Purdue University

Purdue e-Pubs

Department of Biochemistry Faculty Publications

Department of Biochemistry

7-22-2020

The Gcn5 Complexes in Drosophila As A Model for Metazoa

Eliana F. Torres-Zelada

Vikki M. Weake

Follow this and additional works at: https://docs.lib.purdue.edu/biochempubs

Part of the Biochemistry Commons

This document has been made available through Purdue e-Pubs, a service of the Purdue University Libraries. Please contact epubs@purdue.edu for additional information.

TITLE: The Gcn5 complexes in *Drosophila* as a model for metazoa

AUTHORS: Torres-Zelada, Eliana F.[1], and Weake, Vikki M.[1,2,3]

[1]Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907, USA.

[2]Purdue University Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907, USA.

[3]To whom correspondence should be addressed: Vikki M. Weake, Department of Biochemistry, Purdue University, 175 S. University Street, West Lafayette, Indiana 47907, USA, Tel: (765) 496-1730; Fax (765) 494-7897; Email: vweake@purdue.edu

HIGHLIGHTS

► *Drosophila* contain four different Gcn5 complexes. ►Insects share common Gcn5 complexes with other metazoa, but also possess a unique Gcn5 complex. ► The different Gcn5 complexes are nucleated by Ada2 paralogs and splice isoforms. ►The Gcn5 complexes are essential for development in flies.

KEYWORDS

Gcn5, *Drosophila*, chromatin, histone acetylation, histone modification, development, SAGA, ADA, ATAC, CHAT.

17 **ABSTRACT**

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

34 **1. Introduction**

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

52 The fruit fly *Drosophila* has been used as a model organism extensively for genetic studies and
53 developmental biology [6]. The *Drosophila* genome shares 60% homology with humans, and about
54 75% of the genes responsible for human diseases have homologs in flies [7,8]. Moreover, *Drosophila*
55 possess homologs of nearly all of the key factors involved in chromatin modification and transcription,
56 making the fruit fly a powerful model organism for studying chromatin biology [9]. In contrast to
57 mammals, which often possess multiple paralogs of histone modifying enzymes, *Drosophila* usually
58 encodes only a single gene for different histone modifying enzymes, providing a simpler genetic system
59 in which to dissect biological functions of various chromatin-based processes [10]. The *Drosophila* life
60 cycle takes place within 10 days under standard laboratory conditions, beginning with the hatching of
61 an egg into a larval stage, followed by several larval molts, formation of a pupa, metamorphosis
62 (transformation from an immature, larval form to the adult fly), and finally eclosion (emergence from the
63 pupal case) into an adult fly [6]. In this review, we provide a historical perspective on the identification of
64 the *Drosophila* Gcn5 complexes. First, we describe the composition of the Gcn5 complexes in
65 *Drosophila* in comparison to the orthologous complexes in *S. cerevisiae* and human cells. Next, we
66 outline the essential subunits for fly development based on studies in mutant flies, and describe a

67 subset of representative mutant phenotypes that highlight specialized functions for each Gcn5 complex
68 in development. Finally, we describe the genome-wide localization patterns and biochemical roles for
69 each Gcn5 complex in gene expression and other chromatin-based processes, where this has been
70 defined. Last, we briefly discuss Gcn5 complexes in other insect species, and provide an overview of
71 outstanding questions for future studies.

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

73 Immediately after the discovery that the *Tetrahymena* p55 HAT corresponded to *S. cerevisiae* Gcn5, it
74 became clear that Gcn5 was conserved throughout eukaryotes: "*it seems likely that the yeast 55-kDa*
75 *polypeptide is conserved across a wide range of eukaryotes*" [11,12]. Indeed, only three years later, the
76 Allis group identified Gcn5 in the model insect species, *Drosophila* [13]. In contrast to humans, who
77 possess two Gcn5 paralogs (PCAF and Gcn5) [14,15], there is only one Gcn5 homolog in *Drosophila:*
78 Gcn5 (FBgn0020388, CG4107). The gene encoding Gcn5 was historically named *pcaf* in flies [16], but
79 it has since been renamed *gcn5* on FlyBase and in much of the recent literature; we refer to this gene
80 as *gcn5* throughout this review. Gcn5 shares the domains that are common to all Gcn5 homologs
81 including its HAT catalytic domain (469 - 634aa), Gcn5-N-Acetyltransferase (GNAT) domain (514 -
82 598aa), and bromodomain (717 - 795aa) [13], which binds acetylated lysine (Figure 1) [17]. However,
83 *Drosophila* Gcn5 shares higher similarity with both of the human Gcn5 paralogs than with *S. cerevisiae*
84 Gcn5 (yGcn5). Moreover, Gcn5 contains a conserved N-terminal domain that is only found in metazoan
85 Gcn5 homologs like human Gcn5 and PCAF [13]. It has been suggested that this N-terminal PCAF
86 domain in human PCAF has E3 ubiquitin ligase activity [18], but this activity has not been demonstrated
87 for human or *Drosophila* Gcn5 .

88 In all organisms, Gcn5 associates with other proteins that are critical for both its activity and targeting.
89 Although both *S. cerevisiae* and *Drosophila* Gcn5 can acetylate free histone H3 *in vitro*, they are unable
90 to acetylate nucleosomal substrates on their own [13,19]. This lack of nucleosomal acetyltransferase
91 activity is in contrast to human PCAF, which has been shown to acetylate nucleosomal substrates *in*
92 *vitro* [15], and shares substantial homology with *Drosophila* Gcn5 (Figure 1). In *S. cerevisiae*, Gcn5
93 associates tightly with two other proteins, Ada2 and Ada3, forming an heterotrimeric complex *in vitro*
94 [20,21]. A third Gcn5-interacting protein, Sgf29, was later identified in *S. cerevisiae* [22,23]. Together,
95 Gcn5, Ada2, Ada3, and Sgf29 constitute the core Gcn5 HAT module that is sufficient for nucleosomal
96 histone acetylation [19] [*cite Song Tan article within special issue for discussion of core Gcn5 HAT*
97 *module*]. *Drosophila*, like *S. cerevisiae*, has single homologs of Ada3 (FBgn0030891, CG7098) and
98 Sgf29 (FBgn0050390, CG30390), which were readily identified by sequence comparisons with the *S.*

99 *cerevisiae* proteins (Table 1) [24–26]. In contrast, there are two paralogs of Ada2 in *Drosophila*: Ada2a
100 (FBgn0263738, CG43663) and Ada2b (FBgn0037555, CG9638) [25–27]. Both Ada2a and Ada2b share
101 a similar domain structure to *S. cerevisiae* Ada2, possessing conserved ZZ and SANT domains (Figure
102 2) [25–27]. Ada2a also contains a C-terminal SWIRM domain that is present in *S. cerevisiae* Ada2 and
103 human Ada2a and Ada2b [28]. In addition, there are two splice isoforms of Ada2b resulting from
104 alternative usage of splice acceptor sites in the third exon: Ada2b-PA encoding a 418-aa protein
105 (FBppp0081303, also referred to as Ada2bS) and Ada2b-PB encoding a 555-aa protein
106 (FBppp0099776, also referred to as Ada2bL) [27]. These Ada2b splice isoforms are expressed at
107 equivalent levels during different developmental stages in flies, and share both the ZZ and SANT
108 domains, differing only in their C-terminal regions (Figure 2) [27]. The longer Ada2b-PB isoform
109 contains the SWIRM domain in its unique C-terminal region, while the Ada2b-PA isoform lacks this
110 domain (Figure 2). In flies, like in *S. cerevisiae*, the nucleosomal HAT activity of Gcn5 requires
111 interactions with either Ada2a or Ada2b, and Ada3 [13,19,25]. Both Ada2 paralogs, Ada2a and Ada2b,
112 are conserved in other multicellular eukaryotes including *Arabidopsis* and humans [26], providing an
113 early hint that Gcn5's association with other proteins might expand its biological role in multicellular
114 organisms.

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

116 Gcn5 resides within three different multi-subunit complexes in *S. cerevisiae*: the large, highly similar
117 Spt-Ada-Gcn5 Acetyltransferase (SAGA) and SAGA-like (SLIK/SALSA) complexes, and the small
118 Ada2/Gcn5/Ada3 transcription activator (ADA) complex [19,29,30] [*cite Winston review within special*
119 *issue*]. The presence of multiple versions of Ada2 in flies suggested that Gcn5 might reside within
120 additional complexes in *Drosophila*, and raised the question as to which version of Ada2 was present in
121 each complex. During the first decade of the twenty first century, a series of studies led by the Boros
122 and Workman groups revealed the existence of two large multi-subunit Gcn5 complexes in flies. In
123 *Drosophila*, the Ada2b paralog (specifically the Ada2b-PB isoform) is present in the SAGA complex,
124 similar to that found in *S. cerevisiae* [25,26,31]. In contrast, Ada2a resides within a Gcn5 complex that
125 is not present in *S. cerevisiae*, the Ada2a-containing (ATAC) complex [24]. ATAC was first identified in
126 *Drosophila*, and it has since been characterized in mammalian cells and appears to be widely
127 conserved in multicellular eukaryotes [32]. More recently, an ADA-like complex was also identified in
128 *Drosophila* [33], together with an insect-specific Gcn5 complex that contains the shorter Ada2b-PA
129 splice isoform [34]. With the exception of the small ADA complex, which contains only the core HAT
130 subunits, the *Drosophila* Gcn5 complexes each possess additional protein subunits that contribute to
131 their unique biological activities.

132 **3.1 SAGA**

133 The first of the *Drosophila* Gcn5 complexes to be identified, SAGA, is a large 2 MDa complex that
134 contains 20 different protein subunits [25,26]. SAGA has been well characterized in *S. cerevisiae* where
135 its subunits were historically first organized into four major modules: the HAT module (Gcn5, Ada2,
136 Ada3, Sgf29), a deubiquitination module (DUB; Ubp8, Sus1, Sgf11, and Sgf73), the TATA binding
137 protein-Associated Factor (TAF) module (Taf5, Taf6, Taf9, Taf10, and Taf12), and the Suppressor of
138 Ty's (SPT) module (Ada1, Spt3, Spt7, Spt8, and Spt20), with Tra1 originally being classified as a Spt
139 protein, although it was not identified in the original genetic screen [35] . More recent structural studies
140 have resulted in a re-organization of the subunits in the TAF and SPT modules into a structural core
141 (Taf5, Taf6, Taf9, Taf10, Taf12, Spt7, Ada1, and Spt20), a TATA binding protein (TBP) binding module
142 (Spt3 and Spt8), and a transcription factor (TF) binding module consisting only of Tra1 [23,36] [*see*
143 *Tora article within special issue for discussion of structural organization of SAGA*]. The composition of
144 the HAT and DUB modules remains unchanged from the original modular organization. Ubp8 within the
145 DUB module provides SAGA with a second histone modifying activity, catalyzing deubiquitination of
146 monoubiquitinated histone H2B (H2Bub1) [37,38] [*see Mohan article within special issue for discussion*
147 *of DUB module*]. *Drosophila* SAGA (dSAGA) contains orthologs of all *S. cerevisiae* SAGA subunits with
148 the exception of Spt8. In fact, no ortholog of the *Spt8* gene is present in the genome of any metazoan
149 organism [39]. A variant of SAGA, termed SLIK/SALSA, has been purified in *S. cerevisiae*, and
150 contains a C-terminal truncated version of Spt7 and lacks Spt8 [30]. Although dSAGA, like
151 SLIK/SALSA, lacks Spt8, the *in vivo* existence of SLIK/SALSA remains controversial because Spt7 can
152 be cleaved at its C-terminus by the Pep4 protease *in vitro*, resulting in removal of the Spt8-binding
153 domain and subsequent loss of Spt8 [40]. Moreover, the C-terminal domain that is absent from the
154 SLIK/SALSA Spt7 variant is conserved in metazoan Spt7 [41,42], while the N-terminal bromodomain
155 appears to be unique to *S. cerevisiae* Spt7 [43]. Below, we outline the composition of each module of
156 dSAGA, and describe the subunits that differ from their *S. cerevisiae* counterparts.

157 Although some dSAGA subunits could be identified by sequence similarity with their *S. cerevisiae*
158 SAGA counterparts, mass spectrometry of affinity-purified SAGA complexes revealed incorporation of
159 novel subunits that were not predicted by sequence comparisons with the *S. cerevisiae* SAGA
160 components. The HAT module in dSAGA contains Gcn5, Ada3, and Sgf29, which are shared between
161 all of the *Drosophila* Gcn5 complexes (Figure 3) [24–26,33,34]. Although initially both splice isoforms of
162 Ada2b were presumed to be part of the SAGA complex, mass spectrometry of affinity-purified SAGA
163 complexes demonstrated that only the longer Ada2b-PB splice isoform is part of the dSAGA HAT
164 module [31,34]. Orthologs of all four DUB module subunits are also present in flies. The histone

165 deubiquitinase Ubp8 in *S. cerevisiae* corresponds to Nonstop in flies (FBgn0013717, CG4166), which
166 was originally named for the axon targeting defect observed in *nonstop* mutants during neuronal
167 development [44]. Both Nonstop and Sgf11 (FBgn0036804, CG13379) are necessary for
168 deubiquitination of H2Bub1 in flies [45]. The last two DUB module subunits in flies are Ataxin 7
169 (FBgn0031420, CG9866, the ySgf73 ortholog) and E(y)2 (FBgn0000617, CG6474, the ySus1 ortholog)
170 (Table 1) [46–48]. In flies, like *S. cerevisiae*, several of the structural core subunits are shared between
171 the transcription coactivator complex Transcription Factor II D (TFIID) and SAGA, namely E(y)1 (Taf9,
172 FBgn0000617, CG6474), Taf10b (FBgn0026324, CG3069), and Taf12 (FBgn0011290, CG17358)
173 [31,39]. However, other structural core subunits are unique to dSAGA and are not present in *Drosophila*
174 TFIID. For example, the TAF5-like Wda (will decrease acetylation, FBgn0039067, CG4448), and TAF6-
175 like Saf6 (SAGA factor-like TAF6, FBgn0031281, CG3883), are specialized TAF paralogs that are
176 present in SAGA but not in TFIID [31,49]. Similar specialization of TAF proteins has occurred in other
177 metazoan organisms with incorporation of Taf5-like and Taf6-like subunits in mammalian SAGA [39]
178 *[see Timmers article within special issue for more discussion on TAF subunits shared by SAGA and*
179 *TFIID].* Other SAGA subunits in flies are much more conserved, although with considerable variation in
180 some dSAGA subunits at the sequence level compared to their *S. cerevisiae* counterparts. For
181 example, although Tra1 (Nipped-A, FBgn0053554, CG33554), Ada1 (FBgn0051866, CG31866), Spt3
182 (FBgn0037981, CG3169), and Spt7 (FBgn0030874, CG6506) were readily identified by sequence
183 comparison with *S. cerevisiae* [49], Spt20 (FBgn0036374, CG17689) was not identified in flies until
184 mass spectrometry of purified SAGA revealed the presence of this subunit [31]. Tra1/Nipped-A is the
185 largest SAGA subunit (411 kDa in flies) and is shared with another transcriptional coactivator complex,
186 the Tat interactive complex 60 kDa (TIP60; also known as the Nucleosome Acetyltransferase of H4
187 (NuA4) complex), which also possesses HAT activity [39,50]. In contrast to the SAGA HAT module,
188 which preferentially acetylates histone H3, TIP60/NuA4 acetylates histone H4 and H2A.Z [51,52]. Last,
189 SAGA contains two spliceosomal proteins, Sf3b3 (FBgn0035162, CG13900) and Sf3b5 (FBgn0040534,
190 CG11985), that are not present in *S. cerevisiae* SAGA despite the existence of *S. cerevisiae* homologs
191 corresponding to these proteins (Rse1 and Ysf3) [53]. Sf3b3 and Sf3b5 are shared with the Sf3b
192 complex, a component of the U2 small nuclear ribonucleoprotein (snRNP), which recognizes the
193 branch point sequence to facilitate spliceosome assembly [54,55]. Both of these spliceosomal proteins
194 are also present in hSAGA (Table 1) [39,41,56]. Overall, *Drosophila* SAGA resembles the human
195 SAGA complex more closely than either of the *S. cerevisiae* SAGA or SLIK/SALSA complexes,
196 possessing similar specialized Taf-like proteins and containing the two additional spliceosomal proteins.
197 The presence of these additional subunits in the metazoan SAGA complex suggests that SAGA may
198 have gained more specialized roles in gene expression in animals compared to *S. cerevisiae*.

199 Several recent studies have investigated the structure of SAGA, and have provided insight into how
200 each SAGA subunit integrates into the complex as a whole. These studies are described in more depth
201 in another article in this Special Issue by Tora, but are briefly described here to provide context for
202 understanding the organization of *Drosophila* SAGA *[cite Tora article within special issue*]. In *S.*
203 *cerevisiae*, Cryogenic Electron Microscopy (cryoEM) data revealed a existence of a central module
204 containing the structural core and the TBP binding module subunits that forms flexible connections to
205 the HAT and DUB modules, while the large Tra1 subunit exists as a separate module that can bind the
206 activation domain of transcription factors [57–60]. In *S. cerevisiae*, the HAT module is anchored to
207 SAGA by Ada3 binding to Taf6, and HAT module subunits are lost from SAGA when it was purified
208 from Ada3 or Ada2 mutant *S. cerevisiae* [23,58,61]. Similarly, Sgf73 (Ataxin 7) anchors the DUB
209 module to the *S. cerevisiae* SAGA complex, and DUB subunits are lost from SAGA purified from Sgf73
210 mutant *S. cerevisiae* [62,63]. The DUB module requires Sgf73 for activity in *S. cerevisiae* [64], but in
211 flies and plants, an enzymatically active DUB module can exist in the absence of the Sgf73 ortholog
212 Ataxin 7 [47,65]. Notably, there is no ortholog of Sgf73/Ataxin 7 in *Arabidopsis*, suggesting that the
213 DUB module may function independent of SAGA as the major H2Bub1 deubiquitinase [65] [*cite*
214 *Barneche article within special issue*]. In human cells, the protease Caspase 7 has been shown to
215 cleave ATXN7, which could potentially release a free DUB module from SAGA [66]. This mechanism
216 may also exist in *Drosophila*, although it has not yet been demonstrated. Thus, an open question
217 remains as to whether the biological functions attributed to the DUB module subunit in flies (see section
218 6) are due to its role in SAGA or represent its independent activity These questions are discussed
219 further by Mohan et al. in this Special Issue [*cite Mohan article within special issue*].

220 **3.2 ADA**

221 Recently, the Workman group have also identified an ADA-like complex in flies [33]. In S. *cerevisiae*,
222 ADA contains the HAT module and two additional proteins, ADA HAT component 1 and 2 (Ahc1 and
223 Ahc2) [23,29]. Early biochemical studies suggested that an ADA complex might also exist in flies
224 because a small Ada2b-containing complex was detected by glycerol gradients of Ada2b-containing
225 complexes [26]. Indeed, recently Soffers *et al*. showed that there is an ADA complex in flies, which like
226 SAGA contains the Ada2b-PB splice isoform [33]. In contrast to *S. cerevisiae* ADA, the *Drosophila* ADA
227 complex does not contain subunits corresponding to *S. cerevisiae* Ahc1/2, which do not have sequence
228 homologs in flies or humans (Table 2) [33]. Thus, the ADA complex in flies does not possess any
229 unique subunits that can be used to genetically distinguish it from SAGA.

230 **3.3 ATAC**

231 In addition to SAGA and ADA, flies also have an additional 820 kDa multi-subunit Gcn5 complex that is
232 nucleated by the Ada2 paralog Ada2a: ATAC. Size-exclusion chromatography of the *Drosophila* Gcn5
233 complexes provided an early hint that Ada2a and Ada2b resided in distinct complexes [25,26]. Indeed,
234 three years after the identification of the Ada2a paralog, the 13 subunit ATAC complex was first
235 characterized in flies, providing the foundation for studies on this Gcn5 complex in other organisms
236 [24]. ATAC shares the core HAT module subunits (Gcn5, Ada3, and Sgf29) with SAGA. In addition to
237 the HAT module subunits, nine ATAC-specific subunits exist in flies. Six of these ATAC subunits are
238 also present in the mammalian ATAC complex: Atac1 (FBgn0031876, CG9200, human ZZZ3 ortholog),
239 Atac2 (FBgn0032691, CG10414, the human CRBP2 ortholog), D12 (FBgn0027490, CG13400, the
240 human YEATS2 ortholog), Mocs2B (FBgn0039280, CG10238, equivalent to both the human hMoaE
241 and hMBIP proteins), NC2β (FBgn0028926, CG4185, the human NC2B ortholog), and Wds
242 (FBgn0040066, CG17437, the human WDR5 ortholog) (Table 3) [67,68]. The human ortholog of Chrac-
243 14 (FBgn0043002, CG13399) has been detected in some human ATAC purifications [69], but was
244 absent from others [70]. In contrast, two of the *Drosophila* ATAC subunits, Atac3 (FBgn0052343,
245 CG32343) and Hcf (FBgn0039904, CG1710), appear to be specific to the fly ATAC complex and have
246 not been detected in human ATAC [24,69,71]. Like SAGA, *Drosophila* ATAC contains a second histone
247 modifying activity. The Atac2 subunit of ATAC contains a HAT domain, and *Drosophila* Atac2
248 possesses HAT activity toward histone H4 and H2A *in vitro* and *in vivo* [68]. However, the human
249 counterpart for Atac2 (CRBP2) does not possess detectable HAT activity toward histone H4,
250 suggesting that Gcn5 is the only active HAT within the human ATAC complex [70,71]. Thus,
251 *Drosophila* ATAC contains two distinct acetyltransferase enzymes: Gcn5 and Atac2 [68]. Less is known
252 about the modular organization and structure of the ATAC complex compared with SAGA. However,
253 ATAC contains several histone-fold domain proteins, NC2β, D12 and Chrac-14, which may play a
254 structural role in ATAC similar to that involving the structural core subunits in SAGA. While Chrac-14
255 and NC2β fail to form heterodimers, human YEATS2 (the *Drosophila* D12 ortholog) and NC2β interact
256 via their histone-fold domains [68,69]. In addition, both *Drosophila* Chrac-14 and NC2β have the ability
257 to form homodimers [68]. Wds also contains seven WD repeats, and this motif is often involved in
258 protein–protein interactions (Figure 3). In humans, YEATS2 (the *Drosophila* D12 ortholog) and Atac2
259 play a role in the integrity of the ATAC complex [69,70], suggesting that these subunits, together with
260 Wds, Chrac-14 and NC2β, may play a central role in structural organization within the ATAC complex.
261 Like SAGA, several ATAC subunits are shared with other chromatin modifying complexes. For example
262 Chrac-14, Hcf, and Wds are also subunits of the COMPASS-like methyltransferase complexes, which
263 are responsible for the bulk of di- and tri-methylation at histone H3K4 in *Drosophila* [72].

264 **3.4 CHAT**

265 Last, *Drosophila* possess a unique Gcn5 complex that appears to be specific to insects: the Chiffon
266 Histone Acetyltransferase (CHAT) complex. Whereas the Ada2b-PB splice isoform is present in SAGA,
267 the shorter Ada2b-PA splice isoform is not part of the SAGA, ADA or ATAC complexes [31]. Instead,
268 Ada2b-PA nucleates formation of a fourth Gcn5 complex in flies that contains the shared HAT module
269 subunits (Gcn5, Ada3, and Sgf29) together with a fifth protein, Chiffon (FBgn0000307, CG5813) (Table
270 4, Figure 3) [34]. Chiffon is the *Drosophila* homolog of Dbf4, which binds and activates the Cdc7 kinase,
271 forming the Dbf4-dependent kinase (DDK) complex [73]. DDK phosphorylates the Mcm2-7 helicase,
272 activating the initial step in DNA replication [74–76]. In contrast to SAGA and ATAC, the CHAT complex
273 is unlikely to exist in *S. cerevisiae* or humans, because Dbf4 does not co-immunoprecipitate with Gcn5
274 in either of these organisms [34]. Moreover, Chiffon interacts with directly with Gcn5 via its C-terminal
275 domain, and this region of the protein is not conserved outside of insects [34].

276 **4. Substrate specificity of the Gcn5 complexes**

277 In general, the *Drosophila* Gcn5 complexes preferentially acetylate histone H3 *in vitro* and *in vivo*
278 exhibiting the highest activity on K9 and K14 of both recombinant histone H3 peptides and nucleosomal
279 substrates [24,33,34,77]. Although SAGA, ADA, and CHAT show this characteristic HAT activity toward
280 histone H3, the presence of the second HAT in *Drosophila* ATAC expands its activity toward both
281 histones H3 and H4 [24,68]. In fact, *Drosophila* ATAC shows strong specificity for histone H4 in
282 nucleosomal substrates *in vitro* [68]. Moreover, mutations in Atac2 result in reduced global levels of
283 acetylated H4K16 in fly embryos, and *ada2a* mutations decrease levels of acetylated H4K5, H4K12,
284 and H4K16 in polytene chromosomes [68,78,79]. Depletion of Atac2 or Gcn5 from *Drosophila* cells by
285 RNAi revealed that Gcn5 selectively acetylates histone H3, whereas Atac2 has a narrow but not
286 absolute substrate preference for lysines on both H3 and H4 [80]. Other HATs have been shown to
287 work together to deposit particular combinations of acetyl marks on chromatin; for example, CBP,
288 MGEA5, and NAA10 act together to acetylate H4 on both K5 and K8 [80]. Similarly, Atac2's preference
289 for different lysine residues on histones H3 and H4 was modulated by the pre-existing acetylation
290 pattern on those histones [80].These data suggest that both Gcn5 and Atac2 contribute to the
291 expanded HAT activity of the ATAC complex, which is likely influenced *in vivo* by the activity of other
292 HATs.

293 Gcn5 specificity may be altered by its interaction with each Ada2 paralog because rescue experiments
294 with hybrid Ada2 proteins showed that combining the unique C-terminal domain of Ada2a and Ada2b

295 with the N-terminal domain of the other Ada2 paralog was sufficient to rescue the respective mutants
296 and restore histone acetylation patterns [81]. Notably, the two Ada2b splice isoforms also only differ in
297 their C-terminal domains (Figure 2). Thus, the divergent C-terminal domains of the different Ada2
298 paralogs and splice isoforms in *Drosophila* likely contribute to both the formation of the different Gcn5
299 complexes and to the differences in HAT specificity of each complex.

300 In addition to histones, Gcn5 acetylates a number of non-histone targets in flies, which expand the
301 biological functions of the Gcn5 complexes. For example, *Drosophila* Gcn5 acetylates the chromatin
302 remodeling ATPase subunit Imitation SWI (Fbgn0011604, CG8625, Iswi) at K753 both *in vivo* and in
303 *vitro* [82]. This region in Iswi (747 – 756aa) is similar to the N-terminal domain of histone H3,
304 suggesting that Gcn5 may recognize Iswi in a similar fashion to histone H3 [82]. Iswi is part of two
305 nucleosome remodeling complexes in *Drosophila*: Nucleosome remodeling factor (NURF), and the
306 Chromatin accessibility (CHRAC) complex [83]. However, the acetylated form of Iswi is only found in
307 NURF, and is not present in the CHRAC complex [82]. Notably, as discussed in more detail in section
308 7, mutations in the NURF subunit *iswi* or the ATAC subunit *ada2a* show similar phenotypes, and there
309 is a genetic interaction between Ada2a and Iswi in flies [84]. These data suggest that in *Drosophila*,
310 ATAC might target Iswi as a substrate for acetylation by Gcn5, although this has not been tested. In
311 addition to Iswi, *Drosophila* Gcn5 has been shown to acetylate Transcription factor EB (TFEB;
312 FBgn0263112, CG43369), the ortholog of Mtif in flies [85]. Gcn5 acetylates K445 and K450 in Mtif,
313 inhibiting autophagy and lysosomal biogenesis [85]. *Drosophila* Gcn5 also acetylates the Cyclin A
314 associated protein Adenomatous polyposis coli 2, Apc2 (FBgn0026598, CG6193) [86]. Acetylation of
315 Apc2 promotes ubiquitination and degradation of Cyclin A, resulting in its turnover, which regulates the
316 maintenance (both self-renewal and differentiation) of *Drosophila* germline stem cells [86]. More details
317 about acetylation of non-histone substrates by Gcn5 across a variety of organisms including *Drosophila*
318 are described in this Special Issue by [*cite Downey article within special issue*].

319 **5. Gcn5 is essential for development in flies**

320 Although Gcn5 is not essential in for proliferation in *S. cerevisiae*, loss of one of the human Gcn5
321 paralogs, Gcn5 (KAT2A), results in embryonic lethality [87,88]. Thus, the Gcn5 complexes appear to
322 have an essential role in development in multicellular eukaryotes. To characterize the function of Gcn5
323 in *Drosophila*, Antoniewski and colleagues generated several different null *gcn5* alleles (Table 5). Loss
324 of *gcn5* blocks two critical stages in *Drosophila* development: oogenesis (egg development) and
325 metamorphosis. In flies lacking Gcn5, oogenesis is arrested at stage 5 and 6, and zygotic *gcn5* mutants
326 die during the late third instar larval stage (Figure 4) [16]. Moreover, adults with hypomorphic *gcn5*

327 alleles show malformation of appendages such as abnormal elongated metathoracic twisted legs, and
328 also exhibit a reduction in wing size and defects in wing-vein patterning, together with defects in cuticle
329 formation [16]. In addition, null *gcn5* mutants fail to form a puparium, one of the initial steps in
330 metamorphosis, potentially due to defects in expression of genes that respond to the insect hormone
331 ecdysone [16]. Notably, *gcn5* mutants also exhibit severely reduced imaginal discs, suggesting that
332 Gcn5 is required for cell proliferation in flies. Consistent with a potential role in cell proliferation, *gcn5*
333 mutant imaginal discs showed a higher number of cells in S-phase, significantly more cells undergoing
334 mitosis, and higher levels of apoptosis [16]. Mutations in another shared HAT module subunit, *ada3*,
335 result in similar phenotypes to those observed in *gcn5* mutants, with reduced size of imaginal discs and
336 defects in oogenesis [89]. The small imaginal discs in the *ada3* mutant led to the original name *diskette*
337 [89], although this gene has since been renamed *Ada3* on FlyBase*. ada3* mutants also exhibit
338 abnormal structure of polytene chromosomes; in particular showing changes in the banding pattern of
339 the male X chromosome [89].

340 The severe developmental defects in *gcn5* mutants are likely to result from the combinatorial loss of all
341 four *Drosophila* Gcn5 complexes. However, the identification and analysis of mutants that specifically
342 disrupt each of the four Gcn5 complexes in flies suggests that at least three of these Gcn5 complexes
343 are essential for development in flies. For example, mutations in *ada2a* or *ada2b* both result in
344 developmental lethality and oogenesis arrest (Figure 4) [27,77]. Further, mutations that disrupt the
345 SAGA-specific subunits *nonstop*, *sgf11*, *wda*, *taf10b*, and *saf6,* or the CHAT-specific *chiffon* and *ada2b-*
346 *PA* subunits, also result in larval lethality (Table 5, Figure 4) [31,34,45,49,90]. Thus, SAGA, ATAC, and
347 CHAT are essential for fly development. Unfortunately, ADA function cannot be separated genetically
348 from SAGA in flies because both complexes share the Ada2b-PB isoform, and ADA contains no unique
349 subunits in flies [33]. It should be noted that it remains unclear as to why mutations that disrupt different
350 subunits of Gcn5 complexes result in lethality at different developmental stages (Figure 4). Some
351 mutants may exhibit more severe defects and earlier lethality due to their function in complexes outside
352 the Gcn5 complexes, such as *sf3b5*, which is present in both SAGA and the U2 snRNP [55]. In
353 addition, there may be a different amount of maternally supplied gene product that allows some Gcn5
354 complex mutants to survive to a later developmental stage. Germline mutants in several SAGA-specific
355 mutants either fail to complete oogenesis, or cannot progress through embryogenesis (Figure 4),
356 supporting the idea that maternally supplied gene product is required for these zygotic mutants to
357 progress to a later stage in development. However, the level or stability of maternally supplied gene
358 product for different Gcn5 complex subunits has not been examined in flies. Overall, the
359 characterization of mutants that specifically disrupt SAGA, ATAC, or CHAT provides some insight into

360 the different roles of these complexes, and we outline specific biological functions of each complex in
361 the following sections beginning with SAGA.

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

363 SAGA promotes transcription through both its catalytic activities and via interactions with the
364 transcription machinery [91]. In *Drosophila*, SAGA colocalizes extensively with RNA polymerase II (Pol
365 II) and is present at the both the promoter-proximal pause site of lowly expressed or highly regulated
366 genes, and on the gene body of actively transcribed genes [92,93]. Although SAGA colocalizes with Pol
367 II at most actively transcribed genes, gene expression profiling studies of SAGA mutants originally
368 suggested that different SAGA modules might be required for transcription of particular subsets of
369 genes [94]. For example, only a subset of the genes bound by SAGA in embryonic muscle were
370 downregulated in *sgf11* mutants, and these genes showed enriched expression in muscle and functions
371 related to muscle development, suggesting a potential role for the SAGA DUB module in expression of
372 tissue-specific genes [92–94]. However, in human cells SAGA acetylates histone H3K9 and
373 deubiquitinates H2Bub1 on all expressed genes [95], suggesting a much broader role in regulating
374 transcription. This broader role in transcription is consistent with the extensive colocalization of SAGA
375 with Pol II in flies and in human cells [93,95]. Since many of the early gene expression studies on
376 *Drosophila* mutants used microarray analysis approaches that may not have been able to detect global
377 changes in transcription (Table 6), it is possible that a much larger group of genes requires SAGA for
378 proper expression in flies. In addition, studies in *S. cerevisiae* suggest that global changes in
379 transcription can be buffered by changes in mRNA stability [96–98], and most gene expression studies
380 in flies have examined steady-state mRNA levels. Thus, the genes identified in the expression profiling
381 experiments in SAGA mutants may represent those subsets of genes that are most sensitive to loss of
382 particular SAGA activities.

383 Despite the caveat that the gene expression profiling of SAGA mutants in flies may underestimate the
384 number of genes regulated by SAGA, these studies have provided important insight into key
385 developmental processes that require SAGA. Importantly, mutants that disrupt different modules of
386 SAGA show different effects on gene expression, and exhibit specific developmental phenotypes. For
387 example, mutations in *ada2b* disrupt oogenesis, whereas oogenesis progresses normally in *ataxin 7* or
388 *nonstop* mutants [92]. Moreover, genes involved in DNA replication, eggshell formation, and
389 chromosome organization were significantly downregulated in *ada2b* oocytes, but did not change in
390 *ataxin 7* or *nonstop* mutants (Table 6) [92]. While the early zygotic genes are expressed properly in
391 embryos that lack the maternal contribution for *ataxin 7* and *nonstop*, these embryos show later defects

in cellularization and nuclear anchoring [92], 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 [31,34]; 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 (Figure 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 (Figure 4), these mutants show characteristic defects in eye development in the late larval stage just prior to their death [44,45,99]. During the third larval instar, photoreceptor neurons in the developing eye imaginal disc project their axons to specific regions of the developing brain [99]. The SAGA subunit *nonstop* was first identified in a screen for genes involved in this photoreceptor axon targeting process [99]. 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 [44]. This axon targeting defect is caused by loss of *nonstop* or *sgf11* within the glial cells that mark the target layer in the lamina [44]. Transcriptome profiling of these glial cells from *nonstop* and *sgf11* larval brains identified genes involved in axon guidance (Table 6) [100]. 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 [100]. 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 [45,100]. However, in flies the DUB module can bind to chromatin independently of SAGA's HAT or structural core subunits [92], and loss of *ataxin 7* results in decreased H2Bub1 levels due to promiscuous binding of the DUB module [47]. Genes involved in locomotion, organ morphogenesis, and eye and neuronal development were highly regulated by the DUB module [92], 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 [91]. In *S. cerevisiae*, Tra1 recruits SAGA to promoters through interactions with transcription factors [101], allowing Spt3 and Spt8 to interact directly with component of the transcription machinery such as TBP [102]. In flies, mutations in the structural core

425 subunit *Saf6* result in defective expression of SAGA-regulated genes without altering global levels of
426 acetylated histone H3 or H2Bub1 [31]. Likewise, mutations in the *sf3b5* spliceosomal SAGA subunit
427 result in decreased expression of SAGA-regulated genes independent of changes in histone acetylation
428 [53]. Analysis of the relative levels of spliced and unspliced transcripts for genes that are
429 downregulated in *sf3b5* mutants shows that the decreased mRNA levels in *sf3b5* mutants are not
430 necessarily due to changes in splicing efficiency [53]. However, unlike other SAGA mutants, *sf3b5* is
431 required for cell viability in flies, most likely due to its role as part of the U2 snRNP [53,55]. It is unclear
432 how Sf3b5 regulates gene expression as part of SAGA, although it is possible that it may mediate
433 transient interactions between the transcriptional and splicing machinery, which share a common
434 spatial and temporal distribution during the coupled processes of transcription and splicing [93,103]
435 [*see article by Rodriguez-Navarro within special issue for more discussion of moonlighting proteins in*
436 *SAGA*]. Together, these studies suggest that SAGA plays a fundamental role in fly development
437 because it regulates the expression of genes that are required for processes such as oogenesis,
438 metamorphosis, and neuronal development. However, fundamental questions remain as to whether the
439 distinct roles of SAGA in particular developmental processes result from independent activity of
440 particular modules or subunits. In addition, it is unclear as to whether SAGA has overlapping or distinct
441 roles with the ADA and CHAT complexes that are also disrupted in *ada2b* mutants. *Drosophila* SAGA
442 may also regulate a broader set of genes than indicated by past gene expression studies that have
443 profiled steady-state mRNA levels and have not been able to detect global changes in active
444 transcription. In *S. cerevisiae*, data suggests that SAGA regulates expression of all genes [95,98], while
445 in human cells, SAGA deubiquitinates H2Bub1 on the transcribed region of all expressed genes,
446 suggesting a widespread role in transcription regulation [95].

447 **7. ATAC is a double HAT complex required for development**

448 The ATAC complex is exclusive to multicellular eukaryotes, suggesting a potential function unique to
449 development in multicellular organisms. Mutations that disrupt subunits of ATAC show developmental
450 lethality during the larval or pupal stages (Table 5, Figure 4). For example, *ada2a* mutants die during
451 the pupal stage, and Ada2a is also essential for oogenesis [77]. In addition, mutant flies that lack *hcf*,
452 *wds*, *atac3*, and *atac2* die during either the larval or pupal stage of development (Table 5) [68,104–
453 106]. The developmental lethality of ATAC mutants may be due to defects in response to the insect
454 hormone ecdysone, which triggers molting during the larval instars, and is also required for the larval–
455 pupal transition at the onset of metamorphosis [107]. Both ecdysone levels and binding of its receptor
456 to polytene chromosomes are reduced in a*da2a a*nd *ada3* mutants [108]. Moreover, genes required for
457 ecdysone biosynthesis are misregulated in third instar larvae lacking Ada2a and Ada3 (Table 6) [108].

458 Thus, ATAC may be essential for viability in flies in part because it controls levels of hormones that
459 trigger formation of the adult fly.

460 Histone acetyltransferases often act synergistically with nucleosome remodeling complexes to regulate
461 chromatin structure and gene expression [109]. In flies, ATAC interacts genetically and biochemically
462 with the chromatin remodeling complex, NURF [84]. Mutations in the NURF subunit *iswi* or the ATAC
463 subunit *ada2a* show similar defects in eye development, with both mutants exhibiting small and rough
464 eyes [84]. In addition, ATAC and NURF coregulate expression of a subset of genes including
465 *Ultrabithorax* (*Ubx*), *engrailed* (*en*), and *heat-shock protein 70* (*hsp70*) [84]. Moreover, ATAC and
466 NURF are both necessary to maintain proper chromatin structure, particularly on the X chromosome in
467 male flies [84]. In flies, expression of genes on the single male X chromosome is doubled to equal that
468 from the two female X chromosomes in a process termed dosage compensation [110]. During this
469 process, the Males absent on the first (Mof) HAT within the Male Specific Lethal (MSL) complex
470 acetylates H4K16 on the male X chromosome [110]. Mutations in ATAC and NURF subunits such as
471 *ada2a, gcn5*, and *nurf301* show increased frequency of bloated X chromosomes in male flies [84],
472 suggesting that ATAC and NURF maintain proper chromosomal structure of the dosage compensated
473 male X chromosome. Although ATAC acetylates H4K16 [68], the bloated X chromosomes observed in
474 *ada2a* and *gcn5* mutant males show similar levels of acetylated H4K16 compared to their wild-type
475 counterparts [84]. Moreover, X-linked genes are not preferentially misregulated in *ada2a* or *gcn5*
476 mutants [84]. Thus, ATAC and NURF may work together to maintain the chromosomal structure of the
477 dosage compensated male X chromosome, rather than playing a specific role in expression of X-linked
478 genes [84]. Notably, H4K16 acetylation by Mof antagonizes activity of another related chromatin
479 remodeler, Iswi, in flies [111], and negatively regulates interactions between Iswi and its nucleosomal
480 substrate *in vitro* [112]. Thus, the MSL and ATAC complexes may function synergistically with the
481 related NURF and ISWI chromatin remodelers to maintain the structure, acetylation, and expression
482 levels of dosage compensated genes in *Drosophila*. It is possible that the cooperative activity between
483 ATAC and NURF could involve the direct acetylation of one of the NURF subunits, Iswi, by Gcn5 (see
484 Section 4) [82], although this remains to be tested.

485 *Drosophila* ATAC contains three histone-fold domain proteins, D12, Chrac-14 and NC2β, leading to the
486 question as to whether ATAC itself possessed nucleosome remodeling activity because histone-fold
487 domains can bind DNA [113], and Chrac-14, as part of the CHRAC complex, facilitates nucleosome
488 sliding [114]. In addition, the human ortholog of Chrac-14, Chrac-17, enhances nucleosome sliding by
489 the Iswi complex [115]. Purified ATAC does not show remodeling activity by itself on nucleosomal
490 substrates *in vitro* [68]. However, ATAC can stimulate nucleosome sliding by the chromatin remodelers

491 Iswi or SWItch/Sucrose Non-Fermentable (SWI-SNF) *in vitro* [68]. Similarly, recombinant Chrac-14 or
492 NC2β also stimulated nucleosome remodeling by SWI/SNF [68], suggesting that the histone-fold
493 domain proteins in ATAC contributes to its impact on chromatin remodeling. Notably, the inclusion of
494 acetyl-CoA in these *in vitro* nucleosome sliding assays enhanced the effect of ATAC, suggesting that
495 the HAT activity of ATAC also contributes to stimulation of chromatin remodeling by complexes such as
496 Iswi or SWI-SNF [68].

497 In addition to its roles in chromosome structure and interaction with chromatin remodelers, ATAC has
498 been implicated in cell proliferation. Mutations in *gcn5* and *ada3* are associated with reduced size of
499 imaginal discs, which are a highly proliferative tissue, and *gcn5* mutants also show an increased
500 number of cells in S phase [16,89]. However, since Gcn5 and Ada3 are core components of all the
501 Gcn5 complexes in flies, it was not clear whether all or only some of these Gcn5 complexes had roles
502 in cell proliferation. Studies in mammalian cells suggest that ATAC is likely to be responsible for the
503 defects in cell proliferation in *gcn5* and *ada3* mutants due to its role in progression through the G2/M
504 phase of the cell cycle [70]. Knockdown of Atac2 in mouse cells and studies using an *Atac2* knockout
505 mouse model showed that loss of Atac2 results in an increase in the number of apoptotic cells and in
506 an accumulation of cells in G2/M [70]. In addition, Ada2a and Ada3 RNAi knockdown in mouse NIH3T3
507 cells leads to mitotic abnormalities such as centrosome multiplication and defective midbody formation,
508 and ATAC subunits such as Ada2a and Yeats2 localize to the mitotic spindle [116]. Interestingly, SAGA
509 does not appear to share this role in mitosis because deletion of Spt20 does not cause mitotic
510 abnormalities, and Spt20 does not localize to chromatin during mitosis [116]. Although ATAC acetylates
511 H4K16, loss of Ada2a and Ada3 results in the opposite acetylation phenotype in mitotic cells with
512 knockdown cells showing an increase in acetylated H3K14 levels due to an decrease in the activity of
513 the histone deacetylase Sirtuin 2 (SIRT2) [116]. While a role for *Drosophila* ATAC in mitosis has not yet
514 been characterized, it is possible that ATAC shares this function in flies and may be responsible for the
515 decreased cell proliferation observed in *gcn5* and *ada3* mutants.

516 Last, ATAC has been implicated in controlling the expression of genes in stress-induced signaling
517 pathways. Gcn5 complexes have a well characterized role in stress response signaling mediated by
518 mitogen-activated protein kinases (MAPK) [32]. Osmotic stress can activate MAPK cascades, resulting
519 in eventual activation of the c-Jun-NH2-terminal kinase (JNK) [117]. In *Drosophila* S2 cells, sorbitol
520 treatment induces osmotic stress and results in JNK activation [67]. Importantly, ATAC directly interacts
521 with MAPKs via its MBIP/Mocs2B subunits in both humans and flies [67,118]. Moreover, JNK activation
522 in response to osmotic stress is inhibited by the expression of the ATAC subunit Mocs2B in *Drosophila*
523 S2 cells, and ATAC is required for the transcription of JNK target genes such as *chickadee* in these

524 cells [67]. Thus, ATAC appears to directly interact with MAPK signaling proteins to mediate induction of
525 stress response genes in flies, likely through its Mocs2B subunit. This role in stress response for the
526 ATAC complex is reminiscent of *S. cerevisiae* SAGA's function in the endoplasmic reticulum (ER)
527 stress pathway [119]. In mammals, knockdown of the shared SAGA and ATAC subunit Sgf29 results in
528 impaired transcription of ER stress genes, such as *GRP78* [120]. The ER stress response transcription
529 factor ATF6 recruits both SAGA and ATAC to ER stress response genes [121], suggesting that both
530 SAGA and ATAC are involved in induction of stress response genes in metazoan organisms. Analysis
531 of SAGA and ATAC localization on *Drosophila* polytene chromosomes suggest that these Gcn5
532 complexes regulate distinct sets of stress response genes, depending on the type of stress involved
533 [71]. For example, induction of phorbol ester-induced protein kinase C (PKC) pathway genes increased
534 colocalization of ATAC and Pol II without affecting SAGA [71], arguing for a specific role of ATAC in
535 induction of PKC genes in response to stress.

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

538 Whereas the other Gcn5 complexes identified in *Drosophila* are also present in *S. cerevisiae* or
539 humans, the CHAT complex appears to be specific to insects and has an unknown biological function.
540 In addition to the HAT module subunits (Gcn5, Ada3, and Sgf29), CHAT contains the short Ada2b-PA
541 splice isoform and Chiffon, the *Drosophila* ortholog of Dbf4. Chiffon, like other Dbf4 orthologs, binds
542 and activates the cell cycle kinase Cdc7 forming the Dbf4-dependent kinase complex (DDK)
543 [76,122,123]. The DDK complex phosphorylates the Mcm2-7 helicase, activating it to unwind DNA at
544 origins of replication, thus initiating DNA replication [75,76]. Although Dbf4 is highly conserved and is
545 present in most eukaryotes except for plants, Chiffon contains a long C-terminal extension that is
546 specific to insects (Figure 5) [34,73]. The conserved N-terminal domain of Chiffon (1- 400aa) binds and
547 activates Cdc7, while the insect-specific C-terminal domain of Chiffon (401 - 1695aa) is necessary and
548 sufficient to bind Gcn5 and nucleate CHAT formation [34]. Dbf4 is an essential gene in *S. cerevisiae*
549 because of its role in DNA replication, but surprisingly, *chiffon* mutants were originally reported to be
550 viable in *Drosophila* [122]. The *chiffon* gene was first identified in a screen for female sterile mutants,
551 and *chiffon* females lay eggs with a thin and fragile chorion (eggshell) that resembles the fabric of the
552 same name [123]. More recent analysis has shown that indeed, the Cdc7-binding domain of Chiffon is
553 dispensable for fly viability, but surprisingly, the Gcn5-binding domain of *chiffon* is essential for
554 development [34]. In fact, *chiffon* alleles that contain premature stop codons either within, or directly
555 after, the N-terminal Cdc7-binding domain (separating both N- and C- polypeptides) are viable because
556 they still produce a C-terminal product that binds Gcn5 and nucleates CHAT formation [34]. Both

557 domains are encoded by a single large exon in the *chiffon* gene with no evidence of alternative splicing,
558 suggesting that alternative translation start sites and/or proteolytic cleavage may be required to
559 produce these two independent Chiffon polypeptides. These data suggest that *chiffon* could be a
560 dicistronic gene that can independently express two distinct polypeptides that contain either the Cdc7-
561 or Gcn5-binding domains, resulting in DDK or CHAT formation, respectively. It remains unclear as to
562 whether the N- and C-terminal Chiffon polypeptides are expressed at the same time, and little is known
563 about how this process is controlled *in vivo*. The unusual *chiffon* gene structure is somewhat
564 reminiscent of the *ada2a* gene, which also encodes two polypeptides with distinct functions: Ada2a and
565 one of the subunits of RNA polymerase II, Rpb4, in flies and in other insects due to alternative splicing
566 [26].

567 The CHAT complex exhibits *in vitro* and *in vivo* HAT activity toward histone H3, similar to SAGA and
568 ADA [34]. Analysis of histone acetylation levels in somatic mosaics for *chiffon* null alleles showed that
569 loss of Chiffon decreases levels of histone H3 acetylated at K9, K14, and K18, but not K23 [34].
570 Although histone acetylation correlates with, and contributes to a specialized form of DNA re-replication
571 in follicle cells termed gene amplification [124], CHAT-mediated histone acetylation is not required for
572 this type of DNA replication [34]. In *chiffon* mutant cells that lack only its N-terminal Cdc7-binding
573 domain, ovary follicle cells lack the characteristic bromodeoxyuridine (BrDU) foci indicative of chorion
574 gene amplification [34]. However, these DDK-deficient mutant cells retain wild-type histone acetylation
575 levels. In contrast, *chiffon* mutants that lack only its C-terminal domain that binds Gcn5 show decreased
576 histone acetylation, but do not exhibit loss of the characteristic BrDU foci indicative of chorion gene
577 amplification [34]. Similarly, *ada2b* mutant follicle cells show decreased histone acetylation but retain
578 wild-type BrDU incorporation [34]. Together, these data suggest that despite the presence of the Dbf4
579 ortholog Chiffon, the CHAT complex is not required for DNA replication in flies [34]. What then could be
580 the role of the CHAT complex in insects? Currently, CHAT, like SAGA, seems to be essential for both
581 histone H3 acetylation and for development in flies. *chiffon* mutants show decreased histone H3
582 acetylation not only in ovary follicle cells, but also in other tissues such as imaginal discs [34].
583 Moreover, the decreased acetylation at histone H3K14 in *chiffon* mutant cells is similar to that observed
584 in *ada2b* mutants, which lack both the CHAT and SAGA isoforms [34]. Since mutations in the SAGA-
585 specific subunit, *wda*, also reduce acetylation at histone H3K9 in embryos [49], both SAGA and CHAT
586 likely contribute to H3 acetylation in flies. However, expression of the CHAT-specific Ada2b-PA isoform,
587 but not the SAGA/ADA-specific Ada2b-PB isoform, is sufficient to almost fully restore viability to *ada2b*
588 mutants [34,125]. These data suggest that either CHAT might compensate for some of SAGA's
589 essential functions during development, or that the Ada2b-PA splice isoform can incorporate into SAGA

if Ada2b-PB is absent [34]. 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 (Figure 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 (Figure 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 (Figure 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 (Figure 5C) [34,73,122]. 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 [24,33,34]. 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. The finding that alternative splicing of *ada2b* can generate new diversity in HAT complexes [33,34] 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. In light of the fairly recent finding that *Drosophila* possess 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

622 patterns and gene expression profiling will require careful selection of subunits, and should utilize
623 spike-in control approaches that can identify potential global changes in gene expression [126].

624 Over the past 20 years following the identification of Gcn5 in *Drosophila* [13], much insight has been
625 obtained into the structure and function of SAGA from studies in yeast, flies, humans and plants. We
626 refer the reader to the article by Brian Strahl and Scott Briggs in this Special issue [*cite Strahl and*
627 *Briggs article within special issue*] for an in-depth discussion of SAGA's function in transcription, and an
628 outline of key unanswered questions that remain about its function. The exciting new cryo-EM studies
629 of *S. cerevisiae* SAGA illustrate how the different modular parts of the complex function as a whole
630 [57,58,127] [*reviewed within special issue by Tora*], and we look forward to seeing these same
631 approaches applied to the metazoan SAGA and ATAC complexes to elucidate the architectural
632 organization of both complexes. Such studies will provide insight into the similarity and differences
633 between SAGA and ATAC, and show for example, how the two HATs in ATAC might modify histones
634 within the same nucleosome, and how the spliceosomal proteins in metazoan SAGA integrate into the
635 complex. These studies, coupled with functional analysis in model systems such as flies, may help us
636 to understand why the metazoan Gcn5 complexes have diverged in composition from yeast and plants.
637 Plants, like yeast, lack the ATAC complex and do not have the Sf3b3 and Sf3b5 spliceosomal subunits
638 of SAGA [*see Article by Barneche et al. in this Special Issue*]. What, then, is the unique role that the
639 ATAC complex plays in metazoan? Why does metazoan SAGA contain the spliceosomal subunits, and
640 what is their function in the complex?

641 Insects offer a number of advantages over mammalian models to answer these key questions because
642 of their short generation time, and wealth of genetic resources. In addition, since the *Drosophila* SAGA
643 and ATAC complexes largely resemble their mammalian counterparts in terms of composition, flies
644 provide a strong model for the metazoan-specific functions of the Gcn5 complex. *Drosophila* also
645 provide an appropriate biological model to ask questions about Gcn5 complexes that are relevant to
646 human disease. For instance, the neurodegenerative disease Spinocerebellar ataxia type 7 (SCA7)
647 results from polyglutamine expansions in the gene encoding the DUB subunit Ataxin 7 [128,129]. Flies
648 have been used as a model for SCA7 [130,131], and other polyglutamine related neurogenerative
649 diseases such as SCA2 [132]. In humans, SCA7 disease manifests retinal and cerebellar degeneration,
650 and macular dystrophy causing blindness [129]. In *Drosophila*, loss of Ataxin 7 causes neural and
651 retinal degeneration, and impaired movement [47]. Interestingly, similar phenotypes are observed when
652 exogenous polyglutamine-expanded human Ataxin 7 is expressed in *Drosophila* [47]. Thus, *Drosophila*
653 provides a good model organism to study the mechanism of diseases such as SCA7 and could be used
654 to screen compounds suitable for ameliorating symptoms of this neurodegenerative disease [130,133].

655 Last, the finding that alternative splicing of *ada2b* can generate new diversity in HAT complexes [33,34]
656 suggests that there may be other Gcn5 complexes in multicellular organisms that remain to be
657 discovered. It is possible that other novel Gcn5 complexes, like CHAT, may be specific to particular
658 groups of species where they play more specialized roles in developmental processes. *Drosophila*
659 remains an outstanding model for studying function of the Gcn5 complexes, but recent advances in
660 technology allow us to consider examining alternative species outside of traditional model organisms.
661 Expanding the studies on Gcn5 complexes into non-traditional species, including potentially other
662 insects may provide insight into the specialized function of this quintessential HAT in multicellular
663 organisms.

664 **ACKNOWLEDGEMENTS**

665 We thank the National Science Foundation for their support under Award Number 1930237 to V.W.
666 This activity was also funded by Purdue University as part of AgSEED Crossroads funding to support
667 Indiana's Agriculture and Rural Development. E.T.Z. was supported, in part, by a fellowship from the
668 Purdue Research Foundation. We would also like to acknowledge Jerry Workman and Susan Abmayr
669 who have made major contributions to studies on the Gcn5 complexes in *Drosophila*, and whose
670 generosity and kindness have inspired a generation of researchers in chromatin biology.

671 **TABLES**

	FlyBase ID	Annotation Symbol	Gene name	Gene symbol	*S. cerevisiae ortholog*	*H. sapiens* ortholog	DNA/Histone domain/Enzymatic Activity
HAT module	FBgn0030891	CG7098	*transcriptional Adaptor 3 (diskette)*	*Ada3*	*ADA3*	*TADA3*	
	FBgn0020388	CG4107	*Gcn5 acetyltransferase (Pcaf)*	*Gcn5*	*GCN5*	*GCN5/PCAF (KAT2A/KAT2B)*	PCAF, GNAT domain, Bromodomain, Acetyltransferase (EC 2.3.1.48)
	FBgn0050390	CG30390	*SAGA-associated factor 29 kDa*	*Sgf29*	*SGF29*	*SGF29*	Tudor-like domain
	FBgn0037555	CG9638	*transcriptional Adaptor 2b*	*Ada2b (PB isoform)*	*ADA2*	*TADA2B*	Zinc finger ZZ-type, SANT Myb domain
DUB module	FBgn0013717	CG4166	*nonstop*	*Not*	*UBP8*	*USP22 (UBP22)*	Zinc finger-UBP-type, Ubiquitin protease
	FBgn0036804	CG13379	*SAGA associated factor 11 kDa*	*Sgf11*	*SGF11*	*ATXN7L3*	
	FBgn0031420	CG9866	*Ataxin 7*	*Atxn7*	*SGF73*	*ATXN7/ATXN7L1/ATXN7L2*	SCA7 domain
	FBgn0000618	CG15191	*enhancer of yellow 2*	*e(y)2*	*SUS1*	*ENY2*	
Core structural module	FBgn0039067	CG4448	*will decrease acetylation*	*Wda*	*TAF5*	*TAF5L*	WD40 domain
	FBgn0030874	CG6506	*Spt7*	*Spt7*	*SPT7*	*SUPT7L (STAF65G)*	Histone-fold domain
	FBgn0036374	CG17689	*Spt20*	*Spt20*	*SPT20*	*SUPT20H*	
	FBgn0051865	CG31865	*transcriptional Adaptor 1*	*Ada1*	*ADA1*	*TADA1*	Histone-fold domain
	FBgn0031281	CG3883	*SAGA factor-like TAF6*	*Saf6*	*TAF6*	*TAF6L*	Histone-fold domain
	FBgn0000617	CG6474	*enhancer of yellow 1*	*e(y)1*	*TAF9*	*TAF9/TAF9b*	Histone-fold domain
	FBgn0026324	CG3069	*TBP-associated factor 10b*	*Taf10b*	*TAF10*	*TAF10*	Histone-fold domain
	FBgn0011290	CG17358	*TBP-associated factor 12*	*Taf12*	*TAF12*	*TAF12*	Histone-fold domain
TBP binding	FBgn0037981	CG3169	*Spt3*	*Spt3*	*SPT3*	*SUPT3H*	Histone-fold domain
TF-binding module	FBgn0053554	CG33554	*Nipped-A*	*Nipped-A*	*TRA1*	*TRRAP*	PIK-related pseudokinase
Splicing module	FBgn0035162	CG13900	*Splicing factor 3b subunit 3*	*Sf3b3*	-	*SF3B3*	Cleavage/polyadenylation specificity factor
	FBgn0040534	CG11985	*Splicing factor 3b subunit 5*	*Sf3b5*	-	*SF3B5*	

672 **Table 1: *Drosophila* SAGA subunits.** The 20 *Drosophila* SAGA subunits can be organized into HAT,
673 DUB, Core Structural, TBP binding, TF-binding, and splicing modules. The FlyBase ID, Annotation
674 symbol (CG ID number), full gene name, and abbreviated gene symbol are shown for each *Drosophila*
675 subunit, together with the orthologs from *S. cerevisiae* and *H. sapiens* (if present). Paralogous subunits
676 are separated with a "/" sign. Alternative gene names are listed in parentheses. The protein domain and
677 enzymatic activity (E.C. number) are based on FlyBase definitions for each *Drosophila* subunit. Note

678 that Spt7 contains a bromodomain only in *S. cerevisiae*, but not in the metazoan orthologs. In addition,
679 Spt8 is only present in the *S. cerevisiae* SAGA complex and is not listed here.

680

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

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

687

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 (dMoaE, Mocs2)*	*MBIP*	Molybdopterin biosynthesis MoaE

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

693

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

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

	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.	[89]
	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.	[16,45]
SAGA/ADA/CHAT	*Ada2b (PA- and PB isoforms)*	*ada2b*^{1} *ada2b*^{2} *ada2b*^{842}	Null: 1077bp deletion Null: 2.77kb deletion Null: 800bp 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.	[27,34,45, 77]
SAGA	*Nonstop*	*not*^{2}	Null: 538bp deletion	Lethal - pupa	Increased H2Bub1; photoreceptor axon mistargeting during eye development.	[44,45]
	Sgf11	*sgf11*^{e01308}	Null: 5.97kb del etion	Lethal - late larva/early pupa	Increased H2Bub1; photoreceptor axon mistargeting during eye development.	[45,93]
	Ataxin 7	*ataxin 7*^{KG02020}	Null:	Lethal - late larva	Neural and retinal degeneration; reduced locomotion; cellularization defects.	[47,92]
	e(y)2	*e(y)2*^{1}	Null: 167bp deletion	Viable	Short stocky body and separated wings; eyes with altered facets; low fertility.	[46,110]
	Nipped-A	*nipped-A*^{NC186}	Missense: V885D	Lethal - early pupa	Defects in Notch signaling.	[134]
	wda	*wda*^{11} *wda*^{4} *wda*^{8}	Null: 1510bp deletion Null: 857bp deletion Null: 864bp deletion	Lethal - second instar larva	Decreased acetylation at H3K9.	[49]
	Saf6	*saf6*^{303}	Null: 303bp deletion	Lethal - second instar larvae		[31]
	e(y)1	*e(y)1*^{17} *e(y)1*^{190}	Null: 79bp deletion Null: 339bp deletion	Lethal - larva	Dysregulation of ovary follicle cell development.	[135]
	Taf10b	*taf10*^{d25}	Null: 900bp deletion	Lethal - pupae	Decreased acetylation at H3K14; defects in DNA repair efficiency.	[90,136]
	Sf3b5	*sf3b5*^{EY12579}	Transposable element insertion.	Lethal - second instar larva	Reduced cell viability in eyes.	[53]
ATAC	*Ada2a*	*ada2a*^{189}	Null: 720bp deletion	Lethal - pupa	Oogenesis arrested; altered structure of the polytene chromosomes; banding pattern is distorted.	[77]
	hcf	*hcf*^{HR1}	Null: 4348bp deletion	Lethal - pupa	Heterozygous females are Sterile; oogenesis arrested at stage 8; decreased pupae size.	[105]
	wds	*wds*^{G0251} *wds*^{j25}	Not specified	Lethal - larva	Defects in wristles and wing veins; heterozygous male and female are unfertile.	[104]
	Chrac-14	*chrac-14*^{KG01051}	Not specified	Viable	Eclosion defective; flight defective; radiation sensitive.	[137]
	Atac3	*atac3*^{GD4326}	RNAi	Lethal - pupa		[106]
	Atac2	*atac2*^{e03046}	Transposable element insertion.	Lethal - second instar larva	Decreased acetylation at H4K16.	[68]
CHAT	*Chiffon*	*chif*^{Dsred} *chif*^{ETEB3}	Null:5.3kb deletion Null: 6kb deletion	Lethal - third instar larva	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}.	[34,76,122]
		chif^{WF24}	Missense: T521C	Viable		

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.

704

Complex	Gene	Approach	# genes identified	Differentially expressed genes-pathways/processes	Reference
SAGA/ATAC/ ADA/CHAT	*Gcn5*	Microarray; third instar larvae	~284 genes	Morphogenesis.	[84]
	Ada3	Microarray; third instar larvae	~5565 genes	Cuticle formation and ecdysone response.	[108]
SAGA/ADA/CHAT	*Ada2b (PA & PB isoforms)*	RNA-seq; ovaries	>1000 genes	DNA replication, eggshell formation, chromosome organization, and DNA repair.	[92]
		Microarray; third instar larvae	~344 genes	Early ecdysone response genes: glue proteins.	[45]
		Microarray; third instar larvae	~580 genes	Ecdysone-induced genes, cuticle formation, and defense mechanisms.	[108,138]
SAGA	*Nonstop*	RNA-seq; embryos (stage 5)	>6000 genes	Cellularization, embryonic development, and tissue morphogenesis.	[92]
		Microarray; third instar larvae	~987 genes	Early ecdysone-response genes, puparial adhesion, eclosion, signal transduction, and central nervous system remodeling	[45]
		RNA-seq; third instar larvae glia	~1802 genes	Axon guidance, protein folding, cell morphogenesis, axon guidance, synaptic transmission.	[100]
	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.	[93]
		Microarray; third instar larvae	~618 genes	Early ecdysone response genes, puparial adhesion, eclosion, signal transduction, and central nervous system remodeling	[45]
		RNA-seq; third instar larvae glia	~1644 genes	Axon guidance, protein folding, cell morphogenesis, axon guidance synaptic transmission.	[100]
	Ataxin 7	RNA-seq; embryos (stage 5)	>6000 genes	Cellularization, embryonic development, and tissue morphogenesis.	[92]
ATAC	*Ada2a*	Microarrays; third instar larvae	~7306 genes	Cuticle formation and ecdysone pathway response.	[76]

705
706
707
708

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

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. 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] 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.

[7] 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.

[8] 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. https://doi.org/10.1016/j.cell.2014.09.002.

[9] 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.

[10] 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.

[11] 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.

[12] 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.

[13] 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.

[14] 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.

[15] 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.

[16] 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.

[17] 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.
[18] 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.
[19] 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. https://doi.org/10.1101/gad.11.13.1640.
[20] 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.
[21] 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.
[22] 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.
[23] 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.
[24] 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.
[25] 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.
[26] 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.
[27] 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.
[28] 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.
[29] 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.
[30] 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.
[31] 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

814 is required for SAGA-dependent gene expression, Genes Dev. 23 (2009) 2818–2823.
815 https://doi.org/10.1101/gad.1846409.
816 [32] G. Spedale, H.Th.M. Timmers, W.W.M.P. Pijnappel, ATAC-king the complexity of SAGA during
817 evolution, Genes Dev. 26 (2012) 527–541. https://doi.org/10.1101/gad.184705.111.
818 [33] J.H.M. Soffers, X. Li, A. Saraf, C.W. Seidel, L. Florens, M.P. Washburn, S.M. Abmayr, J.L.
819 Workman, Characterization of a metazoan ADA acetyltransferase complex, Nucleic Acids Res. 47
820 (2019) 3383–3394. https://doi.org/10.1093/nar/gkz042.
821 [34] E.F. Torres-Zelada, R.E. Stephenson, A. Alpsoy, B.D. Anderson, S.K. Swanson, L. Florens, E.C.
822 Dykhuizen, M.P. Washburn, V.M. Weake, The Drosophila Dbf4 ortholog Chiffon forms
823 a complex with Gcn5 that is necessary for histone acetylation and viability, J. Cell Sci. 132 (2019)
824 jcs214072. https://doi.org/10.1242/jcs.214072.
825 [35] F. Winston, D.T. Chaleff, B. Valent, G.R. Fink, Mutations affecting Ty-mediated expression of the
826 HIS4 gene of Saccharomyces cerevisiae., Genetics. 107 (1984) 179–197.
827 [36] D. Helmlinger, S. Marguerat, J. Villén, D.L. Swaney, S.P. Gygi, J. Bähler, F. Winston, Tra1 has
828 specific regulatory roles, rather than global functions, within the SAGA co-activator complex,
829 EMBO J. 30 (2011) 2843–2852. https://doi.org/10.1038/emboj.2011.181.
830 [37] J.A. Daniel, M.S. Torok, Z.-W. Sun, D. Schieltz, C.D. Allis, J.R. 3rd Yates, P.A. Grant,
831 Deubiquitination of histone H2B by a yeast acetyltransferase complex regulates transcription., J.
832 Biol. Chem. 279 (2004) 1867–1871. https://doi.org/10.1074/jbc.C300494200.
833 [38] K.W. Henry, A. Wyce, W.-S. Lo, L.J. Duggan, N.C.T. Emre, C.-F. Kao, L. Pillus, A. Shilatifard,
834 M.A. Osley, S.L. Berger, Transcriptional activation via sequential histone H2B ubiquitylation and
835 deubiquitylation, mediated by SAGA-associated Ubp8., Genes Dev. 17 (2003) 2648–2663.
836 https://doi.org/10.1101/gad.1144003.
837 [39] D. Helmlinger, L. Tora, Sharing the SAGA, Trends Biochem. Sci. 42 (2017) 850–861.
838 https://doi.org/10.1016/j.tibs.2017.09.001.
839 [40] G. Spedale, N. Mischerikow, A.J.R. Heck, H.T.M. Timmers, W.W.M.P. Pijnappel, Identification of
840 Pep4p as the protease responsible for formation of the, J. Biol. Chem. 285 (2010) 22793–22799.
841 https://doi.org/10.1074/jbc.M110.108787.
842 [41] E. Martinez, V.B. Palhan, A. Tjernberg, E.S. Lymar, A.M. Gamper, T.K. Kundu, B.T. Chait, R.G.
843 Roeder, Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts
844 with pre-mRNA splicing and DNA damage-binding factors in vivo, Mol. Cell. Biol. 21 (2001) 6782–
845 6795. https://doi.org/10.1128/MCB.21.20.6782-6795.2001.
846 [42] S.V. Antonova, J. Boeren, H.T.M. Timmers, B. Snel, Epigenetics and transcription regulation
847 during eukaryotic diversification: the saga of TFIID, Genes Dev. 33 (2019) 888–902.
848 https://doi.org/10.1101/gad.300475.117.
849 [43] M.A. Demény, E. Soutoglou, Z. Nagy, E. Scheer, À. Jànoshàzi, M. Richardot, M. Argentini, P.
850 Kessler, L. Tora, Identification of a Small TAF Complex and Its Role in the Assembly of TAF-
851 Containing Complexes, PLOS ONE. 2 (2007) e316. https://doi.org/10.1371/journal.pone.0000316.
852 [44] B. Poeck, S. Fischer, D. Gunning, S.L. Zipursky, I. Salecker, Glial Cells Mediate Target Layer
853 Selection of Retinal Axons in the Developing Visual System of Drosophila, Neuron. 29 (2001) 99–
854 113. https://doi.org/10.1016/S0896-6273(01)00183-0.
855 [45] V.M. Weake, K.K. Lee, S. Guelman, C.-H. Lin, C. Seidel, S.M. Abmayr, J.L. Workman, SAGA-
856 mediated H2B deubiquitination controls the development of neuronal connectivity in the
857 Drosophila visual system, EMBO J. 27 (2008) 394–405. https://doi.org/10.1038/sj.emboj.7601966.
858 [46] S. Georgieva, E. Nabirochkina, F.J. Dilworth, H. Eickhoff, P. Becker, L. Tora, P. Georgiev, A.
859 Soldatov, The novel transcription factor e(y)2 interacts with TAF(II)40 and potentiates transcription
860 activation on chromatin templates, Mol. Cell. Biol. 21 (2001) 5223–5231.
861 https://doi.org/10.1128/MCB.21.15.5223-5231.2001.
862 [47] R.D. Mohan, G. Dialynas, V.M. Weake, J. Liu, S. Martin-Brown, L. Florens, M.P. Washburn, J.L.
863 Workman, S.M. Abmayr, Loss of Drosophila Ataxin-7, a SAGA subunit, reduces H2B

864 ubiquitination and leads to neural and retinal degeneration, Genes Dev. 28 (2014) 259–272.
865 https://doi.org/10.1101/gad.225151.113.
866 [48] S. Rodriguez-Navarro, T. Fischer, M.-J. Luo, O. Antunez, S. Brettschneider, J. Lechner, J.E.
867 Perez-Ortin, R. Reed, E. Hurt, Sus1, a functional component of the SAGA histone acetylase
868 complex and the nuclear pore-associated mRNA export machinery., Cell. 116 (2004) 75–86.
869 https://doi.org/10.1016/s0092-8674(03)01025-0.
870 [49] S. Guelman, T. Suganuma, L. Florens, V. Weake, S.K. Swanson, M.P. Washburn, S.M. Abmayr,
871 J.L. Workman, The essential gene wda encodes a WD40 repeat subunit of Drosophila SAGA
872 required for histone H3 acetylation, Mol. Cell. Biol. 26 (2006) 7178–7189.
873 https://doi.org/10.1128/MCB.00130-06.
874 [50] Y. Doyon, W. Selleck, W.S. Lane, S. Tan, J. Cote, Structural and functional conservation of the
875 NuA4 histone acetyltransferase complex from yeast to humans., Mol. Cell. Biol. 24 (2004) 1884–
876 1896. https://doi.org/10.1128/mcb.24.5.1884-1896.2004.
877 [51] S. Allard, R.T. Utley, J. Savard, A. Clarke, P. Grant, C.J. Brandl, L. Pillus, J.L. Workman, J. Cote,
878 NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p
879 and the ATM-related cofactor Tra1p., EMBO J. 18 (1999) 5108–5119.
880 https://doi.org/10.1093/emboj/18.18.5108.
881 [52] J.E. Babiarz, J.E. Halley, J. Rine, Telomeric heterochromatin boundaries require NuA4-dependent
882 acetylation of histone variant H2A.Z in Saccharomyces cerevisiae., Genes Dev. 20 (2006) 700–
883 710. https://doi.org/10.1101/gad.1386306.
884 [53] R. Stegeman, P.J. Spreacker, S.K. Swanson, R. Stephenson, L. Florens, M.P. Washburn, V.M.
885 Weake, The Spliceosomal Protein SF3B5 is a Novel Component of Drosophila SAGA that
886 Functions in Gene Expression Independent of Splicing., J. Mol. Biol. 428 (2016) 3632–3649.
887 https://doi.org/10.1016/j.jmb.2016.05.009.
888 [54] O. Gozani, R. Feld, R. Reed, Evidence that sequence-independent binding of highly conserved U2
889 snRNP proteins upstream of the branch site is required for assembly of spliceosomal complex A.,
890 Genes Dev. 10 (1996) 233–243. https://doi.org/10.1101/gad.10.2.233.
891 [55] N. Herold, C.L. Will, E. Wolf, B. Kastner, H. Urlaub, R. Lührmann, Conservation of the protein
892 composition and electron microscopy structure of Drosophila melanogaster and human
893 spliceosomal complexes, Mol. Cell. Biol. 29 (2009) 281–301. https://doi.org/10.1128/MCB.01415-
894 08.
895 [56] M. Vermeulen, H.C. Eberl, F. Matarese, H. Marks, S. Denissov, F. Butter, K.K. Lee, J.V. Olsen,
896 A.A. Hyman, H.G. Stunnenberg, M. Mann, Quantitative interaction proteomics and genome-wide
897 profiling of epigenetic histone marks and their readers., Cell. 142 (2010) 967–980.
898 https://doi.org/10.1016/j.cell.2010.08.020.
899 [57] H. Wang, C. Dienemann, A. Stützer, H. Urlaub, A.C.M. Cheung, P. Cramer, Structure of the
900 transcription coactivator SAGA, Nature. 577 (2020) 717–720. https://doi.org/10.1038/s41586-020-
901 1933-5.
902 [58] G. Papai, A. Frechard, O. Kolesnikova, C. Crucifix, P. Schultz, A. Ben-Shem, Structure of SAGA
903 and mechanism of TBP deposition on gene promoters, Nature. 577 (2020) 711–716.
904 https://doi.org/10.1038/s41586-020-1944-2.
905 [59] L.M. Díaz-Santín, N. Lukoyanova, E. Aciyan, A.C. Cheung, Cryo-EM structure of the SAGA and
906 NuA4 coactivator subunit Tra1 at 3.7 angstrom resolution, ELife. 6 (2017) e28384.
907 https://doi.org/10.7554/eLife.28384.
908 [60] G. Sharov, K. Voltz, A. Durand, O. Kolesnikova, G. Papai, A.G. Myasnikov, A. Dejaegere, A. Ben
909 Shem, P. Schultz, Structure of the transcription activator target Tra1 within the chromatin
910 modifying complex SAGA, Nat. Commun. 8 (2017) 1556. https://doi.org/10.1038/s41467-017-
911 01564-7.
912 [61] Y. Han, J. Luo, J. Ranish, S. Hahn, Architecture of the Saccharomyces cerevisiae SAGA
913 transcription coactivator complex, EMBO J. 33 (2014) 2534–2546.
914 https://doi.org/10.15252/embj.201488638.

915 [62] A. Kohler, M. Schneider, G.G. Cabal, U. Nehrbass, E. Hurt, Yeast Ataxin-7 links histone
916 deubiquitination with gene gating and mRNA export., Nat. Cell Biol. 10 (2008) 707–715.
917 https://doi.org/10.1038/ncb1733.
918 [63] K.K. Lee, S.K. Swanson, L. Florens, M.P. Washburn, J.L. Workman, Yeast Sgf73/Ataxin-7 serves
919 to anchor the deubiquitination module into both SAGA and Slik(SALSA) HAT complexes.,
920 Epigenetics Chromatin. 2 (2009) 2. https://doi.org/10.1186/1756-8935-2-2.
921 [64] S. Lim, J. Kwak, M. Kim, D. Lee, Separation of a functional deubiquitylating module from the
922 SAGA complex by the proteasome regulatory particle., Nat. Commun. 4 (2013) 2641.
923 https://doi.org/10.1038/ncomms3641.
924 [65] A. Nassrallah, M. Rougée, C. Bourbousse, S. Drevensek, S. Fonseca, E. Iniesto, O. Ait-
925 Mohamed, A.-F. Deton-Cabanillas, G. Zabulon, I. Ahmed, D. Stroebel, V. Masson, B. Lombard, D.
926 Eeckhout, K. Gevaert, D. Loew, A. Genovesio, C. Breyton, G. De Jaeger, C. Bowler, V. Rubio, F.
927 Barneche, DET1-mediated degradation of a SAGA-like deubiquitination module controls H2Bub
928 homeostasis, ELife. 7 (2018) e37892. https://doi.org/10.7554/eLife.37892.
929 [66] S.J. Guyenet, S.S. Mookerjee, A. Lin, S.K. Custer, S.F. Chen, B.L. Sopher, A.R. La Spada, L.M.
930 Ellerby, Proteolytic cleavage of ataxin-7 promotes SCA7 retinal degeneration and neurological
931 dysfunction, Hum. Mol. Genet. 24 (2015) 3908–3917. https://doi.org/10.1093/hmg/ddv121.
932 [67] T. Suganuma, A. Mushegian, S.K. Swanson, S.M. Abmayr, L. Florens, M.P. Washburn, J.L.
933 Workman, The ATAC Acetyltransferase Complex Coordinates MAP Kinases to Regulate JNK
934 Target Genes, Cell. 142 (2010) 726–736. https://doi.org/10.1016/j.cell.2010.07.045.
935 [68] T. Suganuma, J.L. Gutiérrez, B. Li, L. Florens, S.K. Swanson, M.P. Washburn, S.M. Abmayr, J.L.
936 Workman, ATAC is a double histone acetyltransferase complex that stimulates nucleosome
937 sliding, Nat. Struct. Mol. Biol. 15 (2008) 364–372. https://doi.org/10.1038/nsmb.1397.
938 [69] Y.-L. Wang, F. Faiola, M. Xu, S. Pan, E. Martinez, Human ATAC Is a GCN5/PCAF-containing
939 acetylase complex with a novel NC2-like histone fold module that interacts with the TATA-binding
940 protein., J. Biol. Chem. 283 (2008) 33808–33815. https://doi.org/10.1074/jbc.M806936200.
941 [70] S. Guelman, K. Kozuka, Y. Mao, V. Pham, M.J. Solloway, J. Wang, J. Wu, J.R. Lill, J. Zha, The
942 Double-Histone-Acetyltransferase Complex ATAC Is Essential for Mammalian Development, Mol.
943 Cell. Biol. 29 (2009) 1176. https://doi.org/10.1128/MCB.01599-08.
944 [71] Z. Nagy, A. Riss, S. Fujiyama, A. Krebs, M. Orpinell, P. Jansen, A. Cohen, H.G. Stunnenberg, S.
945 Kato, L. Tora, The metazoan ATAC and SAGA coactivator HAT complexes regulate different sets
946 of inducible target genes., Cell. Mol. Life Sci. CMLS. 67 (2010) 611–628.
947 https://doi.org/10.1007/s00018-009-0199-8.
948 [72] M. Mohan, H.-M. Herz, E.R. Smith, Y. Zhang, J. Jackson, M.P. Washburn, L. Florens, J.C.
949 Eissenberg, A. Shilatifard, The COMPASS family of H3K4 methylases in Drosophila, Mol. Cell.
950 Biol. 31 (2011) 4310–4318. https://doi.org/10.1128/MCB.06092-11.
951 [73] J. Tower, Developmental gene amplification and origin regulation., Annu. Rev. Genet. 38 (2004)
952 273–304. https://doi.org/10.1146/annurev.genet.37.110801.143851.
953 [74] P. Pasero, B.P. Duncker, E. Schwob, S.M. Gasser, A role for the Cdc7 kinase regulatory subunit
954 Dbf4p in the formation of initiation-competent origins of replication., Genes Dev. 13 (1999) 2159–
955 2176. https://doi.org/10.1101/gad.13.16.2159.
956 [75] K. Labib, How do Cdc7 and cyclin-dependent kinases trigger the initiation of chromosome
957 replication in eukaryotic cells?, Genes Dev. 24 (2010) 1208–1219.
958 https://doi.org/10.1101/gad.1933010.
959 [76] R. Stephenson, M.R. Hosler, N.S. Gavande, A.K. Ghosh, V.M. Weake, Characterization of a
960 Drosophila Ortholog of the Cdc7 Kinase: A ROLE FOR Cdc7 IN ENDOREPLICATION
961 INDEPENDENT OF CHIFFON, J. Biol. Chem. 290 (2015) 1332–1347.
962 https://doi.org/10.1074/jbc.M114.597948.
963 [77] T. Pankotai, O. Komonyi, L. Bodai, Z. Ujfaludi, S. Muratoglu, A. Ciurciu, L. Tora, J. Szabad, I.
964 Boros, The homologous Drosophila transcriptional adaptors ADA2a and ADA2b are both required

965 for normal development but have different functions, Mol. Cell. Biol. 25 (2005) 8215–8227.
966 https://doi.org/10.1128/MCB.25.18.8215-8227.2005.
967 [78] A. Ciurciu, O. Komonyi, I.M. Boros, Loss of ATAC-specific acetylation of histone H4 at Lys12
968 reduces binding of JIL-1 to chromatin and phosphorylation of histone H3 at Ser10., J. Cell Sci. 121
969 (2008) 3366–3372. https://doi.org/10.1242/jcs.028555.
970 [79] A. Ciurciu, O. Komonyi, T. Pankotai, I.M. Boros, The Drosophila histone acetyltransferase Gcn5
971 and transcriptional adaptor Ada2a are involved in nucleosomal histone H4 acetylation., Mol. Cell.
972 Biol. 26 (2006) 9413–9423. https://doi.org/10.1128/MCB.01401-06.
973 [80] C. Feller, I. Forné, A. Imhof, P.B. Becker, Global and Specific Responses of the Histone
974 Acetylome to Systematic Perturbation, Mol. Cell. 57 (2015) 559–571.
975 https://doi.org/10.1016/j.molcel.2014.12.008.
976 [81] E. E. Vamos, I.M. Boros, The C-terminal domains of ADA2 proteins determine selective
977 incorporation into GCN5-containing complexes that target histone H3 or H4 for acetylation, FEBS
978 Lett. 586 (2012) 3279–3286. https://doi.org/10.1016/j.febslet.2012.06.051.
979 [82] R. Ferreira, A. Eberharter, T. Bonaldi, M. Chioda, A. Imhof, P.B. Becker, Site-specific acetylation
980 of ISWI by GCN5, BMC Mol. Biol. 8 (2007) 73. https://doi.org/10.1186/1471-2199-8-73.
981 [83] D.F.V. Corona, J.W. Tamkun, Multiple roles for ISWI in transcription, chromosome organization
982 and DNA replication., Biochim. Biophys. Acta. 1677 (2004) 113–119.
983 https://doi.org/10.1016/j.bbaexp.2003.09.018.
984 [84] C. Carré, A. Ciurciu, O. Komonyi, C. Jacquier, D. Fagegaltier, J. Pidoux, H. Tricoire, L. Tora, I.M.
985 Boros, C. Antoniewski, The Drosophila NURF remodelling and the ATAC histone acetylase
986 complexes functionally interact and are required for global chromosome organization, EMBO Rep.
987 9 (2008) 187–192. https://doi.org/10.1038/sj.embor.7401141.
988 [85] Y. Wang, Y. Huang, J. Liu, J. Zhang, M. Xu, Z. You, C. Peng, Z. Gong, W. Liu, Acetyltransferase
989 GCN5 regulates autophagy and lysosome biogenesis by targeting TFEB., EMBO Rep. 21 (2020)
990 e48335. https://doi.org/10.15252/embr.201948335.
991 [86] T. Liu, Q. Wang, W. Li, F. Mao, S. Yue, S. Liu, X. Liu, S. Xiao, L. Xia, Gcn5 determines the fate of
992 Drosophila germline stem cells through degradation of Cyclin A, FASEB J. 31 (2017) 2185–2194.
993 https://doi.org/10.1096/fj.201601217R.
994 [87] W. Xu, D.G. Edmondson, Y.A. Evrard, M. Wakamiya, R.R. Behringer, S.Y. Roth, Loss of Gcn5l2
995 leads to increased apoptosis and mesodermal defects during mouse development., Nat. Genet.
996 26 (2000) 229–232. https://doi.org/10.1038/79973.
997 [88] L. Howe, D. Auston, P. Grant, S. John, R.G. Cook, J.L. Workman, L. Pillus, Histone H3 specific
998 acetyltransferases are essential for cell cycle progression, Genes Dev. 15 (2001) 3144–3154.
999 https://doi.org/10.1101/gad.931401.
1000 [89] B. Grau, C. Popescu, L. Torroja, D. Ortuño-Sahagún, I. Boros, A. Ferrús, Transcriptional adaptor
1001 ADA3 of Drosophila melanogaster is required for histone modification, position effect variegation,
1002 and transcription, Mol. Cell. Biol. 28 (2008) 376–385. https://doi.org/10.1128/MCB.01307-07.
1003 [90] Z. Pahi, B.N. Borsos, B. Vedelek, Y.V. Shidlovskii, S.G. Georgieva, I.M. Boros, T. Pankotai,
1004 TAF10 and TAF10b partially redundant roles during Drosophila melanogaster morphogenesis,
1005 Transcription. 8 (2017) 297–306. https://doi.org/10.1080/21541264.2017.1327836.
1006 [91] E. Koutelou, C.L. Hirsch, S.Y. Dent, Multiple faces of the SAGA complex, Nucl. Gene Expr. 22
1007 (2010) 374–382. https://doi.org/10.1016/j.ceb.2010.03.005.
1008 [92] X. Li, C.W. Seidel, L.T. Szerszen, J.J. Lange, J.L. Workman, S.M. Abmayr, Enzymatic modules of
1009 the SAGA chromatin-modifying complex play distinct roles in Drosophila gene expression and
1010 development, Genes Dev. 31 (2017) 1588–1600. https://doi.org/10.1101/gad.300988.117.
1011 [93] V.M. Weake, J.O. Dyer, C. Seidel, A. Box, S.K. Swanson, A. Peak, L. Florens, M.P. Washburn,
1012 S.M. Abmayr, J.L. Workman, Post-transcription initiation function of the ubiquitous SAGA complex
1013 in tissue-specific gene activation, Genes Dev. 25 (2011) 1499–1509.
1014 https://doi.org/10.1101/gad.2046211.

[94] 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.

[95] 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. https://doi.org/10.1101/gad.250225.114.

[96] 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.

[97] 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.

[98] 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. https://doi.org/10.1016/j.molcel.2017.08.016.

[99] 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.

[100] 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. https://doi.org/10.1534/g3.116.031310.

[101] 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.

[102] 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.

[103] 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.

[104] 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. Genet. Genomics. 268 (2002) 425–433. https://doi.org/10.1007/s00438-002-0768-0.

[105] S. Rodriguez-Jato, A. Busturia, W. Herr, Drosophila melanogaster dHCF interacts with both PcG and TrxG epigenetic regulators, PloS One. 6 (2011) e27479–e27479. https://doi.org/10.1371/journal.pone.0027479.

[106] 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.

[107] 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.

[108] 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.

1065 [109]M. Featherstone, Coactivators in transcription initiation: here are your orders, Curr. Opin. Genet.
1066 Dev. 12 (2002) 149–155. https://doi.org/10.1016/S0959-437X(02)00280-0.
1067 [110]P. Georgiev, S. Chlamydas, A. Akhtar, Drosophila dosage compensation: males are from Mars,
1068 females are from Venus, Fly (Austin). 5 (2011) 147–154. https://doi.org/10.4161/fly.5.2.14934.
1069 [111]D.F.V. Corona, C.R. Clapier, P.B. Becker, J.W. Tamkun, Modulation of ISWI function by site-
1070 specific histone acetylation., EMBO Rep. 3 (2002) 242–247. https://doi.org/10.1093/embo-
1071 reports/kvf056.
1072 [112]C.R. Clapier, K.P. Nightingale, P.B. Becker, A critical epitope for substrate recognition by the
1073 nucleosome remodeling ATPase ISWI., Nucleic Acids Res. 30 (2002) 649–655.
1074 https://doi.org/10.1093/nar/30.3.649.
1075 [113]A.D. Baxevanis, G. Arents, E.N. Moudrianakis, D. Landsman, A variety of DNA-binding and
1076 multimeric proteins contain the histone fold motif, Nucleic Acids Res. 23 (1995) 2685–2691.
1077 https://doi.org/10.1093/nar/23.14.2685.
1078 [114]K.F. Hartlepp, C. Fernández-Tornero, A. Eberharter, T. Grüne, C.W. Müller, P.B. Becker, The
1079 histone fold subunits of Drosophila CHRAC facilitate nucleosome sliding through dynamic DNA
1080 interactions, Mol. Cell. Biol. 25 (2005) 9886–9896. https://doi.org/10.1128/MCB.25.22.9886-
1081 9896.2005.
1082 [115]I. Kukimoto, S. Elderkin, M. Grimaldi, T. Oelgeschläger, P.D. Varga-Weisz, The Histone-Fold
1083 Protein Complex CHRAC-15/17 Enhances Nucleosome Sliding and Assembly Mediated by ACF,
1084 Mol. Cell. 13 (2004) 265–277. https://doi.org/10.1016/S1097-2765(03)00523-9.
1085 [116]M. Orpinell, M. Fournier, A. Riss, Z. Nagy, A.R. Krebs, M. Frontini, L. Tora, The ATAC acetyl
1086 transferase complex controls mitotic progression by targeting non-histone substrates, EMBO J. 29
1087 (2010) 2381–2394. https://doi.org/10.1038/emboj.2010.125.
1088 [117]S.-H. Yang, A.D. Sharrocks, A.J. Whitmarsh, Transcriptional regulation by the MAP kinase
1089 signaling cascades., Gene. 320 (2003) 3–21. https://doi.org/10.1016/s0378-1119(03)00816-3.
1090 [118]K. Fukuyama, M. Yoshida, A. Yamashita, T. Deyama, M. Baba, A. Suzuki, H. Mohri, Z. Ikezawa,
1091 H. Nakajima, S. Hirai, S. Ohno, MAPK upstream kinase (MUK)-binding inhibitory protein, a
1092 negative regulator of MUK/dual leucine zipper-bearing kinase/leucine zipper protein kinase., J.
1093 Biol. Chem. 275 (2000) 21247–21254. https://doi.org/10.1074/jbc.M001488200.
1094 [119]A. Welihinda, W. Tirasophon, R. Kaufman, The transcriptional co-activator ADA5 is required for
1095 HAC1 mRNA processing in vivo, J. Biol. Chem. 275 (2000) 3377–81.
1096 https://doi.org/10.1074/jbc.275.5.3377.
1097 [120]A.W. Schram, R. Baas, P.W.T.C. Jansen, A. Riss, L. Tora, M. Vermeulen, H.Th.M. Timmers, A
1098 Dual Role for SAGA-Associated Factor 29 (SGF29) in ER Stress Survival by Coordination of Both
1099 Histone H3 Acetylation and Histone H3 Lysine-4 Trimethylation, PLOS ONE. 8 (2013) e70035.
1100 https://doi.org/10.1371/journal.pone.0070035.
1101 [121]D. Sela, L. Chen, S. Martin-Brown, M.P. Washburn, L. Florens, J.W. Conaway, R.C. Conaway,
1102 Endoplasmic reticulum stress-responsive transcription factor ATF6alpha directs recruitment of the
1103 Mediator of RNA polymerase II transcription and multiple histone acetyltransferase complexes., J.
1104 Biol. Chem. 287 (2012) 23035–23045. https://doi.org/10.1074/jbc.M112.369504.
1105 [122]G. Landis, J. Tower, The Drosophila chiffon gene is required for chorion gene amplification, and is
1106 related to the yeast Dbf4 regulator of DNA replication and cell cycle, Development. 126 (1999)
1107 4281.
1108 [123]T. Schüpbach, E. Wieschaus, Female sterile mutations on the second chromosome of Drosophila
1109 melanogaster. I. Maternal effect mutations., Genetics. 121 (1989) 101.
1110 [124]B.D. Aggarwal, B.R. Calvi, Chromatin regulates origin activity in Drosophila follicle cells, Nature.
1111 430 (2004) 372–376. https://doi.org/10.1038/nature02694.
1112 [125]T. Pankotai, N. Zsindely, E.E. Vamos, O. Komonyi, L. Bodai, I.M. Boros, Functional
1113 characterization and gene expression profiling of Drosophila melanogastershort dADA2b isoform-
1114 containing dSAGA complexes, BMC Genomics. 14 (2013) 44. https://doi.org/10.1186/1471-2164-
1115 14-44.

[126] 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.
[127] 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.
[128] 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.
[129] 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.
[130] 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.
[131] 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. Off. J. Soc. Neurosci. 27 (2007) 2483–2492. https://doi.org/10.1523/JNEUROSCI.5453-06.2007.
[132] 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.
[133] 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.
[134] 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.
[135] 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.
[136] Z. Pahi, Z. Kiss, O. Komonyi, B.N. Borsos, L. Tora, I.M. Boros, T. Pankotai, dTAF10- and dTAF10b-Containing Complexes Are Required for Ecdysone-Driven Larval-Pupal Morphogenesis in Drosophila melanogaster, PloS One. 10 (2015) e0142226–e0142226. https://doi.org/10.1371/journal.pone.0142226.
[137] 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.
[138] 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.

1166 **FIGURE LEGENDS**

1167 **Figure 1. Schematic comparison of *Drosophila* Gcn5 orthologs.** Gcn5 amino acid sequences were
1168 aligned using Clustal Omega, and a schematic comparison of Gcn5 orthologs in *D. melanogaster*, *S.*
1169 *cerevisiae*, and *H. sapiens* was constructed. Accession numbers are as follow: *D. melanogaster* Gcn5,
1170 NP_648586.2; *S. cerevisiae* Gcn5, NP_011768.1; *H. sapiens* Gcn5, XP_006721880.1; *H. sapiens*
1171 PCAF, NP_003875.3. The highly conserved GNAT and Bromodomain, and the metazoan conserved
1172 PCAF domains are boxed in gray, and aligned in each ortholog as indicated by dotted lines. The amino
1173 acid positions for each domain are indicated by the numbers on top of each box. The percentage
1174 identity within the conserved domains in each Gcn5 ortholog relative to the corresponding domains in
1175 *Dm*Gcn5 is indicated by the % within each boxed domain.

1176 **Figure 2. Schematic comparison of *Drosophila* Ada2a and Ada2b orthologs.** Ada2a and Ada2b
1177 amino acid sequences were aligned using Clustal Omega and a schematic comparison of *D.*
1178 *melanogaster* Ada2a and Ada2b (PA and PB isoforms) with *S. cerevisiae* Ada2 and *H. sapiens* Ada2a
1179 and Ada2b was constructed. Accession numbers are as follow: *D. melanogaster* Ada2a
1180 NP_001014636.1, Ada2b-PA NP_649773.1, Ada2b-PB NP_001027151.1; *H. sapiens* Ada2a
1181 NP_001159577.2, Ada2b NP_689506.2; *S. cerevisiae* Ada2 NP_010736.3. The conserved Zinc finger
1182 ZZ-type and SANT domains, and the SWIRM domains are boxed and aligned between the orthologs as
1183 indicated by dotted lines. The C-terminal specific domains for Ada2b-PA and Ada2b-PB are colored in
1184 green or orange, respectively. The amino acid positions for each domain are indicated by the numbers
1185 on top of each box. The percentage identity within the conserved domains in each Ada2a or Ada2b
1186 ortholog relative to the corresponding domains in *Dm*Ada2b or *Dm*Ada2a respectively is indicated by
1187 the % within each boxed domain. The % identity within the SWIRM domain is compared to *Dm*Ada2a.
1188 *Sc*Ada2 was aligned with *Dm*Ada2a.

1189 **Figure 3. Schematic illustration of the subunit composition of SAGA, ATAC, ADA, and CHAT.**
1190 The subunits in the four Gcn5 complexes are shown in the four sections, with shared subunits of the
1191 core Gcn5 HAT module indicated in the central box. The area of each subunit is proportional to its
1192 relative molecular mass. Subunits are colored by complex, or by modules for SAGA, and the yeast
1193 Ada2 orthologs that nucleate formation of each complex shown in orange. Domains present in
1194 individual subunits are shown in the key below the figure.

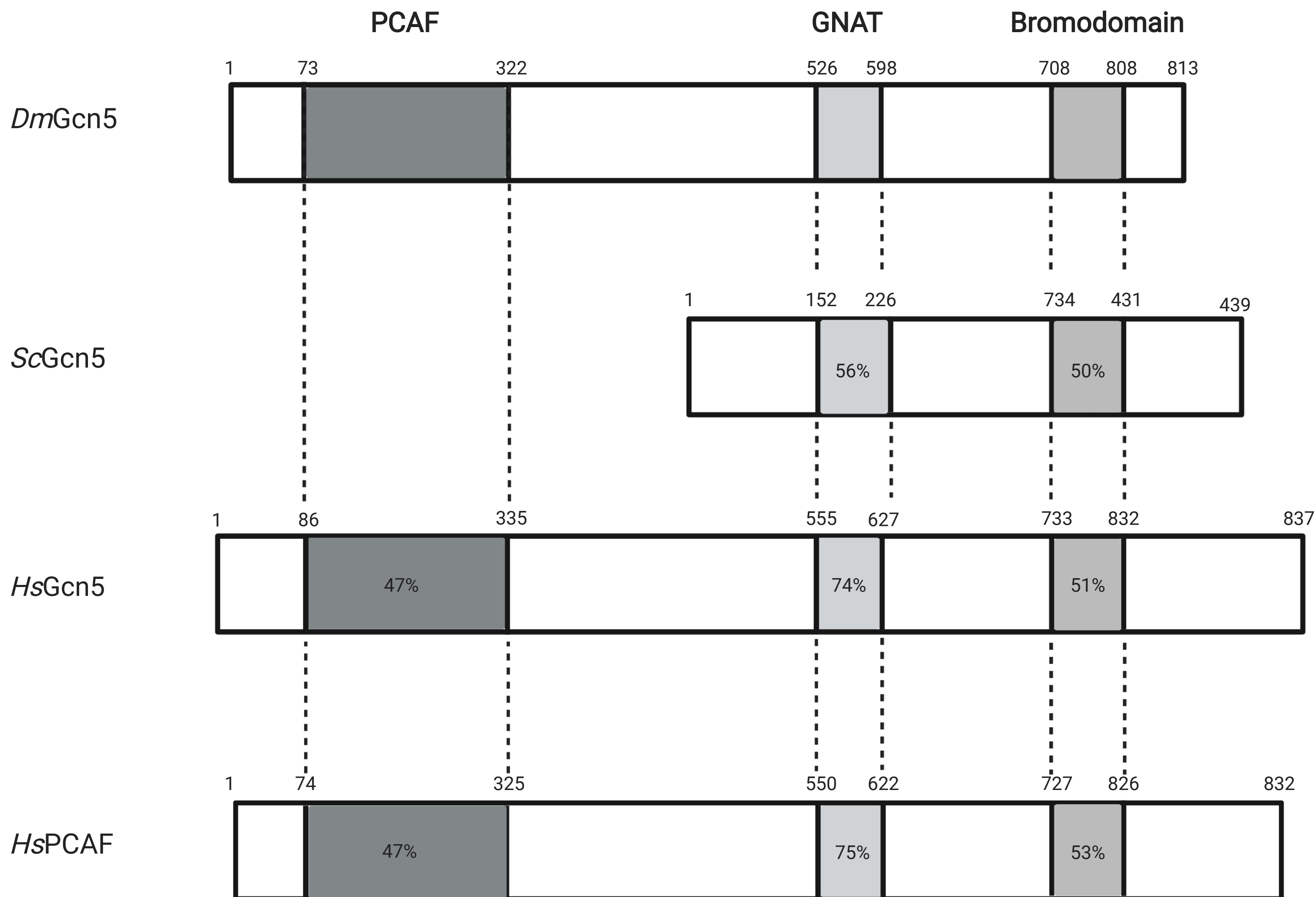
1195 **Figure 4. The *Drosophila* Gcn5 complexes are essential for fly development.** The life cycle of
1196 *Drosophila* comprises four successive stages, namely, egg, larva, pupa, and adult. Twenty-four hours

1197 after a female fly lays her eggs, larvae hatch. Larvae then undergo molting stages known as instars
1198 (three instar stages), during which the head, mouth, cuticle, spiracles, and hooks are shed. After ninety-
1199 six hours, the third instar larva encapsulates itself, forming a pupa. Metamorphosis takes place during
1200 the pupal stage, giving rise to all the structures in the adult fly. Oogenesis takes place within the ovary
1201 of female flies, and consists of 14 stages prior to deposition of the fertilized egg. The mutants shown
1202 disrupt subunits in the SAGA, ADA, ATAC or CHAT complexes, and result in lethality at the indicated
1203 developmental stage of the *Drosophila* life cycle. Mutations that have been shown to impact oogenesis
1204 are also indicated, but this has not been tested for all the mutant alleles shown. The *ada2b* mutant
1205 allele disrupts all three of the SAGA, ADA and CHAT complexes. The mutant alleles shown in this
1206 figure correspond to those listed in Table 5.

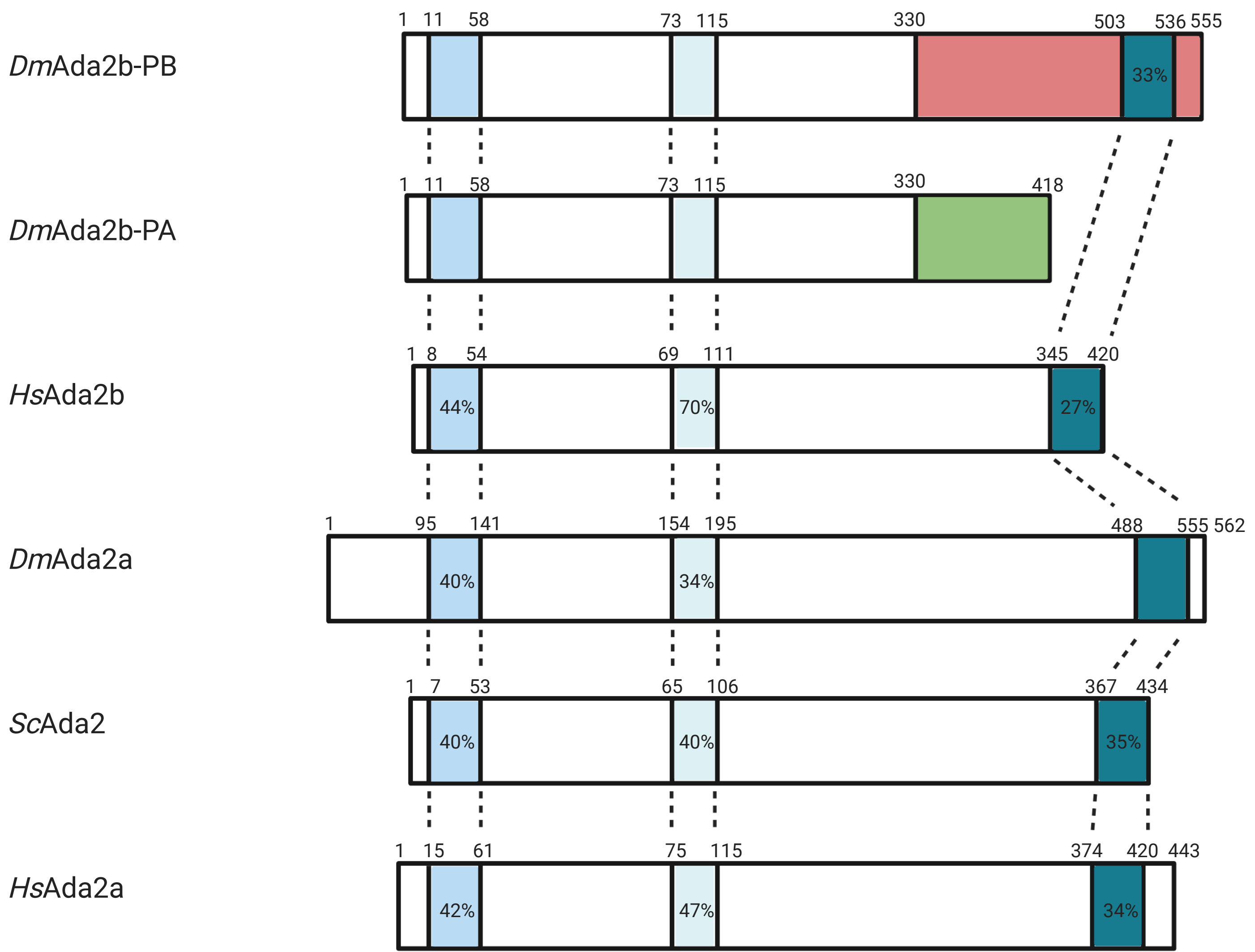
1207 **Figure 5. Insect Gcn5, Ada2, and Chiffon share regions of conservation with *Drosophila*.** Insect
1208 Gcn5, Ada2, and Chiffon homologs were aligned using Clustal Omega. The insect species described in
1209 this figure are: Diptera, *D. melanogaster*, *Musca domestica* (House fly), *Lucilia cuprina* (Australian
1210 sheep blowfly); Coleoptera, *Tribolium castaneum* (Red flour beetle); Lepidoptera, *Danaus plexippus*
1211 (Monarch butterfly); Hymenoptera, *Apis Mellifera* (Western honey bee) and *Linepithema humile*
1212 (Argentine ant). A representative illustration of each insect is shown next to each aligned protein. **A)**
1213 Accession numbers for Gcn5 homologs from the following insect species were used to generate this
1214 alignment: *D. melanogaster* NP_648586.2; *M. domestica* XP_005181707.1*; T. castaneum*
1215 XP_015835856.1; *D. plexippus* DPOGS216125. The GNAT, Bromodomain, and PCAF domains are
1216 boxed in gray. The percentage identity within the conserved domains in each Gcn5 ortholog relative to
1217 the corresponding domains in *Dm*Gcn5 is indicated by the % within each boxed domain. **B)** Accession
1218 numbers for Ada2 homologs from the following insect species were used to generate this alignment: *D.*
1219 *melanogaster* Ada2b-PB NP_001027151.1, Ada2b-PA NP_6497731, Ada2a NP_001014636.1; *M.*
1220 *domestica* Ada2b-PA XP_005186291.1, Ada2b-PB XP_005186290.1, Ada2a XP_019894005.1; *T.*
1221 *castaneum* Ada2b-PA A0A139WFG5, Ada2b-PB XP_008195462, Ada2a XP_015835543.1, *D.*
1222 *plexippus* Ada2b-PA XP_032521398.1, Ada2b-PB XP_032521398.1, Ada2a XP_032528769.1. The
1223 Zinc finger ZZ-type, SANT, and SWIRM domains are boxed. The C-terminal specific domains for
1224 Ada2b-PA and Ada2b-PB are colored in green or orange, respectively. The percentage identity within
1225 the conserved domains in each Ada2a or Ada2b ortholog relative to the corresponding domains in
1226 *Dm*Ada2b or *Dm*Ada2a, respectively, is indicated by the % within each boxed domain. The % identity
1227 within the SWIRM domain is compared to *Dm*Ada2a. **C)** Accession numbers for Dbf4/Chiffon homologs
1228 from the following species were used to generate this alignment: *D. melanogaster* AAD48779.1; *M.*
1229 *domestica* XP_019893793.1; *L. cuprina* A0A0L0CBC7; *T. castaneum* XM_008199666.2; *D. plexippus*

1230 OWR45390.1; *A. mellifera* XP_016770645.1; *L. humile* XP_012229084; *H. sapiens* NP_006707. The
1231 highly conserved region that interacts with Cdc7 (N, M, C domains) and the insect-specific Gcn5-
1232 binding domain are boxed. The percentage identity within the conserved domains in each Dbf4 ortholog
1233 relative to the corresponding domain in *Dm*Chiffon is indicated by the % within each boxed domain.

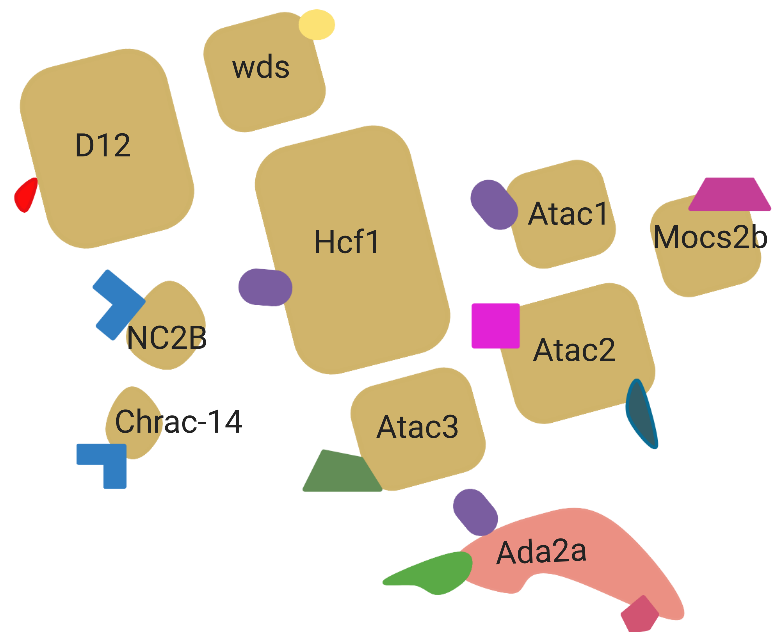
Torres-Zelada_Fig.1



Torres-Zelada_Fig.2



Torres-Zelada_Fig.3

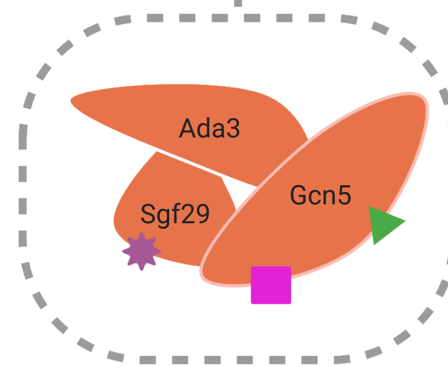


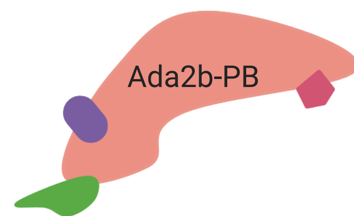
Ada2b-PA

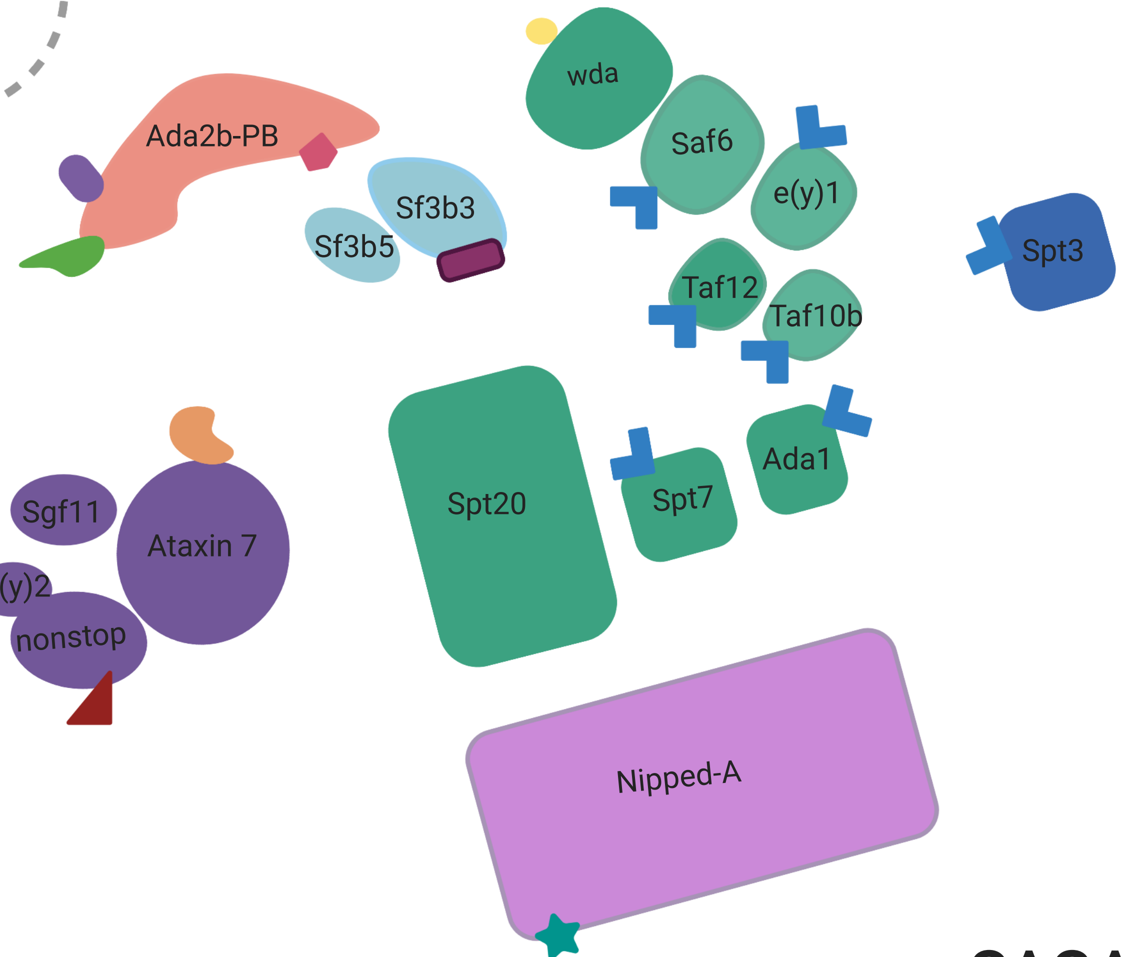
Chiffon

ATAC

CHAT





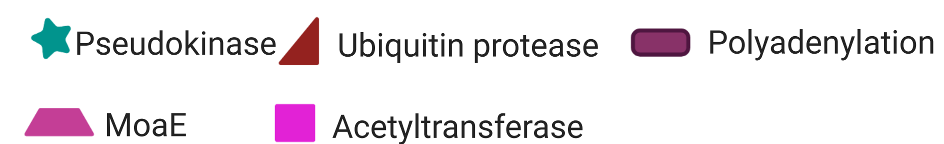


ADA

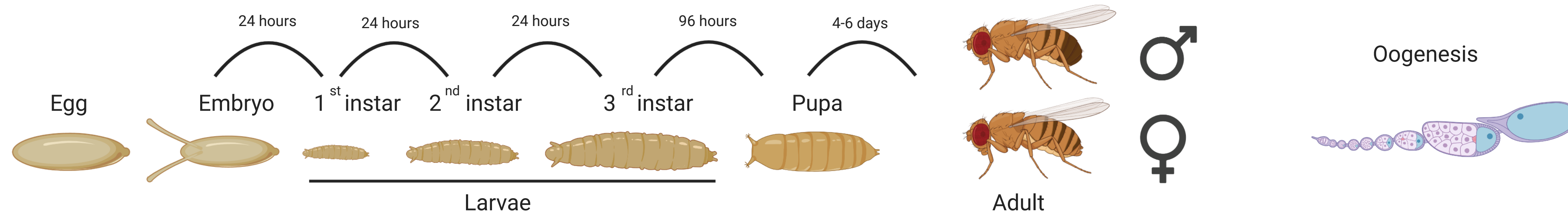
SAGA

DNA/Histone binding domain

Enzymatic domain



Torres-Zelada_Fig.4



Core			*ada3* *gcn5*	Oogenesis arrested in stage 5 and 6 *(gcn5, ada3)*
ATAC	*atac2*	*wds*	*ada2a* *hcf* *atac3*	No signs of oogenesis progression (*ada2a*) Oogenesis arrested in stage 8 (*hcf*)
SAGA	*Saf6* *Sf3b5* *Wda* *Nipped-A*	*e(y)* *Sgf11* *Atxn7*	*Taf10b* *not*	
SAGA / CHAT			*ada2b*	Defects in oogenesis (*ada2b*)
CHAT		*chif*		

Torres-Zelada_Fig.5