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► **To cite this version:**

Juline Auverlot, Avilien Dard, Julio Sáez-Vásquez, Jean-Philippe Reichheld. Redox regulation of epigenetic and epitranscriptomic gene regulatory pathways in plants. *Journal of Experimental Botany*, In press, 10.1093/jxb/erae165 . hal-04555742

HAL Id: hal-04555742

<https://hal.science/hal-04555742>

Submitted on 23 Apr 2024

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1 **Redox regulation of epigenetic and epitranscriptomic gene regulatory pathways in plants**

2

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18 Running title: Redox in epigenetic and epitranscriptomic gene regulation pathways

19

20 Highlight: A review summarizing the current knowledge on the role of redox regulation processes of

21 chromatin remodelling, epigenetic and epitranscriptomic pathways.

22 **Abstract**

23 Developmental and environmental constraints influence genome expression through complex
24 panels of regulatory mechanisms. Epigenetic modifications and remodelling of chromatin are some
25 of the major actors regulating the dynamic of gene expression. Unravelling the factors relaying
26 environmental signals to gene expression reprogramming under stress conditions is an important
27 and fundamental question. Indeed, many enzymes involved in epigenetic and chromatin
28 modifications, are regulated by redox pathways, through post-translational modifications of
29 proteins or by modifications of the flux of metabolic intermediates. Such modifications are potential
30 hubs to relay developmental and environmental changes for gene expression reprogramming. In
31 this review, we aim to update the current knowledge on the interaction between major redox
32 mediators such as ROS, RNS and antioxidant, and epigenetic changes in plants. We will detail how
33 redox status alters the post-translational modifications of proteins, intracellular epigenetic and
34 epitranscriptional modifications, and how redox regulation interplays with DNA methylation,
35 histone acetylation and methylation, miRNA biogenesis, and chromatin structure and remodelling,
36 to reprogram genome expression under environmental constraints.

37

38

39 **Introduction**

40 Genome expression is the driving force of cell growth, development and response to environment
41 of all living organisms. The coordination of spatio-temporal gene expression relies on multiple
42 mechanisms including gene structure and organisation, regulation of transcription factors and
43 epigenetic and epitranscriptomic regulations. Epigenetic and epitranscriptomic processes influence
44 the flow of information between a constant DNA sequence and variable gene expression patterns.
45 This includes various mechanisms like chromatin remodelling, gene transcription and silencing, RNA
46 modifications and maturation, or generation of small RNAs from various precursors (Law and
47 Jacobsen, 2010; Vaucheret and Voinnet, 2024). While epigenetic modifications like DNA
48 methylation can result in heritable effects, they can also be reversed by different erasing enzymes,
49 making these processes highly dynamic. Therefore, they can contribute to the response and
50 adaptation to multiple developmental programs and environmental stimuli (Cui *et al.*, 2023; Lu *et*
51 *al.*, 2023). How epigenetic mechanisms perceive developmental and stress stimuli is a key question.
52 Oxido-reduction (redox) status of the cell is considered as a sensor of environmental stimuli. It
53 influences many enzyme activities through post-translational modifications (PTM), changing their
54 structure and the availability of their co-factors and substrates (Zhang *et al.*, 2022). In particular, the
55 redox status of non-protein and protein thiols is highly responsive to stimuli and can relay signals to
56 downstream pathways. Increasing evidence indicates the responsiveness of chromatin remodelling
57 and epigenetic actors to redox regulation (Wurm and Lindermayr, 2021; Ramakrishnan *et al.*, 2022;
58 Lu *et al.*, 2023).

59 The cellular redox status is defined by the equilibrium between different oxidants and antioxidant
60 system of the cell. Among major oxidants, reactive oxygen species (ROS) and reactive nitrogen
61 species (RNS) are metabolised at different locations inside or outside the cell, depending on the
62 stimuli (Figure 1) (Noctor *et al.*, 2018; Mittler *et al.*, 2022; Hendrix *et al.*, 2023). In addition to their
63 toxic effects on cell metabolism, inducing irreversible oxidation of many cell macromolecules, ROS

64 and RNS play numerous roles in cell signalling (Mittler, 2017; Mittler and Jones, 2023). For example,
65 extracellular H₂O₂ is perceived by plasma membrane receptor kinases to induce intracellular
66 signalling pathways (Wu *et al.*, 2020; Fichman *et al.*, 2022). The interplay between ROS and calcium
67 also signal stress at long distance in plants (Mittler *et al.*, 2022). In many cases, ROS and RNS signal
68 is relayed by different types of proteins oxidation occurring on thiol residues of cysteines or
69 methionines. Redox modifications like disulphide bonds (S-S), S-glutathionylation (S-SG), S-
70 nitrosylation (S-NO), or S-sulfhydration (S-SH) can change protein conformation and activities in a
71 reversible manner which can be readily reduced by different thiol reduction systems like
72 thioredoxins (TRX), glutaredoxins (GRX), protein disulphide isomerase (PDI), S-Nitrosoglutathione
73 reductases (GSNOR) (Mittler *et al.*, 2022; Zhou *et al.*, 2023). These enzymes are themselves
74 dependent on upstream reduction molecules like glutathione (GSH), thioredoxin reductases (NTR),
75 and glutathione reductases (GR), which are dependent on primary reductants like NADPH and NADH
76 (Meyer *et al.*, 2012). These electron donors also provide reducing power to many ROS and RNS
77 detoxification enzymes like different types of peroxidases (PRX), some of them dependent on
78 ascorbate, GSH, GRX or TRX (Meyer *et al.*, 2012; Noctor *et al.*, 2018; Bela *et al.*, 2022).

79 The present review aims to summarise current knowledge on redox regulation and thiol-based PTM
80 modifications of major chromatin remodelling, epigenetic and epitranscriptomic mechanisms,
81 focusing on plants but also on significant evidence from other organisms. Our review will not cover
82 knowledge on redox regulation of transcription factors which can be referred to recent reviews
83 (Mittler *et al.*, 2022; Zhou *et al.*, 2023).

84

85 **Redox regulation of DCL proteins**

86 Epigenetic gene silencing is mediated by RNA-directed DNA methylation (RdDM) pathways. The
87 biogenesis of small RNA is orchestrated by ribonuclease III (RNaseIII) endonucleases, which target
88 double stranded RNA (dsRNA). They are universally involved in processing and maturing almost

89 every class of RNA precursors into functional RNA (Court *et al.*, 2013). In the model plant *Arabidopsis*
90 *thaliana*, two families of RNaseIII are found (Comella *et al.*, 2008; Fukudome and Fukuhara, 2017).
91 The first family corresponds to DICER-LIKE (DCL) enzymes. DCL1 is involved in the production of 21-
92 nt miRNAs from non-perfectly folded hairpin precursors transcribed from *MIR* genes, while DCL2,
93 DCL3 and DCL4 produce 22-, 24- and 21nt siRNAs, respectively, which derive from perfectly paired
94 dsRNA of endogenous or exogenous origins (Vaucheret and Voinnet, 2024). The miRNAs and siRNAs
95 produced by DCL1, DCL2 and DCL4 are loaded onto ARGONAUTE (AGO) proteins that cleave
96 complementary RNA to execute post transcriptional gene silencing (PTGS), while 24-nt siRNAs
97 produced by DCL3 are loaded onto AGO proteins to execute RNA directed DNA methylation (RdDM)
98 and transcriptional gene silencing (TGS) (Borges and Martienssen, 2015). The second RNaseIII family
99 corresponds to RNASE THREE-LIKE (RTL) enzymes (Comella *et al.*, 2008). RTL1 cleaves perfectly
100 paired dsRNA before they are processed by DCL2, DCL3 or DCL4, thus suppressing siRNA production
101 from all endogenous loci during virus infection. RTL1-mediated cleavage of dsRNA precursors are
102 subsequently degraded into nucleotides by exoribonucleases or in some cases can give rise to
103 molecules that are better processed by another DCL than the original dsRNA (Shamandi *et al.*, 2015).
104 Contrasting the broad effect of RTL1, RTL2 specifically modulates the expression of siRNAs spanning
105 the IGS of the 45S rDNA and of ~500 loci producing 24-nt siRNA involved in RdDM (Elvira-Matelot *et*
106 *al.*, 2016).
107 DCL and RTL proteins have in common RNaseIII and RNA binding domains (RBD) as DCL harbour
108 additional upstream domains involved in unwinding (RNA helicases) and specifying siRNAs size (PAZ)
109 (Figure 2A). All these proteins share conserved cysteine residues in each domain. In particular, the
110 RBD domains harbour a cysteine which is conserved in all eukaryotic RTL and DCL proteins. In
111 *Arabidopsis*, RTL1 was shown to be prone to S-SG on a conserved cysteine located in the RBD domain
112 (Figure 2B-C). Treatments with agents inducing S-SG inhibits RTL1 activity, which is restored by
113 glutaredoxins, removing S-SG residue (Figure 2C). Consistently, point mutation of this particular

114 cysteine in serine has been shown to inhibit RNase cleavage activity on target RNA substrate,
115 suggesting that RTL1 is prone to redox regulation (Charbonnel *et al.*, 2017). While this conserved
116 C230 is located on the RNA binding rift, it is also involved in the RNase catalytic activity (Figure 2B).
117 Therefore, it has still to be determined if S-SG affects RNA binding or/and RNase catalytic activities.
118 Interestingly, a similar point mutation of C1742 (the counterpart of C230 of RTL1) in DCL1 affects its
119 RNase activity, suggesting that this Cys residue is also key for DCL proteins (Figure 2D) (Charbonnel
120 *et al.*, 2017). The work by Seta *et al.* (2017) further supports this assumption. DCL3 RNase activity
121 on siRNA generation was shown to be inhibited by oxidation treatment and being restored by thiol
122 reductase like thioredoxins. The identity of the thiol modification in DCL3 has not yet been
123 determined, but the fact that thioredoxin has generally a weak S-SG reduction activity plays against
124 S-SG of DCL3 (Seta *et al.*, 2017). Dimerization of RTL2, a homolog of RTL1, has been proposed to rely
125 on an intermolecular disulphide bond (Comella *et al.*, 2008). Whether this disulphide bond
126 maintains the dimeric structure of RTL2, or is prone to redox regulation is still uncovered.
127 All in all, different evidence points to a thiol-based regulation of small RNA metabolic enzymes which
128 might affect gene expression in function of contrasted redox environment.

129

130 **Redox regulation of miRNA biogenesis**

131 Evidence from several plant species supports the strong connection between the cellular redox
132 environment and the expression of miRNAs biogenesis (Table 1). A well-documented example is
133 *miR398* targeting Cu/Zn superoxide dismutases CSD1 and CSD2, which is regulated under different
134 stress conditions. In response to oxidative stress, *miR398* is transcriptionally down-regulated to
135 release translational suppression of *CSD2* mRNA. Consistently, transgenic plants carrying *miR398*-
136 resistant mutations in *CSD2* show improved tolerance to diverse abiotic stress conditions than
137 plants expressing a *miR398*-susceptible *CSD2* gene (Sunkar *et al.*, 2006). *miR398* was also reported
138 to be rapidly induced by heat stress, targeting *CSD1*, *CSD2* and *CCS* (copper chaperone of CSD1 and

139 CSD2) transcripts and playing a critical role in thermotolerance in Arabidopsis (Guan *et al.*, 2013), a
140 mechanism also involving Natural Antisense Transcripts (NAT) of *MIR398* gene (Li *et al.*, 2020). In
141 different plants, *miR398* is also proposed to be directly linked to cell death (Xu *et al.*, 2014), water
142 deficit (Jovanović *et al.*, 2014), abscisic acid stress (Jia *et al.*, 2009), high sucrose (Dugas and Bartel,
143 2008), and bacterial infection (Katiyar-Agarwal *et al.*, 2006).

144 Expression of miRNAs has also been related to sulphur metabolism. Expression of *miR395* targeting
145 Arabidopsis sulphate transporter 68 (AST68) and three ATP sulphurylases (APS1, APS3 and APS4), is
146 induced by sulphur deficiency. Sulphur deficiency expression of *miR395* is dependent on thiol-
147 reduction, as it is impaired by exogenous GSH treatment or in the *cad2* mutant with low GSH
148 biosynthesis capacities or in *ntra ntrb* mutants inactivated in thioredoxin reductases
149 (Jagadeeswaran *et al.*, 2014). In *Brassica napus*, *miR395* is involved in detoxification of cadmium
150 through regulation of sulphate assimilation, glutathione and other non-protein thiol contents
151 (Zhang *et al.*, 2013). In apple, *miR156* accumulation is induced by GSH but not by H₂O₂, emphasising
152 the specific role of GSH in inducing the expression of *miR156* (Jia *et al.*, 2017). On the other hand,
153 *miRNA156ab* is upregulated by drought stress in apple. *miR156ab* modulate the expression of auxin
154 metabolism and antioxidant enzyme genes by targeting the transcription factor SPL13, therefore
155 enhancing drought stress tolerance (Feng *et al.*, 2023).

156 In rice, *miR528* was shown to be downregulated by H₂O₂, increasing mRNA abundance of the IAA-
157 amino acid conjugates hydrolysis enzyme IAR1. Therefore, *miR528* might be involved in the
158 regulation of auxin homeostasis by controlling IAA release under H₂O₂ stress (Li *et al.*, 2011). In rice
159 infected with the rice stripe virus (RSV), *miR528* is suppressed at both the transcriptional and post-
160 transcriptional levels. RSV infection decreases *miR528* expression and induces the accumulation of
161 AGO18 and AGO1. AGO18 sequesters *miR528* away from AGO1 to block the formation of an RNA-
162 induced silencing complex. This induces the accumulation of the *miR528* target ascorbate oxidase,

163 which catalyses ascorbate oxidation, thereby regulating the redox status and priming ROS-mediated
164 resistance against RSV infection (Wu *et al.*, 2017).

165 Moreover, other examples of H₂O₂-responsive miRNAs have been identified in different plant
166 species (**Table 1**). All in all, these examples reflect the emerging role of miRNAs in fine tuning gene
167 expression through redox regulation.

168

169 **Redox regulation of SAM metabolism and iron chelation.**

170 DNA, RNA and protein methylation are essential for the regulation of gene expression. For example,
171 DNA methylation of cytosines is involved in silencing repetitive sequences and transposable
172 elements in plants (Law and Jacobsen, 2010). A major source of methyl groups is provided by S-
173 adeny methionine (SAM) through the folate and SAM cycle, implying that DNA methylation is
174 closely related to cell metabolism (Shen *et al.*, 2016). The role of folate in methylation processes is
175 evidenced by the fact that plants treated with sulfamethoxazole (SMZ) exhibit substantially reduced
176 levels of DNA methylation and histone H3 Lys-9 dimethylation. SMZ is a structural analog and
177 competitive antagonist to p-aminobenzoic acid (PABA), which is a precursor of folates. SMZ
178 decreased the plant folate pool size and caused methyl deficiency, as demonstrated by reductions
179 in SAM levels and in global DNA methylation (Figure 3) (Zhang *et al.*, 2012).

180 Indeed, several enzymes from the folate and SAM cycle have been shown to be redox modified. The
181 folate enters in the cycle through the bifunctional enzyme dihydrofolate reductase-thymidylate
182 synthases (DHFR-TS) which reduce dihydrofolate (DHF) to tetrahydrofolate (THF) by using NADPH
183 as reducing equivalents. Withing the folate cycle, the conversion of 5,10-methylene-THF to 5,10-
184 methenyl-THF is catalyzed by methylenetetrahydrofolate
185 dehydrogenase/methenyltetrahydrofolate cyclohydrolase (MTHFD), using NADP⁺ for its hydrolase
186 activity. This capacity to regenerate NADPH was proposed to link the folate cycle to GSH recycling
187 rate through the glutathione-ascorbate cycle, and consequently, to the plant capacities to cope with

188 oxidative stress (Gorelova *et al.*, 2017). Indeed, MTHFD1 plays a key role in DNA methylation. A
189 *methfd1-1* loss-of-function mutant is lethal, and an hypomorphic mutant shows accumulation of
190 homocysteine and S-adenosylhomocysteine, genome-wide DNA hypomethylation, loss of H3K9me
191 and transposon derepression (Groth *et al.*, 2016). Within the SAM cycle, homologous gene silencing
192 1 (HOG1)/S-adenosylhomocysteine hydrolase (SAHH) and SAM synthase (SAMS) are prone to S-
193 nitrosylation, as methionine synthase (MS) can be Tyr-nitrated (Lindermayr *et al.*, 2006; Fares *et al.*,
194 2011; Lozano-Juste *et al.*, 2011; Rudolf *et al.*, 2021). Interestingly, the chromosomal distribution of
195 DNA-methylation is similarly altered in *gsnor 1-3* and *sahh1* knock-down, supporting the role of NO-
196 dependent inhibition of SAHH1 on DNA methylation and transposable elements and stress-
197 responsive gene expression (Rudolf *et al.*, 2021). Moreover, HOG1/SAHH has been shown to interact
198 with thiol reductases like the poplar GRXC4 and the Chlamydomonas TRXh1, indicating a possible
199 redox regulation of different steps of the SAM cycle (Rouhier *et al.*, 2005; Pérez-Pérez *et al.*, 2017).
200 Notably, under oxidative conditions, methionine can be oxidised to methionine sulfoxide, which can
201 be reduced by TRX or GRX-dependent methionine sulfoxide reductases (MSR) (Tarrago *et al.*, 2009),
202 potentially adding a further level of redox regulation of the SAM cycle. While redox modifications
203 have been documented for several enzymes of the folate and SAM cycles, their impact on DNA
204 methylation has to be more deeply studied.

205 SAM is the precursor of nicotianamine (NA), a central chelator for intracellular iron in plants (Curie
206 and Briat, 2003). Notably NA is an amino acid derivative, synthesised from three SAM moieties by
207 NICOTIANAMINE SYNTHASE (NAS) (Klatte *et al.*, 2009). As NAS enzymes use SAM to produce NA, it
208 is not excluded that modulation of NAS expression and/or activity might fine-tune SAM availability
209 and subsequently methylation reactions using SAM (Figure 3). Indeed, this seems to be the case in
210 Arabidopsis, where disruption of 3 NAS genes provokes decreased accumulation of NA and affects
211 rDNA and RNA methylation profiles (Montacié *et al.*, 2023). On the top of that, NAS activity might
212 also be regulated by redox-dependent dimerization. Indeed crystal structural of NAS from archaea

213 revealed NAS homodimers are stabilised by intermolecular a disulphide bridge which contributes to
214 the protein thermostability (Dreyfus *et al.*, 2008; Dreyfus *et al.*, 2009). Interestingly, the counterpart
215 cysteines involved in disulphide bonds in archaea are conserved in the NAS protein sequences in
216 Arabidopsis, suggesting that this disulphide bond might have been conserved during evolution.
217 Whether this disulphide bond is prone to redox regulation is not known.

218

219 **Redox regulation of DNA and RNA methylation**

220 DNA methylation involves DNA methyltransferases (DNMT) which transfer methyl groups provided
221 by SAM to cytosines or adenine residues of the DNA backbone. The fact that these enzymes are
222 dependent on different methyl group donors from the folate and SAM cycles suggests that the redox
223 status of the cell may influence DNA methylation. Moreover, both in mammals and plants, increased
224 ROS levels increase DNMT expression and leads to elevated DNA methylation (Zheng *et al.*, 2023;
225 Zhao *et al.*, 2023). In Arabidopsis, GSNO reductase-deficient plants accumulate SAM and affect
226 transmethylation activity and histone methylation status, pointing to the role of GSNOR activity in
227 maintaining DNA methylation and the level of the repressive chromatin marks (Rudolf *et al.*, 2021).
228 However, direct evidence of a redox regulation of DNA methylation enzymes is missing yet.

229 Redox control of DNA methylation may extend to a further level through the presence of iron-
230 sulphur clusters in DNA demethylases enzymes, which remove methylated bases and cleave the
231 DNA backbone at abasic sites. These clusters, contain sulphide-linked di-, tri- and tetra-iron centres
232 that can harbour variable oxidation states. Therefore, these clusters are susceptible to ROS-induced
233 oxidation, which can potentially alter protein structure and function (Lill, 2009). In Arabidopsis, the
234 four DNA demethylases isoforms Repressor of Silencing 1 (ROS1), Demeter (DME), DME-like 2
235 (DML2) and DML3 contain a Fe/S cluster centre. While it has not yet been demonstrated that this
236 Fe/S cluster is redox regulated, it is required for active DNA demethylation activity. The assembly of
237 Fe/S clusters with apoproteins is mediated by the cytosolic Fe/S cluster assembly (CIA) pathway

238 which involves several scaffold and carrier proteins, including MET18 and AE7 (Couturier *et al.*,
239 2013). Duan *et al.* (2015) showed that ROS1 physically associates with MET18 and AE7, and that
240 mutation in *MET18* leads to hypermethylation at thousands of genomic loci, the majority of which
241 overlap with hypermethylated loci identified in *ros1* and *ros1 dml2 dml3* mutants (Duan *et al.*, 2015).
242 Moreover, site-directed mutagenesis of ROS1 indicates that the conserved Fe/S motif is
243 indispensable for ROS1 enzymatic activity. Finally, genes regulated by DME were found to be
244 repressed in mutants of other CIA members (NAR1 and CIA1) through hypermethylation of these
245 loci, suggesting that ROS1-mediated active DNA demethylation requires Fe/S cluster and
246 highlighting an important role of the CIA pathway in epigenetic regulation (Luo *et al.*, 2012;
247 Nakamura *et al.*, 2013; Buzas *et al.*, 2014; Duan *et al.*, 2015).

248 In prokaryotes and eukaryotes, RNA molecules are decorated with various chemical modifications,
249 which are introduced post-transcriptionally by RNA-modifying enzymes. These modifications are
250 required for RNA function, and among more than a hundred of RNA modifications, several modified
251 bases in tRNAs and rRNAs are introduced by RNA-modifying enzymes containing iron-sulphur (Fe/S)
252 clusters (Kimura and Suzuki, 2015). Most Fe/S-containing RNA-modifying enzymes use SAM as a
253 precursor and catalyse chemical reactions like methylation, methylthiolation and
254 carboxymethylation (Edwards *et al.*, 2021). Fe/S clusters react with ROS like O_2^- , resulting in the loss
255 of their ferric ions (Py and Barras, 2010). In *Enterococcus faecalis*, RNA modifications mediated by
256 the Fe/S cluster-containing methyltransferase RlmN, mediate environmental factors through ROS
257 sensing, to fine tune protein translation through N2-methyladenosine (m2A) in both 23S ribosomal
258 RNA and transfer RNA (Lee *et al.*, 2023). The role of Fe/S cluster RNA modification enzymes has been
259 poorly documented in plants. Nevertheless, Inigo *et al.*, 2016 found that several putative Fe/S client
260 proteins directly bind to the Fe/S cluster docking factor GRXS17. Among them are CYTOSOLIC
261 THIOURIDYLASE SUBUNIT1 and CYTOSOLIC THIOURIDYLASE SUBUNIT2, both Fe/S cluster-containing

262 enzymes, essential for the 2-thiolation step of 5-methoxycarbonylmethyl-2thiouridine (mcm5s2U)
263 modification of tRNAs (Iñigo *et al.*, 2016).

264 Among hundreds of known RNA modifications, mRNA oxidation mostly through 8-oxo-7,8-
265 dihydroguanine (8-OHG) is involved in neurodegenerative diseases in mammals (Nunomura *et al.*,
266 1999), prevents oxidative damage to genomic DNA (Kong and Lin, 2010), and causes premature
267 termination of translation (Tanaka *et al.*, 2007). In plants, targeted mRNA oxidation during dry after-
268 ripening of dormant seeds was proposed to govern cell signaling toward germination in the early
269 steps of seed imbibition (Bazin *et al.*, 2011).

270

271 **Redox regulation of histone methylation/demethylation**

272 Histone methylation occurs at different lysine residues in the N-terminal tail of histones. Particularly,
273 different types and location of methylation on histones H3 and H4 have distinct impact on chromatin
274 compaction and gene transcription (Lawrence *et al.*, 2016; Jamge and Berger, 2022). For example,
275 methylation of lysine 4 of histone H3 (H3K4me1) in gene bodies is associated with repressed gene
276 expression, as well as trimethylation of lysine 27 (H3K27me3). However, H3K4me3 in the 5' end of
277 genes, or H3K36me3 in gene bodies are associated with active gene transcription. Other marks such
278 as H3K9me2/3 and H3K27me1 are associated with repetitive sequences and are involved in
279 heterochromatin formation (Jamge and Berger, 2022).

280 Histone methyltransferases (HMTs) contain conserved SET (Suppressor of variegation 3-9, Enhancer
281 of Zeste, and Trithorax) domains and catalyse the methylation of lysine residues by using SAM as a
282 methyl donor. Consistently, decreased SAM levels were shown to reduce H3K9me2 and DNA
283 methylation levels (Zhang *et al.*, 2012). SAM biosynthesis being closely dependent on a redox-
284 dependent mitochondrial and peroxisomal metabolism, it might also link redox regulation to
285 methylation. Furthermore, HMTs are known to affect redox signalling. For example in mammals,
286 inhibition of the histone methyltransferase SETD7 is able to counter NF-κB-induced oxidative stress

287 and pro-inflammatory cytokine production (He *et al.*, 2015). Also in Arabidopsis, mutation in the SET
288 domain-HMT Trithorax 1 (ATX1) is able to suppress the phenotype of the *catalase 2 (cat2)* mutant,
289 which accumulate excessive H₂O₂ and leads to programmed cell death (Kaurilind *et al.*, 2015).
290 Nevertheless, clear evidence of direct redox regulation of HMTs is missing in plants.

291 Histone methylation on arginine residues is catalysed by protein arginine N-methyltransferases
292 (PRMT), which regulate different developmental and stress responses in plants (Wang *et al.*, 2007)
293 (Zhang *et al.*, 2011). In Arabidopsis, the major PRMT5 was proposed to mediate redox signalling to
294 protein methylation. Here, nitric oxide positively regulates the PRMT5 activity through S-NO at
295 Cys125 during stress responses, a function which is proposed to be related to methylation of
296 components of the pre-mRNA splicing machinery rather than histone methylation (Hu *et al.*, 2017).

297 Histone demethylases (HDMs) mediate the removal of methyl group from histone. HDM are
298 composed of two different classes of enzymes, the Jumonji C (JmjC) domain-containing
299 demethylases and the lysine-specific demethylases (LSD). The JmjC demethylases preferentially
300 demethylate mono-, di- and trimethylated lysine of histones, using Fe(ii) and 2-oxoglutarate as co-
301 factors (Tsukada *et al.*, 2006). Oxidation of ferrous to ferric ions led to the inactivation of JmjC
302 demethylases, inducing hypermethylation. In mammals, ROS increase several histone methylation
303 marks including H3K4me₃, H3H9me₂ and H3K27me₃ through modulating the activity of HDM
304 enzymes (Chen *et al.*, 2006; Niu *et al.*, 2015). In Arabidopsis, a *jmj16* mutant accumulates high levels
305 of ROS and H3K4me₃ hypermethylation, inducing leaf senescence through misregulation of
306 senescence gene expression (Liu *et al.*, 2019).

307 In mammals, the mutation of metabolic enzymes of the mitochondrial TCA cycle changes histone
308 methylation levels and induces diseases (Kaelin and McKnight, 2013). For example, mutation of the
309 isocitrate dehydrogenase (IDH) which catalyses the conversion of isocitrate to 2-oxoglutarate,
310 results in decreased JmjC activity and increased histone methylation (Xu *et al.*, 2011). Other
311 intermediates of the TCA cycle like fumarate and succinate are also inhibitors of JmjC. Mutants in

312 succinate dehydrogenase (SDH) and fumarate hydratase (FH) lead to the accumulation of succinate
313 and fumarate and hypermethylation of histone H3 (Xiao *et al.*, 2012). Evidence for redox regulation
314 of the TCA cycle has been documented in plants. In Arabidopsis, partial inactivation of the
315 mitochondrial thioredoxin system leads to increased SDH and FH activities and accumulation of
316 succinate and fumarate (Daloso *et al.*, 2015). In a similar way, plant cytosolic ICDH activity has been
317 proposed to be redox regulated by S-SG (Niazi *et al.*, 2019). Whether such mutations impact histone
318 methylation is unknown, but could link the mitochondrial metabolism to epigenetic state.
319 Lysine-specific demethylases (LSD), the second class of HMD, are amine oxidases using FAD as a co-
320 factor and preferentially reversing mono- and di-methylated lysine of histone (Shi *et al.*, 2004).
321 While the demethylation process involves a redox switch of FAD and release of H₂O₂, to our
322 knowledge there is no evidence of any redox regulation of this class of enzyme in the literature.

323

324 **Redox regulation of histone (de)acetylation**

325 Among various modifications, histone acetylation is predominantly known to affect chromatin
326 conformation and transcription (Nitsch *et al.*, 2021; Shvedunova and Akhtar, 2022). Acetylated
327 histones are generally associated with more relaxed chromatin and more permissive to transcription
328 activation, whereas histone deacetylation generally represses gene expression. The switch between
329 these two states is performed by a panel of writer and eraser enzymes called histone
330 acetyltransferases (HAT) and histone deacetylases (HDAC) (Shvedunova and Akhtar, 2022). In
331 eukaryotes, these enzymes are involved in numerous developmental and stress responses
332 pathways, and are candidates for redox regulation at several levels (Hu *et al.*, 2019; Kumar *et al.*,
333 2021; Cui *et al.*, 2023). The activities of HATs and HDACs are closely connected to the primary
334 metabolism through their precursors and co-factors (Shen *et al.*, 2016; Lu *et al.*, 2023). HAT enzymes
335 use acetyl-CoA to acetylate histone lysine residues. Acetyl-CoA is synthesised in different cellular
336 compartments as in mitochondria, through the oxidative decarboxylation of pyruvate by the

337 mitochondrial pyruvate dehydrogenase (mPDH) complex. Indeed, oxidative decarboxylation of
338 pyruvate is part of the glycolysis pathway, which uses glucose provided by photosynthetic activities.
339 Therefore, it is likely that histone acetylation is controlled by the cellular metabolite fluxes. Indeed,
340 major photosynthetic, glycolysis or TCA cycle enzymes are known to be finely redox regulated
341 (Meyer *et al.*, 2009; Daloso *et al.*, 2015). In Arabidopsis, two enzymes (succinate dehydrogenase and
342 fumarase) of the TCA cycle as well as the cytosolic ATP-citrate lyase (cACL) involved in generation of
343 acetyl-CoA, are redox-regulated by thioredoxins, therefore acting as a direct regulator of carbon
344 flow through the TCA cycle and downstream metabolites (Daloso *et al.*, 2015).

345 While, a direct impact of TCA cycle redox regulation on histone acetylation is still unknown in plants,
346 inactivation of cACL genes decreases acetyl-CoA-derived metabolites and produces plant
347 developmental perturbations similar to a *gcn5* mutation in the major HAT in Arabidopsis (Fatland *et al.*
348 *et al.*, 2005; Benhamed *et al.*, 2006; Bertrand *et al.*, 2003; Xing *et al.*, 2015). Acetyl-CoA is produced
349 via the β -oxidation pathway. Inactivation of peroxisomal acyl-CoA oxidase 4 (ACX4), an enzyme in
350 the fatty acid β -oxidation pathway reduces nuclear histone acetylation and increases DNA
351 methylation at several endogenous genomic loci, which are also targeted by the demethylation
352 enzyme REPRESSOR OF SILENCING 1 (ROS1) (Wang *et al.*, 2019). While the peroxisomal fatty acid β -
353 oxidation pathway is prone to redox regulation, direct evidence of such regulation on histone
354 acetylation is missing.

355 In contrast to the lack of direct evidence of redox regulation on HAT activities, more information is
356 available on HDACs. Among the HDAC family, Sirtuin (SRT) are dependent on NAD⁺, a key redox
357 couple of multiple metabolic pathways like glycolysis, TCA cycle and β -oxidation. The NADH/NAD⁺
358 ratio is dependent on the cellular environment and redox status, and it is known that the activity of
359 several human SRT isoforms is limited by the NAD⁺ levels (Hirschey *et al.*, 2011; Fischer *et al.*, 2012).
360 Also in human, SRT are subjected to various post-translational modifications like phosphorylation
361 (Gerhart-Hines *et al.*, 2011), carbonylation (Caito *et al.*, 2010), S-NO (Kornberg *et al.*, 2010) and S-

362 glutathionylation (Shao *et al.*, 2014) which were shown to regulate SirT1 activity, localisation, and
363 degradation. Shao *et al.* (2014) further showed that oxidative stress conditions inhibit SirT1 activity
364 through S-SG, and inducing cell apoptosis and senescence. S-glutathionylation is reduced by GRX,
365 restoring SirT1 activity (Shao *et al.*, 2014). Other reports in human point to SIRT1 inactivation through
366 S-SG (Zee *et al.*, 2010) and S-NO (Kornberg *et al.*, 2010). In contrast, evidence for thiol redox
367 modifications has not been reported for plant SRTs. This might be due to the lack of conservation in
368 regulatory cysteines between mammals and plants SRT (Pandey *et al.*, 2002). Nevertheless in
369 Arabidopsis, SRT1 regulate stress response through its association with the chromatin, the reduction
370 of H3K9ac levels of the cMyc-Binding Protein 1 (AtMBP-1) locus, and the repression of transcription
371 of two key stress regulators genes *LOS2/ENO2* and *STZ/ZAT10* (Liu *et al.*, 2017). In rice, nuclear
372 sirtuin OsSRT1 represses glycolysis by deacetylating both histones and GAPDH, inhibiting its nuclear
373 accumulation which is enhanced by oxidative stress (Zhang *et al.*, 2017). Again in rice, OsSRT1
374 inactivation induces an increase of histone H3K9 acetylation and a decrease of H3K9 dimethylation,
375 leading to H₂O₂ production, DNA fragmentation, cell death, and lesions mimicking plant
376 hypersensitive responses during incompatible interactions with pathogens, whereas overexpression
377 of OsSRT1 enhanced tolerance to oxidative stress (Huang *et al.*, 2007).

378 Non-sirtuin HDAC, the major group of HDAC are Zn²⁺-dependent and play major roles in various
379 stress responses (Yruela *et al.*, 2021; Cui *et al.*, 2023). Accordingly to these functions, HDAC
380 members are regulated by different stress conditions and are prone to redox-dependent PTMs (Luo
381 *et al.*, 2017). Post-translational modification of HDAC under oxidative stress was first documented
382 in mammals where chemical alkylation at two conserved cysteine residues Cys261 and Cys273 in
383 HDAC1, coincided with attenuation of histone deacetylase activity, changes in histone H3 and H4
384 acetylation patterns and derepression of HDAC-repressed genes (Figure 4A) (Doyle and Fitzpatrick,
385 2010). Intriguingly, Nott *et al.* (2008) showed that S-nitrosylation of HDAC2 occurring at the same
386 two Cys, does not affect deacetylase activity. Rather, it releases the enzyme from the chromatin,

387 resulting in an increased acetylation of histones surrounding the neurotrophin-dependent gene
388 promoters and activating transcription (Nott *et al.*, 2008). HDAC2 inhibition by NO-dependent S-NO
389 is also involved in the pathogenesis of Duchenne muscular dystrophy in mice (Colussi *et al.*, 2008).
390 Oxidative stress also induces tyrosine nitration of HDAC1, 2 and 3, which decreases HDAC activity
391 and promotes gene expression through up-regulation of histone acetylation (Ito *et al.*, 2004; Yang
392 *et al.*, 2006). In human, another redox-switch has been proposed for HDAC8. H₂O₂ induces a
393 reversible formation of a disulphide bridge between two adjacent Cys102 and Cys153, which
394 inactivates deacetylase activity *in vitro* (Jänsch *et al.*, 2019).
395 Redox-regulated Cys are conserved in mammals and plant class I HDAC (HDA6, HDA9 and HDA19)
396 (Figure 4B). In Arabidopsis, proteomic analyses have first identified Cys137 as S-sulfenylated (S-OH)
397 in response to salicylic acid (SA) or pathogen elicitor flg22 in HDA9 and HDA19 (Liu *et al.*, 2015). S-
398 nitrosylation was also found in two members of the plant-specific class HD2A and HD2B (Chaki *et*
399 *al.*, 2015). Mengel *et al.* (2017) suggest that salicylic acid-induced NO affects histone acetylation by
400 inhibiting HDAC complexes, resulting in the hyperacetylation of specific genes involved in plant
401 stress response (Mengel *et al.*, 2017). Further, high light-induced NO decreases histone H3
402 acetylation possibly through a redox regulation of HDA6. Nevertheless, direct evidence of S-NO in
403 HDA6 is missing yet (Ageeva-Kieferle *et al.*, 2021). In Arabidopsis, S-NO of Cys137 in HDA19 was
404 proposed to regulates HDA19 activity and to be required for HDA19 functions in developmental,
405 stress-responsive and epigenetic controls (Zheng *et al.*, 2023).
406 All in all, these examples mirror the multilevel role of redox regulation of histone acetylation, at
407 both at the level of the metabolites and the enzymes regulating histone acetylation.

408

409 **Concluding remarks and future prospects**

410 The cellular response to environmental constraints relies on the dynamic modulation of the genome
411 expression. While many regulators of gene expression such as transcription factors, chromatin

412 dynamics modulators and epigenetic modifications have been identified in recent decades, much
413 remains to be discovered about their means of regulation. The demonstration of fine regulation of
414 enzymes involved in chromatin dynamics and epigenetic modifications, such as DNA methylation
415 and post-translational modifications of histone proteins by metabolic factors and redox
416 modifications (e.g., by thiol-based modifications of cysteines) is a mean of adjusting gene expression
417 according to changes of the environment. Other types of PTM modifications are likely to be
418 discovered. For example, in mammalian cells, histone H3 senses cellular redox changes through
419 glutathionylation of a conserved Cys110 found in different H3 variants, which increases during cell
420 proliferation and decreases during aging. Glutathionylation of histone H3 affects nucleosome
421 stability structure leading to a more open chromatin structure due to decreased levels of DNA
422 helicases associated to nucleosomes (García-Giménez *et al.*, 2013; García-Giménez *et al.*, 2017).
423 Moreover, in *Xenopus leavis*, Cys110 of histone H3 is crucial for binding a Cu²⁺ molecule, and for
424 acting as a copper-dependent antioxidant enzymes (Attar *et al.*, 2020). Despite the large
425 conservation of this Cys110 in eukaryotes, including plants, these functions are still largely
426 unexplored in plants.

427 While proteomic approaches led to the discovery of redox modifications on an increasing number
428 of epigenetic enzymes, little is known about the mechanisms underlying these modifications. For
429 example, the impact of thiol-based redox modifications on chromatin remodelling enzymes activity
430 has only been studied for a limited number of proteins (e.g type I HDAC). The specificity of redox
431 modifications in function to a given stress condition is also poorly documented. Particularly, plant
432 cells exhibit a wide panel of redox pathways that might provide specificity to these regulations.
433 Understanding the interactions between redox enzymes such as thiol reductases and epigenetic
434 regulators should be further investigated. The characterization of specific ROS signatures and redox
435 status of cell compartments in relation to the nature of the stress would allow a better
436 understanding on how environmental signals are transmitted and interpreted by gene regulatory

437 networks (Mittler and Jones, 2023). Imaging techniques using redox-dependent fluorescent
438 proteins are valuable tools to study ROS signatures and redox status in different cellular
439 compartments or more specifically at individual proteins and protein complexes, under different
440 stress constraints (Kritsiligkou *et al.*, 2023). The cytosol located at the junction between the
441 organelles, the apoplast and the nucleus can greatly contribute to signal redox environment to the
442 gene expression machinery in the nucleus. It can also play major role on gene expression via
443 epitranscriptomic modifications and generation of small regulatory RNAs. The regulation of
444 chromatin remodelling and epigenetic enzyme activities by metabolic intermediates (e.g from the
445 TCA or the folate/SAM cycles), which are themselves dependent on redox and primary electron
446 donor of the cell, highlights the close relationships between metabolism, redox and gene
447 expression. These crosstalks likely enable to fine-tune the responses to environmental and
448 developmental signals. ROS, along with ions such as calcium, are also known to signal stress at long
449 distance, in tissues or even from plants to plants (Fichman *et al.*, 2022; Fichman *et al.*, 2023).
450 Whether these signals can also regulate gene expression at long distance, and which are the
451 underlying mechanisms is of great interest (Zandalinas *et al.*, 2020).

452 While epigenetic and epitranscriptomic regulatory mechanisms are under metabolic and redox
453 control, they themselves modulate the expression of stress or metabolism response genes, for
454 example through the maturation of small RNAs. The mechanisms underlying specific gene
455 expression reprogramming under various environmental constraint is still poorly understood. Like
456 redox pathways, epigenetic enzymes like HDAC are composed of multigenic families with specific
457 functions and targets, which might confer some specificity in gene expression reprogramming. The
458 study of these families is still in its infancy, and RNAseq, ChIPseq, interactomics and proteomics
459 methods provide powerful tools to address these questions. Scientific advances to uncover the gene
460 expression regulatory mechanisms are much more developed in model plants such as Arabidopsis,
461 thanks to many tools such as mutant libraries. Nevertheless, the recent development of gene

462 editing techniques now makes it possible to translate these data to plants of agronomic or
463 environmental interest, and to meet the needs of improving plants adaptation to environmental
464 changes.

465

466 **Acknowledgments**

467 This work was funded by the Centre National de la Recherche Scientifique, by the Agence Nationale
468 de la Recherche (grant nos. ANR-REPHARE 19–CE12–0027 and ANR-RoxRNase 20-CE12-0025). This
469 project was funded through Labex AGRO (under I-Site Muse framework) coordinated by the
470 Agropolis Fondation (grant no. Flagship Project 1802-002 - CalClim). This study is set within the
471 framework of the Laboratoires d’Excellence TULIP (ANR-10-LABX-41) and the Ecole Universitaire de
472 Recherche (EUR) TULIP-GS (ANR-18-EUR-0019). JA is supported by a Ph.D. grant from the Université
473 de Perpignan Via Domitia (Ecole Doctorale Energie et Environnement ED305).

474

475 **Author Contributions**

476 Writing, review and editing, JA, AD, JSV and JPR; All authors have read and agree with the present
477 version of the manuscript.

478

479 **Conflict of interest**

480 The authors declare that they have no conflict of interest.

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Figure legends

Figure 1: Stress sensing and ROS-related PTM signalling in plant cells. Stress is sensed in different cellular compartments, leading to accumulation of ROS and second messengers such as Ca²⁺, primary metabolites and proteins involved in signal transduction. The transport of ROS between different compartments is regulated, and different stresses can generate stimulus-specific ROS concentrations within the different cell compartments. This also impacts redox homeostasis in the cytosol and the nucleus, inducing redox-dependent PTMs of proteins that play various roles in chromatin remodelling, gene transcription, RNA processing and protein translation. All these modifications play central roles in stress signalling, acclimatisation and/or defence mechanisms. ER, endoplasmic reticulum ; ROS, reactive oxygen species; PTM, post-translational modifications.

Figure 2: RNaseIII thiol-based redox regulation. (A) Protein domain organisation in Arabidopsis DCL and RTL proteins. The different domains are represented by distinct colors. (B) Protein modelling of AtRTL1 dimer (Swiss Model, template *A. aeolicus* RTL1). Conserved amino acids in the RBD active sites are shown by respective colors. Figure from Charbonnel et al. (2017). (C) Redox regulation of AtRTL1. The active RTL1 dimer (in green) cleaves non-perfectly paired hairpin precursors. S-glutathionylation (GS) e.g. by GSSG inactivates RTL1 (in red) in a GRX-dependent reversible manner. (D) Alignment of RNaseIII domains and dsRBD from DCL and RTL members. RNaseIII domains (blue sequences), dsRBD (red sequences) and active sites (black boxes) are represented. Conserved Cys residues (yellow) in the RNaseIII and RBDs are also pointed by blue arrowheads. The filled arrowhead represent C230 in RTL1. RBD, RNA binding domain. DCL, Dicer-like; GSSG, oxidised glutathione; GRX, glutaredoxin.

Figure 3: Redox components regulate S-adenyl methionine (SAM) synthesis. Dihydrofolate (DHF) is converted to tetrahydrofolate (THF) by the dihydrofolate reductase-thymidylate synthase (DHFR-TS) to enter into the folate cycle. A methyl group is transferred to THF by serine hydroxymethyltransferase (SHMT). The resulting 5,10 methylenetetrahydrofolate (5,10-CH₂-THF) is dehydrogenised to 5,10-CH=THF by methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase (MTHFD), and further reduced to 5-CH₃-THF by Methylenetetrahydrofolate reductase (MTHFR). The methyl group is transferred from 5-CH₃-THF to homocysteine (Hcy) to synthesise methionine (Met) through the methionine synthase (MS) enzyme. Under oxidative conditions, methionine can be oxidised to methionine sulfoxide (MetSO), which is reduced by methionine sulfoxide reductases (MSR), enzymes dependent on TRX or GRX. Methionine synthesizes S-adenyl methionine (SAM) through SAM synthase (SAMS) and is converted to S-adenosyl homocysteine (SAH) which is further processed to homocysteine through S-adenosyl

homocysteine hydrolase/homology-dependent gene silencing 1 (SAHH/HOG1). Nicotianamine (NA) is synthesised from SAM by the nicotianamine synthase (NAS). The 3'-oxo NA intermediate synthesised from NA by nicotianamine aminotransferase (NAAT) is then converted to phytosiderophores via the deoxymugineic acid synthase (DMAS). SAM is known to donate methyl groups to DNA, RNA or proteins, through DNA methyltransferases (DNMT), RNA methyltransferase (RlmN), CYTOSOLIC THIOURIDYLASE SUBUNIT1 (CTU1) and CYTOSOLIC THIOURIDYLASE SUBUNIT2 (CTU2), histone lysine methyltransferases (HKMT) and protein arginine N-methyltransferase (PRMT). The following enzymes are influenced by redox components: MSR, SAMS, MAT, SAHH/HOG1, MS, RlmN, DNMT, HKMT and PRMT. K, lysine; R, arginine; Me, methyl; NO, nitric oxide; NO₂, nitric dioxide; ROS, reactive oxygen species; NADP(H), nicotinamide adenine dinucleotide phosphate; TRX, thioredoxin; GRX, glutaredoxin.

Figure 4: Redox regulated cysteines in class I HDACs. (A) Predicted three-dimensional structure of AtHDA6 (generated by trROSETTA program) showing potential redox regulation sites. Cysteines targeted by redox modifications in human homologs have been highlighted. In purple, C112 and C163 form a disulphide bond in HsHDAC8 which inhibits deacetylase activity (Jänsch et al., 2019). In blue, C273 and C285 are S-NO in HsHDAC2 which impairs chromatin binding. Alkylation of the same cysteines inhibits deacetylase activity in HsHDAC1 (Nott et al., 2008). In green, C285 and C296 are S-NO in AtHDA19 (Zheng et al., 2023). In yellow, other cysteines found in AtHDA6 but without known functions. (B) Redox regulated cysteines are conserved between plants and mammals. Amino acid sequences of human and *A. thaliana* class I HDAs were aligned with Clustalw software. All cysteines present in the alignment are shown and numbered using AtHDA6 as reference. Redox-sensitive cysteines were marked with * and consequence of redox modification is described below. Notice that S-NO C20 and C137 in AtHDA19 are not conserved in AtHDA6, respectively substituted to S22 and A141 in AtHDA6.

Table 1. List of redox-related miRNAs.

miRNA	Species	Targeted genes	Related stress	References
miR156	<i>Malus sieversii</i>	SPL	Drought stress	Feng <i>et al.</i> , 2023
miR160	<i>Arabidopsis thaliana</i> <i>Brachypodium distachyon</i>	Auxin response factor 22 ARF22	H ₂ O ₂ , Ozone-induced oxidative stress	Iyer <i>et al.</i> , 2012, Lv <i>et al.</i> , 2016, Cao <i>et al.</i> , 2019
miR164	<i>Arabidopsis thaliana</i> <i>Brachypodium distachyon</i>	Phytoene dehydrogenase PDs	H ₂ O ₂ , Ozone-induced oxidative stress	Iyer <i>et al.</i> , 2012, Lv <i>et al.</i> , 2016, Cao <i>et al.</i> , 2019
miR395	<i>Arabidopsis thaliana</i> <i>Brassica napus</i> <i>Nicotiana tabacum</i> <i>Oryza sativa</i>	ATPS, SULTR2;1	Nutrient deficiency, Heavy metal	Huang <i>et al.</i> , 2010, Matthewman <i>et al.</i> , 2012, Zhang <i>et al.</i> , 2013a, Jagadeeswaran <i>et al.</i> , 2014, Panda and Sunkar, 2015, Yuan <i>et al.</i> , 2016
miR396b	<i>Poncirus trifoliata</i> <i>Arabidopsis thaliana</i>	ACO	Cold stress	Zhang <i>et al.</i> , 2016
miR397	<i>Lotus japonicus</i> <i>Oryza sativa</i> <i>Brachypodium distachyon</i>	LAC, ARF5	Nutrient deficiency, H ₂ O ₂	Yamasaki <i>et al.</i> , 2009, Li <i>et al.</i> , 2011, De Luis <i>et al.</i> , 2012, Zhang <i>et al.</i> , 2013b, Wang <i>et al.</i> , 2014, Lv <i>et al.</i> , 2016
miR398	<i>Arabidopsis thaliana</i> <i>Medicago truncatula</i> <i>Oryza sativa</i> <i>Phaseolus vulgaris</i> <i>Triticum aestivum</i> <i>Vitis vinifera</i> <i>Solanum lycopersicum</i>	CSD1, CSD2, COX5b, HDT3, Nod19	Nutrient deficiency, H ₂ O ₂ , Heavy metal, Heat stress, Thermotolerance, Salinity stress, Ethylene, Drought stress, Environmental pollutants, Botrytis cinerea infection, Cadmium stress	Sunkar <i>et al.</i> , 2006, Yamasaki <i>et al.</i> , 2009, Lu <i>et al.</i> , 2010, Trindade <i>et al.</i> , 2010, Guan <i>et al.</i> , 2013, Naya <i>et al.</i> , 2014, Kayihan <i>et al.</i> , 2016, Leng <i>et al.</i> , 2017, Li <i>et al.</i> , 2017b, Li <i>et al.</i> , 2017a, Li <i>et al.</i> , 2020b, Li <i>et al.</i> , 2020a, Li <i>et al.</i> , 2022, Liu <i>et al.</i> , 2023, Yan <i>et al.</i> , 2023
miR408	<i>Arabidopsis thaliana</i> <i>Nicotiana tabacum</i> <i>Oryza sativa</i> <i>Medicago truncatula</i>	PCY, PLC, LAC, UCC, UCL	Drought stress, Salinity stress, γ -irradiation, Light, Nutrient deficiency	Trindade <i>et al.</i> , 2010, Zhang <i>et al.</i> , 2017, Pan <i>et al.</i> , 2018, Song <i>et al.</i> , 2018, Gao <i>et al.</i> , 2022
miR474	<i>Citrus sinensis</i> <i>Zea mays</i>	NAD-dependent malic enzyme, PDH	Submergence, Boron deficiency	Zhang <i>et al.</i> , 2008, Lu <i>et al.</i> , 2014, Liang <i>et al.</i> , 2017
miR477	<i>Panicum virgatum</i> <i>Triticum aestivum</i>	Fd-GOGAT	Drought stress	Xie <i>et al.</i> , 2010, Akdogan <i>et al.</i> , 2016
miR528	<i>Agrostis stolonifera</i> <i>Oryza sativa</i>	PCY-like, AO, LAC, MCOs	Salinity stress, Nutrient deficiency, Biotic stress, Heavy metal	Liu <i>et al.</i> , 2015, Yuan <i>et al.</i> , 2015, Wu <i>et al.</i> , 2017
miR531	<i>Panicum virgatum</i> <i>Triticum aestivum</i>	CYP P450, ACO1, HSP 17.9, POD52, POX	Environmental pollutants	Xie <i>et al.</i> , 2010, Li <i>et al.</i> , 2017b
miR1121	<i>Triticum aestivum</i>	CAT-1, POD6, MT3-like	Environmental pollutants	Li <i>et al.</i> , 2017b
miR1436	<i>Oryza sativa</i> <i>Panicum virgatum</i>	POD2	Heat stress	Xie <i>et al.</i> , 2010, Mangrauthia <i>et al.</i> , 2017
miR2102	<i>Oryza sativa</i> <i>Panicum virgatum</i>	CYP P450, ACO1, SOD, COX VI, POD	Heavy metal	Xie <i>et al.</i> , 2010, Sharma <i>et al.</i> , 2015
miR9653b	<i>Triticum aestivum</i>	LOX-like protein	Environmental pollutants	Li <i>et al.</i> , 2017b
miR9773	<i>Triticum aestivum</i>	CYP P450	Environmental pollutants	Li <i>et al.</i> , 2017b
PN-2013	<i>Triticum aestivum</i>	MDHAR	Biotic stress	Feng <i>et al.</i> , 2014
PC-5p-213179-14	<i>Zea mays</i>	POD	Low seed vigour	Gong <i>et al.</i> , 2015
novel_miR_4	<i>Brachypodium distachyon</i>	CYP P450 734A1	H ₂ O ₂	Lv <i>et al.</i> , 2016
novel_miR_120	<i>Brachypodium distachyon</i>	NDH1 α subunit 12	H ₂ O ₂	Lv <i>et al.</i> , 2016
novel_miR_197	<i>Brachypodium distachyon</i>	CYP P450 90D2	H ₂ O ₂	Lv <i>et al.</i> , 2016
novel_miR_234	<i>Brachypodium distachyon</i>	FTR	H ₂ O ₂	Lv <i>et al.</i> , 2016

Abbreviations: ACCO, aminocyclopropanecarboxylate oxidase; ACO, aconitase; AO, ascorbate oxidase; ARF, auxin response factor; ATPS, adenosinetriphosphate sulfurylase; CAT, catalase; COX, cytochrome C oxidase; CSD, copper/zinc superoxide dismutase; CYP, cytochrome P450; Fd-GOGAT, ferredoxin-dependent glutamate synthase; FTR, ferredoxin-dependent thioredoxin reductase; HDT, histone deacetylase; HSP, heat-shock protein; LAC, laccase; LOX, lipoxygenase; MDHAR, monodehydroascorbate reductase; MT, metallothionein; MCO, multicopper oxidase; NDH, NADH dehydrogenase; Nod, nodulin; PCY, plantacyanin; PDH, pyruvate dehydrogenase; POD, peroxidase; POX, peroxidase; SOD, superoxide dismutase; SPL, squamosa promoter binding protein-like; SULTR, sulfate transporter; UCC, uclacyanin; UCL, uclacyanin-like.

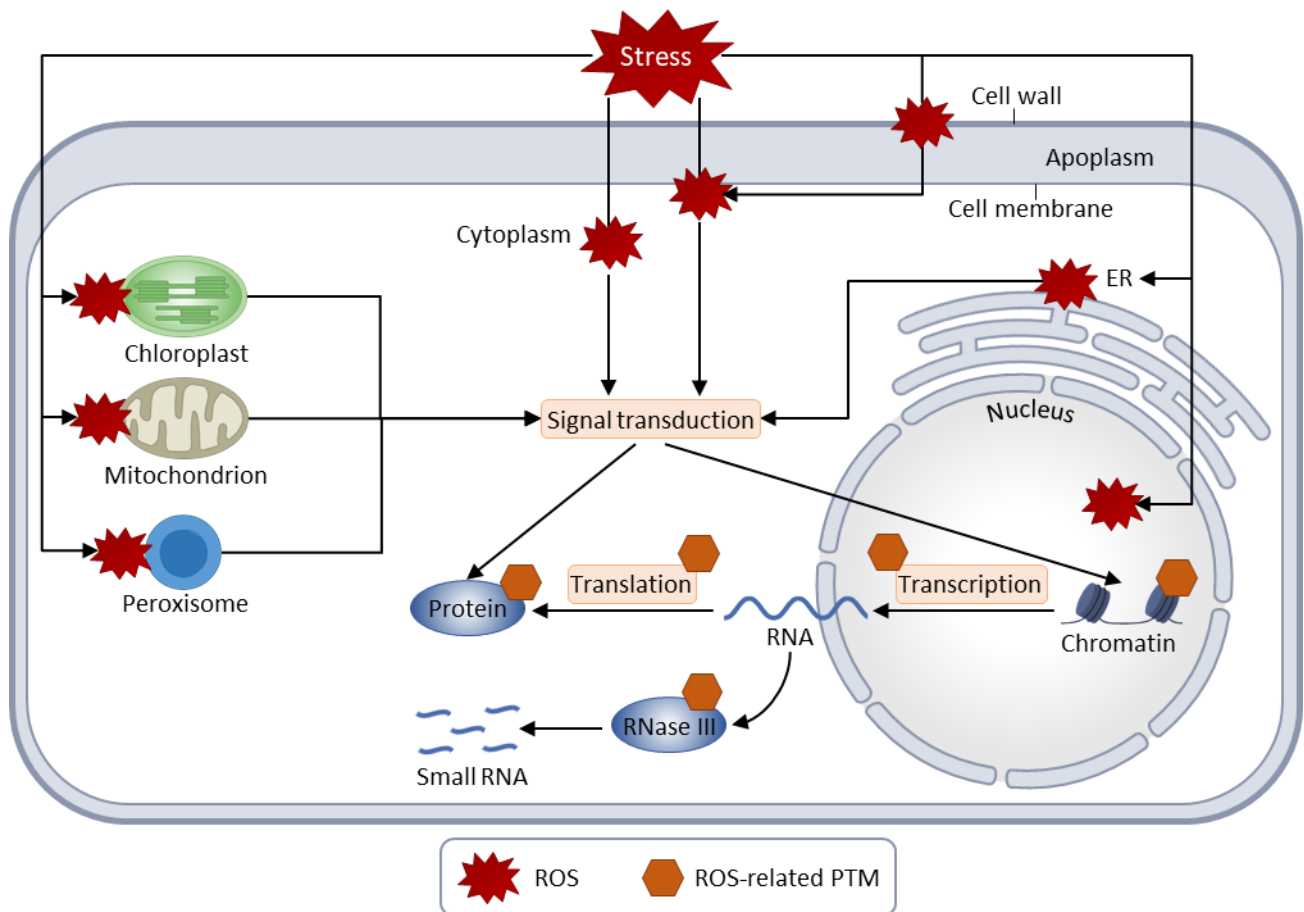


Figure 1: Stress sensing and ROS-related PTM signalling in plant cells. Stress is sensed in different cellular compartments, leading to accumulation of ROS and second messengers such as Ca^{2+} , primary metabolites and proteins involved in signal transduction. The transport of ROS between different compartments is regulated, and different stresses can generate stimulus-specific ROS concentrations within the different cell compartments. This also impacts redox homeostasis in the cytosol and the nucleus, inducing redox-dependent PTMs of proteins that play various roles in chromatin remodelling, gene transcription, RNA processing and protein translation. All these modifications play central roles in stress signalling, acclimatisation and/or defence mechanisms. ER, endoplasmic reticulum ; ROS, reactive oxygen species; PTM, post-translational modifications.

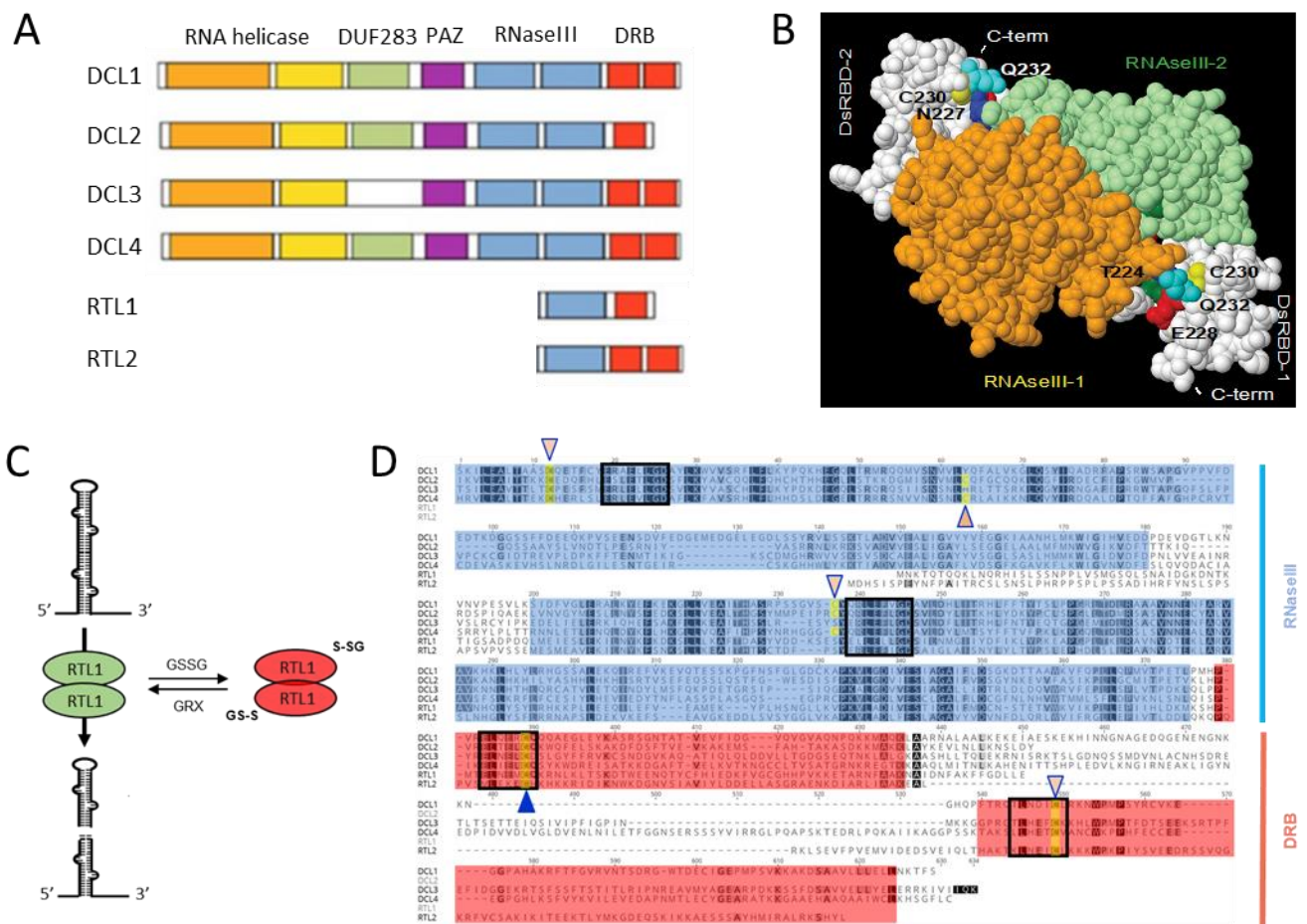


Figure 2: RNaseIII thiol-based redox regulation. (A) Protein domain organisation in Arabidopsis DCL and RTL proteins. The different domains are represented by distinct colors. (B) Protein modelling of AtRTL1 dimer (Swiss Model, template *A. aeolicus* RTL1). Conserved amino acids in the RBD active sites are shown by respective colors. Figure from Charbonnel et al. (2017). (C) Redox regulation of AtRTL1. The active RTL1 dimer (in green) cleaves non-perfectly paired hairpin precursors. S-glutathionylation (S-SG) e.g. by GSSG inactivates RTL1 (in red) in a GRX-dependent reversible manner. (D) Alignment of RNaseIII domains and dsRBD from DCL and RTL members. RNaseIII domains (blue sequences), dsRBD (red sequences) and active sites (black boxes) are represented. Conserved Cys residues (yellow) in the RNaseIII and RBDs are also pointed by blue arrowheads. The filled arrowhead represent C230 in RTL1. RBD, RNA binding domain. DCL, Dicer-like; GSSG, oxidised glutathione; GRX, glutaredoxin.

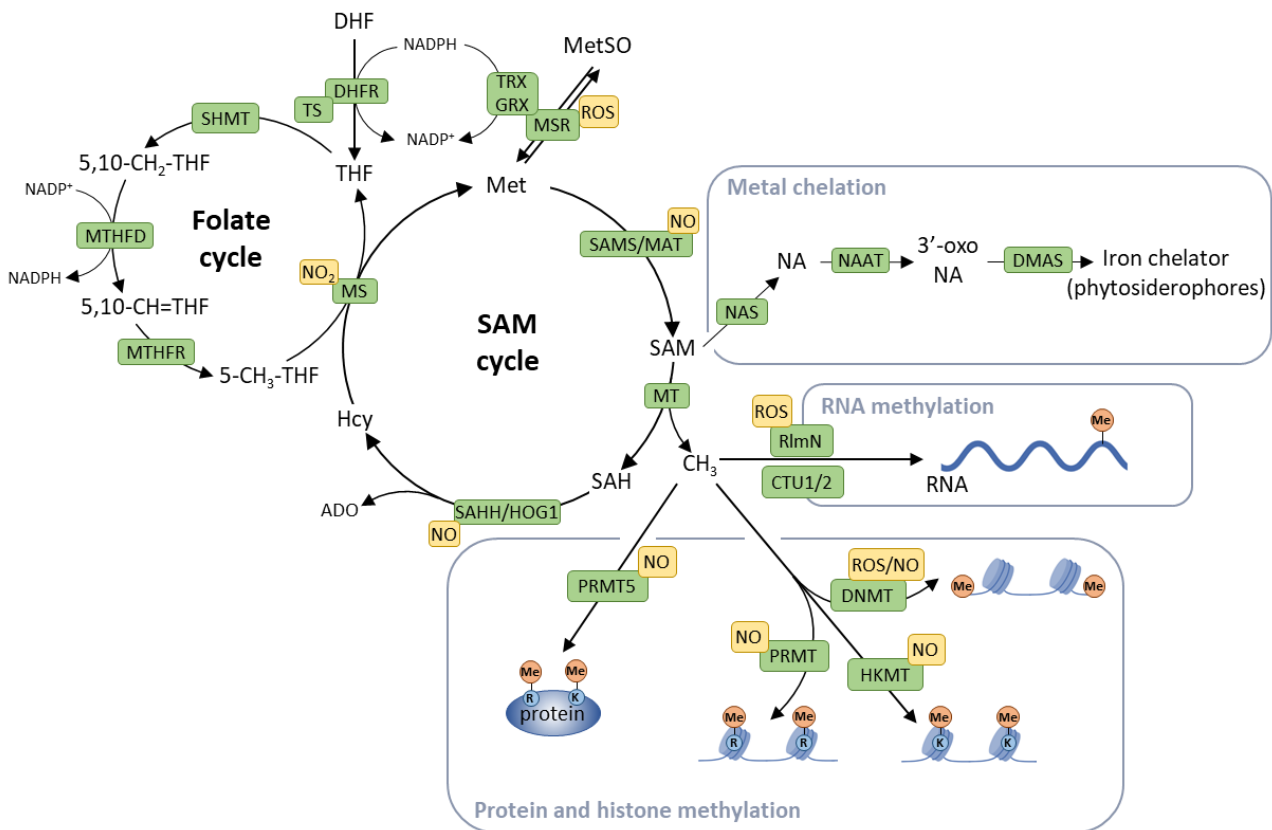
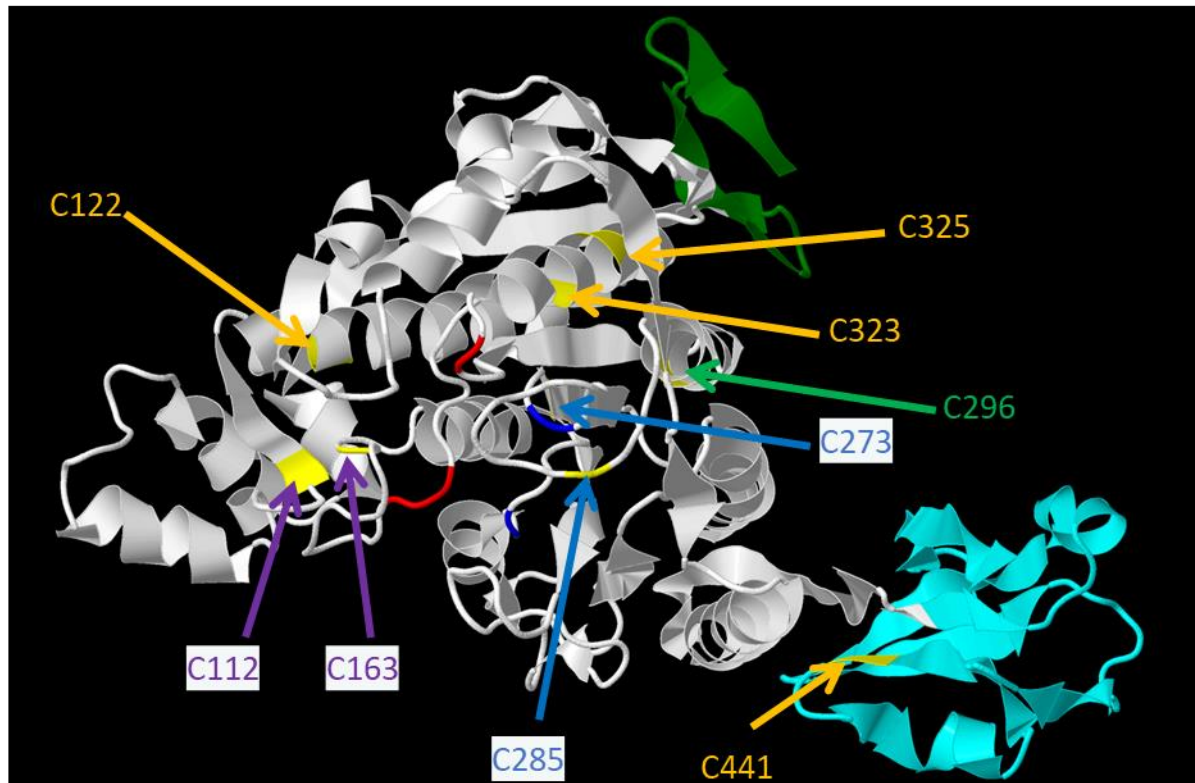


Figure 3: Redox components regulate S-adenyl methionine (SAM) synthesis. Dihydrofolate (DHF) is converted to tetrahydrofolate (THF) by the dihydrofolate reductase-thymidylate synthase (DHFR-TS) to enter into the folate cycle. A methyl group is transferred to THF by serine hydroxymethyltransferase (SHMT). The resulting 5,10 methylenetetrahydrofolate (5,10-CH₂-THF) is dehydrogenised to 5,10-CH=THF by methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase (MTHFD), and further reduced to 5-CH₃-THF by Methylenetetrahydrofolate reductase (MTHFR). The methyl group is transferred from 5-CH₃-THF to homocysteine (Hcy) to synthesise methionine (Met) through the methionine synthase (MS) enzyme. Under oxidative conditions, methionine can be oxidised to methionine sulfoxide (MetSO), which is reduced by methionine sulfoxide reductases (MSR), enzymes dependent on TRX or GRX. Methionine synthesizes S-adenyl methionine (SAM) through SAM synthase (SAMS) and is converted to S-adenosyl homocysteine (SAH) which is further processed to homocysteine through S-adenosyl homocysteine hydrolase/homology-dependent gene silencing 1 (SAHH/HOG1). Nicotianamine (NA) is synthesised from SAM by the nicotianamine synthase (NAS). The 3'-oxo NA intermediate synthesised from NA by nicotianamine aminotransferase (NAAT) is then converted to phytosiderophores via the deoxymugineic acid synthase (DMAS). SAM is known to donate methyl groups to DNA, RNA or proteins, through DNA methyltransferases (DNMT), RNA methyltransferase (RlmN), CYTOSOLIC THIOURIDYLASE SUBUNIT1 (CTU1) and CYTOSOLIC THIOURIDYLASE SUBUNIT2 (CTU2), histone lysine methyltransferases (HKMT) and protein arginine N-methyltransferase (PRMT). The following enzymes are influenced by redox components: MSR, SAMS, MAT, SAHH/HOG1, MS, RlmN, DNMT, HKMT and PRMT. K, lysine; R, arginine; Me, methyl; NO, nitric oxide; NO₂, nitric dioxide; ROS, reactive oxygen species; NADP(H), nicotinamide adenine dinucleotide phosphate; TRX, thioredoxin; GRX, glutaredoxin.

A



B

	22	46	75	112	122	141	157	163	235	273	280	284	285	296	323	325	334	361	419	427	441	461	
AtHDA6	V [*] SY	IRM	IGR	DC [*] PF [*] CR	D [*] AD	KSE	FCY	YAL	Q [*] CG	SGD	LGC	G [*] CF	D [*] CL	RCW	WCY	GV [*] E	-PM	IWS	E	SD	SCR	DN [*] P	
AtHDA7	VSY	IRV	FEK	DGPYCR	EAD	KDK	FGY	YSL	QCG	AGD	FGT	GT [*] F	DCL	RCW	WCY	GEQ	-NR						
AtHDA19	VCY	IRM	LCR	DCPF [*] CQ	LCD	KCE	FCY	YSL	QCG	SGD	LGC	GCF	ECV	RCW	WCY	GV [*] E	-NM	-PD	DD	D	AVE	GCE	
AtHDA9	I	SY	LCM	MAQ	DCPF [*] CQ	LCD	KCE	FCY	YA	QCG	ARD	LGC	GCF	ECV	RCW	WTV	DT [*] E	GHI	QRS	QR	D	AS	
HsHDAC1	VCY	IRM	MTK	DCPF [*] CQ	QTD	KSE	FCY	YA	QCG	SGD	LGC	GCF	KCV	RCW	WTY	DT [*] E	-NM	I	CS	ACE	GGR	KEK	
HsHDAC2	VCY	IRM	MTK	DCPF [*] CQ	QTD	KSE	FCY	YA	QCG	SGD	LGC	GCF	KCV	RCW	WTY	DT [*] E	-NM	I	RA	AC	D	GGR	DKK
HsHDAC3	VAY	LAL	MCR	DCPF [*] CQ	SIC	KCE	FCY	YCL	QCG	GCD	LGC	GCF	ECV	RCW	WTY	EEA	TR	I	RPE	YDG	I		
HsHDAC8	PVY	ASM	MAT	DCPF [*] YAA	MCK	KDE	FCY	YSL	QL	G	AGD	MCS	CSF	KCL	RCW	WTY	GKT	-CR					

C112, C163: Disulphide bond in HsHDAC8 (102, 153) inhibits deacetylase activity

C273, C285: S-NO in HsHDAC2 (262, 274) impairs chromatin binding and alkylation inhibits deacetylase activity HsHDAC1 (261, 273)

C22, C141, C285, C296: S-NO in AtHDA19 (20, 137, 281, 292), C137 S-NO enhance chromatin binding and deacetylase activity

Figure 4: Redox regulated cysteines in class I HDACs. (A) Predicted three-dimensional structure of AtHDA6 (generated by trROSETTA program) showing potential redox regulation sites. Cysteines targeted by redox modifications in human homologs have been highlighted. In purple, C112 and C163 form a disulphide bond in HsHDAC8 which inhibits deacetylase activity (Jänsch et al., 2019). In blue, C273 and C285 are S-NO in HsHDAC2 which impairs chromatin binding. Alkylation of the same cysteines inhibits deacetylase activity in HsHDAC1 (Nott et al., 2008). In green, C285 and C296 are S-NO in AtHDA19 (Zheng et al., 2023). In yellow, other cysteines found in AtHDA6 but without known functions. (B) Redox regulated cysteines are conserved between plants and mammals. Amino acid sequences of human and *A. thaliana* class I HDAs were aligned with Clustalw software. All cysteines present in the alignment are shown and numbered using AtHDA6 as reference. Redox-sensitive cysteines were marked with * and consequence of redox modification is described below. Notice that S-NO C20 and C137 in AtHDA19 are not conserved in AtHDA6, respectively substituted to S22 and A141 in AtHDA6.