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# 1 Multifaceted functions of histone deacetylases in stress response

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19 transcription factors, stress granules

## 20 Glossary

21 **Histone acetylation and deacetylation** are the processes by which histone lysine residues at  
22 the N-terminal tail are acetylated or acetylated lysine residues are deacetylated. Lysine  
23 acetylation (Kac) is the process where an acetyl group is transferred from the donor molecule  
24 acetyl coenzyme A (acetyl-CoA) to a lysine residue. Deacetylation is the reverse reaction where  
25 an acetyl group is removed from an acetylated lysine residue generating acetate. These  
26 reactions are catalyzed by "histone acetyltransferase" (HAT) and "histone deacetylase" (HDAC)  
27 activity, respectively. Histone acetylation marks relaxed chromatin regions and represents an  
28 epigenetic mark for gene activation.

29 **Chromatin remodeling** is the dynamic modification of chromatin architecture by ATP-  
30 dependent chromatin remodeling proteins complexes. These complexes recognize chromatin  
31 modifications such histone acetylation, control chromatin states between relaxation and  
32 condensation, and thus regulate gene activity.

33 **Histone lysine acylations** mirror acetylation, but entail histone acylation of histone lysine  
34 residues with either short-chain fatty acids, such as malonylation (Kmal), propionylation (Kpr),  
35 butyrylation (Kbu), crotonylation (Kcr),  $\beta$ -hydroxybutyrylation (Kbhb), 2-hydroxyisobutyrylation  
36 (Khib), or dicarboxylic acids such as succinylation (Ksu). HAT and HDAC are involved in lysine  
37 acylation and deacylation.

38 **Post-translational modification (PTM)** is the covalent modification of proteins following protein  
39 biosynthesis by ribosomes. As an example, phosphorylation is adding a phosphate to a hydroxyl  
40 group of an amino acid residue. Among other types of PTMs are ubiquitination, acetylation or S-  
41 nitrosylation. PTMs usually respond to cell signaling and regulate the modified protein function.

42 **Stress granules** are dense aggregations composed of proteins and RNAs that can appear in  
43 different cell compartments when the cell is under stress.

44

## 45 **Abstract**

46 Histone deacetylases (HDAC) are important chromatin regulators essential for plant  
47 tolerance to adverse environments. Plants use HDACs as part of diverse strategies to  
48 respond to different stresses. In addition to histone deacetylation and epigenetic  
49 regulation, HDACs deacetylate non-histone proteins and thereby regulate multiple  
50 pathways. Like other post-translational modifications, acetylation/deacetylation is a  
51 reversible switch regulating different cellular processes in plants. Here, by focusing on  
52 results obtained in arabidopsis and rice plants, we analyze the different aspects of  
53 HDAC functions and the underlying regulatory mechanisms in modulating plant  
54 responses to stress. We hypothesize that, in addition to epigenetic regulation of gene  
55 expression, HDACs can also control plant tolerance to stress by regulating transcription,  
56 translation, and metabolic activities and possibly assembly-disassembly of stress  
57 granules through lysine deacetylation of non-histone proteins.

58

## 59 ***HDAC functions beyond histone deacetylation***

60

61 **Histone lysine acetylation (Glossary)** is an important chromatin modification that  
62 promotes chromatin decondensation by neutralizing the positive charges of histones.  
63 Thus, it weakens histone interactions with the negatively charged DNA, and recruits  
64 **chromatin remodeling complexes** to alter chromatin structure [1,2]. **Histone**  
65 **acetyltransferases** and **deacetylases (HDAC)** control the dynamic equilibrium of  
66 histone acetylation levels, affecting chromatin structures and gene activity [1]. Therefore,  
67 like phosphatases/kinases or reductases/oxidases, HDACs/HATs constitute a reversible  
68 pair of enzymes regulating post-translational modifications (PTMs) of histone proteins. In  
69 addition to acetylation, **histone lysine acylation** involves the addition of diverse short-  
70 chain fatty acids as histone marks, which can compete with acetylation and potentially  
71 affect gene expression patterns [2]. The different types of acyl-CoA used for histone  
72 acylation are derived from multiple primary metabolic pathways [3], which vary greatly in  
73 different cell types or in response to adverse environmental conditions. HDAC-regulated  
74 histone deacetylation is essential for epigenetic regulation of plant response to diverse  
75 environmental stress, which has been reviewed in several papers (e.g. [4-7]). On the  
76 other hand, HDACs can also remove the acyl groups from histones and play a role in  
77 regulating chromatin structure and gene expression [2]. Recent results indicated that  
78 HDACs are involved in multiple stress-responses and that different classes of HDACs  
79 may have opposite functions in controlling plant tolerance to stresses by epigenetic  
80 regulation of different sets of genes. HDACs were shown to interact with diverse  
81 chromatin and transcription factors, or with signaling proteins to target specific genomic  
82 loci. In addition, HDAC functions could be regulated by dynamic subcellular localization  
83 and posttranslational modifications. Furthermore, function of HDAC-mediated histone  
84 deacylation in gene regulation has been documented. Importantly, besides histones,  
85 increasing numbers of non-histone proteins have been recognized as substrates for  
86 HDACs. In plants, proteomics analysis uncovered hundreds of lysine acetylated proteins  
87 that are involved in diverse pathways and processes, and exhibiting different subcellular  
88 localizations [8,9]. In this opinion paper, we analyze these new facets of HDAC  
89 functioning in arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*) and discuss the  
90 function of HDAC-mediated deacetylation of actors involved in transcription, translation,

91 and metabolic pathways as well as in cellular organization by modulating membrane-  
92 less organelles.

93

### 94 ***Diverse functions of HDACs in stress responses***

95

96 HDACs are highly conserved enzymes in eukaryotes. In arabidopsis, 18 HDACs have  
97 been identified, of which 12 belong to the Reduced Potassium Dependency3  
98 (RPD3/HDA1) subfamily, 4 belong to the plant-specific HD2 subfamily, and 2 belong to  
99 the SIR2 subfamily [10]. The RPD3/HDA1-like HDACs, which are Zn<sup>2+</sup>-dependent, are  
100 further divided into three classes (I, II and IV) [11]. All HDAC subfamilies are involved in  
101 plant response to diverse stress [4-7], but display agonistic or antagonistic functions in  
102 specific stress-responses (**Table 1**). For instance, arabidopsis RPD3/HDA1-like class I  
103 and class II HDACs oppositely control plant response to salt stress. Class I *hda19* (or  
104 *athd1*) and *hda9* mutants are more tolerant than the wild type to salt stress, whereas  
105 class II quadruple mutants (*hda5, 14, 15, 18*) are hypersensitive to salt stress [12-14].  
106 HDA9 negatively regulates stress-tolerance by inhibiting DNA-binding activity of  
107 WRKY53 that functions as a higher hierarchy transcription activator of stress-responsive  
108 genes [14]. An opposite role in drought stress was also detected for class I and class II  
109 HDACs. For instance, class II *hda15* mutants displayed a reduced ABA sensitivity and  
110 were susceptible to drought stress [15], while class I *hda6* mutants showed a strong  
111 drought tolerance [16]. HDA15 suppresses negative regulators of ABA signaling to  
112 enhance plant tolerance to drought [15], whereas HDA6 regulates metabolic gene  
113 expression for drought stress response [16] (see below). Similarly, class I *hda19* and  
114 *hda9* mutants displayed a warm temperature-insensitive phenotype, which is opposite to  
115 what observed in class II *hda15* mutants [17]. Likely, the opposite functions between  
116 class I and class II HDACs in stress tolerance are based on regulation of distinct cellular  
117 pathways and different sets of stress-responsive genes, which may help to balance  
118 cellular response to stress. This is supported by the comparative transcriptomic analysis  
119 showing that HDA9, HDA19, and HDA15 control a distinct gene expression programs in

120 seedling response to warm (27°C) temperature [17]. HDA15 represses plant response to  
121 warm temperature by deacetylating and repressing thermal responsive marker genes,  
122 whereas HDA9 and HDA19 promote plant response to elevated temperature likely  
123 through repression of metabolic and stress-induced genes, respectively [17]. The  
124 opposite functions of class I and II HDACs in plant response to stresses may also  
125 involve their distinct stress-sensing mechanisms and specific modes of action (see  
126 below).

127  
128 In addition to directly regulating stress-responsive genes, HDAC-mediated stress  
129 responses may also implicate the regulation of metabolic flux by epigenetic regulation of  
130 metabolic gene expression. For instance, HDA15 is shown to repress many primary  
131 metabolic genes in response to elevated temperature [17]. Under normal conditions,  
132 HDA6 directly bound to and repressed acetate biosynthetic pathway genes encoding  
133 pyruvate decarboxylase and acetaldehyde dehydrogenase. However, under drought  
134 stress, HDA6 dissociated from the acetate biosynthetic genes, leading to a metabolic  
135 flux conversion from glycolysis into acetate synthesis [16], which can be recycled to  
136 produce acetyl-CoA, and to support histone acetylation of jasmonate-dependent stress-  
137 responsive genes. Moreover, it was shown that SRT1-controlled plant stress response  
138 regulated glycolysis through epigenetic repression of several glycolytic genes [18,19]. In  
139 addition to histone deacetylation, a few HDACs were also shown to remove different  
140 histone acylation marks. For instance, rice SRT2 selectively catalyzes decrotonylation  
141 [20], while Arabidopsis HDA6 and HDA9 catalyze histone 2-hydroxyisobutyrylation [21].  
142 However, whether HDAC-mediated histone deacylation responds to different metabolic  
143 levels and affects histone acetylation and gene expression in stress response remains  
144 unclear. The mechanism underlying the selective deacylation by individual HDACs is  
145 presently obscure.

146

### 147 ***Controlling HDAC function in stress response***

148

149 As with most chromatin regulators, HDACs do not have DNA-binding activity. Although  
150 the mechanism by which HDACs target to specific genomic loci to undertake histone

151 modification is generally unclear, accumulating results showed that interaction with  
152 DNA-binding transcription factors or chromatin regulators appears to be the principal  
153 manner for HDAC targeting to downstream genes (**Figure 1**). For instance, class I HDA9  
154 interacts with several stress-related transcription factors such as PWR [22], WRKY53  
155 [14]; HOS15 [23], HY5 [24], and ABI4 [25]. HDA6 has been shown to interact with  
156 diverse proteins including BIN2, FLD, MET1, SUVH, AS1, HSI2, transposase, as well as  
157 HD2C [26-31]. Recent results indicated that HD2 proteins are histone chaperones and  
158 are present in complexes with RPD3-like HDACs, increasing their histone deacetylation  
159 activities by bringing histones and HDACs together [27,32,33]. HDA19 (or AtHD1) was  
160 shown to form conserved SIN3-type transcriptional repressor complexes with other  
161 proteins to regulate plant response to abiotic stress through histone deacetylation at  
162 specific target genes [34,35]. In addition, HDA19 interacts with WRKY38 and WRKY62  
163 transcription factors to negatively regulate plant basal response to biotic stress [36].  
164 Class II HDA15 was shown to interact with diverse transcription factors (PIF1/3, NF-  
165 YCs, HY5, HFR1 and MYB86) to control plant response to stress [17,37-40]. AtSRT1  
166 interacts with AtMBP-1 to repress glycolysis and stress tolerance [18]. AtSRT1 and  
167 AtSRT2 interact with ENAP1 to negatively regulate ethylene-responsive genes [41]. The  
168 diverse interacting protein partners of individual HDACs indicate the versatility of their  
169 recruitment to genomic targets and suggest different mechanisms or regulatory  
170 networks of the HDAC proteins in controlling stress responses.

171  
172 Most plant HDAC proteins are localized in the nucleus, while several HDACs are  
173 detected in the cytoplasm, mitochondria, or chloroplasts [42-45] (**Figure 1**). In addition,  
174 arabidopsis HD2 members and HDA15 also function in the nucleolus [46,47]. Some  
175 HDACs show both cytoplasmic and nuclear localization, the partitioning between the two  
176 compartments is likely a regulated process. Dynamic subcellular localization appears to  
177 regulate HDAC function or activity. For instance, HDA9 localizes in both cytoplasm and  
178 nucleus, but is enriched in the nucleus under heat stress at 37 °C, where the zinc-finger  
179 transcription factor YY1 helps to recruit it to target genes and to regulate gene  
180 expression by histone deacetylation [48]. HDA15 localizes in the nucleus in the light and  
181 in the cytosol in the dark [38], but another study showed that HDA15 is sequestered by

182 PIF3 to repress photosynthesis-related genes [37]. HDA5 was previously reported to  
183 reside in the cytoplasm when transiently expressed as a HDA5-YFP fusion protein in  
184 arabidopsis cells [43]. However, another report showed that HDA5 could interact with  
185 FLD and FVE (or MSI4) in the nucleus [49]. Furthermore, the recent characterization of  
186 HDA5 subcellular localization using stable 35S:HDA5-GFP plants revealed its presence  
187 in both the nucleus and cytoplasm [50]. A recent study indicated that HDA6 could be  
188 exported to the cytoplasm by exportin XPO1A, as the *xpo1a* mutation increased nuclear  
189 retention of HDA6, resulting in reduced histone acetylation and enhanced transgene  
190 silencing [51]. Furthermore, the analysis of transient expression in tobacco  
191 demonstrated that HDA14 exhibits dual localization in both mitochondria and  
192 chloroplasts [8]. Thus, subcellular partitioning that can be stress-responsive may control  
193 HDAC activities in gene regulation and other cellular pathways.

194  
195 As with many cellular proteins, HDAC proteins can be subjected to **post-translational**  
196 **modifications (PTMs) (Figure 1)**. It has been shown that HDAC PTMs affect their  
197 activity or function and can be regulated by stress. For instance, HDA9 is  
198 phosphorylated by an unknown mechanism. Under heat stress, the phosphatase  
199 PP2AB $\beta$  dephosphorylates HDA9 which prevents HDA9 from 26S proteasome-  
200 mediated degradation, leading to HDA9 translocating to the nucleus [48]. The  
201 phosphorylation status of HDA15 affects its sub-nuclear localization and enzymatic  
202 activity [47]. Phosphorylation/dephosphorylation of rice HDT701 that negatively regulate  
203 rice immunity is switched in response to *Xanthomonas oryzae pv.oryzae* (*Xoo*) infection  
204 [52-54]. S-nitrosylation is an important PTM that affects protein activity. Proteomic data  
205 have identified arabidopsis HD2B and HD2C as targets of S-nitrosylation, which might  
206 affect their activity [55]. Recently, HDA19 was shown to be modified by S-nitrosylation  
207 which is enhanced under stress and promotes its deacetylase activity [56]. However,  
208 nitric oxide (NO) treatment increases histone acetylation in the plant [57,58], suggesting  
209 that S-nitrosylation is inhibitory for most HDACs catalytic activity. Moreover, HDACs can  
210 be subjected to ubiquitination and proteasome degradation, suggesting a regulation of  
211 their turnover. For instance, cold induces HD2C degradation mediated by the CULLIN4  
212 (CUL4)-based E3 ubiquitin ligase complex [59]. In addition, several rice HDAC proteins



213 are found to be acetylated [60], but the effect of acetylation on the protein activity is not  
214 yet studied. Currently, the importance of PTMs as mechanisms to regulate HDAC  
215 subcellular localization, catalytic activity, and turnover in response to stress is  
216 underestimated.

217

### 218 ***HDAC functions in deacetylation of non-histone proteins***

219 In addition to its occurrence on histones, lysine acetylation (Kac) is a widespread post-  
220 translational modification occurring at a large number of proteins of diverse biological  
221 functions in various organisms [1,61]. Kac is modulated by acetyl-CoA availability in the  
222 cell and is suggested to have a function in protein activity and/or quality control. In  
223 particular, most enzymes of energy metabolism such as glycolysis, tricarboxylic acid  
224 (TCA) cycle and photosynthesis, are modified by Kac, and their acetylation status  
225 affects enzymatic activities and metabolic flux (e.g. [60,62-64]. Because Kac requires  
226 the primary metabolite acetyl-coA, Kac appears to be an additional cellular responsive  
227 mechanism that allows plants to acclimate metabolic and cellular activities to the  
228 environment. Consistently, recent analysis in arabidopsis roots revealed an alteration of  
229 protein lysine acetylation in response to osmotic and salt stress [65]. Homeostasis of  
230 non-histone protein Kac that can occur autonomously in cells with high acetyl-CoA  
231 levels is also regulated by HATs and HDACs. Several plant HDACs were shown to  
232 regulate Kac and activity of proteins involved in diverse cellular pathways including  
233 transcription, translation and metabolism (**Figure 1**).

### 234 **Controlling transcription factor and signaling proteins by lysine deacetylation:**

235 Evidence for HDAC-mediated lysine deacetylation of transcription and signaling proteins  
236 is emerging in plants. For instance, HDA6 can deacetylate BIN2 (a repressor of  
237 brassinosteroid signaling) at lysine 189 to repress its kinase activity in arabidopsis [29].  
238 HDA9 deacetylates and impairs DNA-binding of WRKY53, a stress-responsive  
239 transcription factor [14]. In contrast, arabidopsis SRT1 deacetylates and enhances the  
240 stability of AtMBP-1, a transcriptional repressor of stress tolerance and glycolytic genes  
241 [18]. Similarly, rice SRT1 could deacetylate glyceraldehyde-3-phosphate dehydrogenase  
242 (GAPDH) and inhibit its nuclear localization and moonlighting function as a  
243 transcriptional activator of glycolytic genes under oxidative stress [19]. Thus, controlling

244 transcription factors activity by lysine deacetylation may represent an additional layer of  
245 HDAC-dependent stress-responsive gene regulation. How HDAC senses cellular signals  
246 to deacetylate and regulate activity of transcription factors and signal proteins remains  
247 unclear.

248 **Controlling metabolic and translational activities by lysine deacetylation:** Kac may  
249 affect enzymatic activities and metabolic flux [64], but its effect on most modified  
250 proteins is unclear. It appears that KAc inhibits activities of Rubisco, phosphoglycerate  
251 kinase and GAPDH in *in vitro* assays [64]. Several plant HDACs have been shown to  
252 regulate Kac of metabolic enzymes. Arabidopsis HDA14 located in the chloroplasts,  
253 deacetylates many proteins having functions in photosynthesis [8]. In addition, HDA14  
254 can deacetylate  $\alpha$ -tubulin, associate with  $\alpha/\beta$ -tubulin and is retained on GTP/taxol-  
255 stabilized microtubules [44]. Arabidopsis SRT2 was shown to deacetylate inner  
256 mitochondrial membrane protein complexes to participate in energy metabolism and  
257 metabolite transport [42]. Rice HDA714 and HDA706 function redundantly to maintain  
258 Kac homeostasis of a large number of rice proteins, many of which are metabolic  
259 enzymes [60]. HDA714 is closely related to Arabidopsis HDA14 but is mainly found in  
260 the cytoplasm [60]. Mutation of HDA714 increased Kac of glycolytic and TCA enzymes  
261 as well as chloroplast proteins, suggesting a conserved function between HDA714 and  
262 AtHDA14. *HDA714* mutants showed lower activity of glycolysis [60], corroborating  
263 previous results showing that Kac represses GAPDH enzymatic activity [65]. HDA714-  
264 mediated lysine deacetylation of metabolic enzymes may be related to stress tolerance,  
265 since the *hda714* mutation reduced rice plant tolerance to submergence stress [60].

266 Interestingly, many ribosomal (r-proteins) and translation proteins are extensively  
267 acetylated in rice and many other eukaryotes [60], suggesting that r-protein Kac may be  
268 modulated by intracellular acetyl-CoA levels, which provides a possible link between  
269 translational regulation and cellular metabolic activity. As cellular acetyl-CoA levels are  
270 sensitive to stress, r-protein Kac could connect translational activities to stress  
271 response. HDA714 was found to deacetylate r-proteins in rice plants [60]. HDA714 loss-  
272 of function increases Kac levels but reduces abundance of r-proteins. Further analysis  
273 indicated that the increased Kac of translational proteins resulted in increased ribosome

274 stalling frequency and impaired translation. Thus, HDA714 appears to control  
275 translational activity by regulating r-protein stability and ribosome function through lysine  
276 deacetylation. Whether HDA714-controlled r-protein Kac is involved in stress response  
277 needs further investigation. Nevertheless, the results extend the role of Kac  
278 homeostasis mediated by HDACs in gene expression to protein translational regulation,  
279 These examples illustrate that HDAC may regulate diverse cellular pathways by  
280 deacetylating non-histone proteins. How HDAC activities towards non-histone protein  
281 are controlled await further investigation.

282

### 283 ***Do plant HDACs function in Stress Granules?***

284 **Stress granules** (SGs) are inducible and reversible membraneless biomolecular  
285 condensates and are assembled via liquid-liquid phase separation (LLPS) upon  
286 induction by a variety of endogenous and exogenous stresses [66-68]. In general, SGs  
287 are disassembled during the recovery from the stress condition, allowing the restoration  
288 of active translation [67]. Consistently, SGs contain translationally stalled mRNA,  
289 translation inhibition factors, and RNA binding proteins (RBPs), but also HSPs and other  
290 stress-related proteins [69,70]. In plants, SGs form in response to diverse stress  
291 including heat, hypoxia, high salt, and darkness as well as hormone treatment or  
292 metabolic inhibition [71-74]. Several RBPs have been identified as integral components  
293 of plant SGs [70], and undergo phase separation to promote plant survival in response  
294 to heat stress [75]. While SG has been largely documented in the cytosol, recent works  
295 described formation of chloroplastic SG (cpSG) under heat stress, in which RNA-binding  
296 proteins, ATPases, chaperones, as well as a magnesium chelatase complex, involved in  
297 photosynthetic acclimation to stress was found [76].

298 LLPS is often triggered by proteins with intrinsically disordered regions (IDRs) and/or  
299 prion-like domains that induce the aggregation of complex proteins or protein-RNA  
300 networks, such as SGs. According to the "stickers-and-spacers" model, oligomerization  
301 and condensation is promoted by protein domains called stickers which serve as  
302 adhesion drivers, while the spacers that connect the stickers dictate solvent/protein

303 interactions [77]. PTMs like phosphorylation, can alter the phase separation threshold of  
304 IDRs by changing the charge and hydrophobicity of amino acids, and thus are  
305 recognized as important regulators for condensate formation [78]. For example, Gle1, a  
306 crucial regulator of translation during stress, undergoes phosphorylation by MAPKs and  
307 GSK3. When Gle1 is phosphorylated, it hinders the assembly of SGs and facilitates their  
308 disassembly [79]. Recently, phase separation has been shown to be regulated by  
309 oxidoreduction. In arabidopsis, cysteine residues within the TERMINATING FLOWER  
310 (TMF) transcription factor sense cellular redox to form disulfide bonds that concatenate  
311 multiple TMF molecules and elevate the amount of intrinsically disordered regions to  
312 drive phase separation. This enables TMF to bind and sequester the promoter of a floral  
313 identity gene *ANANTHA* to repress its expression [79]. Among all PTMs, most of lysine  
314 acetylations has a negative effect on phase separation, and appears to play a pivotal  
315 role in inhibiting stress granules assembly [80]. During the dynamics of stress granules  
316 (SGs), the modification of lysine 376 residue in the RNA-binding domain of G3BP1 is  
317 under the regulation of HDAC6 and CBP/p300. When G3BP1 undergoes acetylation, it  
318 negatively affects its ability to bind RNA and interact with other components, thereby  
319 promoting the disassembly of SG [81]. It was shown that the mammalian histone  
320 deacetylase HDAC6 participates in and is required for the formation of SGs during  
321 stress response [82]. Additionally, acetylation of IDR inhibits protein phase separation  
322 and SGs assembly and HDAC6 deacetylates IDRs, promoting SG assembly [83]. The  
323 acetylation of the intrinsically disordered region (IDR) of DDX3X, which is a component  
324 of SGs, hinders the formation of SGs [82] [83]. Plant homologs of G3BP-1 and DDX3  
325 are shown to function in SGs, although no evidence of lysine acetylation has been  
326 explored yet [84,85].

327 Interestingly, many plant cytosolic or chloroplastic SG proteins show lysine acetylation  
328 (**Table 2 and 3**), suggesting that this PTM may also play an important role in SGs in  
329 plant cells. For instance, a HDAC-dependent partitioning of ethylene metabolic proteins  
330 into stress granules might contribute to balance growth and stress responses. No plant  
331 HDAC has been identified yet in SG. However, several plant HDACs localize in the  
332 cytoplasm and in chloroplasts and contain IDRs [11]. Among them, class II HDA14 and

333 HDA15, the closest Arabidopsis homologs of HsHDAC6, and class I HDA6, 7, 9 and 19  
334 harbor IDR both at N- and C-terminal ends. Particularly, the C-terminal IDR is conserved  
335 in the green lineage [11] and is also present in mammalian HDAC2 in which it induces  
336 phase separation *in vitro* [86]. Therefore, HDACs may be potentially implicated in plant  
337 SG formation under stress (**Figure 1**). This is particularly compelling for rice HDA714, as  
338 it targets many translational initiation/elongation proteins, r-proteins, and HSPs as well  
339 as metabolic enzymes for deacetylation in the cytoplasm [60]. Whether HDA714 is  
340 involved in SG dynamics awaits further investigation.

341

### 342 ***Concluding remarks and future perspectives***

343

344 Accumulating evidence indicates that HDACs are essential regulators of plant response  
345 and adaption to adverse environmental conditions. Although HDACs act primarily  
346 through epigenetic regulation of stress-responsive genes by mediating histone  
347 acetylation/deacetylation, increasing data indicate that HDACs are also implicated in the  
348 control of additional cellular processes including metabolism, hormone signaling,  
349 microtubule, transcription, ribosomal biogenesis, and translation to modulate plant stress  
350 tolerance. It appears that some HDACs are specialized to control lysine acetylation of  
351 non-histone proteins in the cytoplasm and/or organelles, while others have a more  
352 polyvalent function in both cytoplasm and nucleus. Several HDACs are involved in  
353 multiple stress pathways and some HDACs respond to and regulate plant response to  
354 the same stress, sometimes with opposing functions. This functional versatility of  
355 HDACs in plant stress responses is mysterious. Whether or how the function of different  
356 HDACs is coordinated in the nucleus and/or in the cytoplasm awaits further investigation  
357 (**Outstanding Questions**). Although HDAC gene expression *per se* may be responsive  
358 to stress, posttranslational modification, protein interaction and subcellular partitioning  
359 are likely the principal way to control HDAC activity in plant response to stress. Whether  
360 and how stress signals regulate HDAC PTMs, protein interaction and/or subcellular  
361 localization remains largely unclear. Stress can trigger metabolic flux conversions, while

362 metabolic activity controls plant adaptation to stress, which partly relies on epigenetic  
363 regulation of gene expression. How HDACs sense metabolic changes and precisely  
364 coordinate metabolic activity to gene expression in specific cell types are challenging  
365 questions to be answered in the future. The function of HDAC in SG dynamics in plant  
366 cells remains to be studied. Several HDACs (e.g., HDA6, HDA9, HDA19, SRT1 as well  
367 as HD2 members) negatively regulate plant immunity and tolerance to heat/salt stress.  
368 Engineering these HDACs or identifying natural inhibitory products targeting specifically  
369 these HDACs would represent an alternative approach to enhance plant/crop stress  
370 tolerance in the global warming era.

371

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375

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581 **Figure legend**

582 **Figure 1.** Multiple modes of action of HDACs in regulating plant stress responses. HDACs  
583 localize in the different cellular compartments. Subcellular partitioning and/or posttranslational  
584 modifications (such as phosphorylation in red and S-nitrosylation in blue) of HDACs can be  
585 regulated by diverse stresses (arrows). In the nucleus, members of all HDAC families  
586 HDA5/HDA15; HDA6/HDA9/HDA19; HD2A/HD2C/HD2D; HDA701/HDA710 and SRT1/OsSRT2)  
587 can be recruited to chromatin by interacting with transcription factors (TFs) and/or chromatin  
588 proteins to deacetylate histones for gene repression. In addition, some HDACs (such as HDA6  
589 and OsSRT2) also remove acyl groups from histones. Besides the histone  
590 deacetylation/deacylation function, HDACs can also deacetylate non-histone proteins such as  
591 TFs and signaling proteins. (i.e. HDA6 deacetylates BIN2, HDA9 deacetylates WRKY53, and  
592 SRT1 deacetylates GAPDH and AtMBP-1). In the cytoplasm, HDACs regulate lysine acetylation  
593 of glycolytic enzymes, microtubules, ribosomal and translational proteins in the cytosol,  
594 photosynthetic proteins in chloroplasts, and tricarboxylic acid cycle (TCA) enzymes and  
595 membrane proteins in mitochondria. A function of HDACs in stress granule assembly and  
596 disassembly is highly possible.

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601 Table 1. Stress functions of HDACs in arabidopsis and rice.

Classes	Arabidopsis Gene	ID	Rice Gene	ID	Functions in stress
<b>Class I</b> RPD3/HDA1 homologs	AtHDA6	At5g63110	OsHDA705	Os08g25570	<i>Athda6</i> mutant is drought tolerant [16], but shows impaired tolerance to heat stress [87]; AtHDA6 regulates scrotomorphogenesis [29]
	AtHDA7	At5g35600			
	AtHDA9	At3g44680	OsHDA711	Os04g33480	<i>Athda9</i> mutant is insensitive to NaCl and polyethylene glycol (PEG) [13]; <i>Athda9</i> mutant shows impaired warm temperature-response [17]; AtHDA9 represses thermotolerance responses [48]
	AtHDA19	At4g38130	OsHDA702 OsHDA703 OsHDA710	Os06g38470 Os02g12350 Os02g12380	<i>Athda19</i> mutant is insensitive to NaCl and polyethylene glycol (PEG); <i>Athda19</i> mutant is salt stress tolerant [12]; <i>hda19</i> mutant shows impaired warm temperature-response [17]; AtHDA19 negatively regulates plant basal defense responses [36] OsHDA710 is required for salt stress tolerance and ABA signaling in rice[88]
			OsHDA701	Os01g40400	OsHDA701 reduces defense gene activation [89]
	AtHDA10	At3g44660			
	AtHDA17	At3g44490			
<b>Class II</b> RPD3/HDA1 homologs	AtHDA5	At5g61060	OsHDA713	Os07g41090	<i>hda5 hda14 hda15 hda18</i> quadruple mutant is hypersensitive to salt stress [12]
	AtHDA8	At1g08460	OsHDA712 OsHDA716	Os05g36920	
	AtHDA14	At4g33470	OsHDA714	Os05g36930 Os12g08220	OsHDA714 represses glycolysis under stress. <i>Oshda714</i> mutant seed germination rates is reduced under water (submergence) stress [60]
	AtHDA15	At3g18520	OsHDA704	Os07g06980	<i>Athda15</i> mutant is insensitive to ABA, susceptible to drought stress but hyposensitive to salinity during seed germination [15] and [90] Tu et al 2022; AtHDA15 overexpression confers salt stress resistance [91]. <i>Athda15</i> mutant shows enhanced warm temperature-response [17]; AtHDA15 regulates responses to light [39]. HDA15 is involved in FR light-regulated hypocotyl elongation [92].
	AtHDA18	At5g61070			
<b>Class III</b> Sir2 homologs	AtSRT1	At5g55760	OsSRT1	Os04g20270	AtSIRT1 has negative function in salt stress and exogenous ABA response [18]; OsSIRT1 is regulates oxidative stress adaptation and represses glycolysis in rice [93,19]
	AtSRT2	At5g09230	OsSRT2	Os12g07950	OsSRT2 is involved in the removal of Kcr.[20]

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<b>Class VI</b> RPD3/HDA1 homologs	AtHDA2	At5g26040	OsHDA706	Os06g37420	HDA706 deacetylates non-histone proteins [60]
<b>Plant-specific HD2</b>	AtHD2A	At3g44750	OsHDT1	Os05g51830	OsHDT1 controls plant immunity [52]
	AtHD2B	At5g22649			
	AtHD2C	At5g03740			AtHD2C is involved in drought stress [94] and heat stress responses [95]; cold induces AtHD2C degradation [96,59]
	AtHD2D	At2g27840			AtHD2D is involved in drought stress response [97,96]

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605 Table 2. List of arabidopsis cytosol SG proteins with lysine-acetylation identified.

Protein ID	Name	Function	Localization	Acetylated Lysine	Putative IDR
AT1G09780	Phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent	Metabolism	cytosol	236,238,402, 463	1-15
AT1G12010	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily	Ethylene Metabolism	peroxisome	63	307-320
AT1G62380	ACO2, ATACO2   ACC oxidase 2	Ethylene Metabolism	golgi	68	308-320
AT1G62390	Phox2   Octicosapeptide/Phox/Bem1p (PB1) domain-containing protein / tetratricopeptide repeat (TPR)-containing protein	Other	nucleus	80, 160,328	1-35, 183-285, 442, 626-676, 751
AT1G63000	NRS/ER, UER1   nucleotide-rhamnose synthase/epimerase-reductase	Rhamnose metabolism	cytosol	179, 263	1-12
AT1G78570	RHM1, ROL1, ATRHM1   rhamnose biosynthesis 1	Rhamnose metabolism	cytosol	49, 290, 551	354-379
AT2G01520	MLP328   MLP-like protein 328	Lipid Binding [putative]	cytosol	15, 136	-
AT2G23350	PAB4, PABP4   poly(A) binding protein 4	RNA	cytosol	130, 131, 139, 216, 334	1-42, 120-133, 253, 302-323, 456, 634-662
AT2G25450	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	Glucosinolate Metabolism	golgi	23,322	-
AT2G42520	P-loop containing nucleoside triphosphate hydrolases superfamily protein	Other	nucleus	390	1-140, 556-600
AT2G45220	Plant invertase/pectin methyl esterase inhibitor superfamily	Pathogen Response	extracellular	373	1-26
AT3G01290	SPFH/Band 7/PHB domain-containing membrane-associated protein family	Pathogen Response	plasma membrane	78, 178	-
AT3G26450	Polyketide cyclase/dehydrase and lipid transport superfamily	Lipid Binding [putative]	cytosol	15, 117	-
AT3G45640	ATMPK3, MPK3, ATMAPK3   mitogen-activated protein kinase 3	Signaling/Regulation	cytosol	69	1-14
AT3G48750	CDKA;1, CDC2AAT, CDK2, CDC, CDC2A, CDKA1   cell division control 2	Signaling/Regulation	cytosol	32, 66	-
AT4G23670	Polyketide cyclase/dehydrase and lipid transport superfamily protein	Lipid Binding [putative]	cytosol	15, 74, 78, 116, 139	-
AT4G23680	Polyketide cyclase/dehydrase and lipid transport superfamily protein	Lipid Binding [putative]	cytosol	116, 136, 139	-
AT4G27500	PPI1   proton pump interactor 1	Transport	endoplasmic reticulum	57, 148, 356, 428, 442	1-70, 154-176, 612
AT4G34110	PAB2, PABP2, ATPAB2   poly(A) binding protein 2	RNA	cytosol	324	1-33, 116-135, 251, 291-313, 354, 615-629
AT4G34870	ROC5, ATCYP1   rotamase cyclophilin 5	Protein Folding	cytosol	49, 78, 96, 142, 147, 151	-
AT4G39520	GTP-binding protein-related	Signaling/Regulation	cytosol	49	44-56
AT5G19510	Translation elongation factor EF1B/ribosomal protein S6 family protein	Translation	cytosol	132	88-131
AT5G20890	TCP-1/cpn60 chaperonin family protein	Protein Folding	cytosol	145	518-527
AT5G59420	ORP3C   OSBP (oxysterol binding protein)-related protein 3C	Lipid Binding [putative]	cytosol	369	1-62, 429-433
AT3G44890	Ribosomal protein L9	Translation	cytosol	98, 158	1-34
AT4G01310	Ribosomal L5P family protein	Translation	cytosol	67, 76, 111, 114	1-47, 245-262
AT3G25920	Ribosomal protein L15	Translation	cytosol	217, 247, 254, 260	1-74, 240-277
AT3G54210	Ribosomal protein L17 family protein	Translation	cytosol	118, 125	1-63
AT3G59400	Protein GENOMES UNCOUPLED 4	Signaling	cytosol	248	1-74, 233-260
AT5G55280	Homolog of bacterial cytokinesis Z-ring protein FTSZ 1-1	Protein binding	cytosol	416	1-66, 385-433
AT5G64050	Glutamate tRNA synthetase	Protein/tRNA binding	cytosol	211, 215, 356, 378, 485, 494	1-51, 557-570
AT2G42220	Rhodanese/Cell cycle control phosphatase superfamily protein	Metabolism	cytosol	73, 123, 129	1-38
AT4G31500	Cytochrome P450, family 83, subfamily B, polypeptide 1	Pathogen response	endoplasmic reticulum	190, 489	1-23, 257-270
AT1G78370	Glutathione S-transferase TAU 20	Glutathione metabolism	cytosol, nucleus	25, 47, 118, 125, 193	-

606 Data of lysine-acetylated proteins were retrieved from [98] and the list of SGs-proteins was from  
 607 [70]. IDR prediction were performed using DISOPRED3 [99]. <http://bioinf.cs.ucl.ac.uk/psipred/>

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609 Table 3. List of arabidopsis plastidial SG proteins with lysine-acetylation identified.

Protein ID	Name	Function	Localization	Acetylated Lysine	Putative IDR
AT1G08520	ALBINA 1	Chlorophyll metabolism	chloroplast	487, 692	1-76, 402-497
AT1G74470	Pyridine nucleotide-disulfide oxidoreductase family protein	Chlorophyll metabolism	chloroplast	137, 383, 423	1-52, 460-467
AT5G55220	trigger factor type chaperone family protein	RNA	chloroplast	162, 221, 254, 382, 476, 532	1-49
AT4G18480	P-loop containing nucleoside triphosphate hydrolases superfamily	Chlorophyll metabolism	chloroplast	122, 308, 374	1-81
AT1G56050	GTP-binding protein-like protein	Metabolism	chloroplast, nucleus	131, 216	1-46
AT2G37660	NAD(P)-binding Rossmann-fold superfamily protein	Rhamnose metabolism	chloroplast	246, 252, 303, 306	1-74
AT5G46580	Pentatricopeptide (PPR) repeat-containing protein	rRNA processing	chloroplast	634	-
AT4G28750	Photosystem I reaction centre subunit IV / PsaE protein	Photosynthesis	chloroplast, plastid	67, 93, 98, 99, 111	1-83
AT5G23020	2-isopropylmalate synthase 2	Glucosinolate Metabolism	chloroplast	217	1-66, 493-503
AT1G76080	Chloroplastic drought-induced stress protein of 32 kD	Pathogen response, heat acclimation	chloroplast	78, 147, 150	1-68
AT4G15560	Deoxyxylulose-5-phosphate synthase	Protein binding	chloroplast	166	1-55
AT1G78630	Ribosomal protein L13 family protein	mRNA binding	plastid	197	1-51
AT5G47890	NADH-ubiquinone oxidoreductase B8 subunit	Unknown	plastid	5, 85	-

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 611 [76]. IDR prediction were performed using DISOPRED3 [99]. <http://bioinf.cs.ucl.ac.uk/psipred/>

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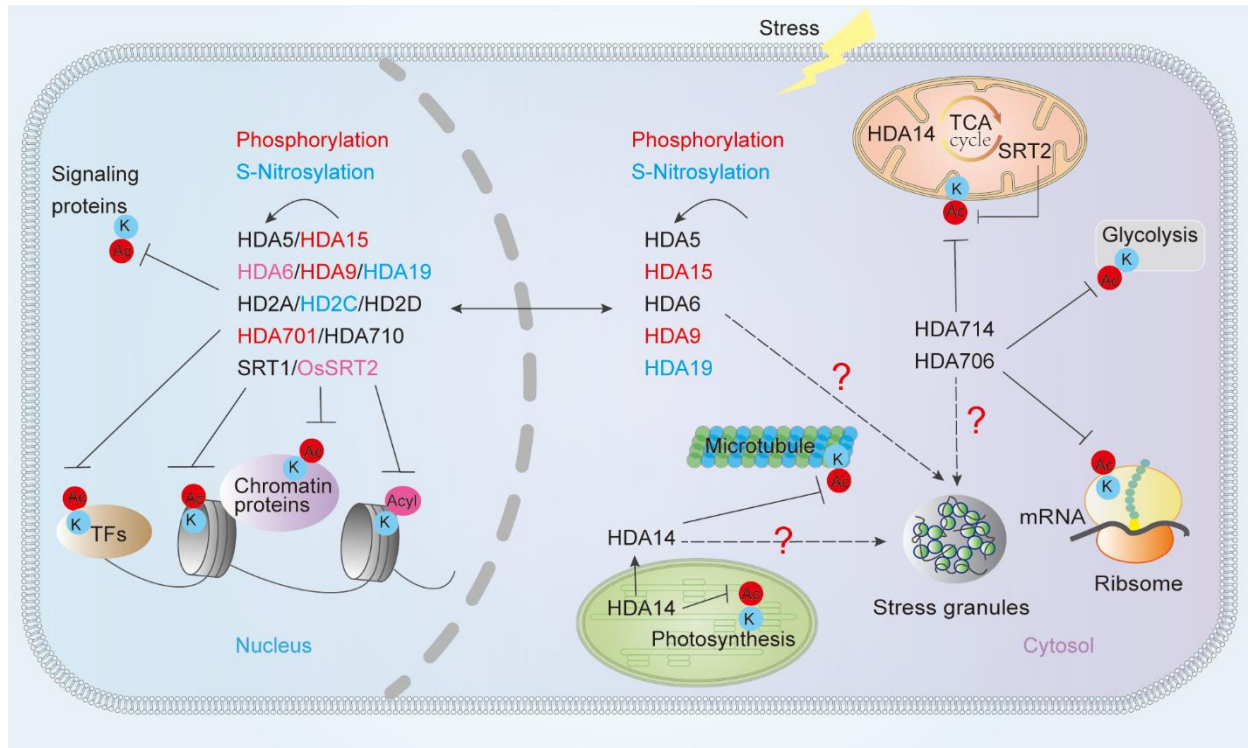
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622 Figure 1

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