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2 **Multifaceted Activities of the Plant SAGA Complex**

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4 **Klaus D. Grasser^{1,*}, Vicente Rubio^{2,*}, Fredy Barneche^{3,*}**

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8 ¹ Cell Biology & Plant Biochemistry, Biochemistry Centre, University of Regensburg, Universitätsstr.

9 31, D-93053 Regensburg, Germany

10 ² Plant Molecular Genetics Dept. Centro Nacional de Biotecnología (CNB-CSIC) Darwin, 3. 28049

11 Madrid, Spain

12 ³ Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Ecole Normale Supérieure, CNRS,

13 INSERM, Université PSL, Paris, France

14

15

16 * Correspondence:

17 Klaus D. Grasser, Klaus.Grasser@ur.de; Tel: +49-941-9433032; Fax: +49-941-9433352

18 Vicente Rubio, vrubio@cnb.csic.es; Tel: +34-91-585-4700; Fax: +34-91-585-4600

19 Fredy Barneche, barneche@biologie.ens.fr; Tel: +33 (0)-144323528; Fax: +33 (0)1 44 32 39 35

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24 **Keywords**

25 SAGA complex; Histone acetyltransferase; GCN5; Deubiquitination module; Plant

26 **Abstract**

27 From yeast to human, the Spt-Ada-GCN5-acetyltransferase (SAGA) gigantic complex modifies
28 chromatin during RNA polymerase II initiation and elongation steps to facilitate transcription. Its
29 enzymatic activity involves a histone acetyltransferase module (HATm) that acetylates multiple lysine
30 residues on the N-terminal tails of histones H2B and H3 and a deubiquitination module (DUBm) that
31 triggers co-transcriptional deubiquitination of histone H2B. With a few notable exceptions described
32 in this review, most SAGA subunits identified in yeast and metazoa are present in plants. Studies
33 from the last 20 years have unveiled that different SAGA subunits are involved in gene expression
34 regulation during the plant life cycle and in response to various types of stress or environmental
35 cues. Their functional analysis in the *Arabidopsis thaliana* model species is increasingly shedding light
36 on their intrinsic properties and how they can themselves be regulated during plant adaptive
37 responses. Recent biochemical studies have also uncovered multiple associations between plant
38 SAGA and chromatin machineries linked to RNA pol II transcription. Still, considerably less is known
39 about the molecular links between SAGA or SAGA-like complexes and chromatin dynamics during
40 transcription in *Arabidopsis* and other plant species. We summarize the emerging knowledge on
41 plant SAGA complex composition and activity, with a particular focus on the best-characterized
42 subunits from its HAT (such as GCN5) and DUB (such as UBP22) modules, and implication of these
43 ensembles in plant development and adaptive responses.

44

45

46 **Highlights**

- 47 • Current knowledge on the SAGA gigantic complex composition in plants.
- 48 • A detailed view on the multiple roles of the SAGA histone acetyltransferase (GCN5) during plant
49 development and adaptive responses to environmental variations.
- 50 • A first overview of the recently identified *Arabidopsis* histone H2B de-ubiquitination module
51 (DUBm) and regulation of its abundance under changing conditions.
- 52 • Emerging questions on plant SAGA function during transcription and impact on gene expression.

53 1. Introduction

54 The large multisubunit transcriptional coactivator complex termed SAGA (Spt-Ada-GCN5-
55 acetyltransferase) is conserved from yeast to mammals. It regulates the initiation and elongation
56 stages of RNA polymerase II (RNA Pol II) transcription mainly by modulating nucleosomal histone
57 post-translational modifications (Koutelou et al., 2010; Weake and Workman, 2012). SAGA displays
58 two enzymatic activities, which involve on one hand a histone acetyltransferase module (HATm)
59 preferentially acetylating lysine residues on the amino-terminal tails of histones H2B and H3 and, on
60 the other hand, a deubiquitination module (DUBm) acting specifically on histone H2B. As detailed in
61 this Special Issue by Espinola Lopez et al. and by Goswami et al., respectively, histone acetylation by
62 HATm has an established role in transcription activation while DUBm is thought to contribute to
63 rapid cycles of monoubiquitination/de-ubiquitination during RNA polymerase progression. SAGA
64 more generally contributes to orchestrate a dynamic choreography of histone modifications linked to
65 transcription (see details on *cis*- and *trans*-histone crosstalks by Strahl and Briggs also in this Special
66 Issue). In addition, it has functions beyond transcription including mRNA export from the nucleus and
67 maintenance of DNA integrity (Baker and Grant, 2007; Rodriguez-Navarro and Hurt, 2011). With a
68 few notable exceptions described below, all SAGA subunits identified in yeast and metazoa were
69 found using either genetics or bioinformatics approaches to be encoded in plant genomes (Moraga
70 and Aquea, 2015; Srivastava et al., 2015). Recent biochemical studies have also shed light on the
71 multiple associations between plant SAGA and other chromatin machineries linked to RNA Pol II
72 transcription (Antosz et al., 2017; Dolan and Chapple, 2017; Koncz et al., 2012). Nevertheless,
73 considering the peculiarities of SAGA module composition and dynamic regulation in plants, we still
74 lack molecular knowledge on the role played by SAGA or SAGA-like complexes on transcription in
75 plants, both at the level of individual genes and at the global cellular level. In the absence of *in vitro*
76 reconstitution and of genome-wide perspectives on SAGA function in plants, most functional studies
77 currently available take advantage of *null* or partial loss-of-function lines of the *Arabidopsis thaliana*
78 model plant species and of a few other crop and tree species.

79 In *Saccharomyces cerevisiae* SAGA is not essential for viability and the deficiency of SAGA
80 subunits in metazoa causes developmental defects up to lethality (Koutelou et al., 2010; Weake and
81 Workman, 2012). In *Arabidopsis* knocking-out the DUBm has little consequences on plant growth
82 capacity while mutations in genes encoding other subunits of the plant SAGA complex trigger severe
83 and pleiotropic developmental defects and impair fertility. For example, knocking-out the *ADA2b*
84 gene encoding a subunit of *Arabidopsis* SAGA HATm triggers dwarfism, impairs root and floral
85 meristem development, and therefore provokes infertility (Sieberer et al., 2003; Vlachonassios et al.,
86 2003; Kornet and Scheres 2009). The HATm catalytic unit GCN5 (General Control Nondepressible 5,
87 also named HAG1) is also essential, notably for the control of *Arabidopsis* floral meristem activity

88 during reproductive organ formation and reproduction, i.e. sepals, petals, stamens and carpels.
89 GCN5 helps to restrict expression of the key regulatory genes *WUSCHEL* (*WUS*), *AGAMOUS* (*AG*), and
90 *LEAFY* (*LFY*) at floral meristems. Accordingly, *GCN5* loss-of-function leads to homeotic
91 transformations of floral organs and the production of terminal flowers (Bertrand et al., 2003; Cohen
92 et al., 2009; Poullos and Vlachonasios 2018). SAGA core module subunits TAF5 and TAF6 are required
93 for viability in *Arabidopsis* (Lago et al., 2005; Mougou et al., 2012), but it should be noted that, like
94 ADA2 and GCN5, these two proteins in addition to being SAGA components are also subunits of
95 other chromatin complexes. Most notably, TAF5 and TAF6 are part of the general transcription factor
96 TFIID (Helmlinger and Tora, 2017). Hence, while various studies indicate that SAGA is involved in
97 plant gene expression in response to various types of stress or environmental cues (Moraga and
98 Aquea, 2015; Srivastava et al., 2015), a systematic functional analysis of each plant SAGA subunit and
99 their molecular links with chromatin dynamics during transcription is missing.

100 Here we summarize the emerging knowledge of SAGA complex composition and activity in
101 plants, with a particular focus on the best-characterized subunits from its HATm and DUBm, and
102 implication of these three ensembles in plant development and adaptive responses. We also
103 envisage plant SAGA activity in conjunction with other chromatin modifiers triggering histone
104 acetylation and H2Bub de-ubiquitination, and in the context of global regulation of the cell
105 transcriptional status.

106

107 **2. Composition of plant SAGA**

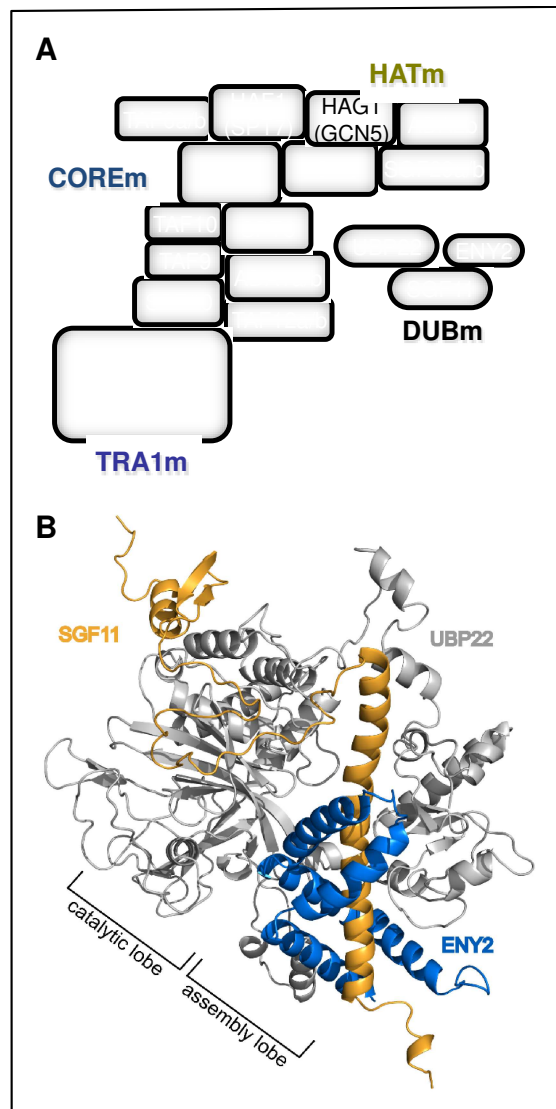
108 In *Arabidopsis* as in yeast and metazoan species, the SAGA complex (approximately 1.6-1.8 MDa) is
109 composed of 18 to 20 subunits divided into four distinct structurally and functionally defined
110 modules (a comparative Table of SAGA known subunits in Human, *D. melanogaster*, *S. cerevisiae* and
111 *Arabidopsis* is given in the accompanying Review by Goswami et al., in this Special Issue). In addition
112 to partial three-dimensional structures (Köhler et al., 2010; Morgan et al., 2016; Samara et al., 2010;
113 Sun et al., 2018), recently the overall structure of the yeast SAGA complex was solved at 3.3-3.9 Å
114 resolution by cryo-electron microscopy (Papai et al., 2020; Wang et al., 2020). Based on this structure
115 the core module (COREm consisting of Taf5, Taf6, Taf9, Taf10, Taf12, Spt3, Spt7, Spt20 and Ada1)
116 occupies a central position in the complex and is connected to the TRA1 module (TRA1m consisting
117 of the ~430-kDa Tra1 protein) via Taf12/Spt20. The TATA-binding protein (TBP) interacting subunits
118 Spt3 and Spt8, the histone acetyltransferase module (HATm consisting of Ada2, Ada3, Gcn5 and
119 Sgf29) and the deubiquitination module (DUBm consisting of Sgf73, Ubp8, Sgf11 and Sus1) are
120 flexibly connected with the core and lined up on one side of the complex. During metazoan
121 evolution, the archetypal SAGA complex as exemplified in yeast, has diverged into different variants
122 (Spedale et al., 2012).

123 *In silico* predictions regarding the composition of the SAGA complex in plants (Moraga and
124 Aquea, 2015; Srivastava et al., 2015) were recently complemented by approaches employing affinity
125 purification of supposed subunits in combination with mass spectrometry (AP-MS) from *Arabidopsis*
126 cells (Nassrallah et al., 2018; Pfab et al., 2018). Analysis of the core subunits SPT3 and TAF10 revealed
127 efficient copurification of subunits of COREm and TRA1m, whereas components of HATm and
128 particularly DUBm were hardly detected (Pfab et al., 2018). Affinity purification of the HATm subunit
129 ADA2b resulted in prominent copurification of other HATm subunits as well as of core components.
130 Isolation of the affinity-tagged DUBm subunits UBP22, SGF11 and ENY2 (*Arabidopsis* orthologues of
131 yeast Ubp8, Sgf11 and Sus1, respectively) resulted in striking reciprocal copurification of other DUBm
132 components, while only few other SAGA components were detected (Nassrallah et al., 2018; Pfab et
133 al., 2018). In conclusion, these analyses suggest that the SAGA core and its association with HATm
134 are essentially conserved, while in plant cells the DUBm interacts with the rest of SAGA only
135 weakly/dynamically (Fig. 1A).

136 Consistently, UBP22 was detected by size-exclusion chromatography analysis of *Arabidopsis*
137 protein extracts mainly in a size range of 100-150 kDa (in agreement with a trimeric DUBm consisting
138 of UBP22, SGF11 and ENY2) and to a lesser extent in the high molecular weight range (consistent
139 with a SAGA-like complex) (Nassrallah et al., 2018). Protein-protein interactions within DUBm were
140 further analysed using yeast two-hybrid, FRET and BiFC analyses. These experiments consistently
141 demonstrated that SGF11 interacts directly with both UBP22 and ENY2, while no interaction was
142 detected between UBP22 and ENY2 (Nassrallah et al., 2018; Pfab et al., 2018).

143 In yeast and metazoa, DUBm contains a fourth subunit, SGF73, which importantly is also part
144 of the CORE module and consequently tethers DUBm to the rest of the SAGA complex (Samara and
145 Wolberger, 2011; Papai et al., 2020; Wang et al., 2020). Surprisingly, plant genomes do not encode a
146 discernible orthologue of the SGF73 protein in term of amino-acids sequence conservation (Moraga
147 and Aquea, 2015; Srivastava et al., 2015) or searching for structural similarities (Nassrallah et al.,
148 2018), and thus the mode and extent of association of the *Arabidopsis* DUBm within a stable SAGA
149 complex is still elusive. Nevertheless, as shown in Figure 1B, building three-dimensional homology
150 models of *Arabidopsis* DUBm demonstrated that the complex consisting of UBP22, SGF11 and ENY2
151 closely resembles the well-known structure of yeast DUBm SAGA (Nassrallah et al., 2018; Pfab et al.,
152 2018). Moreover, extended molecular dynamics simulations indicated that the trimeric *Arabidopsis*
153 DUBm remains stable without SGF73 independently of SAGA (Pfab et al., 2018). Possible functions of
154 individual DUBm complexes independent of SAGA have been reported also for yeast and *Drosophila*
155 (Li et al., 2017; Lim et al., 2013).

156



157
 158 **Figure 1.** Schematic representation of the composition of the Arabidopsis SAGA complex.
 159 **A,** Protein subunits comprising four modules (COREm, TRA1m, HATm and DUBm) of the *Arabidopsis* SAGA complex
 160 established by analogy with the recently solved structures of the yeast SAGA complex (Papai et al., 2020; Wang et al.,
 161 2020). The COREm is also termed main lobe or central module of SAGA (Papai et al., 2020; Wang et al., 2020). While
 162 COREm, TRA1m and HATm efficiently co-purified with each other from *Arabidopsis* cells, the DUBm showed marginal co-
 163 purification (Nassrallah et al., 2018; Pfab et al., 2019), suggesting that it associates only weakly/transiently with the rest of
 164 SAGA. **B,** Cartoon representation of the Arabidopsis DUBm as determined by computational homology modeling (Pfab et
 165 al., 2019) with UBP22, SGF11 and ENY2 color-coded as indicated. The assembly/catalytic lobes of DUBm are indicated
 166 according to the structure of the yeast DUB (Köhler et al., 2010).

167
 168 Based on bioinformatics analyses (Moraga and Aquea, 2015; Srivastava et al., 2015), some
 169 aspects of the composition of SAGA seem to differ in plants when compared with the situation in
 170 yeast and/or metazoa. In addition to the DUBm component SGF73 present in yeast/metazoans but
 171 not in Arabidopsis, the Spt8 protein is part of yeast (but not metazoan) SAGA and also apparently not
 172 encoded in plant genomes. Likewise, several metazoa-specific subunits of the SAGA-related ATAC
 173 complex (e.g. ATAC1, YEATS2) apparently do not have plant orthologs. Furthermore, in *Arabidopsis*

174 several SAGA subunits occur in two paralogous versions, a general property of thousands genes in
175 *Arabidopsis thaliana* as a consequence of ancestral whole-genome duplication events (Blanc et al,
176 Plant Cell 2000). In several cases, both paralogs were identified as SAGA components in AP-MS
177 experiments from *Arabidopsis* cells including SGF29a/b, TRA1a/b and ADA1a/b (Pfab et al., 2018).
178 This is apparently not the case for the HATm subunit ADA2 as two paralogous genes (ADA2a and
179 ADA2b) encode proteins with different function (Hark et al., 2009), ADA2b being the only one
180 detected as a stable SAGA subunit in *Arabidopsis* suspension cultured cells (Figure 1).

181 These findings imply that in plants several versions of SAGA complexes may exist that differ
182 slightly in protein composition. It remains to be seen, whether these different versions of plant SAGA
183 have adopted (partially) different functional specialisation. The AP-MS experiments (Pfab et al.,
184 2018) also revealed association of *Arabidopsis* SAGA with the Mediator complex (Dolan and Chapple,
185 2017) as well as with components of the RNA Pol II elongation complex (Antosz et al., 2017) and
186 spliceosomal factors (Koncz et al., 2012). These interactions suggest that plant SAGA in addition to its
187 well-established role in controlling transcriptional initiation, could be involved in modulating
188 transcriptional elongation and cotranscriptional splicing that is emerging as a critical mechanism
189 regulating plant development and response to stress conditions (Godoy Herz and Kornblihtt, 2019;
190 Van Lijsebettens and Grasser, 2014).

191

192 **3. Plant SAGA-associated acetyltransferase activities**

193 The SAGA-associated histone acetylation activity residing in its so-called HATm is composed of GCN5,
194 ADA2, ADA3 and SGF29 proteins (Fig. 1A). *Arabidopsis* GCN5 holds the catalytic activity towards
195 several lysine residues on histone H3 and H4, including H3K9, K14 and K27 (Benhamed et al., 2006).
196 Non-histone GCN5 targets have also been described, as in the case of the ADA2b subunit in a process
197 of SAGA autoacetylation (Mao et al., 2006). Histone acetylation positively influences transcription,
198 most probably through physical relaxation of the chromatin structure by neutralizing lysine positive
199 charges (Lee and Workman, 2007). This 'open' conformation may facilitate DNA accessibility to
200 transcription factors and RNA polymerases.

201 In plants, the multiple roles of HATm are shown by the number and relevance of the
202 processes it regulates, as revealed by functional characterization of the GCN5 subunit, and, to a
203 lesser extent that of ADA2 proteins. As detailed below, these processes span the plant's entire life
204 cycle, including embryogenesis, root and shoot meristem development, juvenile-to-adult transition,
205 leaf and inflorescence development, trichome formation, as well as specific metabolic or
206 developmental processes, such as wax biosynthesis or wound-induced tissue regeneration. In
207 addition, GCN5 function mediates plant adaptation to environmental and stress signals, including
208 light, DNA-damage, phosphate starvation, iron homeostasis, and salt, heat and cold stresses. Finally,

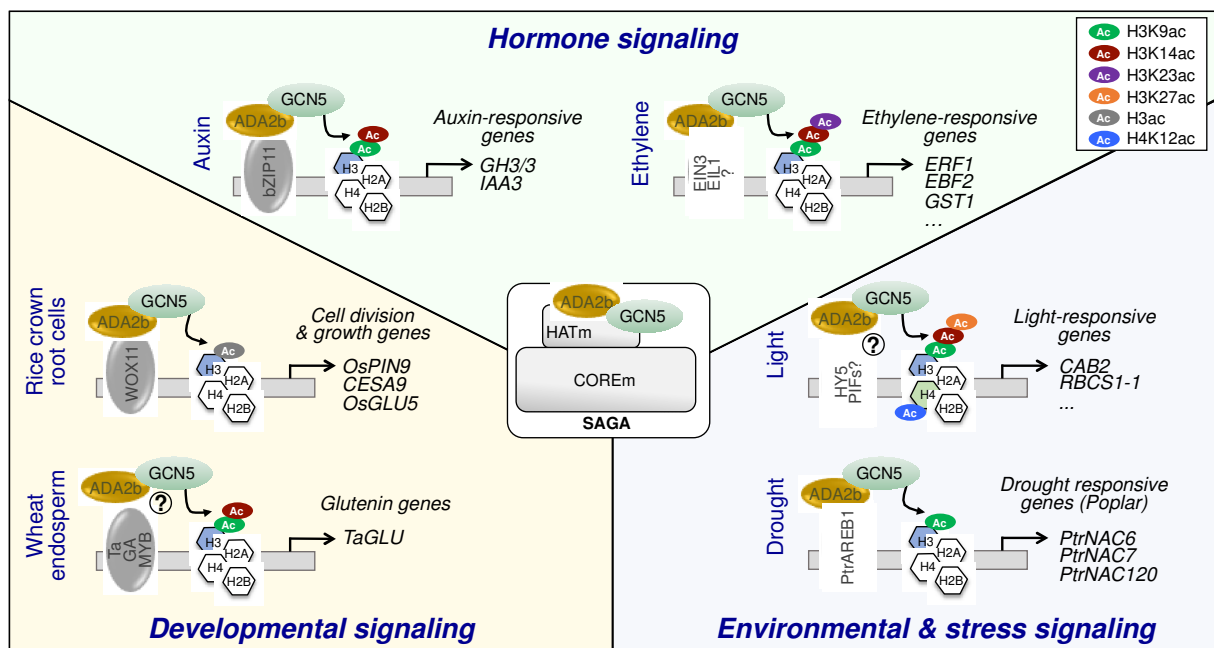
209 the Arabidopsis HATm also has other chromatin-based functional implications (Fig. 2). For example,
 210 ADA2b can recruit the Structural Maintenance of Chromosomes 5 (SMC5) protein for double-strand
 211 break repair (Lai et al., 2018).

212

213 **Multiple roles of GCN5 during plant development**

214 Early studies unveiled that GCN5 loss-of-function mutations trigger severe and pleiotropic
 215 developmental defects as early as during embryogenesis and during the generation of the polar
 216 apical shoot-basal root axis (Long et al., 2006). The first cell divisions leading to embryonic polarity
 217 are tightly regulated by the transcriptional corepressor TOPLESS (TPL). Null mutations in *TPL*
 218 transform the shoot pole into a second root pole, producing a seedling with two roots and no
 219 cotyledons (i.e. embryonic leaves). Interestingly, *gcn5* mutations suppress the *tpl* embryonic
 220 phenotype, indicating that polarity determination requires a tight balance BETWEEN TPL and GCN5
 221 regulation (Long et al., 2006).

222



223

224 **Figure 2.** GCN5-mediated control of plant processes.

225 Representative examples of developmental, hormonal, environmental and stress signaling cascades that involve GCN5-
 226 mediated acetylation of histones and regulated gene expression are shown. In general, upon elicitation by external or
 227 internal signals (indicated on the left), GCN5 and ADA2b subunits of the SAGA histone acetyltransferase module (HATm) are
 228 recruited to the promoter region of responsive genes by specific transcription factors. GCN5-ADA2b activity triggers histone
 229 acetylation favoring RNA Pol II transcription. Question marks indicate that involvement of ADA2b or that of a candidate
 230 transcription factor in GCN5 recruitment has not been fully demonstrated. References are given in the text.

231

232 GCN5 and ADA2b also operate in both cell differentiation and maintenance of
 233 undifferentiated states during root and shoot development (Fig. 2). Accordingly, rice GCN5 and ADA2

234 are expressed in the cells of crown roots, a major root system in cereals. There, the GCN5-ADA2
235 histone acetyltransferase module is recruited by the WUSCHEL-related homeobox protein WOX11 to
236 activate target genes that promote cell division and growth, leading to crown root emergence and
237 elongation (Zhou et al., 2017). The acetyltransferase activity of GCN5, together with that of TFIID
238 complex subunit TAF1/HAF2, is also required for establishing the appropriate cellular patterning of
239 the *Arabidopsis* root epidermis during root growth (Chen et al., 2016). On the other hand, GCN5 and
240 ADA2b allow generation of the protein gradient of PLETHORA (PLT) transcription factors required for
241 *Arabidopsis* root stem cell niche maintenance (Kornet and Scheres, 2009). GCN5 also allows
242 acquisition of pluripotency for the formation of shoots from *Arabidopsis* calli. In this process, GCN5
243 triggers histone acetylation at promoters of genes expressed in root meristems, including genes
244 encoding WOX, PLT, and SCARECROW (SCR) transcription factors (Kim et al., 2018). GCN5 promotes
245 their transcriptional activation, which confers regeneration potential to callus and establishes
246 competence for *de novo* shoot regeneration. Complementary pharmacological studies have shown
247 that inhibition of GCN5-type HATs impairs callus formation upon wounding, a key process during
248 tissue and organ regeneration following severe plant damage (Rymen et al., 2019).

249 At the adult stage, *Arabidopsis* ADA2b is required, upon recruitment by the bZIP11
250 transcription factor, for accurate histone acetylation at auxin-controlled loci, thereby playing a
251 critical role in hormone-mediated morphogenesis (Moulier Anzola et al., 2010; Weiste C and Dröge-
252 Laser W, 2014). Subsequently, GCN5-ADA2b containing complexes contribute to several
253 developmental transitions from the juvenile to the reproductive phases (Kim et al., 2015). This
254 involves the function of SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) factors, whose gene
255 expression is induced by GCN5-ADA2b. Thus, GCN5 and ADA2b determine the transcriptional output
256 of SPLs and promote flowering under optimal photoperiodic conditions (Kim et al., 2015).
257 Characterization of *Arabidopsis gcn5* and *ada2b* mutants has also unveiled specific roles for the
258 HATm in leaf growth by controlling endoreplication levels in leaf cells, which alters leaf size and
259 morphology of trichomes (shoot epidermal appendages; Kotak et al., 2018; Wang et al., 2019a).
260 Unexpectedly, GCN5 and ADA2b seem to play opposite roles in controlling ploidy levels, with GCN5
261 and ADA2b promoting and reducing endoreduplication, respectively. Together with trichomes, the
262 cuticular wax layer of plant cells acts as a barrier against environmental stresses, such as water loss,
263 and is the first defense encountered by herbivores and pathogens. GCN5 regulates stem cuticular
264 wax accumulation by promoting H3K9/14 acetylation at the promoter regions of wax biosynthesis
265 genes, including *ECERIFERUM3* (*CER3*), *CER26*, and *CER1-LIKE1* (*CER1-L1*), which leads to their
266 transcriptional activation and increased cuticular wax production (Wang et al., 2018).

267 GCN5-mediated control of plant metabolic pathways also has an impact on seed protein and
268 fatty acid composition. *GCN5* loss-of-function decreases the ratio of alpha-linolenic acid (ALA) to

269 linoleic acid (LA) in Arabidopsis seed oil. GCN5 targets fatty acid biosynthesis genes, such as *FAD3*,
270 *LACS2*, *LPP3* and *PLAIIIb*, and regulates their H3K9/14 acetylation level and transcriptional
271 expression. High expression of fatty acid desaturase *FAD3* favors conversion of LA into ALA, which
272 accumulates in seeds (Wang et al., 2016). Expression of genes encoding glutenin storage proteins is
273 also regulated by GCN5 in the endosperm of wheat (*Triticum aestivum*) seeds (Fig. 2). This function is
274 mediated by TaGCN5 recruited to the promoter region of high-molecular-weight glutenin subunit
275 genes (*TaGLU*) by the transcription factor TAGAMyB, increasing their H3K9/K14 acetylation and
276 expression (Guo et al., 2015).

277

278 **A role for GCN5 in plant adaptive responses to environmental conditions**

279 Plant development is a plastic process influenced at all stages by both environmental and
280 endogenous cues. The HATm participates in the integration of diverse external and internal signals
281 into physiological and morphological plant responses. First, GCN5, together with acetyltransferase
282 TAF1/HAF2, mediate plant responses to light. In this process, both GCN5 and TAF1/HAF2 control
283 acetylation of H3K9, H3K27, and H4K12 on the promoter of light-responsive genes, whereas GCN5
284 appears to mediate H3K14 acetylation more specifically. Mutations in *GCN5* and *TAF1/HAF2* reduce
285 gene responsiveness to light, resulting in impaired photomorphogenic responses in an additive
286 manner (Benhamed et al., 2006). Interestingly, GCN5 might be targeted to light-inducible genes in a
287 sequence-specific manner through the HY5 transcription factor (Benhamed et al., 2006), a master
288 regulator of plant photomorphogenesis (Fig. 2).

289 GCN5 function is also directly linked to mineral nutrient signaling, by controlling both
290 phosphate starvation responses and iron homeostasis in Arabidopsis. CHIP-PCR assays showed that
291 GCN5 associates with genes controlling accumulation of these essential nutrients. In the case of
292 phosphate, GCN5 targets and mediates H3K9/14 acetylation of *At4*, which expresses a long non-
293 coding RNA that regulates phosphate distribution between shoots and roots (Wang et al., 2019).
294 Similarly, GCN5 targets key genes in iron homeostasis regulation, including *FERRIC REDUCTASE*
295 *DEFECTIVE3 (FRD3)* (Xing et al., 2015). The latter mediates citrate efflux required for iron uptake
296 from the soil and mobilization within the plant. Thus, GCN5 regulates iron distribution by controlling
297 the acetylation level of the *FRD3* target.

298 Plant adaptation to many other abiotic stresses, including salt, heat and cold stresses, also
299 involves GCN5 function. GCN5 mediates H3K9/K14 acetylation and transcriptional activation of
300 *CHITINASE-LIKE1 (CTL1)*, *POLYGALACTURONASE INVOLVED IN EXPANSION3 (PGX3)*, and *MYB54*,
301 which are required for maintenance of Arabidopsis cell wall integrity under high salinity growth
302 conditions (Zheng et al., 2019). GCN5 is also involved in thermotolerance, binding to promoters of
303 heat stress transcription factors *HSFA3* and *UVH6* genes, and facilitating their H3K9/K14 acetylation

304 and expression (Hu et al., 2015). Finally, GCN5 also confers freezing tolerance in *Arabidopsis*. For this,
305 during cold acclimation, the CBF1 transcription factor recruits ADA2b and GCN5 to cold-regulated
306 (*COR*) gene promoters (Stockinger et al., 2001; Mao et al., 2006; Pavangadkar et al., 2010;
307 Vlachonasios et al., 2003). According to this notion, expression of *COR* genes is reduced by loss-of-
308 function mutations of *Arabidopsis ADA2b* and *GCN5*. Interestingly, cold-induced H3 acetylation is
309 unaffected in *ada2b* and *gcn5* mutants, indicating that the ADA2b-GCN5 module is not responsible
310 for histone acetylation at *COR* gene promoters and that other histone acetyltransferases are
311 necessary for cold acclimation (Mao et al., 2006; Pavangadkar et al., 2010).

312 GCN5-mediated histone acetylation also plays a major role in the integration of endogenous
313 and environmental signals. Such a crosstalk is exemplified by abscisic acid (ABA) signaling that
314 regulates seed germination and plant responses to abiotic stresses, such as drought, high salinity,
315 and low temperatures (Chinnusamy et al., 2008). ABA signaling activates AREB1 transcription factors
316 to induce ABA-responsive gene expression, promoting plant adaptation to drought. In poplar trees
317 (*Populus trichocarpa*) this process involves interaction of PtrAREB1 and the PtrGCN5-ADA2b module
318 complex to drought-responsive genes (e.g. *PtrNAC6*, *PtrNAC7*, and *PtrNAC120*), triggering H3K9ac
319 accumulation at their promoters and RNA Pol II recruitment (Li et al., 2019). As shown for
320 *Arabidopsis* response to salt stress where AtPP2C-6-6 phosphatase interacts and dephosphorylates
321 GCN5, GCN5 activity itself might be modulated by ABA signaling components. AtPP2C-6-6 likely
322 functions as a negative regulator of GCN5 activity to fine-tune the plant's responses to high salinity
323 (Servet et al., 2008). As afore-mentioned, shoot apical meristem maintenance requires restricted
324 expression of WUS, a process that is mediated by both CLV1 and GCN5. Of note, *clv1 gcn5* double
325 mutants display constitutive ethylene responses, indicating that GCN5 and CLV1 synergistically
326 repress ethylene responses in *Arabidopsis*. This effect is likely due to increased accumulation of auxin
327 in the *clv1 gcn5* double mutants, which stabilizes ETHYLENE INSENSITIVE 3 (EIN3) and EIN3-LIKE1
328 (EIL1) transcription factors that promote ethylene-induced gene expression (Poulios and
329 Vlachonasios 2018). Nevertheless, GCN5 might act independently of CLV1 to influence the histone
330 acetylation status of ethylene-responsive genes, most likely upon recruitment by EIN3 and EIL1.
331 Altogether, this molecular path allows increasing H3K9/K14/K23 acetylation levels over responsive
332 genes in response to ethylene treatment (Zhang et al., 2016; Wang et al., 2017).

333 In addition to positively regulating the expression of developmental and stress-responsive
334 genes, GCN5 also plays a repressive role on microRNA (miRNA) biosynthesis. miRNAs allow
335 posttranscriptional silencing of a myriad of genes in plants, thereby contributing to determine
336 developmental and environmental transcriptional programs. GCN5-mediated general inhibition of
337 miRNA production likely involves indirect repression of miRNA biosynthetic genes, including *DICER*
338 *LIKE1 (DCL1)*, *SERRATE (SE)*, *HYPONASTIC LEAVES1 (HYL1)* and *ARGONAUTE1 (AGO1)*. However, GCN5

339 is also able to directly activate expression of a specific subset of miRNAs by promoting H3K14
340 acetylation of their loci (Kim et al., 2009).

341 Taken together, these studies highlight the functional relevance of GCN5 in the control of
342 plant development and adaptive responses to the environmental signals. Still, at this stage only in a
343 limited number of cases the identity of the GCN5 gene targets and the mechanism by which it is
344 recruited to its targets is known (Jiang et al. 2020, Ueda and Seki, 2020). It also remains to be
345 determined whether GCN5 acts in these multiple processes as part of the SAGA HATm or, as shown
346 in the case of the yeast ADA complex, as a distinct HAT complex (Eberharter et al., 1999). Studies are
347 further required to test the conservation of the *Arabidopsis* GCN5 and HATm function across other
348 plant species and whether functional specificity exists depending on developmental programs
349 inherent to each plant taxa.

350

351 **4. Plant SAGA-associated deubiquitination activities**

352 Early studies on yeast *S. cerevisiae* have shown that SAGA DUBm is first recruited onto gene
353 promoter regions and travels along the gene body to allow for repeated sequences of histone H2B
354 ubiquitination/de-ubiquitination by opposing Bre1 activity and to facilitate the progression of RNA
355 Pol II (Henry et al., 2003). This mechanism associated to RNA Pol II dynamics is apparently conserved
356 in plants, although DUBm also appears to display several peculiarities described here.

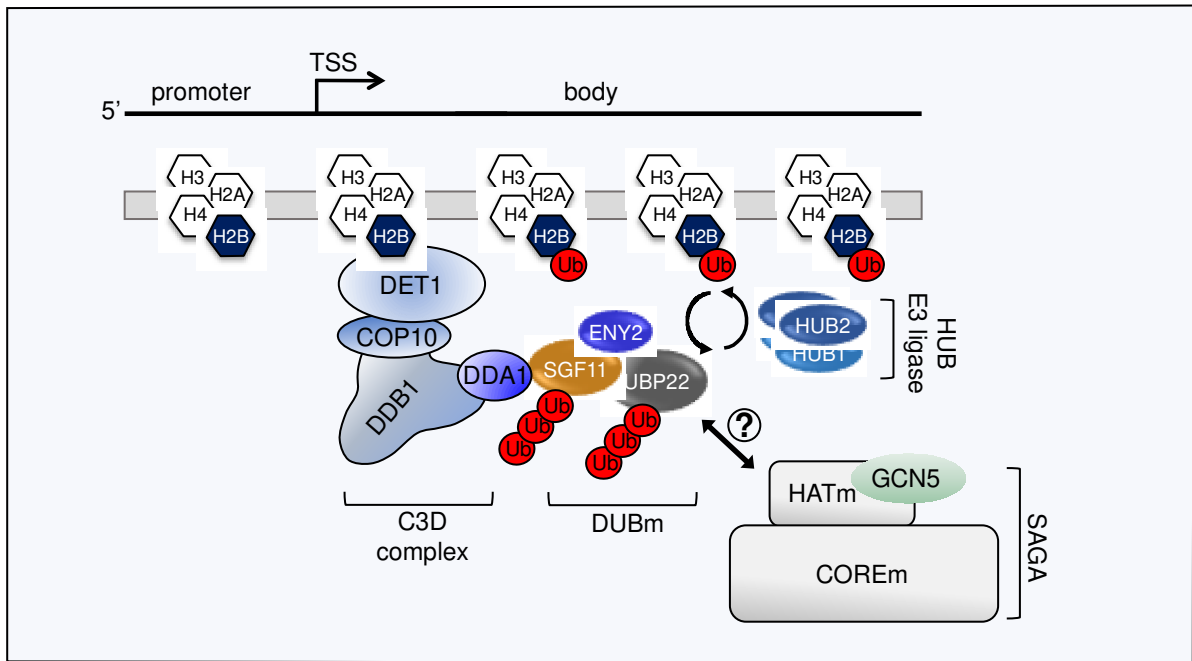
357

358 **Functional impact of the plant SAGA DUBm on chromatin and gene expression**

359 In *Arabidopsis*, the DUBm has a massive impact on the general abundance of the chromatin pool of
360 monoubiquitinated histone H2B. Both *SGF11* and *UBP22* loss-of-function mutations result in 2-3 fold
361 higher H2Bub signals than wild-type plants in immunoblot analyses of chromatin extracts (Nassrallah
362 et al., 2018; Pfab et al., 2018). Upon ectopic expression, *Arabidopsis* UB22 can restore wild-type
363 levels of H2Bub in *Δubp8* mutant yeast cells and can therefore be considered as a *bona fide* homolog
364 of the DUBm catalytic subunit Ubp8 (Nassrallah et al., 2018).

365 Profiling of H2Bub enrichment over the *Arabidopsis* genome revealed that H2Bub is
366 deposited by the HISTONE UBIQUITINATION 1/2 (HUB1/HUB2) E3 ubiquitin ligase along the gene
367 body of thousands of transcribed genes, especially long genes (Roudier et al., 2011; Nassrallah et al.,
368 2018). This further unveiled that H2Bub is present within chromatin domains with other
369 transcriptionally permissive histone marks (e.g., H3K4me2/3, H3K36me3, H3K56ac) (Roudier et al.,
370 2011). Hence, as in other species, plant DUBm may target a vast repertoire of genes to facilitate RNA
371 Pol II progression along nucleosome-packed DNA. Time-series analyses of H2Bub genomic
372 distribution over the *Arabidopsis* genome during the first hours of photomorphogenesis have shown
373 rapid variations over hundreds of genes during their induction by light (Bourbousse et al., 2012).

374 Accordingly, analysis of both *ubp22* and *hub1* null mutant seedlings further showed H2Bub
 375 enrichment to be necessary in *cis* to allow fast and elevated upregulation of responsive genes during
 376 this transition induced by light (Bourbousse et al., 2012; Moraga and Aquea, 2015).
 377



378
 379 **Figure 3.** Working model for DUBm activity in the regulation of H2Bub homeostasis.
 380 After its deposition by the HUB E3 ligase, histone H2Bub can be deubiquitinated along the body of expressed genes by the
 381 protease activity of DUBm subunit UBP22. Co-transcriptional cycles of histone H2B ubiquitination/deubiquitination are
 382 thought to promote RNA Pol II elongation in plants as in other eukaryotes. The "C3D" complex (made of COP10, DET1,
 383 DDB1 and DDA1) controls the stability of DUBm subunits by ubiquitin-mediated proteolytic degradation through a physical
 384 association of its DDA1 subunit to SGF11 (Nassrallah et al., 2018). DET1-based post-translational control of DUBm
 385 abundance may allow the cell to adjust for different transcriptional regimes under unfavorable environmental conditions
 386 such as darkness. The association of DET1 with non-acetylated histone H2B (Benvenuto et al, 2002) and with transcription
 387 factors (Dong et al., 2014) may favor DUBm regulation over specific chromatin domains. DET1 further influences histone
 388 H2B acetylation levels via an unknown mechanism, possibly involving SAGA HATm activity. Alternatively, in the absence of
 389 Sgf73 ortholog in plants, DUBm may act independently from SAGA (Nassrallah et al., 2018; Pfab et al., 2018).

390
 391 Genes encoding the three known subunits of the DUBm are widely expressed in most
 392 *Arabidopsis* organs (Srivastava et al., 2015; Pfab et al., 2018) and their expression also seems to be
 393 correlated with those of other SAGA components (Srivastava et al., 2015). For example, Spt20 and
 394 Chr5, Taf13 and Tra1 and Spt20 are highly co-expressed in different plant organs while Spt20 and
 395 Ubp22, Sgf11 and Tra1, Spt20 and Chr5, Gcn5 and Taf6 are co-expressed in response to stress
 396 (Srivastava et al., 2015).

397 Investigations of DUBm dynamics further unveiled that abundance of SGF11, ENY2 and
 398 UBP22 is tightly regulated at the post-translational level through polyubiquitin-mediated proteolytic

399 degradation. Post-translational control of DUBm stability and abundance was recently shown to
400 comprise part of a general mechanism to modulate H2Bub global level over almost all genes in
401 response to light signals (Fig. 3). DUBm-targeted degradation is more pronounced under dark than
402 light conditions and requires physical association of SGF11 with a subunit of the evolutionarily-
403 conserved and histone H2B-associated DET1 complex (Nassrallah et al., 2018). Still, considering that
404 the trimeric DUBm exists in the absence of SGF73 orthologs in *Arabidopsis*, and therefore
405 independently from the COREm (Nassrallah et al., 2018; Pfab et al., 2018), it remains unclear
406 whether its proteolytic degradation also affects other SAGA functions.

407

408 **Biological impact of DUBm on plant development and adaptive responses**

409 In the absence of Sgf73 orthologue and lack of strong association with the SAGA core module, the
410 biological impact of the plant DUBm as part of a SAGA complex remains poorly understood. Plants
411 over-expressing or bearing loss-of-function alleles of *SGF11* or *UBP22* display no developmental
412 abnormalities, including unchanged rosette diameter and number of inflorescences (Srivastava et al.,
413 2015; Nassrallah et al., 2018; Pfab et al., 2018). The only obvious phenotypic alteration is that the
414 *sgf11* plants are late flowering, but with wild-type leaf numbers (Pfab et al., 2018). *SGF11* mutant
415 analysis further showed that DUBm is required for proper induction of heat, salt and light responsive
416 genes (Srivastava et al., 2015). Differently to the observations made with *UBP22* and *SGF11*, viable
417 *ENY2* null mutant line could not be recovered from public T-DNA/TE insertion collections. Also
418 contrasting with the euchromatic status of *UBP22* and *SGF11* proteins, GFP-*ENY2* subnuclear
419 localization is enriched both in the euchromatic and heterochromatic compartments, sometimes
420 overlapping with nucleolus-associated chromocenters (Nassrallah et al., 2018; Pfab et al., 2018).
421 Considering that epitope-tagged *ENY2* displays a chromatin mobility much lower than that observed
422 for core and linker histones in *Arabidopsis* nuclei (Pfab et al., 2018), these findings suggest that *ENY2*
423 may also have a function outside from SAGA and from DUBm in plants. Still, differently from yeast
424 and metazoa, *Arabidopsis* *ENY2* was found to be unrelated to the TREX-2 mRNA export complex,
425 which localizes at nuclear pores (Pfab et al., 2018).

426 DUBm activity over the genome may further be envisaged in conjunction with other H2Bub
427 de-ubiquitination mechanisms. On the first hand, UBIQUITIN PROTEASE 26 (*UBP26*) erases this mark
428 from several transposable elements (TEs) as part of a DNA methylation-dependent silencing
429 mechanism (Sridhar et al., 2007). On the other hand, *UBP26* depletes the H2Bub mark from genes as
430 shown for *PHERES1* as part of a *Polycomb*-based parental gene imprinting mechanism (Luo et al.,
431 2008) and for the *FLC* developmental regulator (Schmitz et al., 2009). *UBP26* has a very low sequence
432 similarity with *UBP22* and with its yeast homolog *Ubp8* (Wu et al., 2019) and does not co-purify with
433 SAGA subunits (Pfab et al., 2018). Therefore, it presumably does not act in the context of SAGA. On

434 the other hand, rice OTLD1 (OTUBAIN-Like DEUBIQUITINASE), a cysteine protease displaying an
435 Ovarian Tumor protease domain, reduces H2Bub level at ABA-responsive genes during the drought
436 stress response. Interestingly, upon drought recovery OTLD1 is recruited through the OsbZIP46
437 transcription factor (homologous to ABI5 in *Arabidopsis*) to attenuate gene expression in a sequence-
438 specific manner (Ma et al, 2019). In this recovery phase, OTLD1 acts together with a TOPLESS co-
439 repressor complex to deacetylate histones (Tang et al., 2016). In *Arabidopsis*, OTLD1 may also recruit
440 the KDM1C histone demethylase over several target genes (Krichevsky et al., 2011; Keren et al.,
441 2019). In all these studies, H2Bub deubiquitination appears to act in a concerted manner with
442 different chromatin regulatory mechanisms.

443 Collectively, *in silico* and biochemical studies have recently allowed identifying an *Arabidopsis*
444 DUBm comprising only three evolutionarily conserved subunits that has a strong impact on the
445 H2Bub abundance in chromatin. While complete erasure of H2Bub over TEs and *Polycomb*-targeted
446 genes by other DUB machineries such as UBP26 is thought to repress RNA Pol II transcription, UBP22-
447 containing DUBm presumably acts as in yeast and metazoa in transcription facilitation by
448 contributing to rapid ubiquitination/de-ubiquitination cycles that usually result in H2Bub positive
449 steady-state *cis* levels (reviewed in Isono and Nagel 2014; March and Farrona, 2018; Bourbousse and
450 Barneche, 2019). Recent studies also unveiled that environmental signaling pathways target the
451 DUBm for proteolytic degradation (Nassrallah et al., 2018). Hence, constant modulation of DUBm
452 stability and availability may fine tune H2Bub homeostasis in response to different signals.

453

454 **5. Conclusion**

455 Data mining of transcriptomic information showed that basically all *Arabidopsis* and rice SAGA
456 subunit-encoding genes are co-expressed in most plant organs throughout development (Srivastava
457 et al., 2015). Therefore, not surprisingly, mutations in the HATm catalytic subunit GCN5 echo *ada2b*-
458 induced pleiotropic developmental phenotypic defects. Yet they display certain functional specificity
459 or even antagonistic roles in the control of specific plant processes, such as freezing tolerance or
460 regulation of ploidy levels in leaf cells. GCN5, ADA2b and other SAGA subunits may therefore be
461 components of both a common coactivator activity (e.g., HATm) or act independently in distinct
462 complexes (Moraga and Aquea, 2015; Srivastava et al., 2015; Kotak et al., 2018; Vlachonasios et al.,
463 2003). Indeed, null mutations in the SGF11 and UBP22 central subunits of DUBm only result in subtle
464 developmental defects, which is in sharp contrast to the strong and pleiotropic effects induced by
465 loss-of-function of HATm. Another seemingly contradictory observation is that expression of only
466 ~5% of *Arabidopsis* genes is affected by mutations in *GCN5* or *ADA2b* in adult leaves (Vlachonasios et
467 al., 2003). Collectively, this suggests that reaching wild-type mRNA steady-state levels only marginally
468 requires SAGA activity once the leaf has reached an adult morphology whilst its activity on chromatin

469 might be more essential during developmental transitions. Still, in the absence of internal
470 normalization, transcriptomic analyses of RNA steady-state levels performed on entire plants or
471 organs allow probing neither global variations in RNAs abundance per cell nor transcriptional rates.
472 Genome-wide chromatin profiling and measurements of newly-synthesized transcripts after pulse-
473 chase labelling with 4-thiouracil (4tU) allowed disentangling a similar paradox in yeast to unveil the
474 general influence of SAGA on transcription over nearly all genes (Bonnet et al., 2014; Baptista et al.,
475 2018). In addition, reduction of the transcriptional regime in SAGA deficient plants might be buffered
476 by compensatory effects such as reduction in transcript turn-over (Baptista et al., 2018). The recent
477 development of genome-enabled methods to quantify absolute changes of transcript levels and to
478 detect nascent transcripts in *Arabidopsis* should help deciphering the extent of plant SAGA activity.

479 An emerging concept is therefore that SAGA might be critical for efficient developmental and
480 stress responses, especially when the plant is exposed to changing or stressful growth conditions
481 (Srivastava et al., 2015; Nassrallah et al., 2018). Accordingly, the expression of representative salt,
482 heat and light inducible genes is affected in *Arabidopsis* mutant lines of SAGA subunits (Srivastava et
483 al. 2015). In addition, several recent studies indicate that plant SAGA complex abundance and
484 activities are strongly modulated both at transcriptional and post-translational levels (Srivastava et
485 al., 2015; Nassrallah et al., 2018), possibly allowing for an adjustment of the epigenome to the cell
486 transcriptional status. Importantly, the direct targets of SAGA modules over plant genomes have not
487 been determined and therefore, in most instances, we lack information on the primary
488 consequences of SAGA inactivation *in planta*. While GCN5 chromatin association has been
489 determined using an *Arabidopsis* promoter array (Benhamed et al, 2008), the dynamic association of
490 each plant SAGA module with different genomic locations during cell growth and specification
491 remains to be assessed in future studies.

492

493

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507

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823

824 **Figure legends**

825

826 **Figure 1.** Schematic representation of the composition of the Arabidopsis SAGA complex.

827 **A,** Protein subunits comprising four modules (COREm, TRA1m, HATm and DUBm) of the *Arabidopsis*
828 SAGA complex established by analogy with the recently solved structures of the yeast SAGA complex
829 (Papai et al., 2020; Wang et al., 2020). The COREm is also termed main lobe or central module of
830 SAGA (Papai et al., 2020; Wang et al., 2020). While COREm, TRA1m and HATm efficiently co-purified
831 with each other from *Arabidopsis* cells, the DUBm showed marginal co-purification (Nassrallah et al.,
832 2018; Pfab et al., 2019), suggesting that it associates only weakly/transiently with the rest of SAGA.

833 **B,** Cartoon representation of the Arabidopsis DUBm as determined by computational homology
834 modeling (Pfab et al., 2019) with UBP22, SGF11 and ENY2 color-coded as indicated. The
835 assembly/catalytic lobes of DUBm are indicated according to the structure of the yeast DUB (Köhler
836 et al., 2010).

837

838 **Figure 2.** GCN5-mediated control of plant processes.

839 Representative examples of developmental, hormonal, environmental and stress signaling cascades
840 that involve GCN5-mediated acetylation of histones and regulated gene expression are shown. In
841 general, upon elicitation by external or internal signals (indicated on the left), GCN5 and ADA2b
842 subunits of the SAGA histone acetyltransferase module (HATm) are recruited to the promoter region
843 of responsive genes by specific transcription factors. GCN5-ADA2b activity triggers histone
844 acetylation favoring RNA Pol II transcription. Question marks indicate that involvement of ADA2b or
845 that of a candidate transcription factor in GCN5 recruitment has not been fully demonstrated.

846

847 **Figure 3.** Working model for DUBm activity in the regulation of H2Bub homeostasis.

848 After its deposition by the HUB E3 ligase, histone H2Bub can be deubiquitinated along the body of
849 expressed genes by the protease activity of DUBm subunit UBP22. Co-transcriptional cycles of

850 histone H2B ubiquitination/deubiquitination are thought to promote RNA Pol II elongation in plants
851 as in other eukaryotes. The "C3D" complex (made of COP10, DET1, DDB1 and DDA1) controls the
852 stability of DUBm subunits by ubiquitin-mediated proteolytic degradation through a physical
853 association of its DDA1 subunit to SGF11 (Nassrallah et al., 2018). DET1-based post-translational
854 control of DUBm abundance may allow the cell to adjust for different transcriptional regimes under
855 unfavorable environmental conditions such as darkness. The association of DET1 with non-acetylated
856 histone H2B (Benvenuto et al, 2002) and with transcription factors (Dong et al., 2014) may favor
857 DUBm regulation over specific chromatin domains. DET1 further influences histone H2B acetylation
858 levels via an unknown mechanism, possibly involving SAGA HATm activity. Alternatively, in the
859 absence of Sgf73 ortholog in plants, DUBm may act independently from SAGA (Nassrallah et al.,
860 2018; Pfab et al., 2018).