

1 Chloroplast cold-resistance is mediated by the acidic domain of the RNA 2 binding protein CP31A

3 Ayako Okuzaki^{#1}, Marie-Kristin Lehniger^{1§}, Jose M Muino², Benjamin Lenzen¹, Thilo Rühle³, Dario
4 Leister³, Uwe Ohler², Christian Schmitz-Linneweber^{1*}

5 ¹ Molecular Genetics, Humboldt-University Berlin, Philippstr.13, 10115 Berlin, Germany

6 ² Computational Regulatory Genomics, Humboldt-University Berlin / Max Delbrück Centre for Molecular
7 Medicine, Philippstr.13, 10115 Berlin, Germany

8 ³ Plant Molecular Biology, Department of Biology, Ludwig Maximilian University of Munich, Munich,
9 Germany.

10
11 # current address: College of Agriculture, Tamagawa University, Tamagawagakuen 6-1-1, Machida-shi,
12 Tokyo, Japan

13 § equally contributed

14 *corresponding author:

15 Christian Schmitz-Linneweber
16 phone: +49 30 2093-49700
17 fax: +49 30 2093-49701
18 Email: smitzlic@rz.hu-berlin.de

19

20 Running title: An acidic domain for chloroplast cold resistance

21 Key words: chloroplast, RNA processing, RNA binding, acidic domain, RNA editing, RNA stability,
22 organelle, *Arabidopsis thaliana*

23 Author contributions

24 C.S.-L. conceived the original research plan; D.L. supervised the experiments; A.O. generated the
25 transgenic plant lines and performed their analysis. M.-K. L. performed the RIP-Seq and microarray
26 experiments; J.M.M. analyzed the RIP-Seq data. B.L. performed the SSMART analysis; T.R. performed
27 mutant analyses. C.S.-L., D.L. and U.O. supervised the project, designed the experiments and analyzed the
28 data; C.S.-L. wrote the article with contributions of all the authors; C.S.-L. agrees to serve as the author
29 responsible for contact and ensures communication.

30 One sentence summary:

31 Cold exposure induces increased RNA association of the RRM protein CP31A, which mediates cold-
32 resistance of *Arabidopsis thaliana* via its acidic domain

33

34 **Abstract**

35 Chloroplast RNA metabolism is characterized by long-lived mRNAs that undergo a multitude of post-
36 transcriptional processing events. Chloroplast RNA accumulation responds to environmental cues, foremost
37 light and temperature. A large number of nuclear-encoded RNA-binding proteins (RBPs) are required for
38 chloroplast RNA metabolism, but we do not yet know how chloroplast RBPs convert abiotic signals into
39 gene expression changes. Previous studies showed that the chloroplast ribonucleoprotein 31A (CP31A) is
40 required for the stabilization of multiple chloroplast mRNAs in the cold, and that the phosphorylation of
41 CP31A at various residues within its N-terminal acidic domain (AD) can alter its affinity for RNA *in vitro*.
42 Loss of CP31A leads to cold sensitive plants that exhibit bleached tissue at the center of the vegetative
43 rosette. Here, by applying RIP-Seq, we demonstrated that CP31A shows increased affinity for a large
44 number of chloroplast RNAs *in vivo* in the cold. Among the main targets of CP31A were RNAs encoding
45 subunits of the NDH complex and loss of CP31A lead to reduced accumulation of *ndh* transcripts. Deletion
46 analyses revealed that cold-dependent RNA binding and cold resistance of chloroplast development both
47 depend on the AD of CP31A. Together, our analysis established the AD of CP31A as a key mediator of
48 cold acclimation of the chloroplast transcriptome.

49 **Introduction**

50 Chloroplasts contain genetic information that is essential for photosynthesis. The expression of this
51 information is realized by a unique mixture of ancestral bacterial and derived eukaryotic features (Barkan,
52 2011). Chloroplast gene expression adapts to various environmental changes, including light and
53 temperature (e.g. Klein, 1991; Mentzen and Wurtele, 2008; Cho et al., 2009; Castandet et al., 2016).
54 Contributions to such acclimation processes have been described on the transcriptional level (Pfannschmidt,
55 2003; Tsunoyama et al., 2004), but post-transcriptional processes are likely to dominate (Deng and
56 Gruissem, 1987; Eberhard et al., 2002; Udy et al., 2012). One key change in posttranscriptional processes
57 between chloroplast and their bacterial ancestors are the vastly increased RNA half lives in the organelle.
58 In bacteria, transcription and translation are usually directly coupled and mRNAs have short half-lives (in
59 the range of minutes; Selinger et al., 2003). In chloroplasts, the half-lives of mRNAs are long (in the range
60 of hours) and untranslated RNAs accumulate in large amounts (Klaff and Gruissem, 1991; Germain et al.,
61 2012). The turnover rates of chloroplast RNAs change in response to developmental and environmental
62 cues, which is suggesting that RNA stability is regulated in chloroplasts (Deng et al., 1989; Klaff and
63 Gruissem, 1991; Biehl et al., 2005; Bollenbach et al., 2007; Germain et al., 2013; Manavski et al., 2018),
64 but the underlying regulatory factors are largely unknown. Possible candidates for regulators of RNA
65 stability are pentatricopeptide repeat (PPR) proteins and chloroplast ribonucleoproteins (cpRNPs). PPR
66 proteins specifically associate with one or few RNAs (Barkan and Small, 2014), while cpRNPs are

67 generalists that bind to a large number of mRNAs (Kupsch et al., 2012; Teubner et al., 2017). Two cpRNPs
68 named CP29A and CP31A were previously shown to be required for the accumulation of chloroplast RNAs
69 in the cold, which makes them interesting candidates to be mediators of global RNA stability during the
70 acclimation to changing temperature conditions (Kupsch et al., 2012).

71 The cpRNP protein family consists of ten members in *Arabidopsis thaliana* (Ruwe et al., 2011). All cpRNPs
72 are targeted to the chloroplast post-translationally, and dedicated import receptors appear to be responsible
73 for their transport across the chloroplast envelope (Li and Sugiura, 1990; Grimmer et al., 2014). cpRNPs
74 are highly regulated proteins that react to various external and internal signals, particularly light, which
75 controls both their expression and their protein modification state (summarized in Ruwe et al., 2011).
76 Several cpRNPs have been identified as phosphoproteins (Reiland et al., 2009) and an N-terminally
77 acetylated isoform of CP29A was shown to respond rapidly to changes in light and developmental stages
78 (Wang et al., 2006). The phosphorylation of cpRNPs can alter their RNA-binding characteristics *in vitro*
79 (Lisitsky and Schuster, 1995; Loza-Tavera et al., 2006), but evidence for condition-dependent RNA binding
80 of cpRNPs or any other chloroplast RBPs *in vivo* are lacking.

81 Several molecular functions have been suggested for cpRNPs. *In vitro*, a tobacco homolog of *Arabidopsis*
82 CP31A has been shown to support RNA editing of multiple sites (Hirose and Sugiura, 2001). Other cpRNPs
83 have been reported to be required for the 3'-end processing of several mRNAs (Schuster and Gruissem,
84 1991; Hayes et al., 1996; Schuster et al., 1999). They also support the ribozymatic maturation of a viroid
85 RNA genome (Daros and Flores, 2002). The most notable and general function of cpRNPs is however their
86 role in stabilizing mRNAs. *In vitro*, cpRNPs protect the mRNA encoding the D1 subunit of photosystem II
87 against degradation (Nakamura et al., 2001). *In vivo*, they are required for the accumulation of a multitude
88 of mRNAs (Kupsch et al., 2012; Teubner et al., 2017). Co-immunoprecipitation analyses have demonstrated
89 that cpRNPs are associated with multiple chloroplast RNAs (Kupsch et al., 2012; Teubner et al., 2017), and
90 that they prefer unprocessed (unspliced) RNAs over mature forms (Nakamura et al., 1999). Together, the
91 data obtained from these functional analyses show that cpRNPs have a broad target range and contribute to
92 various RNA-processing events and to RNA stabilization.

93 All cpRNPs share a similar design with two RNA recognition motifs (RRMs) that are preceded by a domain
94 rich in acidic residues (acidic domain; AD). While the RRM are well characterized RNA binding domains
95 (Li and Sugiura, 1991; Ye and Sugiura, 1992; Lisitsky et al., 1995), the role of the AD remains to be
96 determined. The cpRNP CP31A stands out from the family in having a particularly long acidic domain,
97 which contains two phosphorylated serine residues within a short repeat element of five amino acids
98 (Reiland et al., 2009). CP31A's AD was suggested to be important for RNA editing based on *in vitro* assays
99 (Hirose and Sugiura, 2001). The AD of the spinach homolog of CP31A is supportive of RNA binding *in*

100 *vitro*, but does not bind RNA itself (Lisitsky and Schuster, 1995). Recently, plastid casein kinase II (pCKII)
101 was demonstrated to phosphorylate the acidic domain of CP31A *in vitro* (Schonberg et al., 2014). Genetic
102 analyses have demonstrated that *Arabidopsis* CP31A supports RNA editing at multiple sites and modulates
103 the stability of multiple mRNAs (Tillich et al., 2009; Kupsch et al., 2012). An RNA strongly affected in
104 *cp31a* mutants was the *ndhF* mRNA, which encodes a subunit of the NDH complex. CP31A binds to the
105 3'-UTR of *ndhF* and is required for the generation of the *ndhF* 3'-terminus (Kupsch et al., 2012). Mutants
106 of CP31A display cold sensitivity: the germination rate of null mutants is reduced and their newly emerging
107 leaf tissue bleaches at 8°C (Kupsch et al., 2012). All analyzed proteins of the photosynthetic apparatus are
108 reduced in this defective tissue, which is at least in part due to multiple defects in RNA processing (e.g.,
109 RNA splicing, RNA editing, and intercistronic processing), but is likely primarily caused by the strong
110 reduction of multiple chloroplast mRNAs (Kupsch et al., 2012). However, it remains unclear how CP31A
111 mechanistically confers cold resistance and whether it directly perceives cold as a signal. We hypothesized
112 that the AD could act as a regulatory domain for RNA binding and cold-responsiveness. We therefore
113 analyzed the ability of CP31A to associate with RNA in response to cold and assessed the role of the AD
114 for CP31A's ability to bind and process chloroplast RNA. Our results demonstrate that the AD of CP31A
115 is essential for plant cold acclimation, at least in part because of its supportive role for cold-dependent RNA
116 binding.

117 **Results**

118 **The RRM domains of CP31A are sufficient for RNA editing of CP31A-dependent sites**

119 The binding of RBPs to their RNA targets can be altered by protein modifications. For several members of
120 the cpRNP family, phosphorylation has been demonstrated to alter RNA binding *in vitro* (Lisitsky and
121 Schuster, 1995; Loza-Tavera et al., 2006; Reiland et al., 2009). As phosphoproteomic analyses identified
122 several phosphorylation sites within the acidic N-terminal domain of CP31A (Reiland et al., 2009;
123 Schonberg et al., 2014), we decided to test the function of this acidic phosphodomain by deletion
124 mutagenesis. We designed three T-DNA based constructs to express CP31A variants in *Arabidopsis* (Fig.
125 1A). The first two expressed variants lack the AD. One was driven by the 35S promoter, while the other
126 was driven by the endogenous genomic region upstream of the transcriptional start site, presumably
127 encompassing the unknown *CP31A* promoter (12769646 to 12767953 of the *Arabidopsis thaliana*
128 chromosome 4 sequence; nucleotides -1 to -1694 relative to the *CP31A* start codon). These constructs were
129 introduced into a *cp31a*-deficient background (homozygous *cp31a-1* null allele) by *Agrobacterium*-
130 mediated gene transfer. In addition, the full-length protein driven by its native promoter was used as a
131 positive control. All plant lines retrieved showed normal development and were indistinguishable from wt
132 plants under normal growth conditions (see below).

133

134 We assessed CP31A protein accumulation
 135 in our transgenic lines by using an antibody
 136 that reacts to two peptides: one situated in
 137 the linker between the two RRM domains
 138 and another at the C-terminus of the protein
 139 (Kupsch et al. 2012). The antibody thus
 140 detects both the full-length as well as the
 141 AD-deletion proteins. We observed
 142 accumulation of proteins that were of the
 143 appropriate size for all transgenic lines (Fig.
 144 1B). These data demonstrate that the full-
 145 length and AD-deficient proteins were
 146 expressed and specifically detected by the
 147 antibody. We quantified signals from three
 148 independent transgenic lines for each
 149 construct (Fig. 1B, C). As expected, there is
 150 variability in expression between individual
 151 lines, which can be attributed to position
 152 effects of the transgene insertion site. In
 153 sum, these analyses demonstrate that all
 154 three constructs were successfully
 155 expressed in a CP31A-deficient
 156 background.

157 Since CP31A has been shown to support
 158 RNA editing at 13 specific sites (Tillich et al., 2009), we investigated these and additional sites in our
 159 complementation lines by performing next-generation sequencing of cloned amplified cDNA (Bentolila et
 160 al., 2013), testing a total of 16 sites on 10 amplicons. This included sites that were previously shown not to
 161 be affected in *cp31a* mutants. Two wt and two *cp31a-1* null mutant plants served as controls. The lowest
 162 read coverage observed for any individual site was above 500 reads per site and was found for site *ndhD*
 163 116281 (number refers to the position in the *Arabidopsis* chloroplast genome), while the average coverage
 164 for all sites and all genotypes analyzed was 3132 reads per site.

165 Of the 13 sites previously described to be dependent on CP31A, we confirmed that RNA editing of 11 sites
 166 was reduced in *cp31a-1* mutants (Fig. 2A). Additional defects were found for two previously unreported

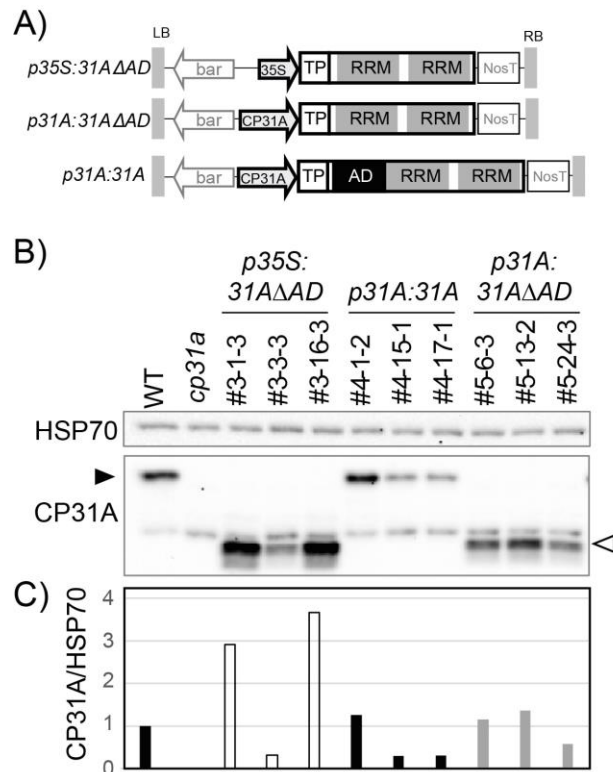


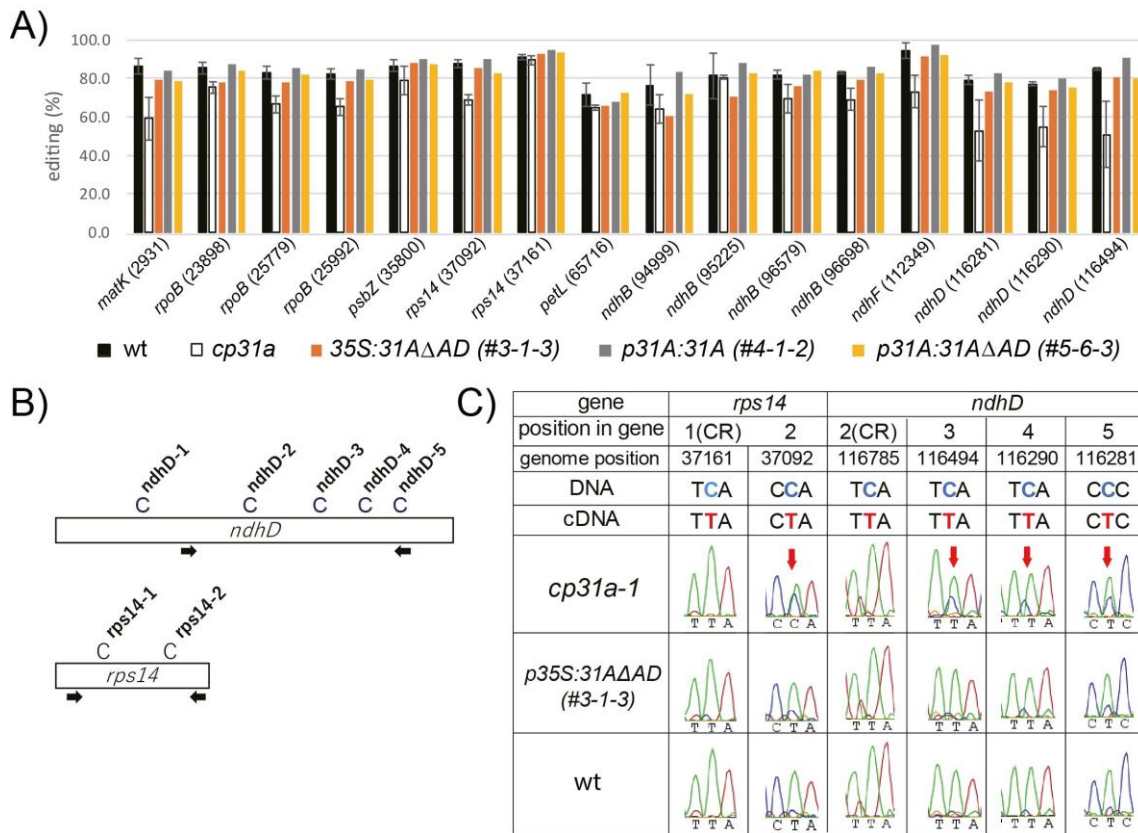
Figure 1: Construction of AD deletion mutants.

A) Schematic overview of constructs used to complement *cp31a* null mutants. *bar* = selectable marker; black arrows indicate promoters. TP = transit peptide. NosT = the terminator of the nos gene. LB/RB = borders of the *Agrobacterium* T-DNA.

B) Western analysis of transgenic lines. Blots were probed with a HSP70 antibody to control for loading and with an antiserum recognizing CP31A. The position of the full-length protein and the truncated, AD-less version are indicated by filled and open arrowheads, respectively.

C) Quantification of CP31A signals normalized to HSP70 signals (from B)).

167 sites in *ndhB* (94999, 96579), whereas no defect was observed for the previously reported sites, *petL* (65716)
 168 and *ndhB* (95225). We speculate that these differences reflect differences in growth conditions: our plants
 169 were grown under long-day conditions (16-h light/8-h dark), whereas the previous study used short-day
 170 conditions (8-h light/16-h dark; Tillich et al., 2009). Importantly, all our complementation lines, irrespective
 171 of the utilized promoter or the presence of the AD showed wt-like editing states at most analyzed sites (Fig.
 172 2A).



173

174 **Figure 2: Analysis of RNA editing sites in *cp31a* complementation lines.**

175 A) Summary of results from amplicon sequencing of RNA editing sites in complementation lines. Editing sites were
 176 amplified by PCR and sequenced on an Illumina platform using barcoded adapters that allowed to eliminate duplicate
 177 amplifications. Each individual cDNA sequence is scored for its editing status and the frequency of edited versus non-
 178 edited cDNAs is shown. Error bars represent the standard deviation calculated from two replicate experiments with
 179 WT and *cp31a* mutants, respectively. Numbers refer to the position of the editing site in the *Arabidopsis thaliana*
 180 chloroplast genome.

181 B) Schematic overview of two genes with their editing sites that were assessed by Sanger sequencing of amplified
 182 cDNAs. Arrows indicate positions of primers used for cDNA amplification.

183 C) Excerpts from electropherograms showing fluorescence signals for base triplets with the editing site always at the
 184 center. Bases marked by red arrows are targets of CP31A. Green traces refer to T signals, blue to C signals and red to
 185 A signals. The expected edited (on cDNA) and unedited (on DNA) triplets are shown above. CR = control sites not
 186 affected by CP31A according to Tillich et al (2009).
 187

188 We confirmed this finding by independent amplification and Sanger sequencing of selected RNA-editing
 189 sites of the *ndhD* and *rps14* mRNAs. The amplicons we sequenced harbored both, CP31A-dependent and

190 CP31A-independent editing sites (Fig. 2B). Our sequencing confirmed that in *cp31a* mutants, sites *ndhD*-
 191 3, 4 and 5, *rps14-2* show reduced editing efficiency when compared to wt. This defect is complemented in
 192 the AD-less CP31A protein (Fig. 2C). The successful complementation demonstrates that the RRM domains
 193 are sufficient for the RNA-editing function of CP31A.

194 **The AD of CP31A is not essential for, but is**
 195 **supportive of the stability of the *ndhF* mRNA**

196 We next analyzed the RNA-stabilizing function of
 197 CP31A by focusing on *ndhF*, which is its major
 198 target under normal growth conditions (Tillich et
 199 al., 2009). Using RNA gel-blot hybridization, we
 200 quantified the amount of *ndhF* mRNA in the
 201 transgenic lines. The accumulation of this transcript
 202 is at least partially restored in all complementing
 203 lines relative to the *cp31a* mutant (Fig. 3A, B). Even
 204 in a line with very little CP31A accumulation and
 205 lacking the AD, a signal for *ndhF* is visible (#3-3-3.
 206 Fig. 1, Fig. 3A,B). This indicates that the RRM
 207 domains were sufficient to stabilize the *ndhF*
 208 mRNA. However, we observed marked differences
 209 in the efficiency of *ndhF* stabilization between the
 210 full-length and AD-deficient proteins. Lines
 211 accumulating large amounts of the AD-less CP31A
 212 protein had only little more *ndhF* mRNA than lines
 213 expressing much less full-length CP31A protein
 214 (compare for example line #3-1-3 with #4-17-1 in
 215 Fig. 1B and Fig. 3A). This was visualized by
 216 calculating the ratio of *ndhF* mRNA levels to
 217 CP31A protein levels (i.e., the stabilization
 218 efficiencies; Fig. 3C), which clearly showed that the full-length proteins outperformed the AD-less proteins
 219 in stabilizing the *ndhF* mRNA. Together, these results show that the AD supports but is not essential for
 220 stabilizing the *ndhF* mRNA and that the RRM domains are capable of performing this task by themselves.

221

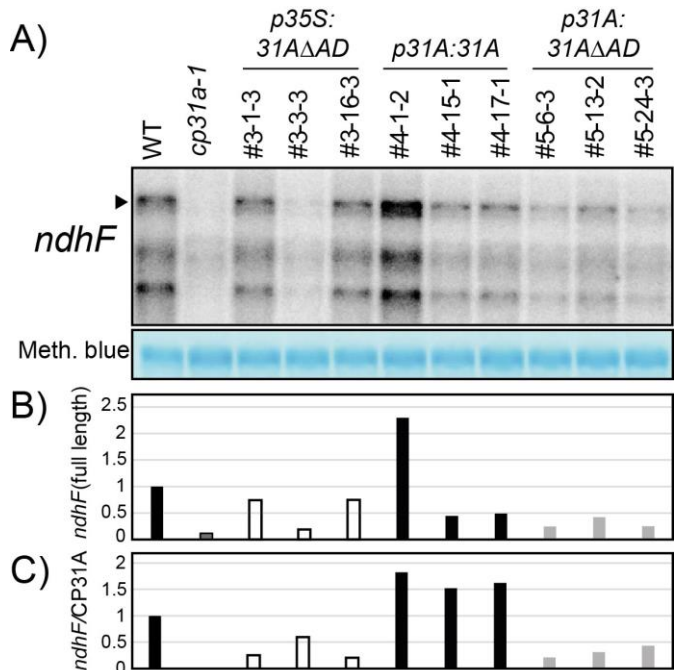


Figure 3: Analysis of the accumulation of the *ndhF* mRNA in *cp31a* complementation lines.

A) RNA gel blot analysis of the *ndhF* mRNA in various *cp31a* complementation lines. 4 μ g total cellular RNA were separated by denaturing agarose gel electrophoresis, transferred to a nylon membrane and probed for *ndhF*. The transcript labelled with an arrowhead represents the full-length *ndhF* mRNA, whereas smaller signals correspond to degradation products (Kupsch et al. 2012). The nylon membrane was labeled with methylene blue to control for equal loading (excerpt with 23S rRNA shown below blot).

B) Quantification of the full-length *ndhF* transcript labeled with an arrowhead in (A).

C) Ratio of *ndhF* mRNA levels over CP31A protein levels (from Fig. 1C).

222 The AD is required for cold-stress tolerance

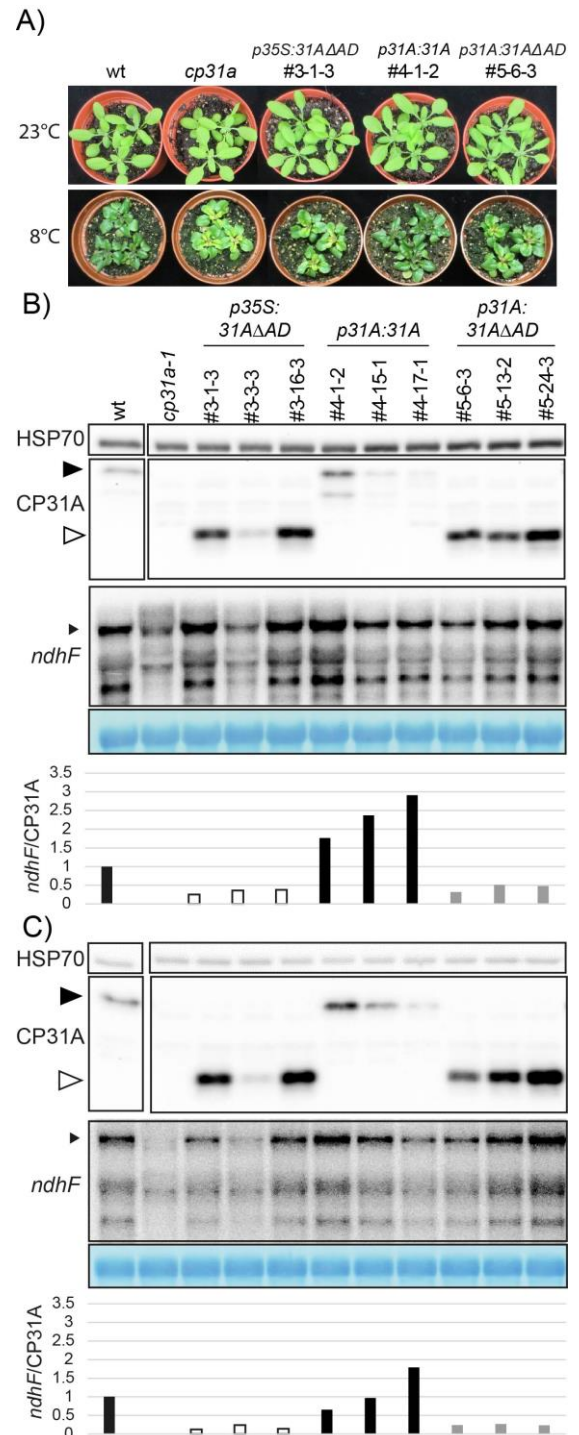
223 To test the impact of the AD on CP31A-mediated cold
 224 tolerance, we challenged 2-week-old plants from the
 225 transgenic lines with low temperature (8°C) for 5 weeks.
 226 Under this treatment, the *cp31a*-null lines are known to display
 227 bleaching of the freshly emerging tissue at the center of the leaf
 228 rosette (Fig. 4A). Similar losses of pigment were also found in
 229 the lines expressing the AD-less CP31A, but not in wt plants
 230 or in mutant plants complemented with the full-length protein
 231 (Fig. 4A). The pigment deficiency has been shown to be
 232 paralleled by a global reduction of the chloroplast-encoded
 233 proteins required for photosynthesis in *cp31a* mutants (Kupsch
 234 et al., 2012). This reduction of photosynthetic proteins was
 235 also observed in plants expressing the AD-less CP31A
 236 proteins, while plants expressing full-length CP31A
 237 accumulated protein amounts similar to those seen in wt plants
 238 (Suppl. Fig. 1). Next, we tested the accumulation of CP31A
 239 and *ndhF* in these lines under cold exposure. We separately
 240 harvested full leaves, as well as the bleached center part of
 241 rosettes in case molecular defects are restricted to the
 242 phenotypically conspicuous tissue. However, both tissues
 243 showed the same tendency: In line with the findings at normal
 244 growth temperatures, the *ndhF*/CP31A ratio was consistently
 245 higher for plants expressing full-length CP31A versus plants
 246 expressing the AD-less protein (Fig. 4B/C and Suppl. Fig. 2).

247 **Figure 4: Analysis of *cp31a* complementation lines during cold** 248 **acclimation.**

249 A) Phenotypes of complementation lines grown at 23°C or
 250 challenged with 8°C for five weeks. Note the bleached tissue in the
 251 center of the rosette in *cp31a* deficient plants and in plants lacking
 252 the AD.

253 B) Analysis of CP31A and HSP70 protein accumulation and *ndhF* mRNA accumulation in *cp31a* complementation
 254 lines. Here, the entire rosette leaves were used for protein and RNA extraction. Panels from top to bottom: immunoblot
 255 analysis of HSP70 proteins as loading control; immunoblot analysis of CP31A proteins; RNA gel blot analysis of
 256 *ndhF*; Methylene blue stain of cytosolic rRNA as loading control; Ratio of quantified *ndhF* and CP31A signals. Filled
 257 and open arrowheads indicate full-length and AD-less CP31A, respectively, while the small arrowhead denotes the
 258 full-length *ndhF* transcript.

259 C) Same analysis as in (B), but with tissue only from the center of the rosette (bleached area in *cp31a* null mutants).



260 These experiments show that the AD of CP31A supports the accumulation of chloroplast RNA and is
261 essential for conferring cold resistance to emerging leaf tissue in *Arabidopsis*.

262 **CP31A associates with multiple mRNAs with a preference for transcripts encoding subunits of the**
263 **NDH complex, and is required for *ndh* mRNA accumulation at standard growth temperatures**

264 RBPs of various origins and organisms show differential expression under temperature changes, and genetic
265 studies indicate that they are important for temperature-dependent RNA processing (Lin et al., 2010; Malay
266 et al., 2011; Gotic et al., 2016). That said, few studies have examined whether the binding of RBPs to their
267 RNA targets is modified in response to external and internal cues *in vivo*. Since CP31A is required for cold
268 resistance, we tested whether its ability to bind RNA is modified at low temperature and also assayed the
269 importance of the AD for this response. Previous studies using low-resolution techniques could only identify
270 whole transcripts as targets of CP31A (Kupsch et al., 2012). We here used a RIP-Seq approach to obtain
271 better resolution (Suppl. Fig. 3A). For this, wt *Arabidopsis* seedlings and plants expressing the AD-less
272 CP31A protein in a *cp31a* background were grown for 14 days under normal growth conditions and then
273 subjected to formaldehyde cross-linking. For cold tests, seedlings were grown for 13 days at standard growth
274 temperature (21°C), transferred to 4°C for 24 hours, and were then subjected to cross-linking and
275 immunoprecipitation (IP; Kupsch et al., 2012). We used such a short incubation time in the cold to avoid
276 pleiotropic effects that can be expected in the bleached tissue resulting from longer cold challenges. The
277 efficiency of precipitation was comparable between samples grown at normal and low temperatures (Suppl.
278 Fig. 3B). RIP-Seq libraries were prepared in duplicate from input and pellet samples (Suppl. Fig. 3C). We
279 tested the reproducibility of the RIP-Seq experiments by calculating pairwise correlation coefficients across
280 all samples (Suppl. Fig. 3D). We found strong correlations among the input samples (average Pearson
281 coefficient (R): 0.95), and the pellet samples from the IP (average Pearson coefficient (R): 0.97). As
282 expected, input and pellet samples formed separate clusters in our correlation analysis (Suppl. Fig. 3D). This
283 analysis demonstrates high reproducibility between biological replicates of our RIP-Seq assay.

284 For the analysis of RIP-Seq reads, we followed established procedures originally developed for ChIP-Seq
285 (Bardet et al., 2011; Muino et al., 2011). We started by identifying transcript regions with an enrichment of
286 RNA in pellet samples over input samples. In total, 75 different binding regions were identified as
287 significant in at least one of the four experiment, which are henceforth called binding sites (BSs). The 75
288 BSs of CP31A are located in 44 transcripts of diverse functionality. Of these 44 transcripts, 31 were also
289 identified by previous RIP-Chip experiments (Suppl. Tab. 1, Suppl. Fig. 4). The difference observed is likely
290 due to methodological dissimilarities. Purified chloroplast stroma was used for the previous RIP-Chip
291 experiments without a cross-link, while here, we used cross-linked frozen total leaf tissue in the RIP-seq.
292 Given these technical differences, it was gratifying to see a similar set of transcripts enriched in the two
293 types of RIP experiments (Suppl. Tab. 1). We next focused on those BSs located within a gene body and

294 thus can be clearly assigned to a specific gene. By contrast, binding sites within intergenic regions cannot
 295 be easily assigned to individual genes due to the polycistronic nature of chloroplast transcripts. Assigned
 296 BSs are found in genes for the photosynthetic complexes as well as for the gene expression apparatus (Tab.
 297 1). We compared the actual distribution of BSs with the calculated numbers of BSs expected for each
 298 functional category (if random binding in the transcriptome is assumed; Tab. 1). This demonstrated that the
 299 NDH complex is the only functional category overrepresented among RIP-Seq targets (12 found versus 3
 300 expected; Tab. 1). We also found an unexpected large number of binding sites antisense to coding regions
 301 of known genes (Tab. 1). Although antisense RNAs are known to exist in chloroplasts, their levels do not
 302 (with a few exceptions) reach those of sense RNAs and their functions (if any) remain unclear (Nakamura
 303 et al., 2003; Hotto et al., 2010; Hotto et al., 2011; Sharwood et al., 2011). Consequently, the functional
 304 significance of CP31A's association with antisense RNAs remains unclear as well.

305 Table 1: Summary of target sites of CP31A across all samples analyzed in RIP-Seq experiments

	Unique CP31A binding sites ¹		Unique CP31A target genes ²		Length of potential target genes (bp) ³		Expected unique binding sites ⁴	
	sense	antisense	sense	antisense	sense	antisense	sense	antisense
NDH complex	12	0	6	0	13002	13002	3	3
Photosynthesis (except <i>ndh</i> genes)	6	3	5	3	23644	23644	6	6
Other metabolism	4	0	2	0	4580	4580	1	1
Ribosome	0	5	0	5	22778	22778	6	6
RNA polymerase	3	1	3	1	11170	11170	3	3
tRNA	2	1	2	1	10196	10196	2	2
Other	2	9	2	4	24177	24177	6	6
All BS	29	19	20	14			27	27
Total BSs in intergenic regions	27		NA		44931		21	
Total BSs	75		34				75	

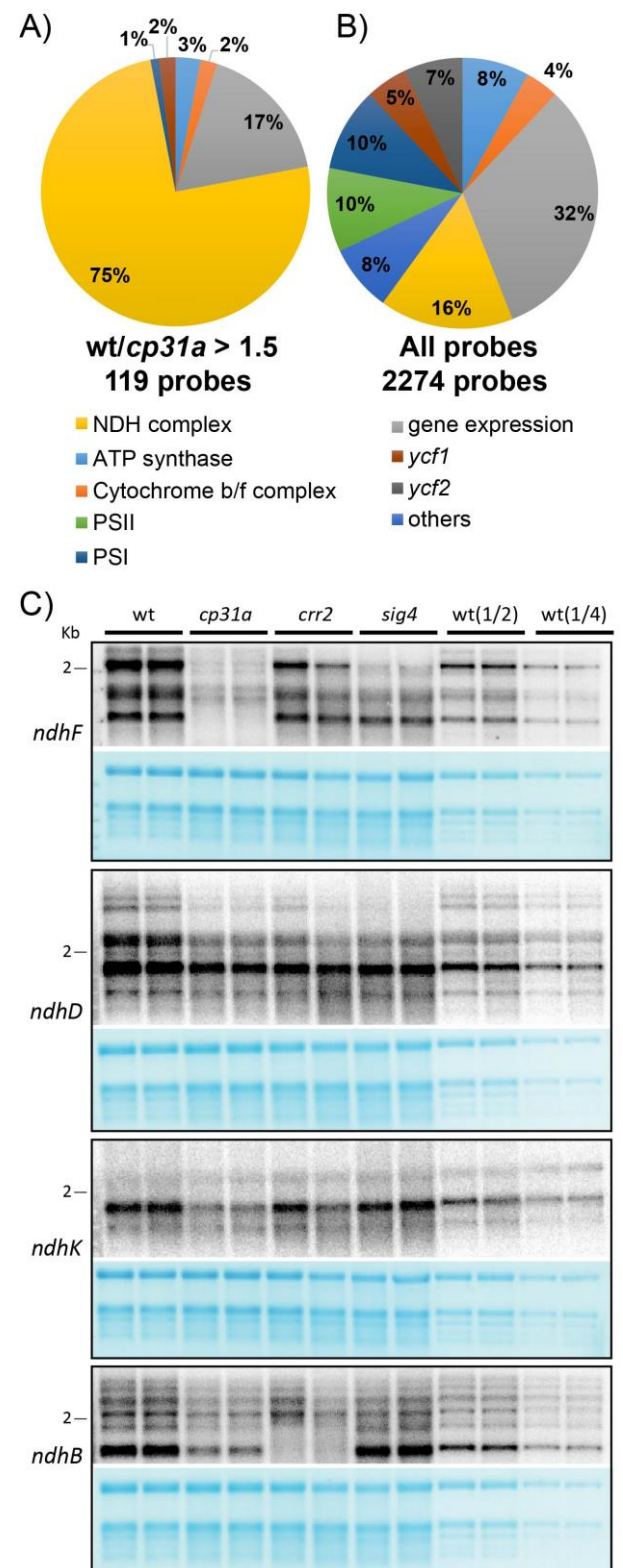
306
 307 ¹ this includes binding sites identified in all samples; rRNAs were excluded from calling binding sites
 308 ² gene annotations according to Araport11
 309 ³ sum of the length of all genes within a category
 310 ⁴ number of target sites expected in a gene class if the 75 binding sites are randomly distributed in the
 311 entire chloroplast genome and if the abundance of transcripts is considered equal between all genes (i.e.
 312 depends only on the combined length of all genes within a class)

313 It is however interesting that at least for the main target category, the *ndh* genes, a preference for sense sites
 314 is evident (Tab. 1), suggesting that CP31A is relevant for *ndh* mRNA expression. We therefore analyzed

315 chloroplast RNA levels in *cp31a* versus wt samples at normal
 316 temperatures using an oligonucleotide tiling array that
 317 represents the entire chloroplast genome of *Arabidopsis*
 318 *thaliana*. We scored only probes whose RNA levels were at least
 319 one third lower in the mutant compared to wt. Of all exon probes
 320 in the array, 16% represent *ndh* sequences. Importantly, among
 321 exon probes whose signals were decreased by at least 0.6-fold in
 322 the mutants, 75% contained *ndh* sequences (Fig. 5A, B). No
 323 other functional category showed enrichment in this analysis. To
 324 confirm these results, we performed RNA gel-blot hybridization
 325 experiments for four *ndh* genes that represent the four *ndh*
 326 operons in the chloroplast genome (Fig. 5C). We analyzed *cp31a*
 327 mutant RNAs alongside RNAs from seedlings with impairment
 328 in the expression of *SIGMA FACTOR 4* (*SIG4*), which is
 329 specifically required for the transcription of the *ndhF* mRNA
 330 (Favory et al., 2005), and *CHLORORESPIRATORY*
 331 *REDUCTION 2* (*CRR2*), which is required for the accumulation
 332 of monocistronic *ndhB* transcripts (Hashimoto et al., 2003). As
 333 expected, the control mutants showed specific defects for their
 334 known target RNAs (Fig. 5C). By contrast, the *ndhK* transcripts
 335 accumulated to normal levels in these two control lines, and the
 336 *ndhD* mRNA was only slightly decreased (Fig. 5C). In contrast
 337 and consistent with our microarray results, the *cp31a* mutants
 338 displayed reductions in all four *ndh* transcripts analyzed. The
 339 decrease was most pronounced for *ndhF*, strong for *ndhK* and
 340 *ndhB*, and somewhat weaker for *ndhD* (Fig. 5C). Collectively,
 341 these findings indicate that CP31A stabilizes mRNAs from all
 342 four *ndh* operons, suggesting that CP31A regulates *ndh* mRNAs
 343 as a group.

344 **Figure 5: Analysis of RNA accumulation in *cp31a* mutants.**

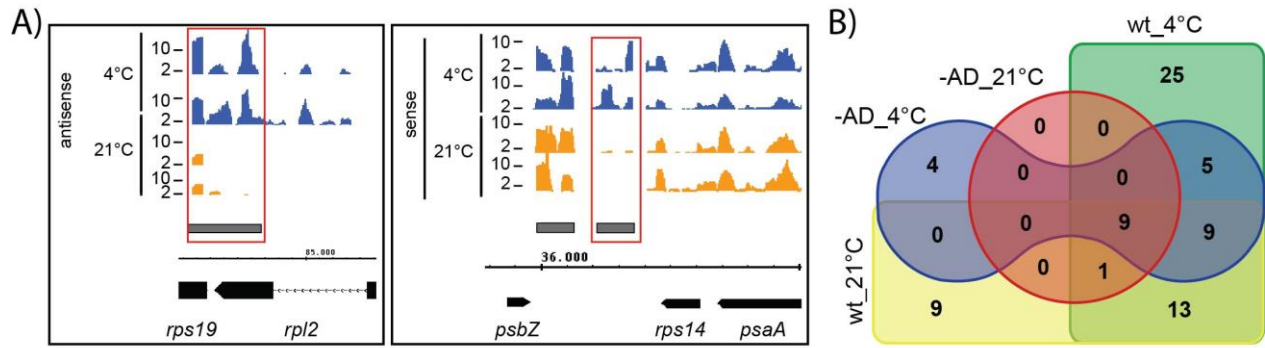
345 A) Summary of microarray analyses of 14 days old wt and *cp31a*
 346 mutant seedlings. Relative abundance of exon probes showing at least 1.5 fold stronger signals in wt versus *cp31a*
 347 mutants. Three replicate microarray hybridizations were analyzed and assigned to different gene categories.
 348 B) Relative distribution of all exon probes on the microarray to different gene categories.
 349 C) RNA gel blot analysis of 14 days old Arabidopsis seedlings. 4 μ g RNA from wt, *cp31a* mutants, and two
 350 control mutants (*crr2* and *sig4*) together with dilutions of wt samples (1/2 and 1/4) were probed with radiolabeled RNA
 351 probes against four different *ndh* genes. The resulting autoradiographs are always shown with the corresponding
 352 methylene blue stains of the membranes (below). The 2 kb marker band is shown as a reference.



367 processing, and/or translation. We therefore compared BSs according to how reliable their enrichment in a
368 given dataset is, based on its FDR value. Using the FDR values, we first focuses on the effect of cold on
369 RNA binding of full-length CP31A and then analyzed the effect of the loss of the AD on binding (next
370 chapter). A majority of BSs were found with high significance in CP31A precipitates in the cold, but were
371 not significantly enriched at higher temperature. This was already visible for some sites in the coverage
372 graphs, as shown in Fig. 7A for binding sites within the *rpl2* mRNAs, or for a site in the intergenic region
373 between the *rps14* and *psbZ* genes. Many BSs show at least some read coverage at both temperatures, but
374 the FDR analysis helped to separate significant from non-significant BSs. The FDR level is displayed for
375 all 75 binding sites as a heat map in Suppl. Fig. 6 and is summarized in Fig. 7B. Most importantly, 30 BSs
376 show significant enrichment with full-length CP31A at 4°C, but not at 21°C (Fig. 7B). Only 9 sites show
377 the opposite behavior, that is, significant enrichment only at 21°C, but not at 4°C. Thus many more sites
378 showed increased versus decreased association with CP31A after cold exposure. Three of the BSs gained in
379 the cold were located in the *ndhF* mRNA, which is known to be a prime target of CP31A (Kupsch et al.,
380 2012). Four more CP31A BSs in *ndh* transcripts were cold-dependent as well (Suppl. Fig. 6). In general,
381 *ndh* mRNAs appeared to be important targets of CP31A under both temperature conditions (Suppl. Fig. 6).
382 Taken together, the results of our temperature-dependent RIP-Seq analysis demonstrate that RNA
383 association of full-length CP31A is higher under cold exposure than at normal temperature, and that *ndh*
384 mRNAs are prime targets under both normal and low temperatures.

385 **The AD of CP31A supports cold-dependent RNA binding**

386 The RRM domain is a highly versatile protein domain that functions in the context of adjacent protein
387 regions. Often, linkers between RRMs or protein domains outside the RRM domains support RNA
388 interactions in addition to the canonical RRM contacts (Lunde et al., 2007). Given this, we used RIP-Seq to
389 test whether the RNA-binding behavior of the AD-less CP31A protein differed from that of the full-length
390 protein. Using the same strategy described for our binding-site analysis with the full-length protein, we
391 scored significant BSs in plants expressing the AD-less variant grown under both normal and cold
392 conditions. We found that the AD-less version of CP31A bound far fewer RNAs than its full-length
393 counterpart (Fig. 7B, Suppl. Fig. 6). Under normal growth temperatures, we identified 41 significant BSs
394 (FDR < 0.05) for the full-length protein and only 10 for the AD-less version (Fig. 7B). Interestingly, the
395 AD-less protein is still responsive to cold: The AD-less protein associated with 18 sites in the cold that are
396 not found to co-precipitate significantly with the protein at normal temperatures (Fig. 7B). On the other
397 hand, AD-less CP31A bound 35 fewer sites than the full-length protein after cold exposure (Fig. 7B). This
398 demonstrates that the AD supports RNA binding under both conditions. Together, our results show (i) that
399 the two RRM domains still react to lowered temperatures in their ability to bind RNA, and (ii) that the AD
400 substantially supports but is not essential for RNA binding.



401
 402 **Figure 7: RIP-Seq uncovers cold-induced RNA binding of CP31A.**
 403 A) Visualization of three examples of temperature-dependent CP31A binding sites. Top track shows the ratio of
 404 precipitated versus input reads of RIP-Seq experiments of wt plants grown at 4°C (blue) or 21°C (orange, for two
 405 biological replicates at each growth temperature). The track below represents in grey boxes the identified binding sites.
 406 The bottom track shows the gene models in black and an intron in *rpl2* as a hatched line. The numbers on the thin
 407 black line refer to positions on the *Arabidopsis* chloroplast genome. The red rectangles indicate temperature-dependent
 408 CP31A BSs.
 409 B) Venn diagram summarizing the occurrence of binding sites in the two genotypes and the two temperature regimes
 410 used for growing the plants. For each genotype-temperature combination, only BSs with an FDR < 0.05 were counted.

411 Discussion

412 CP31A co-regulates *ndh* genes

413 During the evolution of chloroplasts from cyanobacterial ancestors, operon structures were disrupted and
 414 operons were shuffled. Many chloroplast operons therefore include genes with different functions. The *ndh*
 415 genes, for example, are separated into four transcriptional units in *Arabidopsis* and are mixed with genes
 416 from other functional categories. This lack of conservation of operon structures suggests that transcriptional
 417 units are less important than other processes for *ndh* gene regulation in the chloroplast context, giving way
 418 to post-transcriptional processes. Translation plays an important role for regulation (Zoschke and Bock,
 419 2018), but it remains unclear, how post-transcriptional co-regulation is achieved prior to translation. We
 420 herein demonstrate that CP31A associates with many *ndh* mRNAs under both normal and low temperatures.
 421 All analyzed *ndh* mRNAs were reduced in *cp31a* mutants under normal growth conditions, indicating that
 422 the CP31A-*ndh* interaction is functionally important. Since the loss of CP31A does not impede transcription
 423 (Kupsch et al., 2012), we conclude that the protein is required for the stability of *ndh* mRNAs. An alternative
 424 explanation is that the loss of one RNA of the NDH complex has an indirect, hierarchical effect on the other
 425 *ndh* RNAs that yields the observed reduction in all *ndh* mRNAs. This phenomenon has been well described
 426 for hierarchical protein synthesis cascades in chloroplasts (Choquet et al., 2001), but it has not been
 427 previously shown to impact mRNA synthesis or stability. Moreover, the likelihood of this scenario is
 428 weakened by our observation that the losses of *ndhF* or *ndhB* in the *sig4* or *crr2* mutants, respectively, were
 429 not followed by the loss of all *ndh* mRNAs. Taken together, our data indicate that CP31A combines *ndh*

430 mRNAs from different genomic loci into a post-transcriptional operon in the chloroplast. Similar post-
431 transcriptional operons, or “RNA regulons”, have been well-described in fruit flies, budding yeast, and
432 mammalian cells (Keene, 2007). In most cases, these RNA operons function in the combined translation
433 and/or stabilization of the participating RNAs (Gerber et al., 2004; Lykke-Andersen and Wagner, 2005;
434 Townley-Tilson et al., 2006). Given the RNA-stabilizing roles of cpRNPs in general and of CP31A in
435 particular, we propose that CP31A adjusts the stability of the *ndh* transcripts as a group.

436 **The AD contributes to the ability of CP31A to recognize RNA**

437 CP31A has two canonical RRM motifs, which contain the expected aromatic amino acid residues in the
438 RRM’s RNP1 and RNP2 motifs known to be crucial for RNA binding (Ruwe et al., 2011). Here, we
439 show that these RRMs are sufficient to edit chloroplast RNAs and stabilize the *ndhF* mRNA. Still, compared
440 to the wt protein, more AD-less protein is needed to stabilize a similar level of *ndhF* mRNA. Moreover, loss
441 of the AD decreases the ability of CP31A to bind RNA. Thus, the AD domain supports but is not essential
442 for the RNA-binding and RNA-stabilizing functions of CP31A. We do not yet know how the AD achieves
443 its effects at the molecular level. Two non-exclusive possible modes of action are (i) a direct involvement
444 in protein-RNA interactions, and (ii) a role in protein-protein interactions for the recruitment of additional
445 RNA processing factors. Both modes of action were already described in non-plant systems for acidic
446 domains (Lunde et al., 2007). Such functional diversity of an AD has been described for two structural
447 relatives of CP31A, the non-plant RNA binding proteins hnRNP Q and hnRNP R, that also have an N-
448 terminal AD and C-terminal RRM motifs (Geuens et al., 2016). The AD of hnRNP R was shown to support
449 RNA binding and is essential for the role of hnRNP R in the re-initiation of transcription (Fukuda et al.,
450 2013). Similarly, the AD of hnRNP Q forms part of the RNA interaction surface (Hobor et al., 2018) and in
451 addition interacts with the editing factor Apobec (Quaresma et al., 2006). These examples show that it is
452 conceivable that the AD of CP31A contributes to protein-protein interactions (e.g., with other RNA-binding
453 proteins) and also contributes directly to RNA binding. This could serve to increase the affinity for RNA
454 and mediate RNA stabilization and RNA-processing events. A future structural analysis of CP31A bound
455 to RNA would greatly help to understand the mechanistic function of CP31A’s AD.

456 **Cold-dependent RNA association of CP31A**

457 Many RBPs show differential association with RNA depending on physico-chemical conditions *in vitro*,
458 but relatively little is known about how external and internal cues modulate the target spectrum and target
459 affinity of an RBP *in vivo*. Changed binding to RNA due to external signals has been shown for mammalian
460 RNA binding proteins (Rousseau et al., 2002; Cloutier et al., 2018), but even here, rarely on a genome-wide
461 scale (Benegiamo et al., 2018), and comparable data are missing for plants. Our present results on CP31A
462 show that plant RNA-binding proteins can change their RNA target profiles in response to an external signal,

463 here low temperatures, but it remains unclear how this differential RNA binding is induced. Cold is expected
464 to change RNA structures and CP31A can bind dsDNA *in vitro*, albeit with less affinity than ssDNA and
465 ssRNA (Li and Sugiura, 1991). It is also notable that spinach cpRNPs have been implicated in the
466 establishment of the structured 3'-ends of several mRNAs *in vitro* (Schuster and Gruissem, 1991; Lisitsky
467 et al., 1995; Hayes et al., 1996). These RNA termini require the formation of stem-loop RNA structures,
468 and cpRNP-like proteins have been found to associate with such stem-loops in UV cross-linking
469 experiments (Stern et al., 1989; Chen and Stern, 1991; Danon and Mayfield, 1991; Nickelsen and Link,
470 1993). Thus, the binding of CP31A to RNAs under cold exposure could reflect the emergence of RNA
471 structures that become more stable at lower temperatures. An *in vivo* analysis of the structure of CP31A
472 target sites under different temperatures could help address the question of why CP31A associates more
473 extensively with RNA in the cold.

474 What is the functional consequence of the increased RNA association of CP31A under cold exposure? Given
475 that chloroplast RNAs are lost in the bleached tissue of *cp31a* mutants, we propose that an increased
476 interaction with target transcripts could impact the resilience of these transcripts against RNases. An open
477 question however is, which of the many CP31A – transcript interactions in the cold is key to avoid bleaching.
478 Although it is likely that many of these interactions have an incremental effect on the phenotype, we can
479 make some guesses about major contributors. Loss of *ndh* mRNAs, although a prime target of CP31A, is
480 unlikely to be responsible for the bleaching phenotype, since *ndh* genes are downregulated in the cold in
481 *Arabidopsis* (Ivanov et al., 2012), and mutants devoid of the NDH complex do not show bleaching in the
482 cold (Li et al., 2004; Yamori et al., 2011). By contrast, there is a number of other CP31A target RNAs that
483 are known to be essential for chloroplast development and cold-dependent loss of their processing and
484 stabilization could well explain bleaching. This includes mRNAs for subunits of the ATP synthase (*atpA*,
485 *atpB*), of the cytochrome *b₆f* complex (e.g. *petB*, *petD*), of the photosystems (e.g. *psaA*, *psbB*), as well as
486 mRNAs for subunits of the plastid-encoded RNA polymerase (*rpoB*, *rpoC2*). Loss of the expression of any
487 of these genes leads to defective chloroplasts including pale phenotypes (Hajdukiewicz et al., 1997;
488 Rogalski et al., 2006; Stoppel et al., 2011; Manavski et al., 2015; Chen et al., 2016). Furthermore, many
489 mutants affected in chloroplast translation are more sensitive to cold than wt (Rogalski et al., 2008;
490 Fleischmann et al., 2011; Gu et al., 2014; Wang et al., 2016; Zhang et al., 2016; Paieri et al., 2018; Pulido
491 et al., 2018). This includes mutants of chloroplast RNA binding proteins that affect translation and that show
492 cold-dependent bleaching of the center of the *Arabidopsis* rosette (Wang et al., 2016), which is strikingly
493 similar to the phenotype of *cp31a* mutants in the cold. CP31A binds to several mRNAs coding for ribosomal
494 proteins (e.g. *rps18*), thus being potentially important for the production of the translational machinery in
495 the cold. Taken together, we hypothesize that CP31A becomes important for the expression of a combination
496 of its target mRNAs in the cold; their compromised expression in cold-treated *cp31a* mutants leads to loss

497 of chloroplast development and thus bleaching. Complementation analyses and time-resolved analysis of
498 the accumulation of mRNAs in *cp31a* mutants during cold treatment could reveal, which mRNAs are key
499 targets for CP31A during cold acclimation.

500 **Methods**

501 **Plant growth**

502 *Arabidopsis thaliana* Columbia-0, *cp31a-1* T-DNA insertion mutants (Tillich et al., 2009) and AD-deletion
503 mutants were grown on soil with a 16-h-light/8-h-dark cycle at 23°C in a CLF growth cabinet at 120
504 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. For cold stress application, temperatures were lowered to 8°C when plants reached an age of
505 two weeks. Cold exposure was then continued for another five weeks to allow the emergence of sufficient
506 new tissue prior to harvest. For RIP-seq experiments plants were grown on a soil/vermiculite 4:1 mixture at
507 21°C for 14 days (normal conditions). Cold stress was applied after 13 days at 21°C with a 24 h exposure
508 to 4°C.

509 **Vector construction and production of transgenic plants**

510 A full length *Arabidopsis* CP31A cDNA clone was prepared from total *Arabidopsis* RNA. The genomic
511 region encoding the acidic domain (+241 to +447 bp from the start codon of the open reading frame) was
512 deleted as follows: The 5' region of *CP31A* (1-240 bp) and the 3' region of *CP31A* (448-990 bp) were
513 amplified with the primers 31A_gateway_for and A31-minusADrev and A31-minusAD_for and
514 31A_gateway_rev, respectively. These 5' and 3' regions were joined by restriction-based ligation into a
515 pBluescript vector. The *p35SP:31AΔAD* binary vector for constitutive expression of CP31A was constructed
516 as follows: The *31AΔAD* fragment was amplified with the primer set (SmaI-31AF01; 31A-SmaIR01) and
517 ligated into *pJET1.2* cloning vector (Thermo Scientific), and the *SmaI* digested product was integrated into
518 a *SmaI* site between the *35S* promoter and *Nos* terminator sequences of the binary vector *pGL1* (provided
519 by Dr. Boris Hedtke, HU Berlin), which also contains the bar expression cassette. For producing the
520 *p31AP:31A* vector, the *CP31A* 5' UTR and promoter region (1,694 bp of the 5' region of *CP31A*; 12769646
521 to 12767953 of the *Arabidopsis thaliana* chromosome 4 sequence; Acc. No. CP002687.1) was cloned with
522 the specific primer set (CP31A5'up-1694; CP31A-CDS5'_rev). Then, the *CP31A* cDNA was combined with
523 the promoter by Gibson cloning using the primers (XhoI-31Apro F; Cp31A-CDS5'_rev; CP31A-
524 ADtest_for; 31A-SmaIR01) and cloned into *pJET1.2*, yielding *pXhoI-31AP:31A-SmaI*. The 35S promoter
525 was removed and replaced with multi cloning site including *XhoI* and *SmaI* sites, yielding vector *pGL1-
526 MCS*. *XhoI/SmaI* digestion products from *pXhoI-31AP:31A-SmaI* were inserted into *XhoI/SmaI* sites of
527 *pGL1-MCS*, yielding *p31AP:31A* vector. *p31AP:31AΔAD* was constructed in an analogous way. These three
528 vectors were introduced into the *Agrobacterium* GV3103 strain independently, and integrated into the
529 knock-out mutant *cp31a-1* (SALK_109613; Tillich et al., 2009) by the floral-dip method. The T₀ generation

530 was grown on 15 cm diameter plastic plates filled with soil (mixture of horticulture soil and vermiculite).
531 1/2000 diluted BASTA was sprayed on the plants several times in order to select for BASTA resistant plants.
532 BASTA resistant plants were transferred to plastic pots filled with soil and cultivated at 23°C, 8-h-dark/16-
533 h-light at a light intensity of 120-130 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

534 **Immunoblot analysis**

535 Total protein (15 μg) were extracted from fully developed leaves grown at normal conditions and
536 immunological analysis were carried out and antibodies were used as previously reported (Kupsch et al.,
537 2012). Immunoblot analysis for the RIP-seq experiments was performed in the same way with protein
538 samples taken from the input, supernatant and pellet fraction of the co-immunoprecipitations.

539 **RIP-Seq analysis**

540 For RIP-seq experiments *Arabidopsis thaliana* Columbia-0 and AD-deletion mutants under the native
541 promoter (#5-7-1, #5-13-2) were harvested in duplicates after 14 days and flash-frozen in liquid nitrogen.
542 The flash-frozen plant material was grinded under liquid nitrogen to a homogeneous powder. Between 250
543 to 350 mg plant material was suspended in 3 ml RIP-seq lysis buffer containing formaldehyde (50 mM
544 HEPES-KOH pH 8.0, 200 mM KCl, 5 mM MgCl_2 , 5 mM CaCl_2 , 0.5% Nonidet P-40, 0.5% Sodium
545 deoxycholate, 1x cOmpleteTM, EDTA-free Protease Inhibitor Cocktail (Roche), 100 U RiboLock RNase
546 Inhibitor (ThermoFisher Scientific), 1% formaldehyde) per 1 g plant material. Crosslinking was performed
547 for 10 min under rotation at room temperature and stopped by the addition of 125 mM glycine and a 5 min
548 incubation. The plant extract was centrifuged for 10 min at 20,000xg and 4°C to remove insoluble plant
549 material and flash-frozen in liquid nitrogen.

550 For the co-immunoprecipitation (CoIP) 8 μl affinity-purified anti-CP31A antibody (Kupsch et al., 2012)
551 was bound to 50 μl Dynabeads ProteinG (Invitrogen) under rotation (15 rpm). The plant extract was thawed
552 and centrifuged for 10 min at 20,000xg and 4°C. 350 μl of the supernatant were diluted 1:1 with CoIP buffer
553 (150 mM NaCl, 20 mM Tris-HCl pH 7.5, 2 mM MgCl_2 , 0.5% Nonidet P-40, 5 $\mu\text{g}/\text{ml}$ Aprotinin) and
554 incubated with the antibody-coated magnetic beads for 75 min at 15 rpm and 4°C. An aliquot of the
555 antibody-bead solution was taken to serve as the input control. The beads were washed four times in CoIP
556 buffer and resuspended in Proteinase K buffer (100 mM NaCl, 10 mM Tris-HCl pH 7.0, 1 mM EDTA, 0.5%
557 SDS).

558 The crosslink was reversed with 0.1 mg/ml Proteinase K (ThermoFisher Scientific) at 50°C for 1 h and RNA
559 was extracted from input and pellet fractions using TRIzol and RNA Clean and Concentrator Columns
560 (Zymo Research) according to the manufacturer's instructions.

561 Library preparation was performed with the NEBNext[®] Multiplex Small RNA Library Prep Set for Illumina
562 (New England BioLabs) according to the manufacturer's instructions with few deviations. Library
563 preparation was performed for half the volume. Additionally, a 5' adaptor including unique molecular
564 identifiers (UMI) was used (5'-
565 rGrUrUrCrArGrArGrUrUrCrUrArCrArGrUrCrCrGrArCrGrArUrCGATCNNNNNNNN-3'). PCR
566 amplification was performed using the KAPA HiFi HotStart ReadyMix with the cycling protocol for library
567 amplification for Illumina platforms and an annealing temperature of 62°C. The PCR amplified cDNA
568 construct was purified using the GeneJET PCR Purification Kit (ThermoFisher Scientific) according to the
569 manufacturer's instructions and then separated on 6% polyacrylamide gel. Library fragments between
570 160bp and 190bp were extracted from the gel according to the NEBNext[®] protocol and subjected to Illumina
571 sequencing. Read numbers and mapping results are summarized for all libraries in Supple. Table 3.

572 For the bioinformatic identification of the CP31A binding events the following steps were performed:

573 1) Mapping of the Illumina reads. First, reads were split depending on its barcode using
574 *fastx_barcode_splitter* (version 11sep2008) to identify to which sample they correspond, next the UMI
575 barcode was extracted for each read using *umi_tools*, later, reads were trimmed using Trimmomatic version
576 0.36 with the next parameters: *LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:10* in order to
577 eliminate Illumina adapters and bad quality sequence regions. The obtained trimmed reads were aligned to
578 the *Arabidopsis thaliana* genomes (Araport11) using STAR version 2.5.2a with parameter: --
579 *outFilterMultimapNmax 2 --outSAMtype BAM SortedByCoordinate --alignIntronMax 3000 --*
580 *outFilterIntronMotifs RemoveNoncanonical --outSAMstrandField intronMotif --outSAMattrIHstart 0*. At
581 this point, duplicated reads were removed from the BAM file using *umi_tools*. Only reads that mapped to
582 the chloroplast genome with a length bigger than 35 bp were used for downstream analysis.

583 2) Identification of candidate BSs in each condition studied. Reads mapped in rRNA were eliminated.
584 Remaining reads were used to identify BSs using CSAR (version 1.31.0; parameters: *b=10, w=70,*
585 *nper=100, test="Ratio"*) for each strand and sample/replicate independently. Only those regions that
586 showed a significant enrichment in mapped reads when comparing IP versus corresponding input samples
587 at a false discovery rate (FDR) < 0.05 were kept (Muino et al., 2011), which led to the discovery of 371
588 candidate BSs across all samples. In order to obtain a common set of candidate BSs for all samples, the list
589 of BSs for each sample/replicate were merged using the software *mergeBed* from the package *bedtools*
590 (v2.26.0). This resulted in 75 reference BSs.

591 3) Quantification of BSs. The program *featurecounts* (v1.6.0; parameters: *-s 1 -M*) was used to count the
592 number of reads strand-specifically mapping to the common set of candidate BSs obtained from the previous
593 step. DESeq2 (v1.14.1) was used with defaults parameters except *fitType="local"* to obtain normalized

594 number of mapped read in each sample, next it was used to calculate enrichment and significance of number
595 of the candidate BSs comparing IP vs control (two biological replicates). The normalized number of reads
596 were used to calculate Pearson correlation coefficients.

597 **SSMART analysis of CP31A RIP-seq data**

598 SSMART analysis was carried out as described (Munteanu et al., 2018). We used all significant BSs
599 identified in wild type plants grown in cold conditions as input, as in this sequence set most binding events
600 were detected. The input sequences of the BSs were scored using their adjusted p-value according to the
601 actual binding site analysis of the RIP-seq data.

602 **RNA extraction and editing analysis**

603 Total RNA was extracted from fully developed leafs (0.1 g) powdered in liquid nitrogen using Trizol
604 (Thermo Fisher) according to the manufacturer's protocol. DNA was removed from RNA samples by three
605 consecutive DNase I treatments and Phenol/Chloroform/Isoamyl alcohol extractions. DNA removal was
606 checked by PCR with the chloroplast-specific *rps14* primer set. cDNA synthesis was performed with 2 µg
607 RNA using SuperScript III first strand synthesis system (Invitrogen) according to the manufacturer's
608 protocol. A one-to-ten dilution of cDNA was used as a template for amplifying ten cDNAs encompassing
609 16 editing sites (**Fig. 2A**) on nine plastid genes using primer sets previously reported (Tillich et al., 2009).
610 RT-PCR products were purified with MinElute PCR Purification Kit (Qiagen). Bulk RT-PCR products were
611 cloned into the barcoded cloning vector and sequenced using the Illumina MiSeq machine. Sequenced data
612 were sorted according to barcoding of vectors and analyzed with the CLC Genomics Workbench (CLC bio).
613 Selected RT-PCR products were directly analyzed by Sanger Sequencing and analyzed using the Geneious
614 software.

615 **RNA gel blot analysis**

616 Total RNA (4 µg) was fractioned on 1.2% agarose gels containing 1.2% formaldehyde, blotted and
617 hybridized with radiolabeled RNA probes produced by T7 *in vitro* transcription from PCR products
618 generated with primer combinations described in Suppl. Tab. 2.

619 **Acknowledgement**

620 We wish to acknowledge expert technical support by Irina Passow. This work was supported by
621 grants of the Deutsche Forschungsgemeinschaft to CSL (A02 of TRR175), DL (C05 of TRR175)
622 and TR (FOR 2092). Support of MKL by the IRI Life Sciences and the "Frauenförderung des
623 Instituts für Biologie" of the Humboldt University is gratefully acknowledged. AO was supported

624 by JSPS Overseas Research Fellowships. We thank Deserah Strand and Hannes Ruwe for critical
625 discussion of the data.

626 AO, MKL, and TR performed the research and analyzed the data. JMM performed the
627 computational analysis of the RNA seq data. BL performed the analysis of the CP31A BS
628 consensus. DL, UO and CSL designed the research and analyzed the data. CSL wrote the paper
629 with contributions of all authors to the final version.

630 **References**

- 631 **Bardet AF, He Q, Zeitlinger J, Stark A** (2011) A computational pipeline for comparative ChIP-seq
632 analyses. *Nat Protoc* **7**: 45-61<
- 633 **Barkan A** (2011) Expression of plastid genes: organelle-specific elaborations on a prokaryotic scaffold.
634 *Plant Physiol* **155**: 1520-1532
- 635 **Barkan A, Small I** (2014) Pentatricopeptide repeat proteins in plants. *Annu Rev Plant Biol* **65**: 415-442
- 636 **Benegiamo G, Mure LS, Erikson G, Le HD, Moriggi E, Brown SA, Panda S** (2018) The RNA-Binding
637 Protein NONO Coordinates Hepatic Adaptation to Feeding. *Cell Metab* **27**: 404-418 e407
- 638 **Bentolila S, Oh J, Hanson MR, Bukowski R** (2013) Comprehensive high-resolution analysis of the role
639 of an Arabidopsis gene family in RNA editing. *PLoS Genet* **9**: e1003584
- 640 **Biehl A, Richly E, Noutsos C, Salamini F, Leister D** (2005) Analysis of 101 nuclear transcriptomes
641 reveals 23 distinct regulons and their relationship to metabolism, chromosomal gene distribution and co-
642 ordination of nuclear and plastid gene expression. *Gene* **344**: 33-41
- 643 **Bollenbach TJ, Schuster G, Portnoy V, Stern D** (2007) Processing, degradation, and polyadenylation of
644 chloroplast transcripts. *In* R Bock, ed, *Cell and Molecular Biology of Plastids*, Vol 19. Springer, Berlin,
645 Heidelberg, pp 175-211
- 646 **Castandet B, Hotto AM, Strickler SR, Stern DB** (2016) ChloroSeq, an Optimized Chloroplast RNA-Seq
647 Bioinformatic Pipeline, Reveals Remodeling of the Organellar Transcriptome Under Heat Stress. *G3*
648 (Bethesda) **6**: 2817-2827
- 649 **Chen F, Dong G, Wu L, Wang F, Yang X, Ma X, Wang H, Wu J, Zhang Y, Wang H, Qian Q, Yu Y**
650 (2016) A Nucleus-Encoded Chloroplast Protein YL1 Is Involved in Chloroplast Development and Efficient
651 Biogenesis of Chloroplast ATP Synthase in Rice. *Sci Rep* **6**: 32295
- 652 **Chen HC, Stern DB** (1991) Specific ribonuclease activities in spinach chloroplasts promote mRNA
653 maturation and degradation. *J Biol Chem* **266**: 24205-24211
- 654 **Cho WK, Geimer S, Meurer J** (2009) Cluster analysis and comparison of various chloroplast
655 transcriptomes and genes in *Arabidopsis thaliana*. *DNA Res* **16**: 31-44
- 656 **Choquet Y, Wostrikoff K, Rimbault B, Zito F, Girard-Bascou J, Drapier D, Wollman FA** (2001)
657 Assembly-controlled regulation of chloroplast gene translation. *Biochem Soc Trans* **29**: 421-426
- 658 **Cloutier A, Shkreta L, Toutant J, Durand M, Thibault P, Chabot B** (2018) hnRNP A1/A2 and Sam68
659 collaborate with SRSF10 to control the alternative splicing response to oxaliplatin-mediated DNA damage.
660 *Sci Rep* **8**: 2206
- 661 **Danon A, Mayfield APY** (1991) Light regulated translational activators: identification of chloroplast gene
662 specific mRNA binding proteins. *EMBO J*. **10**: 3993-4001
- 663 **Daros JA, Flores R** (2002) A chloroplast protein binds a viroid RNA in vivo and facilitates its hammerhead-
664 mediated self-cleavage. *EMBO J* **21**: 749-759

- 665 **Deng X-W, Gruissem W** (1987) Control of plastid gene expression during development: the limited role
666 of transcriptional regulation. *Cell* **49**: 379-387
- 667 **Deng XW, Tonkyn JC, Peter GF, Thornber JP, Gruissem W** (1989) Post-transcriptional control of
668 plastid mRNA accumulation during adaptation of chloroplasts to different light quality environments. *Plant*
669 *Cell* **1**: 645-654
- 670 **Eberhard S, Drapier D, Wollman F** (2002) Searching limiting steps in the expression of chloroplast-
671 encoded proteins: relations between gene copy number, transcription, transcript abundance and translation
672 rate in the chloroplast of *Chlamydomonas reinhardtii*. *Plant J.* **31**: 149-160
- 673 **Favory JJ, Kobayshi M, Tanaka K, Peltier G, Kreis M, Valay JG, Lerbs-Mache S** (2005) Specific
674 function of a plastid sigma factor for *ndhF* gene transcription. *Nucleic Acids Res* **33**: 5991-5999
- 675 **Fleischmann TT, Scharff LB, Alkatib S, Hasdorf S, Schottler MA, Bock R** (2011) Nonessential plastid-
676 encoded ribosomal proteins in tobacco: a developmental role for plastid translation and implications for
677 reductive genome evolution. *Plant Cell* **23**: 3137-3155
- 678 **Fukuda A, Shimada M, Nakadai T, Nishimura K, Hisatake K** (2013) Heterogeneous nuclear
679 ribonucleoprotein R cooperates with mediator to facilitate transcription reinitiation on the *c-Fos* gene. *PLoS*
680 *One* **8**: e72496
- 681 **Gerber AP, Herschlag D, Brown PO** (2004) Extensive association of functionally and cytotopically
682 related mRNAs with Puf family RNA-binding proteins in yeast. *PLoS Biol* **2**: E79
- 683 **Germain A, Hotto AM, Barkan A, Stern DB** (2013) RNA processing and decay in plastids. *Wiley*
684 *Interdiscip Rev RNA* **4**: 295-316
- 685 **Germain A, Kim SH, Gutierrez R, Stern DB** (2012) Ribonuclease II preserves chloroplast RNA
686 homeostasis by increasing mRNA decay rates, and cooperates with polynucleotide phosphorylase in 3' end
687 maturation. *Plant J* **72**: 960-971
- 688 **Geuens T, Bouhy D, Timmerman V** (2016) The hnRNP family: insights into their role in health and
689 disease. *Hum Genet* **135**: 851-867
- 690 **Gotic I, Omid S, Fleury-Olela F, Molina N, Naef F, Schibler U** (2016) Temperature regulates splicing
691 efficiency of the cold-inducible RNA-binding protein gene *Cirbp*. *Genes Dev* **30**: 2005-2017
- 692 **Grimmer J, Rodiger A, Hoehenwarter W, Helm S, Baginsky S** (2014) The RNA-binding protein RNP29
693 is an unusual Toc159 transport substrate. *Front Plant Sci* **5**: 258
- 694 **Gu L, Xu T, Lee K, Lee K, Kang H** (2014) A chloroplast-localized DEAD-box RNA helicase *AtRH3* is
695 essential for intron splicing and plays an important role in the growth and stress response in *Arabidopsis*
696 *thaliana*. *Plant Physiol Biochem* **82**: 309-318
- 697 **Hajdukiewicz PT, Allison LA, Maliga P** (1997) The two RNA polymerases encoded by the nuclear and
698 the plastid compartments transcribe distinct groups of genes in tobacco plastids. *EMBO J* **16**: 4041-4048
- 699 **Hashimoto M, Endo T, Peltier G, Tasaka M, Shikanai T** (2003) A nucleus-encoded factor, *CRR2*, is
700 essential for the expression of chloroplast *ndhB* in *Arabidopsis*. *Plant J* **36**: 541-549
- 701 **Hayes R, Kudla J, Schuster G, Gabay L, Maliga P, Gruissem W** (1996) Chloroplast mRNA 3'-end
702 processing by a high molecular weight protein complex is regulated by nuclear encoded RNA binding
703 proteins. *EMBO J* **15**: 1132-1141
- 704 **Hirose T, Sugiura M** (2001) Involvement of a site-specific *trans*-acting factor and a common RNA-binding
705 protein in the editing of chloroplast mRNAs: development of a chloroplast in vitro RNA editing system.
706 *EMBO J* **20**: 1144-1152
- 707 **Hobor F, Dallmann A, Ball NJ, Cicchini C, Battistelli C, Ogradowicz RW, Christodoulou E, Martin**
708 **SR, Castello A, Tripodi M, Taylor IA, Ramos A** (2018) A cryptic RNA-binding domain mediates Syncrip
709 recognition and exosomal partitioning of miRNA targets. *Nat Commun* **9**: 831
- 710 **Hotto AM, Huston ZE, Stern DB** (2010) Overexpression of a natural chloroplast-encoded antisense RNA
711 in tobacco destabilizes 5S rRNA and retards plant growth. *BMC Plant Biol* **10**: 213

- 712 **Hotto AM, Schmitz RJ, Fei Z, Ecker JR, Stern DB** (2011) Unexpected Diversity of Chloroplast
713 Noncoding RNAs as Revealed by Deep Sequencing of the Arabidopsis Transcriptome. *G3* (Bethesda) **1**:
714 559-570
- 715 **Ivanov AG, Rosso D, Savitch LV, Stachula P, Rosembert M, Oquist G, Hurry V, Huner NP** (2012)
716 Implications of alternative electron sinks in increased resistance of PSII and PSI photochemistry to high
717 light stress in cold-acclimated Arabidopsis thaliana. *Photosynth Res* **113**: 191-206
- 718 **Keene JD** (2007) RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet* **8**: 533-543
- 719 **Klaff P, Gruissem W** (1991) Changes in chloroplast mRNA stability during leaf development. *Plant Cell*
720 **3**: 517-529
- 721 **Klein RR** (1991) Regulation of light-induced chloroplast transcription and translation in eight-day- old
722 dark-grown barley seedlings. *Plant Physiol.* **97**: 335-342
- 723 **Kupsch C, Ruwe H, Gusewski S, Tillich M, Small I, Schmitz-Linneweber C** (2012) Arabidopsis
724 Chloroplast RNA Binding Proteins CP31A and CP29A Associate with Large Transcript Pools and Confer
725 Cold Stress Tolerance by Influencing Multiple Chloroplast RNA Processing Steps. *Plant Cell* **10**: 4266-
726 4280
- 727 **Li XG, Duan W, Meng QW, Zou Q, Zhao SJ** (2004) The function of chloroplastic NAD(P)H
728 dehydrogenase in tobacco during chilling stress under low irradiance. *Plant Cell Physiol* **45**: 103-108
- 729 **Li YQ, Sugiura M** (1990) Three distinct ribonucleoproteins from tobacco chloroplasts: each contains a
730 unique amino terminal acidic domain and two ribonucleoprotein consensus motifs. *EMBO J* **9**: 3059-3066
- 731 **Li YQ, Sugiura M** (1991) Nucleic acid-binding specificities of tobacco chloroplast ribonucleoproteins.
732 *Nucleic Acids Res* **19**: 2893-2896
- 733 **Lin BC, Defenbaugh DA, Casey JL** (2010) Multimerization of hepatitis delta antigen is a critical
734 determinant of RNA binding specificity. *J Virol* **84**: 1406-1413
- 735 **Lisitsky I, Liveanu V, Schuster G** (1995) RNA-Binding Characteristics of a Ribonucleoprotein from
736 Spinach Chloroplast. *Plant Physiol.* **107**: 933-941
- 737 **Lisitsky I, Schuster G** (1995) Phosphorylation of a chloroplast RNA-binding protein changes its affinity
738 to RNA. *Nucl. Acids Res.* **23**: 2506-2511
- 739 **Loza-Tavera H, Vargas-Suarez M, Diaz-Mireles E, Torres-Marquez ME, Gonzalez de la Vara LE,**
740 **Moreno-Sanchez R, Gruissem W** (2006) Phosphorylation of the spinach chloroplast 24 kDa RNA-binding
741 protein (24RNP) increases its binding to petD and psbA 3' untranslated regions. *Biochimie* **88**: 1217-1228
- 742 **Lunde BM, Moore C, Varani G** (2007) RNA-binding proteins: modular design for efficient function. *Nat*
743 *Rev Mol Cell Biol* **8**: 479-490
- 744 **Lykke-Andersen J, Wagner E** (2005) Recruitment and activation of mRNA decay enzymes by two ARE-
745 mediated decay activation domains in the proteins TTP and BRF-1. *Genes Dev* **19**: 351-361
- 746 **Malay AD, Watanabe M, Heddle JG, Tame JR** (2011) Crystal structure of unliganded TRAP:
747 implications for dynamic allostery. *Biochem J* **434**: 427-434
- 748 **Manavski N, Schmid LM, Meurer J** (2018) RNA-stabilization factors in chloroplasts of vascular plants.
749 *Essays Biochem* **62**: 51-64
- 750 **Manavski N, Torabi S, Lezhneva L, Arif MA, Frank W, Meurer J** (2015) HIGH CHLOROPHYLL
751 FLUORESCENCE145 Binds to and Stabilizes the psbA 5' UTR via a Newly Defined Repeat Motif in
752 Embryophyta. *Plant Cell* **27**: 2600-2615
- 753 **Mentzen WI, Wurtele ES** (2008) Regulon organization of Arabidopsis. *BMC Plant Biol* **8**: 99
- 754 **Muino JM, Kaufmann K, van Ham RC, Angenent GC, Krajewski P** (2011) ChIP-seq Analysis in R
755 (CSAR): An R package for the statistical detection of protein-bound genomic regions. *Plant Methods* **7**: 11
- 756 **Munteanu A, Mukherjee N, Ohler U** (2018) SSMART: sequence-structure motif identification for RNA-
757 binding proteins. *Bioinformatics* **34**: 3990-3998

- 758 **Nakamura T, Furuhashi Y, Hasegawa K, Hashimoto H, Watanabe K, Obokata J, Sugita M, Sugiura**
759 **M** (2003) Array-based analysis on tobacco plastid transcripts: preparation of a genomic microarray
760 containing all genes and all intergenic regions. *Plant Cell Physiol* **44**: 861-867
- 761 **Nakamura T, Ohta M, Sugiura M, Sugita M** (1999) Chloroplast ribonucleoproteins are associated with
762 both mRNAs and intron-containing precursor tRNAs. *FEBS Lett* **460**: 437-441
- 763 **Nakamura T, Ohta M, Sugiura M, Sugita M** (2001) Chloroplast ribonucleoproteins function as a
764 stabilizing factor of ribosome-free mRNAs in the stroma. *J Biol Chem* **276**: 147-152
- 765 **Nickelsen J, Link G** (1993) The 54 kDa RNA-binding protein from mustard chloroplasts mediates
766 endonucleolytic transcript 3' end formation in vitro. *Plant J* **3**: 537-544
- 767 **Paieri F, Tadini L, Manavski N, Kleine T, Ferrari R, Morandini P, Pesaresi P, Meurer J, Leister D**
768 (2018) The DEAD-box RNA Helicase RH50 Is a 23S-4.5S rRNA Maturation Factor that Functionally
769 Overlaps with the Plastid Signaling Factor GUN1. *Plant Physiol* **176**: 634-648
- 770 **Pfannschmidt T** (2003) Chloroplast redox signals: how photosynthesis controls its own genes. *Trends Plant*
771 *Sci* **8**: 33-41
- 772 **Pulido P, Zagari N, Manavski N, Gawronski P, Matthes A, Scharff LB, Meurer J, Leister D** (2018)
773 CHLOROPLAST RIBOSOME ASSOCIATED Supports Translation under Stress and Interacts with the
774 Ribosomal 30S Subunit. *Plant Physiol* **177**: 1539-1554
- 775 **Quaresma AJ, Oyama S, Jr., Barbosa JA, Kobarg J** (2006) The acidic domain of hnRNPQ (NSAP1) has
776 structural similarity to Barstar and binds to Apobec1. *Biochem Biophys Res Commun* **350**: 288-297
- 777 **Reiland S, Messerli G, Baerenfaller K, Gerrits B, Endler A, Grossmann J, Gruissem W, Baginsky S**
778 (2009) Large-scale Arabidopsis phosphoproteome profiling reveals novel chloroplast kinase substrates and
779 phosphorylation networks. *Plant Physiol* **150**: 889-903
- 780 **Rogalski M, Ruf S, Bock R** (2006) Tobacco plastid ribosomal protein S18 is essential for cell survival.
781 *Nucleic Acids Res* **34**: 4537-4545
- 782 **Rogalski M, Schottler MA, Thiele W, Schulze WX, Bock R** (2008) Rpl33, a nonessential plastid-encoded
783 ribosomal protein in tobacco, is required under cold stress conditions. *Plant Cell* **20**: 2221-2237
- 784 **Rousseau S, Morrice N, Peggie M, Campbell DG, Gaestel M, Cohen P** (2002) Inhibition of SAPK2a/p38
785 prevents hnRNP A0 phosphorylation by MAPKAP-K2 and its interaction with cytokine mRNAs. *EMBO J*
786 **21**: 6505-6514
- 787 **Ruwe H, Kupsch C, Teubner M, Schmitz-Linneweber C** (2011) The RNA-recognition motif in
788 chloroplasts. *J Plant Physiol* **168**: 1361-1371
- 789 **Schonberg A, Bergner E, Helm S, Agne B, Dunschede B, Schunemann D, Schutkowski M, Baginsky**
790 **S** (2014) The peptide microarray "ChloroPhos1.0" identifies new phosphorylation targets of plastid casein
791 kinase II (pCKII) in Arabidopsis thaliana. *PLoS One* **9**: e108344
- 792 **Schuster G, Gruissem W** (1991) Chloroplast mRNA 3' end processing requires a nuclear-encoded RNA-
793 binding protein. *EMBO J*. **10**: 1493-1502
- 794 **Schuster G, Lisitsky I, Klaff P** (1999) Polyadenylation and degradation of mRNA in the chloroplast. *Plant*
795 *Physiol* **120**: 937-944
- 796 **Selinger DW, Saxena RM, Cheung KJ, Church GM, Rosenow C** (2003) Global RNA half-life analysis
797 in Escherichia coli reveals positional patterns of transcript degradation. *Genome Res* **13**: 216-223
- 798 **Sharwood RE, Hotto AM, Bollenbach TJ, Stern DB** (2011) Overaccumulation of the chloroplast
799 antisense RNA AS5 is correlated with decreased abundance of 5S rRNA in vivo and inefficient 5S rRNA
800 maturation in vitro. *RNA* **17**: 230-243
- 801 **Stern DB, Jones H, Gruissem W** (1989) Function of plastid mRNA 3'inverted repeats. RNA stabilization
802 and gene specific protein binding. *J. Biol. Chem.* **264**: 18742-18750
- 803 **Stoppel R, Lezhneva L, Schwenkert S, Torabi S, Felder S, Meierhoff K, Westhoff P, Meurer J** (2011)
804 Recruitment of a ribosomal release factor for light- and stress-dependent regulation of petB transcript
805 stability in Arabidopsis chloroplasts. *Plant Cell* **23**: 2680-2695

- 806 **Teubner M, Fuss J, Kuhn K, Krause K, Schmitz-Linneweber C** (2017) The RNA recognition motif
807 protein CP33A is a global ligand of chloroplast mRNAs and is essential for plastid biogenesis and plant
808 development. *Plant J* **89**: 472-485
- 809 **Tillich M, Hardel SL, Kupsch C, Armbruster U, Delannoy E, Gualberto JM, Lehwark P, Leister D,**
810 **Small ID, Schmitz-Linneweber C** (2009) Chloroplast ribonucleoprotein CP31A is required for editing and
811 stability of specific chloroplast mRNAs. *Proc Natl Acad Sci U S A* **106**: 6002-6007
- 812 **Townley-Tilson WH, Pendergrass SA, Marzluff WF, Whitfield ML** (2006) Genome-wide analysis of
813 mRNAs bound to the histone stem-loop binding protein. *RNA* **12**: 1853-1867
- 814 **Tsunoyama Y, Ishizaki Y, Morikawa K, Kobori M, Nakahira Y, Takeba G, Toyoshima Y, Shiina T**
815 (2004) Blue light-induced transcription of plastid-encoded psbD gene is mediated by a nuclear-encoded
816 transcription initiation factor, AtSig5. *Proc Natl Acad Sci U S A* **101**: 3304-3309
- 817 **Udy DB, Belcher S, Williams-Carrier R, Gualberto JM, Barkan A** (2012) Effects of reduced chloroplast
818 gene copy number on chloroplast gene expression in maize. *Plant Physiol* **160**: 1420-1431
- 819 **Wang BC, Wang HX, Feng JX, Meng DZ, Qu LJ, Zhu YX** (2006) Post-translational modifications, but
820 not transcriptional regulation, of major chloroplast RNA-binding proteins are related to Arabidopsis
821 seedling development. *Proteomics* **6**: 2555-2563
- 822 **Wang S, Bai G, Wang S, Yang L, Yang F, Wang Y, Zhu JK, Hua J** (2016) Chloroplast RNA-Binding
823 Protein RBD1 Promotes Chilling Tolerance through 23S rRNA Processing in Arabidopsis. *PLoS Genet* **12**:
824 e1006027
- 825 **Yamori W, Sakata N, Suzuki Y, Shikanai T, Makino A** (2011) Cyclic electron flow around photosystem
826 I via chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during
827 photosynthesis and plant growth at low temperature in rice. *Plant J* **68**: 966-976
- 828 **Ye L, Sugiura M** (1992) Domains required for nucleic acid binding activities in chloroplast
829 ribonucleoproteins. *Nucleic Acids Res* **20**: 6275-6279
- 830 **Zhang J, Yuan H, Yang Y, Fish T, Lyi SM, Thannhauser TW, Zhang L, Li L** (2016) Plastid ribosomal
831 protein S5 is involved in photosynthesis, plant development, and cold stress tolerance in Arabidopsis. *J Exp*
832 *Bot* **67**: 2731-2744
- 833 **Zoschke R, Bock R** (2018) Chloroplast Translation: Structural and Functional Organization, Operational
834 Control, and Regulation. *Plant Cell* **30**: 745-770