Photosystem II repair and plant immunity: Lessons learned from Arabidopsis mutant lacking the THYLAKOID LUMEN PROTEIN 18.3

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In review

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

SJ, JI, SK, JS and FM contributed to acquisition, analysis and drafting the work, while MS and EMA designed the work and contributed to acquisition, analysis and drafting the work.

Keywords

Arabidopsis thaliana, Defense, Photosynthesis, Photosystem II repair cycle, thylakoid lumen, Trancriptomics

In review

Abstract

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Chloroplasts play an important role in the cellular sensing of abiotic and biotic stress. Signals originating from photosynthetic light reactions, in the form of redox and pH changes, accumulation of reactive oxygen and electrophile species or stromal metabolites are of key importance in chloroplast retrograde signaling. These signals initiate plant acclimation responses to both abiotic and biotic stresses. To reveal the molecular responses activated by rapid fluctuations in growth light intensity, gene expression analysis was performed with Arabidopsis thaliana wild type and the tlp18.3 mutant plants, the latter showing a stunted growth phenotype under fluctuating light conditions (Biochem. J, 406, 415-425). Expression pattern of genes encoding components of the photosynthetic electron transfer chain did not differ between fluctuating and constant light conditions, neither in wild type nor in tlp18.3, and the composition of the thylakoid membrane protein complexes likewise remained unchanged. Nevertheless, the fluctuating light conditions repressed in wild type plants a broad spectrum of genes involved in immune responses, which likely resulted from shade-avoidance responses and their intermixing with hormonal signaling. On the contrary, in the tlp18.3 mutant plants there was an imperfect repression of defense-related transcripts upon growth under fluctuating light, possibly by signals originating from minor malfunction of the PSII repair cycle, which directly or indirectly modulated the transcript abundances of genes related to light perception via phytochromes. Consequently, a strong allocation of resources to defense reactions in the tlp18.3 mutant plants presumably results in the stunted growth phenotype under fluctuating light.

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Ethics statement

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In review

Abstract

Chloroplasts play an important role in the cellular sensing of abiotic and biotic stress. Signals originating from photosynthetic light reactions, in the form of redox and pH changes, accumulation of reactive oxygen and electrophile species or stromal metabolites are of key importance in chloroplast retrograde signaling. These signals initiate plant acclimation responses to both abiotic and biotic stresses. To reveal the molecular responses activated by rapid fluctuations in growth light intensity, gene expression analysis was performed with *Arabidopsis thaliana* wild type and the *tlp18.3* mutant plants, the latter showing a stunted growth phenotype under fluctuating light conditions (Biochem. J, 406, 415-425). Expression pattern of genes encoding components of the photosynthetic electron transfer chain did not differ between fluctuating and constant light conditions, neither in wild type nor in *tlp18.3*, and the composition of the thylakoid membrane protein complexes likewise remained unchanged. Nevertheless, the fluctuating light conditions repressed in wild type plants a broad spectrum of genes involved in immune responses, which likely resulted from shade-avoidance responses and their intermixing with hormonal signaling. On the contrary, in the *tlp18.3* mutant plants there was an imperfect repression of defense-related transcripts upon growth under fluctuating light, possibly by signals originating from minor malfunction of the PSII repair cycle, which directly or indirectly modulated the transcript abundances of genes related to light perception via phytochromes. Consequently, a strong allocation of resources to defense reactions in the *tlp18.3* mutant plants presumably results in the stunted growth phenotype under fluctuating light.

1.Introduction

Photosystem II (PSII), embedded in the thylakoid membranes, catalyzes light-dependent water splitting with concomitant oxygen evolution and electron transfer to the plastoquinone pool. PSII consists of the chloroplast-encoded core subunits D1, D2, CP43 and CP47, as well as numerous other subunits, encoded by both the chloroplast and nuclear genomes. Of these proteins, the nuclear-encoded proteins PsbO, PsbP and PsbQ together with the manganese-

50 calcium cluster form the so called oxygen evolving complex (OEC), located at the lumenal
51 surface of the PSII complex. In higher plants, the functional PSII complex is formed of PSII
52 dimer, to which nuclear-encoded light-harvesting complex (LHC) II proteins, Lhcb1-6, are
53 tightly connected forming PSII-LHCII supercomplexes.
54
55 Photosynthetic water splitting and evolution of one oxygen molecule require four consequent
56 excitations and subsequent charge separations in the reaction center chlorophyll (Chl) P680,
57 thus producing extremely oxidizing, and potentially hazardous reactive oxygen species (ROS),
58 which enhance oxidative damage to PSII as well as to other thylakoid proteins (Krieger-
59 Liszkay et al., 2008; Pospisil, 2009). Despite the existence of detoxification systems for
60 scavenging of ROS, damage to PSII is unavoidable (Aro et al., 1993; Tyystjärvi and Aro, 1996;
61 Takahashi and Badger, 2011). In particular, the PSII core protein D1 is prone to light-induced
62 damage, and thus an efficient repair cycle has evolved for PSII, which includes proteolytic
63 degradation of damaged D1 protein and its replacement with a newly-synthetized D1 copy
64 (reviewed in Baena-Gonzalez and Aro, 2002; Edelman and Mattoo, 2008; Nixon et al., 2010).
65 These processes involve reversible monomerization of the PSII-LHCII supercomplexes
66 (Danielsson et al., 2006), as well as dynamic changes in grana diameter and in lumen volume
67 (Kirchhoff et al., 2011; Herbstova et al., 2012). A vast number of auxiliary proteins, such as
68 kinases, phosphatases, proteases, transporters and chaperones have been shown to assist the
69 PSII repair cycle (reviewed in Mulo et al., 2008; Chi et al., 2012; Nickelsen and Rengstl, 2013;
70 Järvi et al., 2015). One of these, the THYLAKOID LUMEN PROTEIN OF 18.3 kDa
71 (TLP18.3) has been shown to be required for efficient degradation of the damaged D1 protein
72 and dimerization of the PSII complex (Sirpiö et al., 2007). Notably, high light treatment
73 challenging the PSII repair cycle triggered only a moderate damage of PSII in *tlp18.3* (Sirpiö
74 et al., 2007), which suggest that TLP18.3 is not a crucial component of the repair cycle but
75 instead plays a role in fine tuning the repair cycle. Based on structural data, TLP18.3 has been
76 suggested to be an acidic phosphatase, but only low phosphatase activity was measured for
77 TLP18.3 (Wu et al., 2011). Recently, the regulatory role of PSII repair cycle has been extended
78 to consist also the maintenance of photosystem I (PSI) and indeed, insufficient regulation of
79 the PSII repair cycle seems to exert an effect also on the function of PSI (Tikkanen et al., 2014).
80 Moreover, PSII is crucial for plant immunity through production of ROS, which are not only
81 damaging the components of the photosynthetic electron transfer chain, but also act as
82 important retrograde signaling molecules (Rodríguez-Herva et al., 2012; de Torres Zabala et
83 al., 2015). In line with this, a functional connection between PSII repair and regulation of cell
84 death in tobacco leaves infected by tobacco mosaic virus has been established (Seo et al., 2000).
85
86 While the exact role of photosynthetic components in sensing and signaling the pathogen
87 infection is only emerging, a wealth of information has accumulated during the past few years
88 on the consequences of fluctuating light on the activity of the photosynthetic machinery (Grieco
89 et al., 2012; Suorsa et al., 2012; Allahverdiyeva et al., 2013; Kono and Terashima, 2014).
90 Nevertheless, we still lack knowledge on how the rapid fluctuations in growth light intensity
91 affect the acclimation processes at the level of nuclear gene expression, and even less is known
92 about potential cross-talk between light acclimation, PSII repair cycle and disease resistance
93 under fluctuating light. Here, we investigated how the constantly fluctuating growth light
94 intensity modulates the transcript profile of wild type Arabidopsis plants, and how such an
95 acclimation response is further affected by the deficiency of the thylakoid lumen protein
96 TLP18.3. Five-week old plants grown either under constant or fluctuating light conditions for
97 their entire life span were used as material to study the late stage of the acclimation process.

In review

98 **2. Material and methods**
99
100 **2.1. Plant material and growth conditions**
101
102 *Arabidopsis thaliana* (Arabidopsis), ecotype Columbia 0, wild type and *tlp18.3* (GABI-Kat
103 459D12) plants (Sirpiö et al., 2007) were used in all experiments. Plants were grown in 8 h
104 light regime at 23ºC either under a photon flux density of 120 µmol photons m^{-2} s^{-1} or under
105 fluctuating light intensities, in which plants were exposed to 50 µmol photons m^{-2} s^{-1} for five
106 minutes and subsequently to high light of 500 µmol photons m^{-2} s^{-1} for one minute (Tikkanen
107 et al., 2010), the cycles being repeated during the entire photoperiod. Osram HQI-BT 400 W/D
108 Metal Halide lamps with spectral power distribution from 350 to 800 nm were used as a light
109 source. Five-week old plants were used for all experiments.
110
111 **2.2. Gene expression analyses**
112
113 Microarray analyses of wild type and *tlp18.3* plants were performed essentially as in (Konert
114 et al., 2015). In short, leaf material was harvested four hours after the onset of light period in
115 order to be sure that the plants were in photosynthetically active state and that the PSII repair
116 cycle was properly ongoing and immediately frozen in liquid nitrogen. RNA was isolated
117 using an Agilent Plant RNA isolation mini kit according to manufacturer`s instructions. Cy-3
118 labelled RNA samples were hybridized to Agilent Arabidopsis Gene Expression Microarrays,
119 4x44K (Design ID 021169) and scanned with Agilent Technologies Scanner G2565CA with
120 a profile AgilentHD _GX_1Color. Numeric data were produced with Agilent Feature
121 Extraction program, version 10.7.3.
122
123 Pre-processing of microarrays was performed using Limma's normexp background correction
124 method to avoid negative or zero corrected intensities, followed by between-array
125 normalization using the quantile method to make all array distributions to have the same
126 empirical distribution. Control probes were filtered and then within-array replicate spots were
127 replaced with their average. Pair-wise comparisons between groups were conducted using the
128 Linear Models for Microarray Data (Limma) package Version 3.26.1 from Bioconductor
129 (http://www. bioconductor.org/). The false discovery rate of differentially expressed gene list
130 for treatment/control and between-treatment comparisons was based on the Benjamini and
131 Hochberg (BH) procedure. Genes with a score below an adjusted p-value threshold of 0.01 and
132 which also showed a minimum of twofold change in expression between conditions or
133 genotype were selected as significantly differentially expressed genes. Gene annotations were
134 obtained from the Arabidopsis Information Resource (TAIR; http://www.arabidopsis.org/).
135 Functional clustering and analysis was performed using the Database for Annotation,
136 Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/home.jsp)
137 version 6.7. Differentially expressed genes were compared against gene sets collected from
138 various sources such as publications using the Plant GeneSet Enrichment Analysis Toolkit
139 (PlantGSEA) (http://structuralbiology.cau.edu.cn/PlantGSEA/).
140
141 To detect co-regulated gene sets, a cluster analysis of the differentially expressed genes was
142 carried out using data from (Georgii et al., 2012), consisting of microarray data downloaded
143 from NASCArrays (http://affymetrix.arabidopsis.info/narrays/experimentbrowse.pl),
144 ArrayExpress (http://www.ebi.ac.uk/microarrayas/ae/), Gene Expression Omnibus
145 (http://www.ncbi.nlm.nih.gov/geo/), and The Integrated Microarray Database System
146 (http://ausubellab.mgh.harvard.edu/imds). Arrays were normalised with Robust Multi-array
147 Average (RMA), and log2 ratio of the mean of treatment and control expressions across

In review

148 biological replicates was computed. Bayesian Hierarchical Clustering was carried out using R
149 package BHC (Cooke et al., 2011) using log2 fold change ±1 as discretization threshold. Gene
150 set enrichment analysis of the co-regulated gene clusters was carried out using StringDB
151 (http://string-db.org/) (Szklarczyk et al., 2015).
152
153 **2.3. Isolation of the thylakoid membrane and separation of protein complexes**
154
155 Thylakoid isolation and the blue native (BN)-PAGE were performed essentially as described
156 in Järvi et al., 2011. Sodium fluoride was included in thylakoid isolation buffers for samples
157 intended to BN-PAGE, whilst excluded from thylakoids used for spectroscopy analyses (see
158 below). For BN-PAGE, the thylakoid membrane (4 µg Chl) was resuspended into ice-cold
159 25BTH20G buffer (25 mM BisTris/HCl (pH 7.0), 20% (w/v) glycerol and 0.25 mg ml−1
160 Pefabloc) to a Chl concentration of 1.0 mg ml−1. An equal volume of 2.0% (w/v) detergent (n-
161 Dodecyl β-D-maltoside, Sigma) solution (diluted in 25BTH20G) was added to the sample and
162 thylakoid membrane was solubilized in darkness for 5 min on ice. Traces of insoluble material
163 were removed by centrifugation at 18,000 g at 4ºC for 20 min. Prior to loading, the samples
164 were supplemented with a one-tenth volume of Serva Blue G buffer (100 mM BisTris/HCl (pH
165 7.0), 0.5 M ACA, 30% (w/v) sucrose and 50 mg ml−1 Serva Blue G).
166
167 **2.4. Spectroscopic quantitation of PSI and PSII**
168
169 Room temperature continuous wave electron paramagnetic resonance (EPR) spectroscopy was
170 performed essentially as described in Danielsson et al., 2004 and Suorsa et al., 2015.
171 Measurements were performed at the Chl concentration of 2 mg ml^{-1}.
172
173 **2.5. Photosynthetic activity measurements**
174
175 The Dual-PAM-100 (Walz, http://www.walz.com/) was used for the measurement of PSII
176 quantum yields. Quantum yields of PSII (F_V/F_M, F_{II}, F_{NPQ} and F_{NO}) were determined from
177 leaves dark adapted for 30 min before the measurements. Saturating pulse (800 ms, 6000 µmol
178 photons $m^{-2}s^{-1}$) was applied to determine the maximal fluorescence. Measurements were done
179 in actinic red light of 50, 120 or 500 µmol photons $m^{-2}s^{-1}$.
180
181 **2.6. Statistical Analyses**
182
183 The numerical data were subjected to statistical analysis by Student's t test with statistical
184 significance at the p values < 0.05.
185
186 **3. Results**
187
188 **3.1 Fluctuating growth light only slightly modified the photosynthetic light reactions**
189
190 Accumulating evidence during recent years has demonstrated that sudden, abrupt changes in
191 light intensity threaten particularly PSI, not PSII (Grieco et al., 2012; Suorsa et al., 2012;
192 Allahverdiyeva et al., 2013; Kono and Terashima, 2014). Indeed, quantitation of the functional
193 PSI/PSII ratios from wild type plants with EPR revealed a PSI/PSII ratio of 1.12 for plants
194 grown under constant light conditions (Suorsa et al., 2015), whereas plants grown under
195 fluctuating light conditions exhibited a clearly lower value, 1.02.
196

In review

197 The *tlp18.3* plants showed a distinct stunted phenotype upon growth under fluctuating white
198 light and the dry weight of the *tlp18.3* plants (12.2±5.7 mg) was markedly decreased as
199 compared to wild type (29.9±4.7 mg) (n=6). This observation prompted us to monitor whether
200 the oligomeric structure of the thylakoid membrane protein complexes of wild type and *tlp18.3*
201 plants grown either under constant or fluctuating light conditions is altered. Malfunction of the
202 PSII repair cycle is often evidenced by a low amount of the most active PSII complexes, the
203 PSII-LHCII complexes, accompanied by a high amount of PSII monomers, which are under
204 the repair cycle (Danielsson et al., 2006). To that end, the BN-PAGE separation of thylakoid
205 protein complexes according to their molecular mass was applied. In line with earlier results
206 (Sirpiö et al., 2007), the *tlp18.3* thylakoids accumulated slightly less of the PSII-LHCII
207 complexes under constant light (Figure 1). Similar result was also evident under fluctuating
208 light intensities, the amount of PSII-LHCII being somewhat lower in *tlp18.3* as compared to
209 wild type. However, no significant differences were observed in heterogeneity of the
210 photosynthetic protein complexes, when WT and mutant plants grown either under constant or
211 fluctuating light were compared (Figure 1). Previous report has shown that the maximal PSII
212 quantum yield is not changed in *tlp18.3* grown under constant growth light conditions as
213 compared to wild type (Sirpiö et al., 2007). In line with this, the maximum quantum yield and
214 effective quantum yields of PSII remained rather similar, when the *tlp18.3* and wild type plants
215 grown their entire life span under fluctuating light were compared (Table 1). Indeed, the PSII
216 activity was only slightly down-regulated in *tlp18.3* as compared to wild type. Thus, the growth
217 defect shown by the *tlp18.3* plants under fluctuating light intensities does not originate from
218 the diminished pool of active PSII complexes.
219
220 **3.2 Consequences of fluctuating growth light intensity on gene expression**
221
222 To further characterize plant acclimation to fluctuating light, we performed transcript profiling
223 of the wild type and *tlp18.3* plants grown under constant and fluctuating light intensities and
224 compared the four datasets: (*i*) wild type plants grown under fluctuating versus constant growth
225 light, (*ii*) *tlp18.3* plants grown under fluctuating versus constant growth light, (*iii*) *tlp18.3*
226 versus wild type plants grown under fluctuating light and (*iv*) *tlp18.3* versus wild type plants
227 grown under constant light. Gene enrichment analysis and functional annotation clustering of
228 differentially expressed genes were performed using the Database for Annotation,
229 Visualization and Integrated Discovery (DAVID) bioinformatic resource (the cutoff was set to
230 logFC > 1 and the adjusted p-value threshold to a minimum of 0.01).
231
232 Wild type plants grown under fluctuating light showed significantly different transcript
233 abundance for 406 genes as compared to wild type grown under constant light, whereas in
234 *tlp18.3* mutant, 321 genes responded differentially to fluctuating light as compared to growth
235 light (Figure 2). When the transcript abundances between the genotypes was compared, 237
236 genes showed significantly different transcript abundance in *tlp18.3* compared to wild type
237 when grown under fluctuating light conditions, whereas under constant growth light the
238 number of differentially expressed genes between wild type and *tlp18.3* was 102 (Figure 2).
239 Thus, it can be concluded that the growth light condition altered the number of differentially
240 regulated genes more pronouncedly than the genotype. Moreover, the wild type plants showed
241 more profound changes at their gene expression level as a response to fluctuating growth light
242 than the *tlp18.3* plants.
243
244 **3.2.1 Plants grown under fluctuating light did not show differential abundance of**
245 **photosynthesis related transcripts**
246

In review

247 Examination of differentially expressed genes revealed no photosynthesis-related gene
248 ontologies in any of the four datasets analyzed (Table 2, 3). Indeed, no gene ontologies related
249 to photosynthetic light reactions, Calvin-Benson-Bassham cycle or biosynthesis of
250 photosynthetic pigments was observed in the gene enrichment analysis. Presumably, regulation
251 of the photosynthetic machinery at transcriptional level does not play an important role during
252 acclimation to relatively mild light intensity fluctuations, being designed such that the total
253 amount of photons hitting the leaf remained nearly unchanged during the 8 h light period, when
254 constant and fluctuating light conditions were compared. Likewise, deficient function of the
255 TLP18.3 protein had only minor effects on transcript abundance of various photosynthesis
256 genes.
257
258 **3.2.2 Fluctuating light conditions induced transcriptional adjustments in immunity**
259 **related genes both in wild type and *tlp18.3* plants**
260
261 Bioinformatic analysis revealed that the majority of differentially expressed gene ontologies
262 between plants grown under fluctuating and constant light conditions were linked to biotic or
263 abiotic stress responses (Table 2A, B). In wild type, growth under fluctuating light resulted in
264 decreased transcript abundance within numerous gene ontologies related to plant immunity, as
265 compared to wild type grown under constant light (Table 2A). These genes included mitogen-
266 activated protein kinases (MAPKs) involved in early defense signaling, Toll / Interleukin-1
267 receptor-nucleotide binding site (TIR-NBS) class resistance (R) proteins mediating effector-
268 triggered immunity (ETI) as well as pathogen related defense proteins, such as plant defensins
269 (Supplementary Table 1). In contrast, the *tlp18.3* mutants showed both decreased and increased
270 transcript abundance within gene ontologies related to plant immunity, when fluctuating and
271 constant light grown plants were compared to each other (Table 2B). For example, ankyrin
272 *BDA1* (*AT5G54610*), which is induced by salicylic acid (SA) and is involved in innate
273 immunity (Blanco et al., 2005; Yang et al., 2012) showed cumulative repression in the
274 transcript abundance in response to fluctuating light and deficient function of the TLP18.3
275 protein. In contrast, plant defensin *PDF2.1* (*AT2G02120*) and defensin-like (*AT2G43535*)
276 genes, which are activated in response to fungal infection, were induced in *tlp18.3* under
277 fluctuating light.
278
279 With respect to abiotic stress, gene ontologies "response to UV" and "response to light
280 stimulus" were enriched in the transcriptome of *tlp18.3*, when plants grown under fluctuating
281 and constant light were compared (Table 2B). For example, increased abundance of transcripts
282 for *EARLY LIGHT-INDUCED PROTEIN2* (*ELIP2*; *AT4G14690*), which modulates Chl
283 biosynthesis to prevent photo-oxidative stress (Tzvetkova-Chevolleau et al., 2007; Hayami et
284 al., 2015), was observed in the fluctuating light grown *tlp18.3* (Supplementary Table 1). In
285 contrast, no gene ontologies related to light perception showed differential expression in the
286 wild type plants as a response to fluctuating light (Table 2A). Decreased transcript abundance
287 of gene ontologies associated with lipid localization and lipid transport were also observed as
288 response to fluctuating light specifically in *tlp18.3*. Several genes encoding lipid-transfer
289 proteins such as *LIPID TRANSFER PROTEIN 3* (*LTP3*; *AT5G59320*), which mediates freezing
290 and drought stress in Arabidopsis (Guo et al., 2013), were down-regulated in the *tlp18.3*
291 mutants, when plants were grown under fluctuating light as compared to constant growth light
292 (Supplementary Table 1).
293
294 When fluctuating-light-grown *tlp18.3* and wild type plants were compared to each other,
295 increased transcript abundance of genes related to the defense mechanisms in *tlp18.3* was again
296 the most prominent result (Table 3A). Enrichment analysis and functional annotation clustering

In review

of the differentially expressed gene ontologies in *tlp18.3* and wild type plants also revealed that several gene clusters related to abiotic stresses were differentially expressed in *tlp18.3* as compared to wild type under fluctuating light. Decreased transcript abundance of gene ontologies “response to light stimulus” and “response to oxidative stress” was observed in *tlp18.3* as compared to wild type. Closer look at the genes among these categories pinpointed that the transcript abundance for cytosolic and chloroplastic *COPPER/ZINC SUPEROXIDE DISMUTASES 1* (*AT1G08830*) and 2 (*AT2G28190*), respectively, was repressed in *tlp18.3* as compared to wild type under fluctuating light conditions (Supplementary Table 1).

Finally, when constant-light-grown *tlp18.3* and wild type plants were compared, only a few gene ontologies related to biotic or abiotic stresses were identified (Table 3B). This result is consistent with the postulated role of TLP18.3 specifically during the dynamic light acclimation process, as evidenced by the distinct growth phenotype of the mutant plants under fluctuating light.

3.2.3 Adjustments in immunity related genes under fluctuating light are linked to plant hormones

Plant acclimation to various stresses, including the light stress, is regulated by signaling cascades, which include plant hormones as central components (Karpiński et al., 2013; Müller and Munne-Bosch, 2015). In wild type plants, growth under fluctuating light as compared to constant light resulted in reduced transcript abundance of several genes related to SA signaling cascades (Table 2A). For example, expression of a gene encoding SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1 (SARD1; AT1G73805), a key regulator of ISOCHORISMATE SYNTHASE 1, a rate-limiting enzyme in pathogen-induced SA biosynthesis (Zhang et al., 2010), was shown to be down-regulated in wild type plants grown under fluctuating light. Also expression of a gene encoding BENZOIC ACID/SA CARBOXYL METHYLTRANSFERASE 1 (BSMT1; AT3G11480), which synthetizes methyl salicylate (a mobile signal molecule for plant systemic acquired resistance) from SA (Park et al., 2007), was down-regulated in fluctuating light. In line with these results, *WALL-ASSOCIATED KINASE 2* (*WAK2; AT1G21270*) and *L-TYPE LECTIN RECEPTOR KINASE IV.1* (*LecRK-IV.1; AT2G37710*), which are both induced by SA, showed reduced transcript abundance in wild type plants as response to fluctuating light (He et al., 1999; Blanco et al., 2005) (Supplementary Table 1). Also the *tlp18.3* plants grown under fluctuating light showed decreased abundance of gene transcripts related to SA signaling as compared to plants grown under constant light (Table 2B). However, the number of repressed genes was lower in *tlp18.3* as compared to wild type and no differential expression of SARD1 or BSMT1 were observed in *tlp18.3* as response to fluctuating light (Table 2, Supplementary Table 1). Decreased amount of transcripts related to SA signaling was also evident when *tlp18.3* plants grown under constant light were compared to wild type (Table 3B), while no difference in SA signaling was observed between *tlp18.3* and wild type plants grown under fluctuating light (Table 3A). To that end, the fluctuating light condition and to a lesser extent deficient function of the TLP18.3 protein repressed the SA responsive genes.

Similarly, ethylene (ET) and jasmonate (JA) related defense pathways showed reduced transcript abundance in wild type plants grown under fluctuating light as compared to constant light (Table 2A), while in the *tlp18.3* mutant no difference was observed in ET/JA defense reactions between the light conditions (Table 2B). It seems that the repression of ET/JA responsive gene expression under fluctuating light is blocked in the *tlp18.3* mutants, which

In review

346 became apparent when ET/JA responses between fluctuating light grown *tlp18.3* and wild type
347 plants were compared (Table 3A).
348
349 The most prominent alteration in the gene ontology level, when the transcript abundances of
350 constant light grown *tlp18.3* and wild type plants were compared, was an increase in transcripts
351 of six genes encoding CC-type glutaredoxins (*ROXY 5, ROXY 11-15*) and two of those, *ROXY*
352 *5* and *ROXY 13*, were up-regulated in *tlp18.3* as compared to wild type also under fluctuating
353 light (Table 3 and 4, Supplementary Table 1). As CC-type glutaredoxins have been suggested
354 to be capable of suppressing the JA and ET -induced defense genes (Zander et al., 2012), a
355 causal connection might exist between expression of JA and ET -responsive genes and
356 differential expression of *ROXY* genes. It can be concluded that alteration in the gene
357 expression patterns of SA, ET and JA signaling are taking place during plant acclimation to
358 fluctuating light and that these alterations are strongly affected by the deficient function of the
359 TLP18.3 protein.
360
361 **3.2.4 Phytochrome-mediated light signaling is likely to be altered in *tlp18.3***
362
363 Next, we wanted to further explore which Arabidopsis genes showed a differential expression
364 pattern in the *tlp18.3* plants both under constant and fluctuating light conditions. In addition to
365 ROXY5 and ROXY13 located in the endomembrane system, genes encoding cold (DELTA-9
366 DESATURASE 1) and drought-repressed (DROUGHT-REPRESSED 4) proteins, acid
367 phosphatase (AT4G29270), and two putative membrane transporters (AT5G62730,
368 AT2G16660) showed differential expression in *tlp18.3*. Interestingly, two genes encoding
369 bHLH class phytochrome A-signaling components, LONG HYPOCOTYL IN FAR-RED 1
370 (HFR1; AT1G02340) and PHYTOCHROME INTERACTING FACTOR 3-LIKE 1 (PIL1;
371 AT2G46970) (Fairchild et al., 2000; Salter et al., 2003), showed decreased transcript
372 abundance in *tlp18.3* as compared to wild type (Table 4). Instead, expression of gene encoding
373 EARLY FLOWERING 4 (ELF4; AT2G40080), a phytochrome-controlled regulator of
374 circadian clock was induced in *tlp18.3* mutant as compared to wild type. Taking together, the
375 deficient function of TLP18.3 is likely to change the phytochrome-mediated light signaling
376 both under constant and fluctuating light intensities.
377
378 **3.2.5. Decreased transcript abundance of dark-induced genes suggest that nitrogen to**
379 **carbon and/or phosphorus to carbon ratios might be altered in *tlp18.3* under fluctuating**
380 **light**
381
382 Nutrient availability plays an important regulatory role in growth and development of plants,
383 but also cross-talk between nutrient availability and disease resistance exist (Huber, 1980;
384 Hermans et al., 2006). Interestingly, *GLUTAMINE-DEPENDENT ASPARAGINE SYNTHASE*
385 *1 / DARK-INDUCED 6* (*ASN1/DIN6; AT3G47340)* and *DARK-INDUCED 1 / SENESCENCE*
386 *1* (*DIN1/SEN1; AT4G35770*) genes showed strong down-regulation in fluctuating light grown
387 *tlp18.3* as compared to either fluctuating light grown wild type or constant light grown *tlp18.3*
388 (Supplementary Table 1). ASN1/DIN6 regulates the flow of nitrogen into asparagine, which
389 acts as nitrogen storage and transport compound in darkness and its gene expression is
390 regulated by nitrogen to carbon ratio (Lam et al., 1994). DIN1/SEN1, which has been suggested
391 to contribute to enhanced susceptibility to plant viruses, is induced by phosphate starvation and
392 repressed by sugars (Fernández-Calvino et al., 2015). The differential expression of
393 *ASN1/DIN6* and *DIN1/SEN1* is linked to deficient function of TLP18.3 under fluctuating light
394 but the exact mechanism behind transcriptional repression of these two genes remains to be
395 verified.

In review

3.4.6. Cluster analysis of genes whose expression in fluctuating light requires functionality of TLP18.3

Finally, to shed light on gene expression changes that depend on the functionality of TLP18.3 under fluctuating light, the expression profiles of genes differentially expressed in wild type but not in *tlp18.3* upon growth under fluctuating light were clustered using publicly available datasets (Figure 3). These wild type specific genes grouped into 13 co-expression clusters, which were further analyzed for enrichment of gene ontology categories (Supplementary Table 2). Clusters 3-13 contained genes with increased transcript abundance in different abiotic stress conditions including salinity and drought as well as methyl viologen (Paraquat; PQ) and the SA analog BTH (Figure 3). Under UV-B stress, in contrast, the expression of these genes was generally down-regulated (Figure 3). This pattern of gene expression was particularly evident within the gene clusters 5, 6 and 9, which showed significant enrichment of gene ontology categories related to plant immunity, such as “response to chitin”, “ethylene-activated signaling pathway” or “systemic acquired resistance” (Supplementary Table 2). In wild type the genes belonging to clusters 5, 6 and 9 were generally down-regulated, showing a similar pattern to UV-B stress.

4 Discussion

During the past few years evidence has been accumulated concerning the role of photosynthesis in plant immunity. Here, we have provided new insights into the linkage between light acclimation and plant immunity at the level of gene expression as well as addressed the role of TLP18.3 protein within these processes. Chloroplasts, in addition to their main task in conversion of solar energy into chemical energy, participate in a number of other reactions like biosynthesis of amino acids, hormones and secondary metabolites as well as cellular sensing of abiotic and biotic stress signals. Indeed, signals originating from photosynthetic light reaction, such as redox state of the electron transfer chain, accumulation of stromal metabolites as well as ROS and reactive electrophilic species are key components of chloroplast retrograde signaling (Fey et al., 2005; Piippo et al., 2006; Queval and Foyer, 2012; Szechyńska-Hebda and Karpiński, 2013; Bobik and Burch-Smith, 2015; Gollan et al., 2015). These signals respond rapidly to changes in perception of light by the two photosystems.

Here, we focused on plants grown under either constant or fluctuating light conditions for their entire life span in order to unravel how the rapid fluctuations in the growth light intensity affect the acclimation processes at the level of nuclear gene expression. In short, neither photosynthesis related genes nor the photosynthetic protein complexes showed significant alterations as a response to fluctuating light (Figure 1, Table 1, 2, 3). Instead, EPR spectroscopy revealed that the relative amount of functional PSI complexes was lowered in fluctuating light as compared to plants grown under constant light. Most prominently, in wild type plants fluctuations in growth light suppressed the expression of genes related to defense reactions (Table 2A). Despite the high light peaks of one minute, the low-light phase is dominant in our fluctuating light setup. Hence, it is highly likely that decreased transcript abundance of the defense genes in wild type Arabidopsis under fluctuating light is linked to shade-avoidance and is mediated by plant hormones (Vandenbussche et al., 2005; Wit et al., 2013). The experimental setup, in which the gene expression was studied from plants grown their entire life span either under constant or fluctuating light did not allow us to identify specific immune responses activated by the fluctuations in the growth light intensity. Instead, this experimental setup shed

In review

445 light into late stages of the plant acclimation process, in which a vast number of defense
446 pathways were affected.
447
448 Contrary to wild type, in *tlp18.3* the alterations in the overall gene expression pattern, as a
449 response to fluctuating light, were less evident and indeed, the *tlp18.3* plants were less capable
450 of turning off the gene expression related to plant immunity under fluctuating light conditions
451 (Table 2B, Figure 2, Figure 3). It is known that the photoreceptor-derived signals activate the
452 shade-avoidance responses and reduce the defense reactions against pathogens and pests to
453 save resources for the growth of the plant (Ballare, 2014). Interestingly, the gene expression of
454 two components of phytochrome-mediated light signaling, *HFR1* and *PIL1*, was shown to be
455 altered in *tlp18.3* (Table 4). *HFR1* and *PIL1* genes are involved in transcriptional regulation
456 pathways downstream of phytochromes, which integrate light and hormonal signals and play a
457 role in shade-avoidance responses (Jiao et al., 2007). Of these, HFR1 also contributes to the
458 crosstalk between the light signaling and plant innate immunity (Tan et al., 2015). Based on
459 these results, it is evident that the functionality of TLP18.3 protein modifies the light perception
460 and/or signaling network, and possibly also the signaling related to nutrient availability
461 (Supplementary Table 1). Allocation of resources to defense reactions in *tlp18.3* is likely
462 associated with the lower biomass of mutant plants as compared to wild type plants under low-
463 light dominant fluctuating light. It should be noted that the *tlp18.3* plants also had lower
464 biomass as compared to wild type when grown under high-light dominant fluctuating light with
465 longer, one hour light pulses (Sirpiö et al., 2007). It remains to be studied whether the growth
466 phenotype of *tlp18.3* under high-light dominant fluctuating light originates directly from the
467 diminished pool of active PSII complexes. Indeed, duration, frequency and intensity of
468 fluctuating light regimes have been shown to affect the acclimation responses in Arabidopsis
469 (Alter et al., 2012). To that end, it would be interesting to compare how the gene expression
470 patterns of low-light and high-light dominant fluctuating light conditions differ from each
471 other.
472
473 Defective degradation of the D1 core protein of PSII in *tlp18.3* is a promising system for search
474 of chloroplast-derived retrograde signals, which affect to gene expression related to plant
475 immunity. In line with this, low amount of the D1 degrading protease FtsH has been earlier
476 observed to accelerate the hypersensitive reaction in tobacco (Seo et al., 2000). Recently, a link
477 between PsbS-mediated photoprotection and pathogen resistance has also been shown to exist
478 (Göhre et al., 2012; Johansson Jänkänpää et al., 2013). Further, as the PSII repair cycle and
479 maintenance of PSI are interconnected (Tikkanen et al., 2014), also PSI and/or PSI electron
480 acceptors might act as a source of retrograde signaling components under fluctuating light. It
481 should be noted that the pool of active PSII was not changed in *tlp18.3* as compared to wild
482 type under low-light dominant fluctuating light (Table 1) and thus the effect might be indirect.
483 We postulate that the compensation mechanisms activated in the *tlp18.3* are likely to alter the
484 chloroplast-derived retrograde signals. Taken together, our results demonstrate that light
485 acclimation and plant immunity are interconnected and the proper repair cycle of PSII plays a
486 key role in the process.
487
488 **Author Contributors**
489
490 SJ, JI, SK, JS and FM contributed to acquisition, analysis and drafting the work, while MS
491 and EMA designed the work and contributed to acquisition, analysis and drafting the work.
492
493 **Conflict of Interest**
494

In review

495 The authors declare that the research was conducted in the absence of any commercial or
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In review

Tables and Figures

Table 1. PSII quantum yields of wild type and *tlp18.3* plants grown under fluctuating light.

Photosynthetic parameter	Wild type	*tlp18.3*
Effective PSII quantum yield, F_{II}		
50 µmol photons $m^{-2}s^{-1}$	0.50±0.02	0.47±0.04
120 µmol photons $m^{-2}s^{-1}$	0.28±0.06	0.26±0.03
500 µmol photons $m^{-2}s^{-1}$	0.04±0.01	0.03±0.01
Non-photochemical energy dissipation, F_{NPQ}		
50 µmol photons $m^{-2}s^{-1}$	0.13±0.02	0.15±0.04
120 µmol photons $m^{-2}s^{-1}$	0.48±0.07	0.47±0.03
500 µmol photons $m^{-2}s^{-1}$	0.68±0.01	0.66±0.01*
Yield of non-regulated non-photochemical energy lost, F_{NO}		
50 µmol photons $m^{-2}s^{-1}$	0.37±0.01	0.38±0.03
120 µmol photons $m^{-2}s^{-1}$	0.24±0.01	0.27±0.00*
500 µmol photons $m^{-2}s^{-1}$	0.28±0.00	0.31±0.02
Maximal quantum yield of PSII, F_V/F_M	0.78±0.01	0.76±0.02*

The values are the means ± SD, n = 4-5, except for F_V/F_M n=12. Statistically significant differences comparing the mutant plants to that of the corresponding wild type are marked with asterix (*). See text for details.

Table 2. Classification of significantly differently expressed genes base on gene enrichment analysis of plants grown either under fluctuating light (FL) or constant growth light (CL). (A) Gene enrichment analysis of wild type plants grown either under fluctuating or constant light. (B) Gene enrichment analysis of *tlp18.3* plants grown either under fluctuating or constant light. Categories, which co-exist in A. and B., are italicized.

(A) Wild type FL vs wild type CL

Term		Count	*P*-Value
Increased transcript abundance			
GOTERM_MF_FAT	GO:0005507 copper ion binding	5	0.0055
GOTERM_CC_FAT	GO:0031225 anchored to membrane	6	0.0076
Decreased transcript abundance			
GOTERM_BP_FAT	GO:0006952 defense response	43	3.26E-14
GOTERM_MF_FAT	GO:0004672 *protein kinase activity*	40	7.91E-12
GOTERM_BP_FAT	GO:0010033 *response to organic substance*	42	1.18E-11
GOTERM_BP_FAT	GO:0006468 *protein amino acid phosphorylation*	39	4.18E-11
GOTERM_BP_FAT	GO:0009751 *response to salicylic acid stimulus*	16	8.69E-11
GOTERM_BP_FAT	GO:0006955 immune response	20	4.62E-10
GOTERM_BP_FAT	GO:0016310 *phosphorylation*	39	7.75E-10
GOTERM_BP_FAT	GO:0010200 response to chitin	14	1.24E-09
GOTERM_MF_FAT	GO:0004674 *protein serine/threonine kinase activity*	33	4.75E-09
GOTERM_BP_FAT	GO:0006796 *phosphate metabolic process*	39	6.76E-09
GOTERM_BP_FAT	GO:0006793 *phosphorus metabolic process*	39	6.91E-09
GOTERM_BP_FAT	GO:0045087 innate immune response	18	8.31E-09

In review

GOTERM_BP_FAT	GO:0009617 *response to bacterium*	17	1.02E-08
GOTERM_BP_FAT	GO:0009611 *response to wounding*	13	7.11E-08
GOTERM_BP_FAT	GO:0042742 *defense response to bacterium*	14	1.10E-07
GOTERM_BP_FAT	GO:0009743 response to carbohydrate stimulus	14	2.74E-07
GOTERM_MF_FAT	GO:0032559 *adenyl ribonucleotide binding*	49	1.85E-06
GOTERM_MF_FAT	GO:0030554 *adenyl nucleotide binding*	50	5.06E-06
GOTERM_MF_FAT	GO:0001883 *purine nucleoside binding*	50	5.06E-06
GOTERM_MF_FAT	GO:0001882 *nucleoside binding*	50	5.54E-06
GOTERM_MF_FAT	GO:0005524 ATP binding	47	7.74E-06
GOTERM_BP_FAT	GO:0009814 defense response, incompatible interaction	9	9.97E-06
GOTERM_BP_FAT	GO:0009873 ethylene mediated signaling pathway	11	1.73E-05
GOTERM_BP_FAT	GO:0009723 response to ethylene stimulus	13	2.44E-05
GOTERM_MF_FAT	GO:0032555 purine ribonucleotide binding	49	3.11E-05
GOTERM_MF_FAT	GO:0032553 ribonucleotide binding	49	3.11E-05
GOTERM_BP_FAT	GO:0009753 response to jasmonic acid stimulus	10	5.35E-05
GOTERM_BP_FAT	GO:0009719 response to endogenous stimulus	26	5.38E-05
GOTERM_MF_FAT	GO:0017076 purine nucleotide binding	50	7.16E-05
GOTERM_BP_FAT	GO:0000160 two-component signal transduction system	11	1.41E-04
GOTERM_MF_FAT	GO:0005529 sugar binding	8	3.13E-04
GOTERM_MF_FAT	GO:0000166 nucleotide binding	52	0.0016
GOTERM_MF_FAT	GO:0004713 protein tyrosine kinase activity	11	0.0016
GOTERM_BP_FAT	GO:0009725 response to hormone stimulus	21	0.0021
GOTERM_BP_FAT	GO:0009816 defense response to bacterium	4	0.0028
GOTERM_BP_FAT	GO:0009620 *response to fungus*	13	0.0031
GOTERM_MF_FAT	GO:0005509 calcium ion binding	12	0.0034
GOTERM_BP_FAT	GO:0009863 salicylic acid mediated signaling pathway	4	0.0038
GOTERM_BP_FAT	GO:0006979 response to oxidative stress	10	0.0043
GOTERM_BP_FAT	GO:0043900 regulation of multi-organism process	3	0.0050
GOTERM_CC_FAT	GO:0005618 cell wall	15	0.0057
GOTERM_BP_FAT	GO:0009867 jasmonic acid mediated signaling pathway	4	0.0065
GOTERM_CC_FAT	GO:0030312 external encapsulating structure	15	0.0065
GOTERM_BP_FAT	GO:0016265 death	9	0.0068
GOTERM_BP_FAT	GO:0008219 cell death	9	0.0068
GOTERM_CC_FAT	GO:0012505 endomembrane system	59	0.0073
GOTERM_MF_FAT	GO:0030246 carbohydrate binding	8	0.0073
GOTERM_BP_FAT	GO:0009625 response to insect	3	0.0099

(B) ***tlp18.3*** **FL vs** ***tlp18.3*** **CL**

Term		Count	*P*-Value
Increased transcript abundance			
GOTERM_BP_FAT	GO:0009611 *response to wounding*	8	1.33E-04
GOTERM_BP_FAT	GO:0010224 response to UV-B	5	4.47E-04
GOTERM_MF_FAT	GO:0080030 methyl indole-3-acetate esterase activity	3	0.0013
GOTERM_BP_FAT	GO:0009628 response to abiotic stimulus	20	0.0017
GOTERM_BP_FAT	GO:0009411 response to UV	5	0.0022
GOTERM_MF_FAT	GO:0030414 peptidase inhibitor activity	4	0.0032

In review

GOTERM_BP_FAT	GO:0009620 *response to fungus*	10	0.0064
GOTERM_MF_FAT	GO:0004857 enzyme inhibitor activity	6	0.0081
GOTERM_BP_FAT	GO:0009416 response to light stimulus	10	0.0094
GOTERM_MF_FAT	GO:0005385 zinc ion transmembrane transporter activity	3	0.0099
Decreased transcript abundance			
GOTERM_BP_FAT	GO:0009751 *response to salicylic acid stimulus*	8	4.23E-06
GOTERM_BP_FAT	GO:0009617 *response to bacterium*	9	1.14E-05
GOTERM_MF_FAT	GO:0004672 *protein kinase activity*	15	8.88E-05
GOTERM_MF_FAT	GO:0004674 *protein serine/threonine kinase activity*	13	3.89E-04
GOTERM_BP_FAT	GO:0006468 *protein amino acid phosphorylation*	14	6.61E-04
GOTERM_BP_FAT	GO:0006793 *phosphorus metabolic process*	15	0.0011
GOTERM_BP_FAT	GO:0042742 *defense response to bacterium*	6	0.0013
GOTERM_BP_FAT	GO:0016310 *phosphorylation*	14	0.0017
GOTERM_BP_FAT	GO:0006796 *phosphate metabolic process*	14	0.0033
GOTERM_BP_FAT	GO:0006869 lipid transport	5	0.0041
GOTERM_BP_FAT	GO:0010033 *response to organic substance*	13	0.0049
GOTERM_BP_FAT	GO:0010876 lipid localization	5	0.0061
GOTERM_MF_FAT	GO:0030554 *adenyl nucleotide binding*	19	0.0078
GOTERM_MF_FAT	GO:0001883 *purine nucleoside binding*	19	0.0078
GOTERM_MF_FAT	GO:0001882 *nucleoside binding*	19	0.0081
GOTERM_MF_FAT	GO:0032559 *adenyl ribonucleotide binding*	18	0.0092

702 Gene enrichment analysis was performed using DAVID (adjusted p-value threshold
703 minimum 0.01). % indicates the percentage of genes differentially regulated over the number
704 of total genes within the term. BP, biological process, CC, cellular component, GO, gene
705 ontology, MF, molecular function.

In review

706 **Table 3. Classification of significantly differentially expressed genes base on gene**
707 **enrichment analysis in wild type and *tlp18.3* plants.** (A) Gene enrichment analysis of in
708 *tlp18.3* plants as compared to wild type plants grown under fluctuating light (FL). (B) Gene
709 enrichment analysis of in *tlp18.3* plants as compared to wild type plants grown under constant
710 light (CL).
711

(A) *tlp18.3* FL vs wild type FL

Term		Count	*P*-Value
Increased transcript abundance			
GOTERM_BP_FAT	GO:0009611 response to wounding	12	1.75E-10
GOTERM_BP_FAT	GO:0010033 response to organic substance	24	7.66E-09
GOTERM_BP_FAT	GO:0010200 response to chitin	10	1.45E-08
GOTERM_BP_FAT	GO:0009743 response to carbohydrate stimulus	11	5.85E-08
GOTERM_BP_FAT	GO:0009719 response to endogenous stimulus	18	5.33E-06
GOTERM_BP_FAT	GO:0009725 response to hormone stimulus	16	4.05E-05
GOTERM_BP_FAT	GO:0009723 response to ethylene stimulus	9	4.41E-05
GOTERM_BP_FAT	GO:0006952 defense response	16	1.66E-04
GOTERM_BP_FAT	GO:0000160 two-component signal transduction system	7	8.21E-04
GOTERM_BP_FAT	GO:0009628 response to abiotic stimulus	16	8.28E-04
GOTERM_BP_FAT	GO:0009409 response to cold	7	0.0012
GOTERM_BP_FAT	GO:0009873 ethylene mediated signaling pathway	6	0.0017
GOTERM_BP_FAT	GO:0009612 response to mechanical stimulus	3	0.0029
GOTERM_BP_FAT	GO:0009631 cold acclimation	3	0.0045
GOTERM_BP_FAT	GO:0006869 lipid transport	5	0.0066
GOTERM_BP_FAT	GO:0009620 response to fungus	8	0.0066
GOTERM_CC_FAT	GO:0012505 endomembrane system	29	0.0072
GOTERM_BP_FAT	GO:0009753 response to jasmonic acid stimulus	5	0.0081
GOTERM_BP_FAT	GO:0009266 response to temperature stimulus	7	0.0090
GOTERM_BP_FAT	GO:0010876 lipid localization	5	0.0098
Decreased transcript abundance			
GOTERM_BP_FAT	GO:0009642 response to light intensity	5	5.96E-05
GOTERM_BP_FAT	GO:0006979 response to oxidative stress	7	1.73E-04
GOTERM_MF_FAT	GO:0004784 superoxide dismutase activity	3	2.66E-04
GOTERM_MF_FAT	GO:0016721 oxidoreductase activity.	3	2.66E-04
GOTERM_BP_FAT	GO:0009628 response to abiotic stimulus	12	4.88E-04
GOTERM_BP_FAT	GO:0000302 response to reactive oxygen species	5	7.28E-04
GOTERM_BP_FAT	GO:0006801 superoxide metabolic process	3	7.45E-04
GOTERM_BP_FAT	GO:0010035 response to inorganic substance	8	8.78E-04
GOTERM_MF_FAT	GO:0005507 copper ion binding	5	0.0013
GOTERM_BP_FAT	GO:0009416 response to light stimulus	7	0.0022
GOTERM_BP_FAT	GO:0009314 response to radiation	7	0.0026
GOTERM_BP_FAT	GO:0009617 response to bacterium	5	0.0055
GOTERM_BP_FAT	GO:0009063 cellular amino acid catabolic process	3	0.0073
GOTERM_BP_FAT	GO:0009644 response to high light intensity	3	0.0073
GOTERM_BP_FAT	GO:0009310 amine catabolic process	3	0.0083

In review

(B) *tlp18.3* CL vs wild type CL

Term		Count	*P*-Value
Increased transcript abundance			
GOTERM_MF_FAT	GO:0030614 oxidoreductase activity.	5	1.92E-09
GOTERM_MF_FAT	GO:0008794 arsenate reductase (glutaredoxin) activity	5	1.92E-09
GOTERM_MF_FAT	GO:0030613 oxidoreductase activity.	5	1.92E-09
GOTERM_MF_FAT	GO:0030611 arsenate reductase activity	5	2.62E-09
GOTERM_MF_FAT	GO:0015035 protein disulfide oxidoreductase activity	6	5.97E-09
GOTERM_MF_FAT	GO:0015036 disulfide oxidoreductase activity	6	1.21E-08
GOTERM_MF_FAT	GO:0016667 oxidoreductase activity.	6	1.84E-07
GOTERM_BP_FAT	GO:0045454 cell redox homeostasis	6	8.27E-07
GOTERM_BP_FAT	GO:0022900 electron transport chain	6	2.05E-06
GOTERM_BP_FAT	GO:0019725 cellular homeostasis	6	8.08E-06
GOTERM_BP_FAT	GO:0042592 homeostatic process	6	2.07E-05
GOTERM_BP_FAT	GO:0006091 generation of precursor metabolites and energy	6	1.23E-04
GOTERM_MF_FAT	GO:0009055 electron carrier activity	6	0.0012
Decreased transcript abundance			
GOTERM_BP_FAT	GO:0009751 response to salicylic acid stimulus	5	4.07E-04
GOTERM_MF_FAT	GO:0004672 protein kinase activity	8	0.0038
GOTERM_BP_FAT	GO:0010033 response to organic substance	9	0.0050
GOTERM_MF_FAT	GO:0004674 protein serine/threonine kinase activity	7	0.0086

712 Gene enrichment analysis was performed using DAVID (adjusted p-value threshold
713 minimum 0.01). % indicates the percentage of genes differentially regulated over the number
714 of total genes within the term. BP, biological process, CC, cellular component, GO, gene
715 ontology, MF, molecular function.
716
717 **Table 4. List of genes, which are significantly differentially expressed in *tlp18.3* as**
718 **compared to wild type both under fluctuating (FL) and constant light (CL) conditions**
719 **((logFC > 1)).**

Gene		logFC FL	logFC CL
Drought-repressed 4	AT1G73330	2.06	1.15
ELF4	AT2G40080	1.72	1.60
Major facilitator superfamily protein	AT5G62730	1.46	1.25
Major facilitator superfamily protein	AT2G16660	1.32	1.18
Monothiol glutaredoxin-S4 / ROXY 13	AT4G15680	1.21	1.57
Putative glutaredoxin-C12 / ROXY 5	AT2G47870	1.18	1.23
Delta-9 acyl-lipid desaturase 1	AT1G06080	-1.35	-1.01
HAD superfamily. subfamily IIIB acid phosphatase	AT4G29270	-1.94	-1.54
Transcription factor PIL1	AT2G46970	-2.23	-1.37
Transcription factor HFR1	AT1G02340	-2.31	-1.29
TLP18.3	AT1G54780	-7.13	-7.07

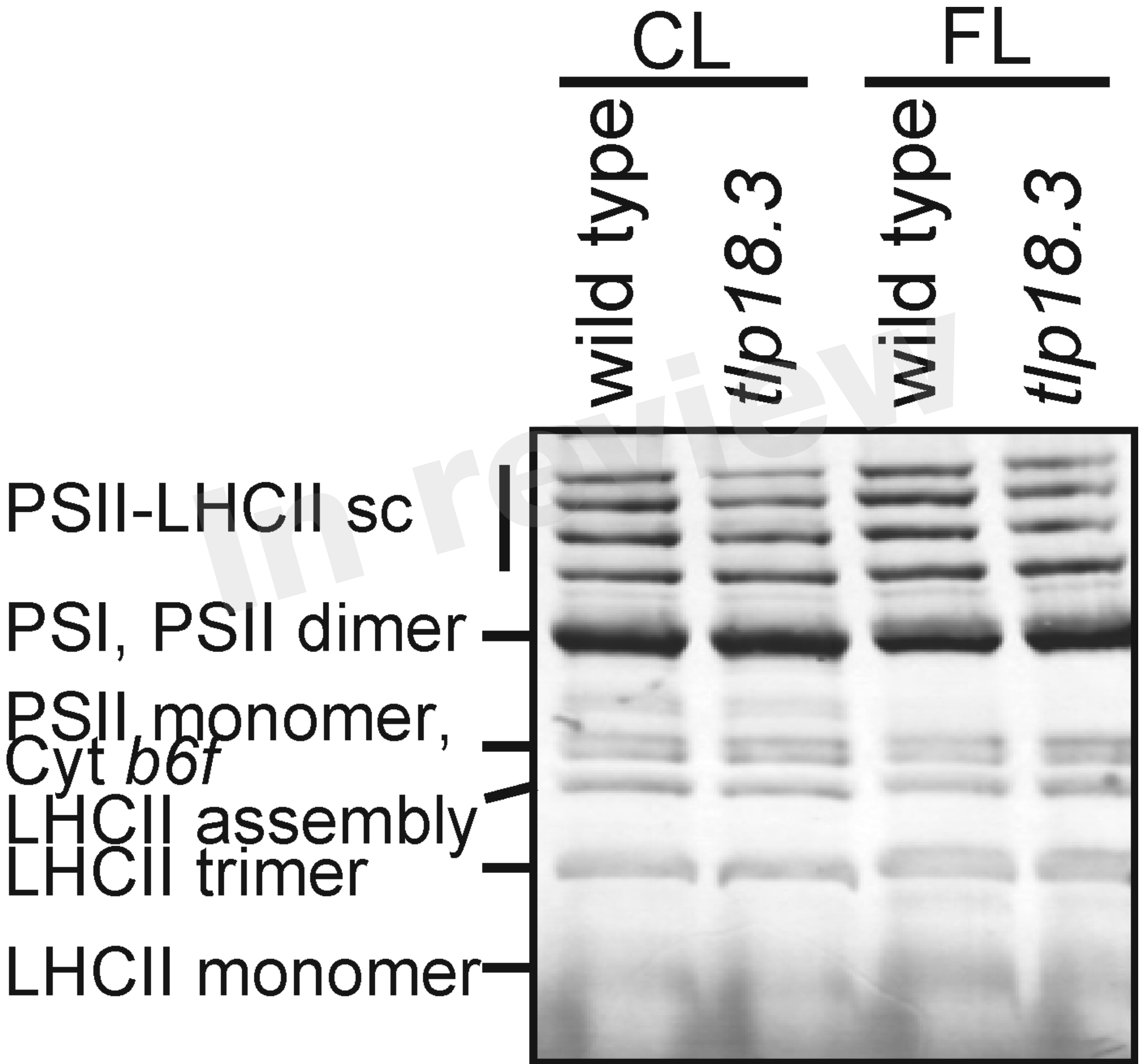
720

In review

721 **Figure 1: Accumulation of thylakoid protein complexes in wild type and *tlp18.3* plants.**
722 Plants were grown in 8h light regime either in a photon flux density of 120 µmol photons m^{-2}
723 s^{-1} (constant growth light; CL) or 50 µmol photons $m^{-2}s^{-1}$ for five minutes and 500 µmol µmol
724 photons $m^{-2}s^{-1}$ for one minute (fluctuating light; FL). sc. supercomplex. A representative
725 example from three independent biological replications is shown.
726
727 **Figure 2: Venn diagram showing the overlap of significantly differentially regulated**
728 **genes (logFC > 1) in response to either fluctuating light (FL) as compared to constant**
729 **growth light (CL) or deficient function of the TLP18.3 protein.**
730
731 **Figure 3. Cluster analysis of genes differentially expressed in the wild type but not in**
732 ***tlp18.3* in response to fluctuating light as compared to constant growth light.** Bayesian
733 hierarchical clustering of genes, which are significantly differentially regulated (logFC > 1) in
734 wild type under fluctuating light as compared to constant light, is presented. Data sets used
735 include abiotic and biotic stress experiments. Blue and red indicate decreased and increased
736 expression as compared to untreated plants, respectively.
737
738 **Supplementary material**
739
740 **Supplementary Table l. Adjustments in gene expression in wild type and *tlp18.3***
741 **Arabidopsis plants grown either under fluctuating light (FL) or constant light (CL) in 8h**
742 **light regime.** Values are the means of three independent biological replicates. Statistically
743 significant values with logFC >1 are indicated.
744
745 **Supplementary Table 2. Gene clusters and GO enrichments among genes differentially**

In review

Figure 1.TIF



In review

Figure 2.TIF

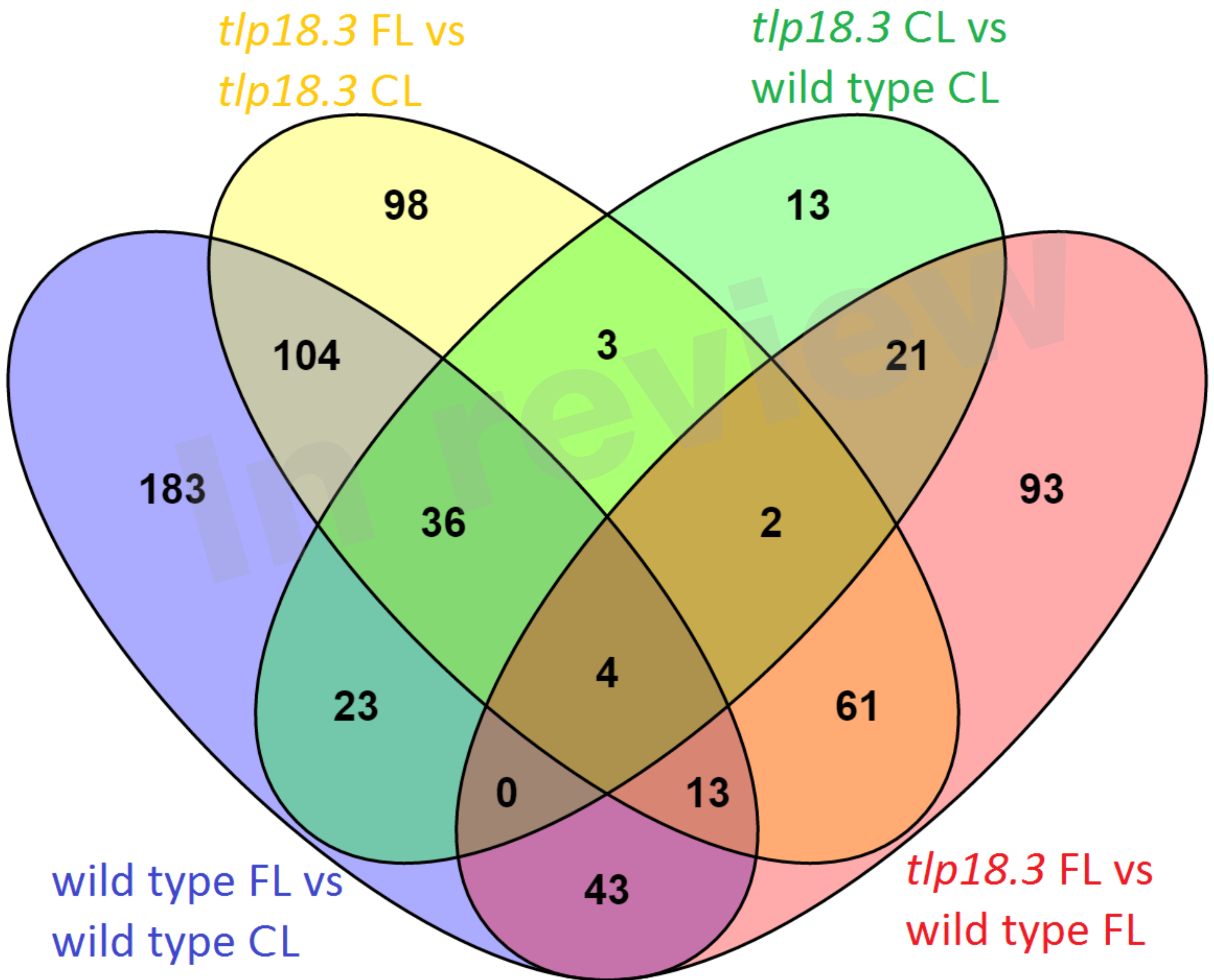


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Figure 3.TIF