexes such as Iswi or SWI-SNF [70].

In addition to its roles in chromosome structure and interaction with chromatin remodelers, ATAC has been implicated in cell proliferation. Mutations in *gcn5* and *ada3* are associated with reduced size of imaginal discs, which are a highly proliferative tissue, and *gcn5* mutants also show an increased number of cells in S phase [17,91]. However, since Gcn5 and Ada3 are core components of all the Gcn5 complexes in flies, it was not clear whether all or only some of these Gcn5 complexes had roles in cell proliferation. Studies in mammalian cells suggest that ATAC is likely to be responsible for the defects in cell proliferation in *gcn5* and *ada3* mutants due to its role in progression through the G2/M phase of the cell cycle [72]. Knockdown of Atac2 in mouse cells and studies using an *Atac2* knockout mouse model showed that loss of Atac2 results in an increase in the number of apoptotic cells and in an accumulation of cells in G2/M [72]. In addition, Ada2a and Ada3 RNAi knockdown in mouse NIH3T3 cells leads to mitotic abnormalities such as centrosome multiplication and defective midbody formation, and ATAC subunits such as Ada2a and Yeats2 localize to the mitotic spindle [118]. Interestingly, SAGA does not appear to share this role in mitosis because deletion of Spt20 does not cause mitotic abnormalities, and Spt20 does not localize to chromatin during mitosis [118]. Although ATAC acetylates H4K16, loss of Ada2a and Ada3 results in the opposite acetylation phenotype in mitotic cells with knockdown cells showing an increase in acetylated H3K14 levels due to an decrease in the activity of the histone deacetylase Sirtuin 2 (SIRT2) [118]. While a role for *Drosophila* ATAC in mitosis has not yet been characterized, it is possible that ATAC shares this function in flies and may be responsible for the decreased cell proliferation observed in *gcn5* and *ada3* mutants.

Last, ATAC has been implicated in controlling the expression of genes in stress-induced signaling pathways. Gcn5 complexes have a well characterized role in stress response signaling mediated by mitogen-activated protein kinases (MAPK) [33]. Osmotic stress can activate MAPK cascades, resulting in eventual activation of the c-Jun-NH2-terminal kinase (JNK) [119]. In *Drosophila* S2 cells, sorbitol treatment induces osmotic stress and results in JNK activation [69]. Importantly, ATAC directly interacts with MAPKs *via* its MBIP/Mocs2B subunits in both humans and flies [69,120]. Moreover, JNK activation in response to osmotic stress is inhibited by the expression of the ATAC subunit Mocs2B in *Drosophila* S2 cells, and ATAC is required for the transcription of JNK target genes such as *chickadee* in these cells [69]. Thus, ATAC appears to directly interact with MAPK signaling proteins to mediate induction of stress response genes in flies, likely through its Mocs2B subunit. This role in stress response for the ATAC complex is reminiscent of *S. cerevisiae* SAGA's function in the endoplasmic reticulum (ER) stress pathway [121]. In mammals, knockdown of the shared SAGA and ATAC subunit Sgf29 results in impaired transcription of ER stress genes, such as *GRP78* [122]. The ER stress response transcription factor ATF6 recruits both SAGA and ATAC to ER stress response genes [123], suggesting that both SAGA and ATAC are involved in induction of stress response genes in metazoan organisms. Analysis of SAGA and ATAC localization on *Drosophila* polytene chromosomes suggest that these Gcn5 complexes regulate distinct sets of stress response genes, depending on the type of stress involved [73]. For example, induction of phorbol ester-induced protein kinase C (PKC) pathway genes increased colocalization of ATAC and Pol II without affecting SAGA [73], arguing for a specific role of ATAC in induction of PKC genes in response to stress.

8. CHAT is an insect-specific Gcn5 complex that contains a protein associated with DNA replication

Whereas the other Gcn5 complexes identified in *Drosophila* are also present in *S. cerevisiae* or humans, the CHAT complex appears to be specific to insects and has an unknown biological function. In addition to the HAT module subunits (Gcn5, Ada3, and Sgf29), CHAT contains the short Ada2b-PA splice isoform and Chiffon, the *Drosophila* ortholog of Dbf4. Chiffon, like other Dbf4 orthologs, binds and activates the cell cycle kinase Cdc7 forming the Dbf4-dependent kinase complex (DDK) [78,124,125]. The DDK complex phosphorylates the Mcm2–7 helicase, activating it to unwind DNA at origins of replication, thus initiating DNA replication [77,78]. Although Dbf4 is highly conserved and is present in most eukaryotes except for plants, Chiffon contains a long C-terminal extension that is specific to insects (Fig. 5) [35,75]. The conserved N-terminal domain of Chiffon (1- 400aa) binds and activates Cdc7, while the insect-specific C-terminal domain of Chiffon (401 - 1695aa) is necessary and sufficient to bind Gcn5 and nucleate CHAT formation [35]. Dbf4 is an essential gene in *S. cerevisiae* because of its role in DNA replication, but surprisingly, *chiffon* mutants were originally reported to be viable in *Drosophila* [124]. The *chiffon* gene was first identified in a screen for female sterile mutants, and *chiffon* females lay eggs with a thin and fragile chorion (eggshell) that resembles the fabric of the same name [125]. More recent analysis has shown that indeed, the Cdc7-binding domain of Chiffon is dispensable for fly viability, but surprisingly, the Gcn5-binding domain of *chiffon* is essential for development [35]. In fact, *chiffon* alleles that contain premature stop codons either within, or directly after, the N-terminal Cdc7-binding domain (separating both N- and C- polypeptides) are viable because they still produce a C-terminal product that binds Gcn5 and nucleates CHAT formation [35]. Both domains are encoded by a single large exon in the *chiffon* gene with no evidence of alternative splicing, suggesting that alternative translation start sites and/or proteolytic cleavage may be required to produce these two independent Chiffon polypeptides. These data suggest that *chiffon* could be a dicistronic gene that can independently express two distinct polypeptides that contain either the Cdc7- or Gcn5-binding domains, resulting in DDK or CHAT formation, respectively. It remains unclear as to whether the N- and C-terminal Chiffon polypeptides are expressed at the same time, and little is known about how this process is controlled *in vivo*. The unusual *chiffon* gene structure is somewhat reminiscent of the *ada2a* gene, which also encodes two polypeptides with distinct functions: Ada2a and one of the subunits of RNA polymerase II, Rpb4, in flies and in other insects due to alternative splicing [27].

The CHAT complex exhibits *in vitro* and *in vivo* HAT activity toward histone H3, similar to SAGA and ADA [35]. Analysis of histone acetylation levels in somatic mosaics for *chiffon* null alleles showed that loss of Chiffon decreases levels of histone H3 acetylated at K9, K14, and K18, but not K23 [35]. Although histone acetylation correlates with, and contributes to a specialized form of DNA re-replication in follicle cells termed gene amplification [126], CHAT-mediated histone acetylation is not required for this type of DNA replication [35]. In *chiffon* mutant cells that lack only its N-terminal Cdc7-binding domain, ovary follicle cells lack the characteristic bromodeoxyuridine (BrDU) foci indicative of chorion gene amplification [35]. However, these DDK-deficient mutant cells retain wild-type histone acetylation levels. In contrast, *chiffon* mutants that lack only its C-terminal domain that binds Gcn5 show decreased histone acetylation, but do not exhibit loss of the characteristic BrDU foci indicative of chorion gene amplification [35]. Similarly, *ada2b* mutant follicle cells show decreased histone acetylation but retain wild-type BrDU incorporation [35]. Together, these data suggest that

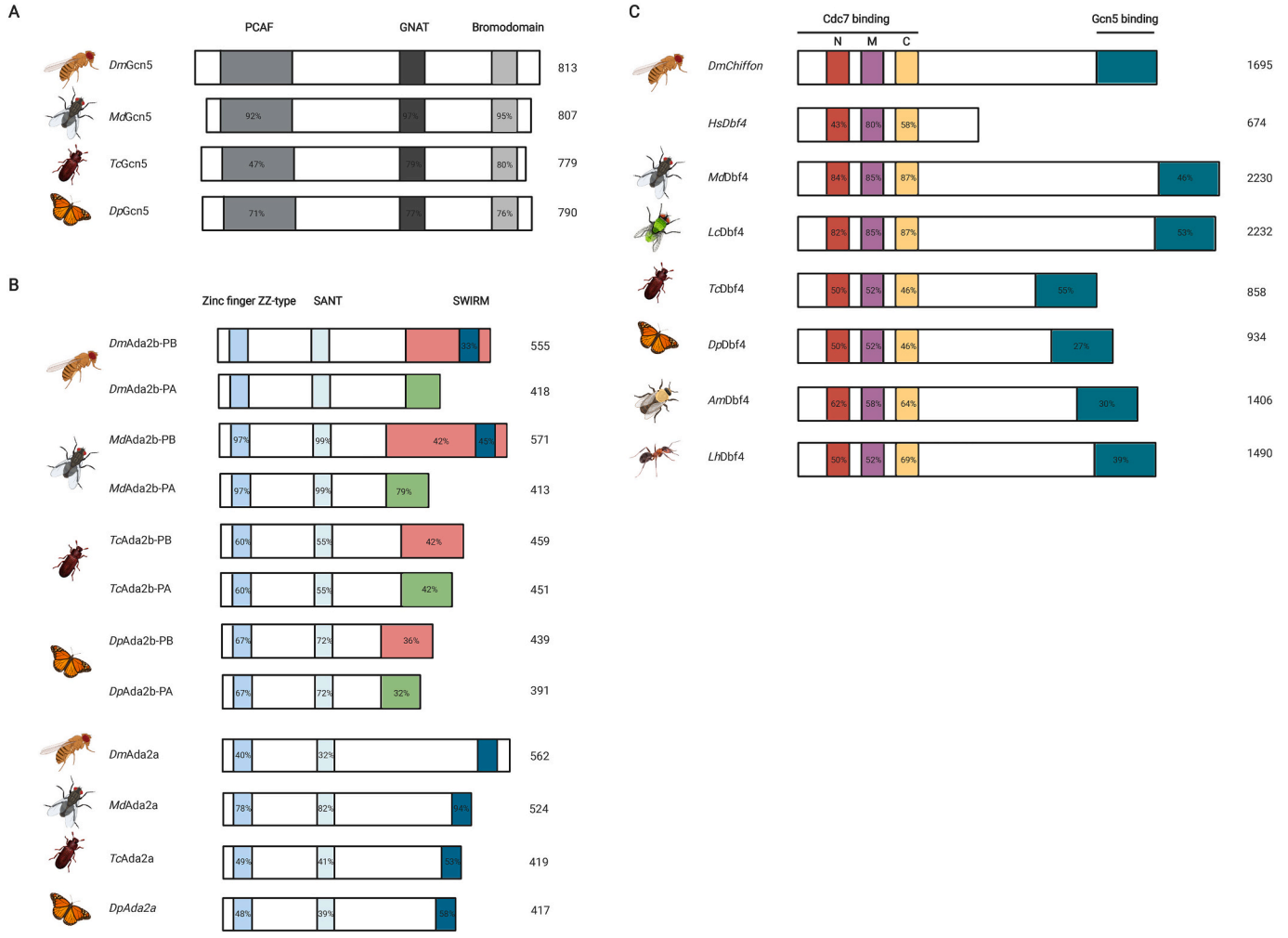

Fig. 5. Insect Gcn5, Ada2, and Chiffon share regions of conservation with *Drosophila*. Insect Gcn5, Ada2, and Chiffon homologs were aligned using Clustal Omega. The insect species described in this figure are: Diptera, *D. melanogaster*, *Musca domestica* (House fly), *Lucilia cuprina* (Australian sheep blowfly); Coleoptera, *Tribolium castaneum* (Red flour beetle); Lepidoptera, *Danaus plexippus* (Monarch butterfly); Hymenoptera, *Apis mellifera* (Western honey bee) and *Linepithema humile* (Argentine ant). A representative illustration of each insect is shown next to each aligned protein. **A)** Accession numbers for Gcn5 homologs from the following insect species were used to generate this alignment: *D. melanogaster* NP_648586.2; *M. domestica* XP_005181707.1; *T. castaneum* XP_015835856.1; *D. plexippus* DPOGS216125. The GNAT, Bromodomain, and PCAF domains are boxed in gray. The percentage identity within the conserved domains in each Gcn5 ortholog relative to the corresponding domains in *Dm*Gcn5 is indicated by the % within each boxed domain. **B)** Accession numbers for Ada2 homologs from the following insect species were used to generate this alignment: *D. melanogaster* Ada2b-PB NP_001027151.1, Ada2b-PA NP_6497731, Ada2a NP_001014636.1; *M. domestica* Ada2b-PA XP_005186291.1, Ada2b-PB XP_005186290.1, Ada2a XP_019894005.1; *T. castaneum* Ada2b-PA A0A139WFG5, Ada2b-PB XP_008195462, Ada2a XP_015835543.1, *D. plexippus* Ada2b-PA XP_032521398.1, Ada2b-PB XP_032521398.1, Ada2a XP_032528769.1. The Zinc finger ZZ-type, SANT, and SWIRM domains are boxed. The C-terminal specific domains for Ada2b-PA and Ada2b-PB are colored in green or orange, respectively. The percentage identity within the conserved domains in each Ada2a or Ada2b ortholog relative to the corresponding domains in *Dm*Ada2b or *Dm*Ada2a, respectively, is indicated by the % within each boxed domain. The % identity within the SWIRM domain is compared to *Dm*Ada2a. **C)** Accession numbers for Dbf4/Chiffon homologs from the following species were used to generate this alignment: *D. melanogaster* AAD48779.1; *M. domestica* XP_019893793.1; *L. cuprina* A0A0L0CBC7; *T. castaneum* XM_008199666.2; *D. plexippus* OWR45390.1; *A. mellifera* XP_016770645.1; *L. humile* XP_012229084; *H. sapiens* NP_006707. The highly conserved region that interacts with Cdc7 (N, M, C domains) and the insect-specific Gcn5-binding domain are boxed. The percentage identity within the conserved domains in each Dbf4 ortholog relative to the corresponding domain in *Dm*Chiffon is indicated by the % within each boxed domain.

despite the presence of the Dbf4 ortholog Chiffon, the CHAT complex is not required for DNA replication in flies [35]. What then could be the role of the CHAT complex in insects? Currently, CHAT, like SAGA, seems to be essential for both histone H3 acetylation and for development in flies. *Chiffon* mutants show decreased histone H3 acetylation not only in ovary follicle cells, but also in other tissues such as imaginal discs [35]. Moreover, the decreased acetylation at histone H3K14 in *chiffon* mutant cells is similar to that observed in *ada2b* mutants, which lack both the CHAT and SAGA isoforms [35]. Since mutations in the SAGA-specific subunit, *wda*, also reduce acetylation at histone H3K9 in embryos [51], both SAGA and CHAT likely contribute to H3 acetylation in flies. However, expression of the CHAT-specific Ada2b-PA isoform, but not the SAGA/ADA-specific Ada2b-PB isoform, is sufficient to almost fully restore viability to *ada2b* mutants [35,127]. These data suggest that either CHAT might compensate for some of SAGA's essential functions during development, or that the Ada2b-PA splice isoform can incorporate into SAGA if Ada2b-PB is absent [35]. It remains unclear whether CHAT is necessary for gene expression, and if so, whether CHAT regulates common or distinct gene targets compared to SAGA and the other Gcn5 complexes in flies.

9. Roles for Gcn5 complexes in other insects

The Gcn5 complexes have been best studied in the model insect

Drosophila melanogaster, and no Gcn5 complexes have been described in other insects yet. However, other insect species, like *Drosophila*, possess a single Gcn5 ortholog with shared domain structure including the metazoan-specific N-terminal domain (Fig. 5A). Both Ada2a and Ada2b are also widely conserved throughout insects suggesting that the ADA, SAGA, and ATAC complexes are likely present in all insect species (Fig. 5B). Further, like in *Drosophila*, Ada2b in most insect species has two splice isoforms that share a common N-terminal domain, which includes the Zinc finger ZZ-type and SANT domain, and have the specific C-terminal regions corresponding to the *Drosophila* Ada2b-PA and Ada2b-PB splice isoforms (Fig. 5B). The presence of both Ada2b splice isoforms in other insect species supports the idea that the CHAT complex is likely conserved across insect species. In addition, the Chiffon C-terminal extension that directly binds Gcn5 *in vitro* is conserved in a wide range of insect species from beetles to ants (Fig. 5C) [35,75,124]. Currently, the biological function of the CHAT complex is unknown, but it is possible that this complex plays a specialized role in insects due to some unique aspect of their development or physiology.

10. Conclusion and future directions

During evolution there has been a divergence and diversification of the Gcn5 complexes. *Drosophila* has provided a powerful model in which to identify and characterize these novel Gcn5 complexes, and was the first multicellular organism shown to contain the ADA, ATAC and CHAT complexes [25,34,35]. The expanded repertoire of Gcn5 complexes in flies and in other metazoan organisms appears to result from divergence of the Ada2 subunit. While *S. cerevisiae* only has one Ada2 ortholog, flies have at least three versions of Ada2: Ada2a and the two splice isoforms of Ada2b. In light of the fairly recent finding that *Drosophila* possesses four Gcn5 complexes rather than just SAGA and ATAC, it may be necessary to re-interpret some of the conclusions from previous studies showing specific roles for SAGA, or particular modules of SAGA, in developmental processes. New genome-wide studies of Gcn5 complex localization patterns and gene expression profiling will require careful selection of subunits, and should utilize spike-in control approaches that can identify potential global changes in gene expression [128].

Over the past 20 years following the identification of Gcn5 in *Drosophila* [14], much insight has been obtained into the structure and function of SAGA from studies in yeast, flies, humans and plants. We refer the reader to the article by Brian Strahl and Scott Briggs in this Special issue [147] for an in-depth discussion of SAGA's function in transcription, and an outline of key unanswered questions that remain about its function. The exciting new cryo-EM studies of *S. cerevisiae* SAGA illustrate how the different modular parts of the complex function as a whole [59,60,129,144], and we look forward to seeing these same approaches applied to the metazoan SAGA and ATAC complexes to elucidate the architectural organization of both complexes. Such studies will provide insight into the similarity and differences between SAGA and ATAC, and show for example, how the two HATs in ATAC might modify histones within the same nucleosome, and how the spliceosomal proteins in metazoan SAGA integrate into the complex. These studies, coupled with functional analysis in model systems such as flies, may help us to understand why the metazoan Gcn5 complexes have diverged in composition from yeast and plants. Plants, like yeast, lack the ATAC complex and do not have the Sf3b3 and Sf3b5 spliceosomal subunits of SAGA [143]. What, then, is the unique role that the ATAC complex plays in metazoan? Why does metazoan SAGA contain the spliceosomal subunits, and what is their function in the complex?

Insects offer a number of advantages over mammalian models to answer these key questions because of their short generation time, and wealth of genetic resources. In addition, since the *Drosophila* SAGA and ATAC complexes largely resemble their mammalian counterparts in terms of composition, flies provide a strong model for the metazoan-specific functions of the Gcn5 complex. *Drosophila* also provides an appropriate biological model to ask questions about Gcn5 complexes that are relevant to human disease. For instance, the neurodegenerative disease Spinocerebellar ataxia type 7 (SCA7) results from polyglutamine expansions in the gene encoding the DUB subunit Ataxin 7 [130,131]. Flies have been used as a model for SCA7 [132,133], and other polyglutamine related neurogenerative diseases such as SCA2 [134]. In humans, SCA7 disease manifests retinal and cerebellar degeneration, and macular dystrophy causing blindness [131]. In *Drosophila*, loss of Ataxin 7 causes neural and retinal degeneration, and impaired movement [49]. Interestingly, similar phenotypes are observed when exogenous polyglutamine-expanded human Ataxin 7 is expressed in *Drosophila* [49]. Thus, *Drosophila* provides a good model organism to study the mechanism of diseases such as SCA7 and could be used to screen compounds suitable for ameliorating symptoms of this neurodegenerative disease [132,135].

Last, the finding that alternative splicing of *ada2b* can generate new diversity in HAT complexes [34,35] suggests that there may be other Gcn5 complexes in multicellular organisms that remain to be discovered. It is possible that other novel Gcn5 complexes, like CHAT, may be specific to particular groups of species where they play more specialized roles in developmental processes. *Drosophila* remains an outstanding model for studying function of the Gcn5 complexes, but recent advances in technology allow us to consider examining alternative species outside of traditional model organisms. Expanding the studies on Gcn5 complexes into non-traditional species, including potentially other insects may provide insight into the specialized function of this quintessential HAT in multicellular organisms.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] J.E. Brownell, C.D. Allis, Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation, Curr. Opin. Genet. Dev. 6 (1996) 176–184. doi:https://doi.org/10.1016/s0959-437x(96)80048-7.
[2] B. Li, M. Carey, J.L. Workman, The role of chromatin during transcription, Cell. 128 (2007) 707–719, https://doi.org/10.1016/j.cell.2007.01.015.
[3] C.D. Allis, T. Jenuwein, The molecular hallmarks of epigenetic control, Nat. Rev. Genet. 17 (2016) 487–500, https://doi.org/10.1038/nrg.2016.59.
[4] P.D. Gregory, K. Wagner, W. Horz, Histone acetylation and chromatin remodeling, Exp. Cell Res. 265 (2001) 195–202, https://doi.org/10.1006/excr.2001.5187.
[5] A.P. Wolffe, Nucleosome positioning and modification: chromatin structures that potentiate transcription, Trends Biochem. Sci. 19 (1994) 240–244, https://doi.org/10.1016/0968-0004(94)90148-1.
[6] P.A. Grant, F. Winston, S.L. Berger, The biochemical and genetic discovery of the SAGA complex, Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 194669, 2020, https://doi.org/10.1016/j.bbagrm.2020.194669.
[7] B.H. Jennings, Drosophila – a versatile model in biology & medicine, Mater. Today 14 (2011) 190–195, https://doi.org/10.1016/S1369-7021(11)70113-4.

[8] M.F. Wangler, S. Yamamoto, H.J. Bellen, Fruit flies in biomedical research, Genetics. 199 (2015) 639, https://doi.org/10.1534/genetics.114.171785.

[9] S. Yamamoto, M. Jaiswal, W.-L. Charng, T. Gambin, E. Karaca, G. Mirzaa, W. Wiszniewski, H. Sandoval, N.A. Haelterman, B. Xiong, K. Zhang, V. Bayat, G. David, T. Li, K. Chen, U. Gala, T. Harel, D. Pehlivan, S. Penney, L.E.L.M. Vissers, J. de Ligt, S.N. Jhangiani, Y. Xie, S.H. Tsang, Y. Parman, M. Sivaci, E. Battaloglu, D. Muzny, Y.-W. Wan, Z. Liu, A.T. Lin-Moore, R.D. Clark, C.J. Curry, N. Link, K.L. Schulze, E. Boerwinkle, W.B. Dobyns, R. Allikmets, R.A. Gibbs, R. Chen, J.R. Lupski, M.F. Wangler, H.J. Bellen, A drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases, Cell. 159 (2014) 200–214. doi:https://doi.org/10.1016/j.cell.2014.09.002.

[10] A.M. Karnay, F. Elefant, Chapter 14 - Drosophila epigenetics, in: T.O. Tollefsbol (Ed.), Handb. Epigenetics, Second ed., Academic Press, 2017, pp. 205–229, https://doi.org/10.1016/B978-0-12-805388-1.00014-6.

[11] K.A. Saravanan, H. Kumar, S. Chhotaray, A.L. Preethi, A.J. Talokar, A. Natarajan, S. Parida, B. Bhushan, M. Panigrahi, Drosophila melanogaster: a promising model system for epigenetic research, Biol. Rhythm. Res. (2019) 1–19, https://doi.org/10.1080/09291016.2019.1685216.

[12] J.E. Brownell, C.D. Allis, An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in Tetrahymena macronuclei, Proc. Natl. Acad. Sci. 92 (1995) 6364, https://doi.org/10.1073/pnas.92.14.6364.

[13] J.E. Brownell, J. Zhou, T. Ranalli, R. Kobayashi, D.G. Edmondson, S.Y. Roth, C. D. Allis, Tetrahymena histone acetyltransferase a: a homolog to yeast Gcn5p linking histone acetylation to gene activation, Cell. 84 (1996) 843–851, https://doi.org/10.1016/S0092-8674(00)81063-6.

[14] E.R. Smith, J.M. Belote, R.L. Schiltz, X.J. Yang, P.A. Moore, S.L. Berger, Y. Nakatani, C.D. Allis, Cloning of Drosophila GCN5: conserved features among metazoan GCN5 family members, Nucleic Acids Res. 26 (1998) 2948–2954, https://doi.org/10.1093/nar/26.12.2948.

[15] R. Candau, P.A. Moore, L. Wang, N. Barlev, C.Y. Ying, C.A. Rosen, S.L. Berger, Identification of human proteins functionally conserved with the yeast putative adaptors ADA2 and GCN5, Mol. Cell. Biol. 16 (1996) 593–602, https://doi.org/10.1128/mcb.16.2.593.

[16] X.J. Yang, V.V. Ogryzko, J. Nishikawa, B.H. Howard, Y. Nakatani, A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A, Nature. 382 (1996) 319–324, https://doi.org/10.1038/382319a0.

[17] C. Carré, D. Szymczak, J. Pidoux, C. Antoniewski, The histone H3 acetylase dGcn5 is a key player in *Drosophila melanogaster* metamorphosis, Mol. Cell. Biol. 25 (2005) 8228, https://doi.org/10.1128/MCB.25.18.8228-8238.2005.

[18] C. Dhalluin, J.E. Carlson, L. Zeng, C. He, A.K. Aggarwal, M.-M. Zhou, M.-M. Zhou, Structure and ligand of a histone acetyltransferase bromodomain, Nature. 399 (1999) 491–496, https://doi.org/10.1038/20974.

[19] L.K. Linares, R. Kiernan, R. Triboulet, C. Chable-Bessia, D. Latreille, O. Cuvier, M. Lacroix, L. Le Cam, O. Coux, M. Benkirane, Intrinsic ubiquitination activity of PCAF controls the stability of the oncoprotein Hdm2, Nat. Cell Biol. 9 (2007) 331–338, https://doi.org/10.1038/ncb1545.

[20] P.A. Grant, L. Duggan, J. Côté, S.M. Roberts, J.E. Brownell, R. Candau, R. Ohba, T. Owen-Hughes, C.D. Allis, F. Winston, S.L. Berger, J.L. Workman, Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an ADA complex and the SAGA (Spt/Ada) complex., Genes Dev. 11 (1997) 1640–1650. doi:https://doi.org/10.1101/gad.11.13.1640.

[21] T. Georgakopoulos, N. Gounalaki, G. Thireos, Genetic evidence for the interaction of the yeast transcriptional co-activator proteins GCN5 and ADA2, Mol. Gen. Genet. MGG 246 (1995) 723–728, https://doi.org/10.1007/bf00290718.

[22] J. Horiuchi, N. Silverman, G.A. Marcus, L. Guarente, ADA3, a putative transcriptional adaptor, consists of two separable domains and interacts with ADA2 and GCN5 in a trimeric complex, Mol. Cell. Biol. 15 (1995) 1203–1209, https://doi.org/10.1128/mcb.15.3.1203.

[23] S.L. Sanders, J. Jennings, A. Canutescu, A.J. Link, P.A. Weil, Proteomics of the eukaryotic transcription machinery: identification of proteins associated with components of yeast TFIID by multidimensional mass spectrometry, Mol. Cell. Biol. 22 (2002) 4723–4738, https://doi.org/10.1128/mcb.22.13.4723-4738.2002.

[24] K.K. Lee, M.E. Sardiu, S.K. Swanson, J.M. Gilmore, M. Torok, P.A. Grant, L. Florens, J.L. Workman, M.P. Washburn, Combinatorial depletion analysis to assemble the network architecture of the SAGA and ADA chromatin remodeling complexes, Mol. Syst. Biol. 7 (2011) 503, https://doi.org/10.1038/msb.2011.40.

[25] S. Guelman, T. Suganuma, L. Florens, S.K. Swanson, C.L. Kiesecker, T. Kusch, S. Anderson, J.R. Yates, M.P. Washburn, S.M. Abmayr, J.L. Workman, Host cell factor and an uncharacterized SANT domain protein are stable components of ATAC, a novel dAda2A/dGcn5-containing histone acetyltransferase complex in *Drosophila*, Mol. Cell. Biol. 26 (2006) 871, https://doi.org/10.1128/MCB.26.3.871-882.2006.

[26] T. Kusch, S. Guelman, S.M. Abmayr, J.L. Workman, Two Drosophila Ada2 homologues function in different multiprotein complexes, Mol. Cell. Biol. 23 (2003) 3305–3319, https://doi.org/10.1128/mcb.23.9.3305-3319.2003.

[27] S. Muratoglu, S. Georgieva, G. Pápai, E. Scheer, I. Enünlü, O. Komonyi, I. Cserpán, L. Lebedeva, E. Nabirochkina, A. Udvardy, L. Tora, I. Boros, Two different *Drosophila* ADA2 homologues are present in distinct GCN5 histone acetyltransferase-containing complexes, Mol. Cell. Biol. 23 (2003) 306, https://doi.org/10.1128/MCB.23.1.306-321.2003.

[28] D. Qi, J. Larsson, M. Mannervik, Drosophila Ada2b is required for viability and normal histone H3 acetylation, Mol. Cell. Biol. 24 (2004) 8080, https://doi.org/10.1128/MCB.24.18.8080-8089.2004.

[29] A.M. Gamper, J. Kim, R.G. Roeder, The STAGA subunit ADA2b is an important regulator of human GCN5 catalysis, Mol. Cell. Biol. 29 (2009) 266–280, https://doi.org/10.1128/MCB.00315-08.

[30] A. Eberharter, D.E. Sterner, D. Schieltz, A. Hassan, J.R. Yates, S.L. Berger, J. L. Workman, The ADA complex is a distinct histone acetyltransferase Complex in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 19 (1999) 6621, https://doi.org/10.1128/MCB.19.10.6621.

[31] D.E. Sterner, R. Belotserkovskaya, S.L. Berger, SALSA, a variant of yeast SAGA, contains truncated Spt7, which correlates with activated transcription, Proc. Natl. Acad. Sci. 99 (2002) 11622, https://doi.org/10.1073/pnas.182021199.

[32] V.M. Weake, S.K. Swanson, A. Mushegian, L. Florens, M.P. Washburn, S. M. Abmayr, J.L. Workman, A novel histone fold domain-containing protein that replaces TAF6 in Drosophila SAGA is required for SAGA-dependent gene expression, Genes Dev. 23 (2009) 2818–2823, https://doi.org/10.1101/gad.1846409.

[33] G. Spedale, H.Th.M. Timmers, W.W.M.P. Pijnappel, ATAC-king the complexity of SAGA during evolution, Genes Dev. 26 (2012) 527–541, https://doi.org/10.1101/gad.184705.111.

[34] J.H.M. Soffers, X. Li, A. Saraf, C.W. Seidel, L. Florens, M.P. Washburn, S. M. Abmayr, J.L. Workman, Characterization of a metazoan ADA acetyltransferase complex, Nucleic Acids Res. 47 (2019) 3383–3394, https://doi.org/10.1093/nar/gkz042.

[35] E.F. Torres-Zelada, R.E. Stephenson, A. Alpsoy, B.D. Anderson, S.K. Swanson, L. Florens, E.C. Dykhuizen, M.P. Washburn, V.M. Weake, The *Drosophila* Dbf4 ortholog Chiffon forms a complex with Gcn5 that is necessary for histone acetylation and viability, J. Cell Sci. 132 (2019) jcs214072. doi:https://doi.org/10.1242/jcs.214072.

[36] F. Winston, D.T. Chaleff, B. Valent, G.R. Fink, Mutations affecting Ty-mediated expression of the HIS4 gene of Saccharomyces cerevisiae, Genetics. 107 (1984) 179–197.

[37] D. Helmlinger, S. Marguerat, J. Villén, D.L. Swaney, S.P. Gygi, J. Bähler, F. Winston, Tra1 has specific regulatory roles, rather than global functions, within the SAGA co-activator complex, EMBO J. 30 (2011) 2843–2852, https://doi.org/10.1038/emboj.2011.181.

[38] J.A. Daniel, M.S. Torok, Z.-W. Sun, D. Schieltz, C.D. Allis, J.R. Yates 3rd, P.A. Grant, Deubiquitination of histone H2B by a yeast acetyltransferase complex regulates transcription., J. Biol. Chem. 279 (2004) 1867–1871. doi:https://doi.org/10.1074/jbc.C300494200.

[39] D.V. Cornelio-Parra, R. Goswami, K. Costanzo, P. Morales-Sosa, R.D. Mohan, Function and regulation of the Spt-Ada-Gcn5-Acetyltransferase (SAGA) deubiquitinase module, Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 2020, p. 194630, https://doi.org/10.1016/j.bbagrm.2020.194630.

[40] K.W. Henry, A. Wyce, W.-S. Lo, L.J. Duggan, N.C.T. Emre, C.-F. Kao, L. Pillus, A. Shilatifard, M.A. Osley, S.L. Berger, Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8, Genes Dev. 17 (2003) 2648–2663, https://doi.org/10.1101/gad.1144003.

[41] D. Helmlinger, L. Tora, Sharing the SAGA, Trends Biochem. Sci. 42 (2017) 850–861, https://doi.org/10.1016/j.tibs.2017.09.001.

[42] G. Spedale, N. Mischerikow, A.J.R. Heck, H.T.M. Timmers, W.W.M.P. Pijnappel, Identification of Pep4p as the protease responsible for formation of the, J. Biol. Chem. 285 (2010) 22793–22799, https://doi.org/10.1074/jbc.M110.108787.

[43] E. Martinez, V.B. Palhan, A. Tjernberg, E.S. Lymar, A.M. Gamper, T.K. Kundu, B. T. Chait, R.G. Roeder, Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors in vivo, Mol. Cell. Biol. 21 (2001) 6782–6795, https://doi.org/10.1128/MCB.21.20.6782-6795.2001.

[44] S.V. Antonova, J. Boeren, H.T.M. Timmers, B. Snel, Epigenetics and transcription regulation during eukaryotic diversification: the saga of TFIID, Genes Dev. 33 (2019) 888–902, https://doi.org/10.1101/gad.300475.117.

[45] M.A. Demény, E. Soutoglou, Z. Nagy, E. Scheer, À. Jànoshàzi, M. Richardot, M. Argentini, P. Kessler, L. Tora, Identification of a small TAF complex and its role in the assembly of TAF-containing complexes, PLoS One 2 (2007), e316, https://doi.org/10.1371/journal.pone.0000316.

[46] B. Poeck, S. Fischer, D. Gunning, S.L. Zipursky, I. Salecker, Glial cells mediate target layer selection of retinal axons in the developing visual system of Drosophila, Neuron. 29 (2001) 99–113, https://doi.org/10.1016/S0896-6273(01)00183-0.

[47] V.M. Weake, K.K. Lee, S. Guelman, C.-H. Lin, C. Seidel, S.M. Abmayr, J. L. Workman, SAGA-mediated H2B deubiquitination controls the development of neuronal connectivity in the Drosophila visual system, EMBO J. 27 (2008) 394–405, https://doi.org/10.1038/sj.emboj.7601966.

[48] S. Georgieva, E. Nabirochkina, F.J. Dilworth, H. Eickhoff, P. Becker, L. Tora, P. Georgiev, A. Soldatov, The novel transcription factor e(y)2 interacts with TAF(II)40 and potentiates transcription activation on chromatin templates, Mol. Cell. Biol. 21 (2001) 5223–5231, https://doi.org/10.1128/MCB.21.15.5223-5231.2001.

[49] R.D. Mohan, G. Dialynas, V.M. Weake, J. Liu, S. Martin-Brown, L. Florens, M. P. Washburn, J.L. Workman, S.M. Abmayr, Loss of Drosophila Ataxin-7, a SAGA subunit, reduces H2B ubiquitination and leads to neural and retinal degeneration, Genes Dev. 28 (2014) 259–272, https://doi.org/10.1101/gad.225151.113.

[50] S. Rodriguez-Navarro, T. Fischer, M.-J. Luo, O. Antunez, S. Brettschneider, J. Lechner, J.E. Perez-Ortin, R. Reed, E. Hurt, Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery, Cell. 116 (2004) 75–86, https://doi.org/10.1016/s0092-8674(03)01025-0.

[51] S. Guelman, T. Suganuma, L. Florens, V. Weake, S.K. Swanson, M.P. Washburn, S. M. Abmayr, J.L. Workman, The essential gene wda encodes a WD40 repeat subunit of Drosophila SAGA required for histone H3 acetylation, Mol. Cell. Biol. 26 (2006) 7178–7189, https://doi.org/10.1128/MCB.00130-06.
[52] Y. Doyon, W. Selleck, W.S. Lane, S. Tan, J. Cote, Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans, Mol. Cell. Biol. 24 (2004) 1884–1896, https://doi.org/10.1128/mcb.24.5.1884-1896.2004.
[53] S. Allard, R.T. Utley, J. Savard, A. Clarke, P. Grant, C.J. Brandl, L. Pillus, J. L. Workman, J. Cote, NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p, EMBO J. 18 (1999) 5108–5119, https://doi.org/10.1093/emboj/18.18.5108.
[54] J.E. Babiarz, J.E. Halley, J. Rine, Telomeric heterochromatin boundaries require NuA4-dependent acetylation of histone variant H2A.Z in *Saccharomyces cerevisiae*, Genes Dev. 20 (2006) 700–710. doi:https://doi.org/10.1101/gad.1386306.
[55] R. Stegeman, P.J. Spreacker, S.K. Swanson, R. Stephenson, L. Florens, M.P. Washburn, V.M. Weake, The spliceosomal protein SF3B5 is a novel component of drosophila SAGA that functions in gene expression independent of splicing, J. Mol. Biol. 428 (2016) 3632–3649. doi:https://doi.org/10.1016/j.jmb.2016.05.009.
[56] O. Gozani, R. Feld, R. Reed, Evidence that sequence-independent binding of highly conserved U2 snRNP proteins upstream of the branch site is required for assembly of spliceosomal complex A, Genes Dev. 10 (1996) 233–243, https://doi.org/10.1101/gad.10.2.233.
[57] N. Herold, C.L. Will, E. Wolf, B. Kastner, H. Urlaub, R. Lührmann, Conservation of the protein composition and electron microscopy structure of Drosophila melanogaster and human spliceosomal complexes, Mol. Cell. Biol. 29 (2009) 281–301, https://doi.org/10.1128/MCB.01415-08.
[58] M. Vermeulen, H.C. Eberl, F. Matarese, H. Marks, S. Denissov, F. Butter, K.K. Lee, J.V. Olsen, A.A. Hyman, H.G. Stunnenberg, M. Mann, Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers, Cell. 142 (2010) 967–980, https://doi.org/10.1016/j.cell.2010.08.020.
[59] H. Wang, C. Dienemann, A. Stützer, H. Urlaub, A.C.M. Cheung, P. Cramer, Structure of the transcription coactivator SAGA, Nature. 577 (2020) 717–720, https://doi.org/10.1038/s41586-020-1933-5.
[60] G. Papai, A. Frechard, O. Kolesnikova, C. Crucifix, P. Schultz, A. Ben-Shem, Structure of SAGA and mechanism of TBP deposition on gene promoters, Nature. 577 (2020) 711–716, https://doi.org/10.1038/s41586-020-1944-2.
[61] L.M. Díaz-Santín, N. Lukoyanova, E. Aciyan, A.C. Cheung, Cryo-EM structure of the SAGA and NuA4 coactivator subunit Tra1 at 3.7 angstrom resolution, ELife. 6 (2017) e28384. doi:https://doi.org/10.7554/eLife.28384.
[62] G. Sharov, K. Voltz, A. Durand, O. Kolesnikova, G. Papai, A.G. Myasnikov, A. Dejaegere, A. Ben Shem, P. Schultz, Structure of the transcription activator target Tra1 within the chromatin modifying complex SAGA, Nat. Commun. 8 (2017) 1556, https://doi.org/10.1038/s41467-017-01564-7.
[63] Y. Han, J. Luo, J. Ranish, S. Hahn, Architecture of the *Saccharomyces cerevisiae* SAGA transcription coactivator complex, EMBO J. 33 (2014) 2534–2546. doi:10.15252/embj.201488638.
[64] A. Kohler, M. Schneider, G.G. Cabal, U. Nehrbass, E. Hurt, Yeast Ataxin-7 links histone deubiquitination with gene gating and mRNA export, Nat. Cell Biol. 10 (2008) 707–715. doi:https://doi.org/10.1038/ncb1733.
[65] K.K. Lee, S.K. Swanson, L. Florens, M.P. Washburn, J.L. Workman, Yeast Sgf73/Ataxin-7 serves to anchor the deubiquitination module into both SAGA and Slik (SALSA) HAT complexes, Epigenetics Chromatin 2 (2009) 2, https://doi.org/10.1186/1756-8935-2-2.
[66] S. Lim, J. Kwak, M. Kim, D. Lee, Separation of a functional deubiquitylating module from the SAGA complex by the proteasome regulatory particle, Nat. Commun. 4 (2013) 2641, https://doi.org/10.1038/ncomms3641.
[67] A. Nassrallah, M. Rougée, C. Bourbousse, S. Drevensek, S. Fonseca, E. Iniesto, O. Ait-Mohamed, A.-F. Deton-Cabanillas, G. Zabulon, I. Ahmed, D. Stroebel, V. Masson, B. Lombard, D. Eeckhout, K. Gevaert, D. Loew, A. Genovesio, C. Breyton, G. De Jaeger, C. Bowler, V. Rubio, F. Barneche, DET1-mediated degradation of a SAGA-like deubiquitination module controls H2Bub homeostasis, ELife. 7 (2018), e37892, https://doi.org/10.7554/eLife.37892.
[68] S.J. Guyenet, S.S. Mookerjee, A. Lin, S.K. Custer, S.F. Chen, B.L. Sopher, A.R. La Spada, L.M. Ellerby, Proteolytic cleavage of ataxin-7 promotes SCA7 retinal degeneration and neurological dysfunction, Hum. Mol. Genet. 24 (2015) 3908–3917, https://doi.org/10.1093/hmg/ddv121.
[69] T. Suganuma, A. Mushegian, S.K. Swanson, S.M. Abmayr, L. Florens, M. P. Washburn, J.L. Workman, The ATAC Acetyltransferase complex coordinates MAP kinases to regulate JNK target genes, Cell. 142 (2010) 726–736, https://doi.org/10.1016/j.cell.2010.07.045.
[70] T. Suganuma, J.L. Gutiérrez, B. Li, L. Florens, S.K. Swanson, M.P. Washburn, S. M. Abmayr, J.L. Workman, ATAC is a double histone acetyltransferase complex that stimulates nucleosome sliding, Nat. Struct. Mol. Biol. 15 (2008) 364–372, https://doi.org/10.1038/nsmb.1397.
[71] Y.-L. Wang, F. Faiola, M. Xu, S. Pan, E. Martinez, Human ATAC Is a GCN5/PCAF-containing acetylase complex with a novel NC2-like histone fold module that interacts with the TATA-binding protein., J. Biol. Chem. 283 (2008) 33808–33815. doi:https://doi.org/10.1074/jbc.M806936200.
[72] S. Guelman, K. Kozuka, Y. Mao, V. Pham, M.J. Solloway, J. Wang, J. Wu, J.R. Lill, J. Zha, The double-histone-acetyltransferase complex ATAC is essential for mammalian development, Mol. Cell. Biol. 29 (2009) 1176, https://doi.org/10.1128/MCB.01599-08.
[73] Z. Nagy, A. Riss, S. Fujiyama, A. Krebs, M. Orpinell, P. Jansen, A. Cohen, H. G. Stunnenberg, S. Kato, L. Tora, The metazoan ATAC and SAGA coactivator HAT complexes regulate different sets of inducible target genes, Cell. Mol. Life Sci. CMLS. 67 (2010) 611–628, https://doi.org/10.1007/s00018-009-0199-8.
[74] M. Mohan, H.-M. Herz, E.R. Smith, Y. Zhang, J. Jackson, M.P. Washburn, L. Florens, J.C. Eissenberg, A. Shilatifard, The COMPASS family of H3K4 methylases in Drosophila, Mol. Cell. Biol. 31 (2011) 4310–4318, https://doi.org/10.1128/MCB.06092-11.
[75] J. Tower, Developmental gene amplification and origin regulation, Annu. Rev. Genet. 38 (2004) 273–304, https://doi.org/10.1146/annurev.genet.37.110801.143851.
[76] P. Pasero, B.P. Duncker, E. Schwob, S.M. Gasser, A role for the Cdc7 kinase regulatory subunit Dbf4p in the formation of initiation-competent origins of replication, Genes Dev. 13 (1999) 2159–2176, https://doi.org/10.1101/gad.13.16.2159.
[77] K. Labib, How do Cdc7 and cyclin-dependent kinases trigger the initiation of chromosome replication in eukaryotic cells? Genes Dev. 24 (2010) 1208–1219, https://doi.org/10.1101/gad.1933010.
[78] R. Stephenson, M.R. Hosler, N.S. Gavande, A.K. Ghosh, V.M. Weake, Characterization of a Drosophila ortholog of the Cdc7 kinase: a role FOR Cdc7 IN endoreplication independent of chiffon, J. Biol. Chem. 290 (2015) 1332–1347, https://doi.org/10.1074/jbc.M114.597948.
[79] T. Pankotai, O. Komonyi, L. Bodai, Z. Ujfaludi, S. Muratoglu, A. Ciurciu, L. Tora, J. Szabad, I. Boros, The homologous Drosophila transcriptional adaptors ADA2a and ADA2b are both required for normal development but have different functions, Mol. Cell. Biol. 25 (2005) 8215–8227, https://doi.org/10.1128/MCB.25.18.8215-8227.2005.
[80] A. Ciurciu, O. Komonyi, I.M. Boros, Loss of ATAC-specific acetylation of histone H4 at Lys12 reduces binding of JIL-1 to chromatin and phosphorylation of histone H3 at Ser10, J. Cell Sci. 121 (2008) 3366–3372, https://doi.org/10.1242/jcs.028555.
[81] A. Ciurciu, O. Komonyi, T. Pankotai, I.M. Boros, The Drosophila histone acetyltransferase Gcn5 and transcriptional adaptor Ada2a are involved in nucleosomal histone H4 acetylation., Mol. Cell. Biol. 26 (2006) 9413–9423. doi:https://doi.org/10.1128/MCB.01401-06.
[82] C. Feller, I. Forné, A. Imhof, P.B. Becker, Global and specific responses of the histone acetylome to systematic perturbation, Mol. Cell 57 (2015) 559–571, https://doi.org/10.1016/j.molcel.2014.12.008.
[83] E.E. Vamos, I.M. Boros, The C-terminal domains of ADA2 proteins determine selective incorporation into GCN5-containing complexes that target histone H3 or H4 for acetylation, FEBS Lett. 586 (2012) 3279–3286, https://doi.org/10.1016/j.febslet.2012.06.051.
[84] R. Ferreira, A. Eberharter, T. Bonaldi, M. Chioda, A. Imhof, P.B. Becker, Site-specific acetylation of ISWI by GCN5, BMC Mol. Biol. 8 (2007) 73, https://doi.org/10.1186/1471-2199-8-73.
[85] D.F.V. Corona, J.W. Tamkun, Multiple roles for ISWI in transcription, chromosome organization and DNA replication, Biochim. Biophys. Acta 1677 (2004) 113–119, https://doi.org/10.1016/j.bbaexp.2003.09.018.
[86] C. Carré, A. Ciurciu, O. Komonyi, C. Jacquier, D. Fagegaltier, J. Pidoux, H. Tricoire, L. Tora, I.M. Boros, C. Antoniewski, The Drosophila NURF remodelling and the ATAC histone acetylase complexes functionally interact and are required for global chromosome organization, EMBO Rep. 9 (2008) 187–192, https://doi.org/10.1038/sj.embor.7401141.
[87] Y. Wang, Y. Huang, J. Liu, J. Zhang, M. Xu, Z. You, C. Peng, Z. Gong, W. Liu, Acetyltransferase GCN5 regulates autophagy and lysosome biogenesis by targeting TFEB, EMBO Rep. 21 (2020) e48335. doi:10.15252/embr.201948335.
[88] T. Liu, Q. Wang, W. Li, F. Mao, S. Yue, S. Liu, X. Liu, S. Xiao, L. Xia, Gcn5 determines the fate of Drosophila germline stem cells through degradation of Cyclin A, FASEB J. 31 (2017) 2185–2194, https://doi.org/10.1096/fj.201601217R.
[89] W. Xu, D.G. Edmondson, Y.A. Evrard, M. Wakamiya, R.R. Behringer, S.Y. Roth, Loss of Gcn5l2 leads to increased apoptosis and mesodermal defects during mouse development, Nat. Genet. 26 (2000) 229–232, https://doi.org/10.1038/79973.
[90] L. Howe, D. Auston, P. Grant, S. John, R.G. Cook, J.L. Workman, L. Pillus, Histone H3 specific acetyltransferases are essential for cell cycle progression, Genes Dev. 15 (2001) 3144–3154, https://doi.org/10.1101/gad.931401.
[91] B. Grau, C. Popescu, L. Torroja, D. Ortuño-Sahagún, I. Boros, A. Ferrús, Transcriptional adaptor ADA3 of Drosophila melanogaster is required for histone modification, position effect variegation, and transcription, Mol. Cell. Biol. 28 (2008) 376–385, https://doi.org/10.1128/MCB.01307-07.
[92] Z. Pahi, B.N. Borsos, B. Vedelek, Y.V. Shidlovskii, S.G. Georgieva, I.M. Boros, T. Pankotai, TAF10 and TAF10b partially redundant roles during Drosophila melanogaster morphogenesis, Transcription. 8 (2017) 297–306, https://doi.org/10.1080/21541264.2017.1327836.
[93] E. Koutelou, C.L. Hirsch, S.Y. Dent, Multiple faces of the SAGA complex, Nucl. Gene Expr. 22 (2010) 374–382, https://doi.org/10.1016/j.ceb.2010.03.005.
[94] X. Li, C.W. Seidel, L.T. Szerszen, J.J. Lange, J.L. Workman, S.M. Abmayr, Enzymatic modules of the SAGA chromatin-modifying complex play distinct roles in Drosophila gene expression and development, Genes Dev. 31 (2017) 1588–1600, https://doi.org/10.1101/gad.300988.117.
[95] V.M. Weake, J.O. Dyer, C. Seidel, A. Box, S.K. Swanson, A. Peak, L. Florens, M. P. Washburn, S.M. Abmayr, J.L. Workman, Post-transcription initiation function of the ubiquitous SAGA complex in tissue-specific gene activation, Genes Dev. 25 (2011) 1499–1509, https://doi.org/10.1101/gad.2046211.
[96] V.M. Weake, J.L. Workman, SAGA function in tissue-specific gene expression, Trends Cell Biol. 22 (2012) 177–184, https://doi.org/10.1016/j.tcb.2011.11.005.
[97] J. Bonnet, C.-Y. Wang, T. Baptista, S.D. Vincent, W.-C. Hsiao, M. Stierle, C.-F. Kao, L. Tora, D. Devys, The SAGA coactivator complex acts on the whole transcribed

genome and is required for RNA polymerase II transcription, Genes Dev. 28 (2014) 1999–2012. doi:https://doi.org/10.1101/gad.250225.114.
[98] G. Haimovich, D.A. Medina, S.Z. Causse, M. Garber, G. Millán-Zambrano, O. Barkai, S. Chávez, J.E. Pérez-Ortín, X. Darzacq, M. Choder, Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis, Cell. 153 (2013) 1000–1011, https://doi.org/10.1016/j.cell.2013.05.012.
[99] J.B. Rodríguez-Molina, S.C. Tseng, S.P. Simonett, J. Taunton, A.Z. Ansari, Engineered covalent inactivation of TFIIH-kinase reveals an elongation checkpoint and results in widespread mRNA stabilization, Mol. Cell 63 (2016) 433–444, https://doi.org/10.1016/j.molcel.2016.06.036.
[100] T. Baptista, S. Grünberg, N. Minoungou, M.J.E. Koster, H.T.M. Timmers, S. Hahn, D. Devys, L. Tora, SAGA is a general cofactor for RNA polymerase II transcription, Mol. Cell. 68 (2017) 130–143.e5. doi:https://doi.org/10.1016/j.molcel.2017.08.016.
[101] K.A. Martin, B. Poeck, H. Roth, A.J. Ebens, L.C. Ballard, S.L. Zipursky, Mutations disrupting neuronal connectivity in the Drosophila visual system, Neuron. 14 (1995) 229–240, https://doi.org/10.1016/0896-6273(95)90281-3.
[102] J. Ma, K.J. Brennan, M.R. D'Aloia, P.E. Pascuzzi, V.M. Weake, Transcriptome profiling identifies multiplexin as a target of SAGA deubiquitinase activity in glia required for precise axon guidance during Drosophila visual development, G3 GenesGenomesGenetics 6 (2016) 2435. doi:https://doi.org/10.1534/g3.116.031310.
[103] C.E. Brown, L. Howe, K. Sousa, S.C. Alley, M.J. Carrozza, S. Tan, J.L. Workman, Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit, Science. 292 (2001) 2333, https://doi.org/10.1126/science.1060214.
[104] D.E. Sterner, P.A. Grant, S.M. Roberts, L.J. Duggan, R. Belotserkovskaya, L. A. Pacella, F. Winston, J.L. Workman, S.L. Berger, Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction, Mol. Cell. Biol. 19 (1999) 86–98, https://doi.org/10.1128/mcb.19.1.86.
[105] L. Herzel, D.S.M. Ottoz, T. Alpert, K.M. Neugebauer, Splicing and transcription touch base: co-transcriptional spliceosome assembly and function, Nat. Rev. Mol. Cell Biol. 18 (2017) 637–650, https://doi.org/10.1038/nrm.2017.63.
[106] M. Hollmann, E. Simmerl, U. Schäfer, M. Schäfer, The essential Drosophila melanogaster gene Wds (will die slowly) codes for a WD-repeat protein with seven repeats, Mol. Gen. Genomics. 268 (2002) 425–433, https://doi.org/10.1007/s00438-002-0768-0.
[107] S. Rodriguez-Jato, A. Busturia, W. Herr, *Drosophila melanogaster* dHCF Interacts with both PcG and TrxG epigenetic regulators, PLOS ONE 6 (12) (2011) e27479, https://doi.org/10.1371/journal.pone.0027479.
[108] G. Dietzl, D. Chen, F. Schnorrer, K.-C. Su, Y. Barinova, M. Fellner, B. Gasser, K. Kinsey, S. Oppel, S. Scheiblauer, A. Couto, V. Marra, K. Keleman, B.J. Dickson, A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila, Nature. 448 (2007) 151–156, https://doi.org/10.1038/nature05954.
[109] E.T. Danielsen, M.E. Moeller, K.F. Rewitz, Chapter two - nutrient signaling and developmental timing of maturation, in: A.E. Rougvie, M.B. O'Connor (Eds.), Curr. Top. Dev. Biol, Academic Press, 2013, pp. 37–67, https://doi.org/10.1016/B978-0-12-396968-2.00002-6.
[110] T. Pankotai, C. Popescu, D. Martín, B. Grau, N. Zsindely, L. Bodai, L. Tora, A. Ferrús, I. Boros, Genes of the ecdysone biosynthesis pathway are regulated by the dATAC histone acetyltransferase complex in Drosophila, Mol. Cell. Biol. 30 (2010) 4254, https://doi.org/10.1128/MCB.00142-10.
[111] M. Featherstone, Coactivators in transcription initiation: here are your orders, Curr. Opin. Genet. Dev. 12 (2002) 149–155, https://doi.org/10.1016/S0959-437X(02)00280-0.
[112] P. Georgiev, S. Chlamydas, A. Akhtar, Drosophila dosage compensation: males are from Mars, females are from Venus, Fly (Austin). 5 (2011) 147–154, https://doi.org/10.4161/fly.5.2.14934.
[113] D.F.V. Corona, C.R. Clapier, P.B. Becker, J.W. Tamkun, Modulation of ISWI function by site-specific histone acetylation, EMBO Rep. 3 (2002) 242–247, https://doi.org/10.1093/embo-reports/kvf056.
[114] C.R. Clapier, K.P. Nightingale, P.B. Becker, A critical epitope for substrate recognition by the nucleosome remodeling ATPase ISWI, Nucleic Acids Res. 30 (2002) 649–655, https://doi.org/10.1093/nar/30.3.649.
[115] A.D. Baxevanis, G. Arents, E.N. Moudrianakis, D. Landsman, A variety of DNA-binding and multimeric proteins contain the histone fold motif, Nucleic Acids Res. 23 (1995) 2685–2691, https://doi.org/10.1093/nar/23.14.2685.
[116] K.F. Hartlepp, C. Fernández-Tornero, A. Eberharter, T. Grüne, C.W. Müller, P. B. Becker, The histone fold subunits of Drosophila CHRAC facilitate nucleosome sliding through dynamic DNA interactions, Mol. Cell. Biol. 25 (2005) 9886–9896, https://doi.org/10.1128/MCB.25.22.9886-9896.2005.
[117] I. Kukimoto, S. Elderkin, M. Grimaldi, T. Oelgeschläger, P.D. Varga-Weisz, The histone-fold protein complex CHRAC-15/17 enhances nucleosome sliding and assembly mediated by ACF, Mol. Cell 13 (2004) 265–277, https://doi.org/10.1016/S1097-2765(03)00523-9.
[118] M. Orpinell, M. Fournier, A. Riss, Z. Nagy, A.R. Krebs, M. Frontini, L. Tora, The ATAC acetyl transferase complex controls mitotic progression by targeting non-histone substrates, EMBO J. 29 (2010) 2381–2394, https://doi.org/10.1038/emboj.2010.125.
[119] S.-H. Yang, A.D. Sharrocks, A.J. Whitmarsh, Transcriptional regulation by the MAP kinase signaling cascades, Gene. 320 (2003) 3–21, https://doi.org/10.1016/s0378-1119(03)00816-3.
[120] K. Fukuyama, M. Yoshida, A. Yamashita, T. Deyama, M. Baba, A. Suzuki, H. Mohri, Z. Ikezawa, H. Nakajima, S. Hirai, S. Ohno, MAPK upstream kinase (MUK)-binding inhibitory protein, a negative regulator of MUK/dual leucine zipper-bearing kinase/leucine zipper protein kinase, J. Biol. Chem. 275 (2000) 21247–21254, https://doi.org/10.1074/jbc.M001488200.
[121] A. Welihinda, W. Tirasophon, R. Kaufman, The transcriptional co-activator ADA5 is required for HAC1 mRNA processing in vivo, J. Biol. Chem. 275 (2000) 3377–3381, https://doi.org/10.1074/jbc.275.5.3377.
[122] A.W. Schram, R. Baas, P.W.T.C. Jansen, A. Riss, L. Tora, M. Vermeulen, H.Th. M. Timmers, A dual role for SAGA-associated factor 29 (SGF29) in ER stress survival by coordination of both histone H3 acetylation and histone H3 lysine-4 trimethylation, PLoS One 8 (2013), e70035, https://doi.org/10.1371/journal.pone.0070035.
[123] D. Sela, L. Chen, S. Martin-Brown, M.P. Washburn, L. Florens, J.W. Conaway, R. C. Conaway, Endoplasmic reticulum stress-responsive transcription factor ATF6alpha directs recruitment of the mediator of RNA polymerase II transcription and multiple histone acetyltransferase complexes, J. Biol. Chem. 287 (2012) 23035–23045, https://doi.org/10.1074/jbc.M112.369504.
[124] G. Landis, J. Tower, The Drosophila chiffon gene is required for chorion gene amplification, and is related to the yeast Dbf4 regulator of DNA replication and cell cycle, Development. 126 (1999) 4281.
[125] T. Schüpbach, E. Wieschaus, Female sterile mutations on the second chromosome of Drosophila melanogaster. I. Maternal effect mutations, Genetics. 121 (1989) 101.
[126] B.D. Aggarwal, B.R. Calvi, Chromatin regulates origin activity in Drosophila follicle cells, Nature. 430 (2004) 372–376, https://doi.org/10.1038/nature02694.
[127] T. Pankotai, N. Zsindely, E.E. Vamos, O. Komonyi, L. Bodai, I.M. Boros, Functional characterization and gene expression profiling of Drosophila melanogastershort dADA2b isoform-containing dSAGA complexes, BMC Genomics 14 (2013) 44, https://doi.org/10.1186/1471-2164-14-44.
[128] L. Jiang, F. Schlesinger, C.A. Davis, Y. Zhang, R. Li, M. Salit, T.R. Gingeras, B. Oliver, Synthetic spike-in standards for RNA-seq experiments, Genome Res. 21 (2011) 1543–1551, https://doi.org/10.1101/gr.121095.111.
[129] G. Liu, X. Zheng, H. Guan, Y. Cao, H. Qu, J. Kang, X. Ren, J. Lei, M.-Q. Dong, X. Li, H. Li, Architecture of Saccharomyces cerevisiae SAGA complex, Cell Discov. 5 (2019) 25, https://doi.org/10.1038/s41421-019-0094-x.
[130] M. Holmberg, C. Duyckaerts, A. Dürr, G. Cancel, I. Gourfinkel-An, P. Damier, B. Faucheux, Y. Trottier, E.C. Hirsch, Y. Agid, A. Brice, Spinocerebellar ataxia type 7 (SCA7): a neurodegenerative disorder with neuronal intranuclear inclusions, Hum. Mol. Genet. 7 (1998) 913–918, https://doi.org/10.1093/hmg/7.5.913.
[131] G. David, N. Abbas, G. Stevanin, A. Dürr, G. Yvert, G. Cancel, C. Weber, G. Imbert, F. Saudou, E. Antoniou, H. Drabkin, R. Gemmill, P. Giunti, A. Benomar, N. Wood, M. Ruberg, Y. Agid, J.-L. Mandel, A. Brice, Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion, Nat. Genet. 17 (1997) 65–70, https://doi.org/10.1038/ng0997-65.
[132] R.D. Mohan, J.L. Workman, S.M. Abmayr, Drosophila models reveal novel insights into mechanisms underlying neurodegeneration, Fly (Austin). 8 (2014) 148–152, https://doi.org/10.4161/19336934.2014.969150.
[133] M. Latouche, C. Lasbleiz, E. Martin, V. Monnier, T. Debeir, A. Mouatt-Prigent, M.-P. Muriel, L. Morel, M. Ruberg, A. Brice, G. Stevanin, H. Tricoire, A conditional pan-neuronal Drosophila model of spinocerebellar ataxia 7 with a reversible adult phenotype suitable for identifying modifier genes, J. Neurosci. 27 (2007) 2483–2492, https://doi.org/10.1523/JNEUROSCI.5453-06.2007.
[134] A.C. Elden, H.-J. Kim, M.P. Hart, A.S. Chen-Plotkin, B.S. Johnson, X. Fang, M. Armakola, F. Geser, R. Greene, M.M. Lu, A. Padmanabhan, D. Clay-Falcone, L. McCluskey, L. Elman, D. Juhr, P.J. Gruber, U. Rüb, G. Auburger, J. Q. Trojanowski, V.M.-Y. Lee, V.M. Van Deerlin, N.M. Bonini, A.D. Gitler, Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS, Nature. 466 (2010) 1069–1075, https://doi.org/10.1038/nature09320.
[135] T.L. Burke, J.L. Miller, P.A. Grant, Direct inhibition of Gcn5 protein catalytic activity by polyglutamine-expanded ataxin-7, J. Biol. Chem. 288 (2013) 34266–34275, https://doi.org/10.1074/jbc.M113.487538.
[136] M. Gause, J.C. Eissenberg, A.F. Macrae, M. Dorsett, Z. Misulovin, D. Dorsett, Nipped-A, the Tra1/TRRAP subunit of the Drosophila SAGA and Tip60 complexes, has multiple roles in notch signaling during wing development, Mol. Cell. Biol. 26 (2006) 2347–2359, https://doi.org/10.1128/MCB.26.6.2347-2359.2006.
[137] G. Xie, Z. Yu, D. Jia, R. Jiao, W.-M. Deng, E(y)1/TAF9 mediates the transcriptional output of Notch signaling in Drosophila, J. Cell Sci. 127 (2014) 3830, https://doi.org/10.1242/jcs.154583.
[138] Z. Pahi, Z. Kiss, O. Komonyi, B.N. Borsos, L. Tora, et al., dTAF10- and dTAF10b-containing complexes are required for ecdysone-driven larval-pupal morphogenesis in *Drosophila melanogaster*, PLOS ONE 10 (11) (2015) e0142226, https://doi.org/10.1371/journal.pone.0142226.
[139] V. Mathew, A.-L. Pauleau, N. Steffen, A. Bergner, P.B. Becker, S. Erhardt, The histone-fold protein CHRAC14 influences chromatin composition in response to DNA damage, Cell Rep. 7 (2014) 321–330, https://doi.org/10.1016/j.celrep.2014.03.008.
[140] N. Zsindely, T. Pankotai, Z. Ujfaludi, D. Lakatos, O. Komonyi, L. Bodai, L. Tora, I. M. Boros, The loss of histone H3 lysine 9 acetylation due to dSAGA-specific dAda2b mutation influences the expression of only a small subset of genes, Nucleic Acids Res. 37 (2009) 6665–6680, https://doi.org/10.1093/nar/gkp722.
[141] J.E. Brownell, C.D. Allis, HAT discovery: heading toward an elusive goal with a key biological assist, Biochim. Biophys. Acta (BBA) - Gene Regul. Mech. (2020) 194605, https://doi.org/10.1016/j.bbagrm.2020.194605.

[142] E. Koutelou, A.T. Farria, S.Y. Dent, Complex functions of Gcn5 and Pcaf in development and disease, Biochim. Biophys. Acta (BBA) - Gene Regul. Mech. (2020) 194609, https://doi.org/10.1016/j.bbagrm.2020.194609.
[143] K.D. Grasser, V. Rubio, F. Barneche, Multifaceted activities of the plant SAGA complex, Biochim. Biophys. Acta (BBA) - Gene Regul. Mech. (2020) 194613, https://doi.org/10.1016/j.bbagrm.2020.194613.
[144] D. Helmlinger, G. Papai, D. Devys, L. Tora, What do the structures of GCN5-containing complexes teach us about their function? Biochim. Biophys. Acta (BBA) - Gene Regul. Mech. (2020) 194614, https://doi.org/10.1016/j.bbagrm.2020.194614.
[145] H.T.M. Timmers, SAGA and TFIID: friends of TBP drifting apart, Biochim. Biophys. Acta (BBA) - Gene Regul. Mech. (2020) 194604, https://doi.org/10.1016/j.bbagrm.2020.194604.
[146] C. Nuño-Cabanes, S. Rodríguez-Navarro, The promiscuity of the SAGA complex subunits: multifunctional or moonlighting proteins? Biochim. Biophys. Acta (BBA) - Gene Regul. Mech. (2020) 194607, https://doi.org/10.1016/j.bbagrm.2020.194607.
[147] B.D. Strahl, S.D Briggs, The SAGA continues: the rise of cis-and trans-histone crosstalk pathways, Biochim. Biophys. Acta (BBA) - Gene Regul. Mech. (2020) 194600, https://doi.org/10.1016/j.bbagrm.2020.194600.
[148] M. Downey, Non-histone protein acetylation by the evolutionarily conserved GCN5 and PCAF acetyltransferases, Biochim. Biophys. Acta (BBA) - Gene Regul. Mech. (2020) 194608, https://doi.org/10.1016/j.bbagrm.2020.194608.