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 plants, 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 photosystem II (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.

**Keywords:** Arabidopsis thaliana, defense, photosynthesis, photosystem II repair cycle, thylakoid lumen, transcriptomics
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-calcium cluster form the so-called oxygen-evolving complex (OEC), located at the lumenal surface of the PSII complex. In higher plants, the functional PSII complex is formed as a PSII dimer, to which nuclear-encoded light-harvesting complex (LHC) II proteins, Lhcb1-6, are tightly connected forming PSII-LHCII supercomplexes.

Photosynthetic water splitting and evolution of one oxygen molecule require four sequential excitations and subsequent charge separations in the reaction center chlorophyll (Chl) P680, thus producing extremely oxidizing, and potentially hazardous reactive oxygen species (ROS), which enhance oxidative damage to PSII as well as to other thylakoid proteins (Krieger-Liszkay et al., 2008; Pospisil, 2009). Despite the existence of detoxification systems for scavenging of ROS, damage to PSII is unavoidable (Aro et al., 1993; Tyystjärvi and Aro, 1996; Takahashi and Badger, 2011). In particular, the PSII core protein D1 is prone to light-induced damage, and thus an efficient repair cycle has evolved for PSII, which includes proteolytic degradation of damaged D1 protein and its replacement with a newly-synthesized D1 copy (reviewed in Baena-Gonzalez and Aro, 2002; Edelman and Mattoo, 2008; Nixon et al., 2010). These processes involve reversible monomerization of the PSII-LHCII supercomplexes (Danielsson et al., 2006), as well as dynamic changes in grana diameter and in lumen volume (Kirchhoff et al., 2011; Herbstova et al., 2012). A vast number of auxiliary proteins, such as kinases, phosphatases, proteases, transporters, and chaperones have been shown to assist the PSII repair cycle (reviewed in Mulo et al., 2008; Chi et al., 2012; Nickelsen and Rengel, 2013; Järvi et al., 2015). One of these, the THYLAKOID LUMEN PROTEIN OF 18.3 kDa (TLP18.3) has been shown to be required for efficient degradation of the damaged D1 protein and dimerization of the PSII complex (Sirpiö et al., 2007). Notably, high light treatment challenging the PSII repair cycle triggered only a moderate damage of PSII in tlp18.3 plants (Sirpiö et al., 2007), which suggest that TLP18.3 is not a crucial component of the repair cycle but instead plays a role in fine tuning the repair cycle. Based on structural data, TLP18.3 has been shown to be an acidic phosphatase, but only low phosphatase activity was measured for TLP18.3 (Wu et al., 2011). Recently, the regulatory role of the PSII repair cycle has been extended to include the maintenance of photosystem I (PSI) and indeed, insufficient regulation of the PSII repair cycle seems to exert an effect also on the function of PSI (Tikkkanen et al., 2014). Moreover, PSI is crucial for plant immunity through production of ROS, which are not only damaging the components of the photosynthetic electron transfer chain, but also act as important retrograde signaling molecules (Rodríguez-Herva et al., 2012; de Torres Zabala et al., 2015). In line with this, a functional connection between PSII repair and regulation of cell death in tobacco leaves infected by tobacco mosaic virus has been established (Seo et al., 2000).

While the exact role of photosynthetic components in sensing and signaling the pathogen infection is only emerging, a wealth of information has accumulated during the past few years on the consequences of fluctuating light on the activity of the photosynthetic machinery (Grieco et al., 2012; Suorsa et al., 2012; Allahverdiyeva et al., 2013; Kono and Terashima, 2014). Nevertheless, we still lack knowledge on how the rapid fluctuations in growth light intensity affect the acclimation processes at the level of nuclear gene expression, and even less is known about potential cross-talk between light acclimation, the PSII repair cycle and disease resistance under fluctuating light. Here, we investigated how the constantly fluctuating growth light intensity modulates the transcript profile of wild-type Arabidopsis thaliana (hereafter Arabidopsis) plants, and how such an acclimation response is further affected by the deficiency of the thylakoid lumen protein TLP18.3. Five-week old plants grown either under constant or fluctuating light conditions for their entire life span were used as material to study the late stage of the acclimation process.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis, ecotype Columbia 0, wild-type and tlp18.3 (GABI-Kat 459D12) plants (Sirpiö et al., 2007) were used in all experiments. Plants were grown in 8 h light regime at 23°C either under a photon flux density of 120 μmol photons m−2 s−1 or under fluctuating light intensities, in which plants were exposed to 50 μmol photons m−2 s−1 for 5 min and subsequently to high-light of 500 μmol photons m−2 s−1 for 1 min (Tikkkanen et al., 2010), the cycles being repeated during the entire photoperiod. Osram HQI-BT 400 W/D Metal Halide lamps with spectral power distribution from 350 to 800 nm were used as a light source. Five-week-old plants were used for all experiments.

Gene Expression Analyses

Microarray analyses of wild-type and tlp18.3 plants were performed essentially as in Konert et al. (2015). In short, leaf material was harvested 4 h after the onset of the light period in order to be sure that the plants were in a photosynthetically active state and that the PSII repair cycle was properly ongoing and immediately frozen in liquid nitrogen. RNA was isolated using an Agilent Plant RNA isolation mini kit according to manufacturer’s instructions. Cy-3 labeled RNA samples were hybridized to Arabidopsis Gene Expression Microarrays, 4 × 44 K (Design ID 021169) and scanned with Agilent Technologies Scanner G2565CA with a profile AgilentHD _GX_1Color. Numeric data were produced with Agilent Feature Extraction program, version 10.7.3.

Pre-processing of microarrays was performed using Limma’s normexp background correction method to avoid negative or zero corrected intensities, followed by between-array normalization using the quantile method to make all array distributions to have the same empirical distribution. Control probes were filtered and then within-array replicate spots were
replaced with their average. Pair-wise comparisons between groups were conducted using the Linear Models for Microarray Data (Limma) package Version 3.26.1 from Bioconductor (http://www.bioconductor.org/). The false discovery rate of differentially expressed genes for treatment/control and between-treatment comparisons was based on the Benjamini and Hochberg (BH) procedure. Genes with a score below an adjusted $p$-value threshold of 0.01 and which also showed a minimum of twofold change in expression between conditions or genotype were selected as significantly differentially expressed genes. Gene annotations were obtained from the Arabidopsis Information Resource (TAIR; http://www.arabidopsis.org/). Functional clustering and analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/home.jsp) version 6.7. Differentially expressed genes were compared against gene sets collected from various sources such as publications using the Plant GeneSet Enrichment Analysis Toolkit (PlantGSEA) (http://structuralbiology.cau.edu.cn/PlantGSEA/).

To detect co-regulated gene sets, a cluster analysis of the differentially expressed genes was carried out using data from (Georgii et al., 2012), consisting of microarray data downloaded from NASCArrays (ftp://ultraparabid.nottingham.ac.uk/NASCArrays/By_Experiment_ID/), ArrayExpress (http://www.ebi.ac.uk/microarraysae/), Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), and The Integrated Microarray Database System (http://ausubellab.mgh.harvard.edu/). Arrays were normalized with Robust Multi-array Average (RMA), and log2 ratio of the mean of treatment and control expressions across biological replicates was computed. Bayesian Hierarchical Clustering was carried out using R package BHC (Cooke et al., 2011) using log2 fold change $\pm 1$ as discretization threshold. Gene set enrichment analysis of the co-regulated gene clusters was carried out using StringDB (http://string-db.org/; Szklarczyk et al., 2015).

Isolation of the Thylakoid Membrane and Separation of Protein Complexes

Thylakoid isolation and blue native (BN)-PAGE were performed essentially as described in Järvi et al. (2011). Sodium fluoride was included in thylakoid isolation buffers for samples intended for BN-PAGE, whilst excluded from thylakoids used for spectroscopy analyses (see below). For BN-PAGE, the thylakoid membrane (4 $\mu$g Chl) was resuspended into ice-cold 25BTH20G buffer [25 mM BisTris/HCl (pH 7.0), 20% (w/v) glycerol and 0.25 mg ml$^{-1}$ Pefabloc] to a Chl concentration of 1.0 mg ml$^{-1}$. An equal volume of 2.0% (w/v) detergent (n-dodecyl $\beta$-D-maltoside, Sigma) solution (diluted in 25BTH20G) was added to the sample and thylakoid membrane was solubilized in darkness for 5 min on ice. Traces of insoluble material were removed by centrifugation at 18,000 g at 4°C for 20 min. Prior to loading, the samples were supplemented with a one-tenth volume of Serva Blue G buffer [100 mM BisTris/HCl (pH 7.0), 0.5 M ACA, 50% (w/v) sucrose, and 50 mg ml$^{-1}$ Serva Blue G].

Spectroscopic Quantitation of PSI and PSII

Room temperature continuous wave electron paramagnetic resonance (EPR) spectroscopy was performed essentially as described in Danielsson et al. (2004) and Suorsa et al. (2015). Measurements were performed at the Chl concentration of 2 mg ml$^{-1}$.

Photosynthetic Activity Measurements

The Dual-PAM-100 (Walz, http://www.walz.com/) was used for the measurement of PSII quantum yields. Quantum yields of PSII ($F_v/F_m$, $\Phi_{II}$, $\Phi_{NPQ}$, and $\Phi_{NO}$) were determined from leaves dark adapted for 30 min before the measurements. Saturating pulse (800 ms, 6000 $\mu$mol photons m$^{-2}$s$^{-1}$) was applied to determine the maximal fluorescence. Measurements were done in actinic red light of 50, 120, or 500 $\mu$mol photons m$^{-2}$s$^{-1}$.

Statistical Analyses

The numerical data were subjected to statistical analysis by Student's $t$-test with statistical significance at the $p < 0.05$.

RESULTS

Fluctuating Growth Light Only Slightly Modified the Photosynthetic Light Reactions

Accumulating evidence during recent years has demonstrated that sudden, abrupt changes in light intensity threaten particularly PSI, not PSII (Grieco et al., 2012; Suorsa et al., 2012; Allahverdiyeva et al., 2013; Kono and Terashima, 2014). Indeed, quantitation of the functional PSI/PSII ratios from wild-type plants with EPR revealed a PSI/PSII ratio of 1.12 for plants grown under constant light conditions (Suorsa et al., 2015), whereas plants grown under fluctuating light conditions exhibited a clearly lower value, 1.02.

The $tlp18.3$ plants showed a distinct stunted phenotype upon growth under fluctuating white light and the dry weight of the $tlp18.3$ plants ($12.2 \pm 5.7$ mg) was markedly decreased as compared to wild type ($29.9 \pm 4.7$ mg; $n = 6$). This observation prompted us to monitor whether the oligomeric structure of the thylakoid membrane protein complexes of wild-type and $tlp18.3$ plants grown either under constant or fluctuating light conditions is altered. Malfunction of the PSI repair cycle is often evidenced by a low amount of the most active PSII complexes, the PSII-LHCII complexes, accompanied by a high amount of PSI monomers, which are under the repair cycle (Danielsson et al., 2006). To that end, the BN-PAGE separation of thylakoid protein complexes according to their molecular mass was applied. In line with earlier results (Sirpiö et al., 2007), the $tlp18.3$ thylakoids accumulated slightly less of the PSI-LHCII complexes under constant light (Figure 1). Similar result was also evident under fluctuating light intensities, the amount of PSII-LHCII being somewhat lower in $tlp18.3$ plants as compared to wild type. However, no significant differences were observed in heterogeneity of the photosynthetic protein complexes, when wild-type and mutant plants grown either under constant or fluctuating light were compared (Figure 1). A previous report has shown that the maximal PSII quantum yield is not changed...
in tlp18.3 plants grown under constant growth light conditions as compared to wild type (Siripiö et al., 2007). In line with this, the maximum quantum yield and effective quantum yields of PSII remained rather similar, when the tlp18.3 and wild-type plants grown their entire life span under fluctuating light were compared (Table 1). Indeed, the PSII activity was only slightly down-regulated in tlp18.3 plants as compared to wild type. Thus, the growth defect shown by the tlp18.3 plants under fluctuating light intensities does not originate from the diminished pool of active PSII complexes.

Consequences of Fluctuating Growth Light Intensity on Gene Expression

To further characterize plant acclimation to fluctuating light, we performed transcript profiling of the wild-type and tlp18.3 plants grown under constant and fluctuating light intensities and compared the four datasets: (i) wild-type plants grown under fluctuating vs. constant growth light, (ii) tlp18.3 plants grown under fluctuating vs. constant growth light, (iii) tlp18.3 vs. wild-type plants grown under fluctuating light, and (iv) tlp18.3 vs. wild-type plants grown under constant light. Gene enrichment analysis and functional annotation clustering of differentially expressed genes were performed using the DAVID bioinformatic resource (the cutoff was set to logFC > 1 and the adjusted p-value threshold to a minimum of 0.01).

Wild-type plants grown under fluctuating light showed significantly different transcript abundance for 406 genes as compared to wild type grown under constant light, whereas in the tlp18.3 mutant, 321 genes responded differentially to fluctuating light as compared to wild type grown under constant light (Figure 2). When the transcript abundances between the genotypes was compared, 237 genes showed significantly different transcript abundance in tlp18.3 plants compared to wild type when grown under fluctuating light conditions, whereas under constant growth light the number of differentially expressed genes between wild type and the tlp18.3 mutant was 102 (Figure 2). Thus, it can be concluded that the growth light condition altered the number of differentially regulated genes more pronouncedly than the genotype. Moreover, the wild-type plants showed more profound changes at their gene expression level as a response to fluctuating growth light than the tlp18.3 plants.

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, $\Phi_{U}$ | | |
| 50 $\mu$mol photons m$^{-2}$s$^{-1}$ | $0.50 \pm 0.02$ | $0.47 \pm 0.04$ |
| 120 $\mu$mol photons m$^{-2}$s$^{-1}$ | $0.28 \pm 0.06$ | $0.26 \pm 0.03$ |
| 500 $\mu$mol photons m$^{-2}$s$^{-1}$ | $0.04 \pm 0.01$ | $0.03 \pm 0.01$ |

| NON-PHOTOCHEMICAL ENERGY DISSIPATION, $\Phi_{NPQ}$ | | |
| 50 $\mu$mol photons m$^{-2}$s$^{-1}$ | $0.13 \pm 0.02$ | $0.15 \pm 0.04$ |
| 120 $\mu$mol photons m$^{-2}$s$^{-1}$ | $0.48 \pm 0.07$ | $0.47 \pm 0.03$ |
| 500 $\mu$mol photons m$^{-2}$s$^{-1}$ | $0.68 \pm 0.01$ | $0.66 \pm 0.01^*$ |

| YIELD OF NON-REGULATED NON-PHOTOCHEMICAL ENERGY LOST, $\Phi_{NO}$ | | |
| 50 $\mu$mol photons m$^{-2}$s$^{-1}$ | $0.37 \pm 0.01$ | $0.38 \pm 0.03$ |
| 120 $\mu$mol photons m$^{-2}$s$^{-1}$ | $0.24 \pm 0.01$ | $0.27 \pm 0.00^*$ |
| 500 $\mu$mol photons m$^{-2}$s$^{-1}$ | $0.28 \pm 0.00$ | $0.31 \pm 0.02$ |

| MAXIMAL QUANTUM YIELD OF PSII, $F_{MV}$ | | |
| | $0.78 \pm 0.01$ | $0.76 \pm 0.02^*$ |

The values are the means $\pm$ SD, n = 4–5, except for $F_{MV}$, n = 12. Statistically significant differences comparing the mutant plants to that of the corresponding wild type are marked with asterix (*). See text for details.
Plants Grown under Fluctuating Light did not Show Differential Abundance of Photosynthesis Related Transcripts

Examination of differentially expressed genes revealed no photosynthesis-related gene ontologies in any of the four datasets analyzed (Tables 2, 3). Indeed, no gene ontologies related to photosynthetic light reactions, Calvin-Benson-Bassham cycle, or biosynthesis of photosynthetic pigments was observed in the gene enrichment analysis. Presumably, regulation of the photosynthetic machinery at transcriptional level does not play an important role during acclimation to relatively mild light intensity fluctuations, being designed such that the total amount of photons hitting the leaf remained nearly unchanged during the 8 h light period, when constant and fluctuating light conditions were compared. Likewise, deficient function of the TLP18.3 protein had only minor effects on transcript abundance of various photosynthesis genes.

Fluctuating Light Conditions Induced Transcriptional Adjustments in Immunity Related Genes Both in Wild-Type and tlp18.3 Plants

Bioinformatic analysis revealed that the majority of differentially expressed gene ontologies between plants grown under fluctuating and constant light conditions were linked to biotic or abiotic stress responses (Tables 2A, B). In wild type, growth under fluctuating light resulted in decreased transcript abundance within numerous gene ontologies related to plant immunity, as compared to wild type grown under constant light (Table 2A). These genes included mitogen-activated protein kinases (MAPKs) involved in early defense signaling, Toll/Interleukin-1 receptor-nucleotide binding site (TIR-NBS) class resistance (R) proteins mediating effector-triggered immunity (ETI) as well as pathogen related defense proteins, such as plant defensins (Supplementary Table 1). In contrast, the tlp18.3 mutant showed both decreased and increased transcript abundance within gene ontologies related to plant immunity, when fluctuating and constant light grown plants were compared to each other (Table 2B). For example, ankyrin BDA1 (AT5G54610), which is induced by salicylic acid (SA) and is involved in innate immunity (Blanco et al., 2005; Yang et al., 2012) showed cumulative repression in the transcript abundance in response to fluctuating light and deficient function of the TLP18.3 protein. In contrast, plant defensin PDF2.1 (AT2G02120) and defensin-like (AT2G43535) genes, which are activated in response to fungal infection, were induced in tlp18.3 plants under fluctuating light.

With respect to abiotic stress, gene ontologies “response to UV” and “response to light stimulus” were enriched in the transcriptome of tlp18.3 leaves, when plants grown under fluctuating and constant light were compared (Table 2B). For example, increased abundance of transcripts for EARLY LIGHT-INDUCED PROTEIN2 (ELIP2; AT4G14690), which modulates Chl biosynthesis to prevent photo-oxidative stress (Tzetkova-Chevolleau et al., 2007; Hayami et al., 2015), was observed in the fluctuating-light-grown tlp18.3 plants (Supplementary Table 1). In contrast, no gene ontologies related to light perception showed differential expression in the wild-type plants as a

| Term | Count | P-value |
|------|-------|---------|
| GO:0005507 copper ion binding | 5 | 0.0055 |
| GO:0031225 anchored to membrane | 6 | 0.0076 |
| GO:0006952 defense response | 43 | 3.26E-14 |
| GO:004672 protein kinase activity | 40 | 7.91E-12 |
| GO:0010033 response to organic substance | 42 | 1.18E-11 |
| GO:006488 protein amino acid phosphorylation | 39 | 4.18E-11 |
| GO:009751 response to salicylic acid stimulus | 16 | 8.69E-11 |
| GO:0069655 immune response | 20 | 4.82E-10 |
| GO:0016310 phosphorylation | 39 | 7.75E-10 |
| GO:0010200 response to chitin | 14 | 1.24E-09 |
| GO:004674 protein serine/threonine kinase activity | 33 | 4.75E-09 |
| GO:006796 phosphate metabolic process | 39 | 6.76E-09 |
| GO:006793 phosphorus metabolic process | 39 | 6.91E-09 |
| GO:0045087 innate immune response | 18 | 8.31E-09 |
| GO:0009617 response to bacterium | 17 | 1.02E-08 |
| GO:0009611 response to wounding | 13 | 7.11E-08 |
| GO:0042742 defense response to bacterium | 14 | 1.10E-07 |
| GO:0009743 response to carbohydrate stimulus | 14 | 2.74E-07 |
| GO:0032559 adenylyl ribonucleotide binding | 49 | 1.85E-06 |
| GO:0030554 adenylyl nucleotide binding | 50 | 5.06E-06 |
| GO:0001883 purine nucleoside binding | 50 | 5.06E-06 |
| GO:0001882 nucleoside binding | 50 | 5.54E-06 |
| GO:0055524 ATP binding | 47 | 7.74E-06 |
| GO:0098814 defense response, incompatible interaction | 9 | 9.97E-06 |
| GO:0009873 ethylene mediated signaling pathway | 11 | 1.73E-05 |
| GO:0009723 response to ethylene stimulus | 13 | 2.44E-05 |
| GO:0032555 purine ribonucleotide binding | 49 | 3.11E-05 |
| GO:0032553 ribonucleotide binding | 49 | 3.11E-05 |

(Continued)
response to fluctuating light (Table 2A). Decreased transcript abundance of gene ontologies associated with lipid localization and lipid transport were also observed as response to fluctuating light specifically in tlp18.3 leaves. Several genes encoding lipid-transfer proteins such as LIPID TRANSFER PROTEIN 3 (LTP3; AT3G59320), which mediates freezing and drought stress in Arabidopsis (Guo et al., 2013), were down-regulated in the tlp18.3 mutant, when plants were grown under fluctuating light compared to constant growth light (Supplementary Table 1).

When fluctuating-light-grown tlp18.3 and wild-type plants were compared to each other, increased transcript abundance of genes related to the defense mechanisms in the tlp18.3 mutant was again the most prominent result (Table 3A). Enrichment analysis and functional annotation clustering of the differentially expressed gene ontologies in tlp18.3 and wild-type plants also revealed that several gene clusters related to abiotic stresses were
TABLE 3 | Classification of significantly differentially expressed genes base on gene enrichment analysis in wild-type and tlpl8.3 plants: (A) Gene enrichment analysis of in tlpl8.3 plants as compared to wild-type plants grown under fluctuating light (FL); (B) Gene enrichment analysis of in tlpl8.3 plants as compared to wild-type plants grown under constant light (CL).

| Term | Count | P-value |
|------|-------|---------|
| **(A) tlpl8.3 FL vs. WILD TYPE FL** | | |
| **Increased Transcript Abundance** | | |
| G0TERM_BP_FAT GO:0009611 response to wounding | 12 | 1.75E-10 |
| G0TERM_BP_FAT GO:0010033 response to organic substance | 24 | 7.66E-09 |
| G0TERM_BP_FAT GO:0010200 response to chitin | 10 | 1.45E-08 |
| G0TERM_BP_FAT GO:0009743 response to carbohydrate stimulus | 11 | 5.85E-08 |
| G0TERM_BP_FAT GO:0009719 response to endogenous stimulus | 18 | 5.33E-06 |
| G0TERM_BP_FAT GO:0009725 response to hormone stimulus | 16 | 4.05E-05 |
| G0TERM_BP_FAT GO:0009723 response to ethylene stimulus | 9 | 4.41E-05 |
| G0TERM_BP_FAT GO:0009652 defense response | 16 | 1.66E-04 |
| G0TERM_BP_FAT GO:000160 two-component signal transduction system | 7 | 8.21E-04 |
| G0TERM_BP_FAT GO:0009628 response to abiotic stimulus | 16 | 8.28E-04 |
| G0TERM_BP_FAT GO:0009409 response to cold | 7 | 0.0012 |
| G0TERM_BP_FAT GO:0009873 ethylene mediated signaling pathway | 6 | 0.0017 |
| G0TERM_BP_FAT GO:0009612 response to mechanical stimulus | 3 | 0.0029 |
| G0TERM_BP_FAT GO:0009631 cold acclimation | 3 | 0.0045 |
| G0TERM_BP_FAT GO:0008869 lipid transport | 5 | 0.0066 |
| G0TERM_BP_FAT GO:0009620 response to fungus | 8 | 0.0066 |
| G0TERM_CC_FAT GO:0012505 endomembrane system | 29 | 0.0072 |
| G0TERM_BP_FAT GO:0009753 response to jasmonic acid stimulus | 5 | 0.0081 |
| G0TERM_BP_FAT GO:0009266 response to temperature stimulus | 7 | 0.0090 |
| G0TERM_BP_FAT GO:0010876 lipid localization | 5 | 0.0098 |
| **Decreased Transcript Abundance** | | |
| G0TERM_BP_FAT GO:0009642 response to light intensity | 5 | 5.96E-05 |
| G0TERM_BP_FAT GO:0009879 response to oxidative stress | 7 | 1.73E-04 |
| G0TERM_MF_FAT GO:0004784 superoxide dismutase activity | 3 | 2.66E-04 |
| G0TERM_MF_FAT GO:0016721 oxidoreductase activity | 3 | 2.66E-04 |
| G0TERM_BP_FAT GO:0009628 response to abiotic stimulus | 12 | 4.88E-04 |
| G0TERM_BP_FAT GO:0000302 response to reactive oxygen species | 5 | 7.28E-04 |
| G0TERM_BP_FAT GO:0009801 superoxide metabolic process | 3 | 7.45E-04 |
| G0TERM_BP_FAT GO:0010035 response to inorganic substance | 8 | 8.78E-04 |

**(B) tlpl8.3 CL vs. WILD TYPE CL**

| Term | Count | P-value |
|------|-------|---------|
| **Increased Transcript Abundance** | | |
| G0TERM_MF_FAT GO:0003614 oxidoreductase activity | 5 | 1.92E-09 |
| G0TERM_MF_FAT GO:0008794 arsenate reductase (glutaredoxin) activity | 5 | 1.92E-09 |
| G0TERM_MF_FAT GO:0020613 oxidoreductase activity | 5 | 1.92E-09 |
| G0TERM_MF_FAT GO:0030611 arsenate reductase oxidoreductase activity | 5 | 1.92E-09 |
| G0TERM_MF_FAT GO:0015036 disulfide oxidoreductase activity | 6 | 5.97E-09 |
| G0TERM_MF_FAT GO:0015038 disulfide oxidoreductase activity | 6 | 1.21E-08 |
| G0TERM_MF_FAT GO:0015086 oxidoreductase activity | 6 | 1.84E-07 |
| G0TERM_BP_FAT GO:0045454 cell redox homeostasis | 6 | 8.27E-07 |
| G0TERM_BP_FAT GO:0022900 electron transport chain | 6 | 2.05E-06 |
| G0TERM_BP_FAT GO:0019725 cellular homeostasis | 6 | 8.08E-06 |
| G0TERM_BP_FAT GO:0024592 homeostatic process | 6 | 2.07E-05 |
| G0TERM_BP_FAT GO:0006979 generation of precursor metabolites and energy | 6 | 1.23E-04 |
| G0TERM_MF_FAT GO:0009055 electron carrier activity | 6 | 0.0012 |
| **Decreased Transcript Abundance** | | |
| G0TERM_BP_FAT GO:0009751 response to salicylic acid stimulus | 5 | 4.07E-04 |
| G0TERM_MF_FAT GO:0004672 protein kinase activity | 8 | 0.0038 |
| G0TERM BP_FAT GO:0010033 response to organic substance | 9 | 0.0050 |
| G0TERM_MF_FAT GO:0004674 protein serine/threonine kinase activity | 7 | 0.0086 |

Gene enrichment analysis was performed using DAVID (adjusted p-value threshold minimum 0.01). % indicates the percentage of genes differentially regulated over the number of total genes within the term. BP, biological process; CC, cellular component; GO, gene ontology; MF, molecular function.
differentially expressed in *tlp18.3* plants 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 the *tlp18.3* mutant 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* plants 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.

**Adjustments in Immunity-Related Genes under Fluctuating Light are Linked to Plant Hormones**

Plant acclimation to various stresses, including light stress, is regulated by signaling cascades, which include plant hormones as central components (Karpinski et al., 2013; Müller and Munné-Bosch, 2015). In wild-type plants, growth under fluctuating light results in decreased transcript abundance of several genes related to SA signaling cascade (Table 2A). For example, expression of a gene encoding SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1 (SARD1; AT1G37380), a key regulator of ISOCHORISMATE SYNTATHASE 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 the *tlp18.3* mutant as compared to wild type and no differential expression of SARD1 or BSMT1 were observed in *tlp18.3* plants 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 became apparent when ET/JA responses between fluctuating light grown *tlp18.3* and wild-type plants were compared (Table 3A).

The most prominent alteration in the gene ontology level, when the transcript abundances of constant light grown *tlp18.3* and wild-type plants were compared, was an increase in transcripts of six genes encoding CC-type glutaredoxins (ROXY 5, ROXY 11-15) and two of those, ROXY 5 and ROXY 13, were up-regulated in *tlp18.3* as compared to wild type also under fluctuating light (Tables 3, 4, Supplementary Table 1). As CC-type glutaredoxins have been suggested to be capable of suppressing the JA and ET-induced defense genes (Zander et al., 2012), a causal connection might exist between expression of JA and ET-responsive genes and differential expression of ROXY genes. It can be concluded that alteration in the gene expression patterns of SA, ET, and JA signaling are taking place during plant acclimation to fluctuating light and that these alterations are strongly affected by the deficient function of the TLP18.3 protein.

**Phytochrome-Mediated Light Signaling is Likely to be Altered in *tlp18.3* Plants**

Next, we wanted to further explore which Arabidopsis genes showed a differential expression pattern in the *tlp18.3* plants both under constant and fluctuating light conditions. In addition to ROXY5 and ROXY13 located in the endomembrane system, genes encoding cold (DELTA-9 DESATURASE 1)
and drought-repressed (DROUGHT-REPRESSED 4) proteins, acid phosphatase (AT4G29270), and two putative membrane transporters (AT5G62730, AT2G16660) showed differential expression in the tlp18.3 mutant. Interestingly, two genes encoding bHLH class phytochrome A-signaling components, LONG HYPOCOTYL IN FAR-RED 1 (HFR1; AT1G02340) and PHYTOCHROME INTERACTING FACTOR 3-LIKE 1 (PIL1; AT2G46970; Fairchild et al., 2000; Salter et al., 2003), showed decreased transcript abundance in tlp18.3 plants as compared to wild type (Table 4). Instead, expression of the gene encoding EARLY FLOWERING 4 (ELF4; AT2G40080), a phytochrome-controlled regulator of circadian clock was induced in the tlp18.3 mutant as compared to wild type. Taken together, the deficient function of TLP18.3 is likely to change the phytochrome-mediated light signaling both under constant and fluctuating light intensities.

Decreased Transcript Abundance of Dark-Induced Genes Suggest that Nitrogen to Carbon and/or Phosphorus to Carbon Ratios Might be Altered in tlp18.3 Plants under Fluctuating Light

Nutrient availability plays an important regulatory role in growth and development of plants, but also cross-talk between nutrient availability and disease resistance exist (Huber, 1980; Hermans et al., 2006). Interestingly, GLUTAMINE-DEPENDENT ASPARAGINE SYNTHASE 1/DARK-INDUCED 6 (ASN1/DIN6; AT3G47340) and DARK-INDUCED 1/SENCENCE 1 (DIN1/SEN1; AT4G35770) genes showed strong down-regulation in fluctuating light grown tlp18.3 plants as compared to either fluctuating light grown wild type or constant light grown tlp18.3 plants (Supplementary Table 1). ASN1/DIN6 regulates the flow of nitrogen into asparagine, which acts as a nitrogen storage and transport compound in darkness and its gene expression is regulated by the nitrogen to carbon ratio (Lam et al., 1994). DIN1/SEN1, which has been suggested to contribute to enhanced susceptibility to plant viruses, is induced by phosphate starvation and repressed by sugars (Fernández-Calvino et al., 2015). The differential expression of ASN1/DIN6 and DIN1/SEN1 is linked to deficient function of TLP18.3 under fluctuating light but the exact mechanism behind transcriptional repression of these two genes remains to be verified.

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.

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 the 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 the photosynthetic light reactions 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, Tables 1–3). 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 1 min, 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 light into late stages of the plant acclimation process, in which a vast number of defense pathways were affected.

Contrary to wild type, in the tlp18.3 mutant the alterations in the overall gene expression pattern, as a response to fluctuating light, were less evident and indeed, the tlp18.3 plants were less capable of turning off the gene expression...
related to plant immunity under fluctuating light conditions (Table 2B, Figures 2, 3). It is known that the photoreceptor-derived signals activate the shade-avoidance responses and reduce the defense reactions against pathogens and pests to save resources for the growth of the plant (Ballare, 2014). Interestingly, the gene expression of two components...
of phytochrome-mediated light signaling, HFR1 and PIL1, was shown to be altered in tlp18.3 leaves (Table 4). HFR1 and PIL1 genes are involved in transcriptional regulation pathways downstream of phytochromes, which integrate light and hormonal signals and play a role in shade-avoidance responses (Jiao et al., 2007). Of these, HFR1 also contributes to the crosstalk between light signaling and plant innate immunity (Tan et al., 2015). Based on these results, it is evident that the functionality of TLP18.3 protein modifies the light perception and/or signaling network, and possibly also the signaling related to nutrient availability (Supplementary Table 1). Allocation of resources to defense reactions in the tlp18.3 mutant is likely associated with the lower biomass of mutant plants as compared to wild-type plants under low-light dominant fluctuating light. It should be noted that the tlp18.3 plants also had lower biomass as compared to wild type when grown under high-light dominant fluctuating light with longer, 1 h light pulses (Sirpiö et al., 2007). It remains to be studied whether the growth phenotype of tlp18.3 plants under high-light dominant fluctuating light originates directly from the diminished pool of active PSII complexes. Indeed, duration, frequency, and intensity of fluctuating light regimes have been shown to affect the acclimation responses in Arabidopsis (Alter et al., 2012). To that end, it would be interesting to compare how the gene expression patterns of low-light and high-light dominant fluctuating light conditions differ from each other.

Defective degradation of the D1 core protein of PSII in tlp18.3 plants is a promising system for the search of chloroplast-derived retrograde signals which affect gene expression related to plant immunity. In line with this, low amount of the D1 degrading protease FtsH has been earlier observed to accelerate the hypersensitive reaction in tobacco (Seo et al., 2000). Recently, a link between PsbS-mediated photoprotection and pathogen resistance has also been shown to exist (Göhre et al., 2012; Johansson Jänkänp et al., 2013). Further, as the PSII repair cycle and maintenance of PSI are interconnected (Tikkkanen et al., 2014), also PSI and/or PSI electron acceptors might act as a source of retrograde signaling components under fluctuating light. It should be noted that the pool of active PSII was not changed in tlp18.3 plants as compared to wild type under low-light dominant fluctuating light (Table 1) and thus the effect might be indirect. We postulate that the compensation mechanisms activated in the tlp18.3 mutant are likely to alter the chloroplast-derived retrograde signals. Taken together, our results demonstrate that light acclimation and plant immunity are interconnected and the proper repair cycle of PSII plays a key role in the process.

**AUTHOR CONTRIBUTORS**

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

**FUNDING**

Our research was financially supported by the Academy of Finland (project numbers 272424, 271832, and 275870), TEKES LIF 40128/14, the Swedish Research Council, the Swedish Energy Agency, the Knut and Alice Wallenberg Foundation and the Initial Training Networks (ITN) CALIPSO (607607), and PHOTOCOMM (317814).

**ACKNOWLEDGMENTS**

Microarray and sequencing unit of the Turku Centre for Biotechnology is thanked for assistance with microarray hybridizations. Kurt Ståle, Mika Keränne, Virpi Paakkarinen, Marjaana Rantala, Sanna Rantala, Ville Käpylä, and Saara Mikola are acknowledged for their excellent technical assistance.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2016.00405

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Conflict of Interest Statement: 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.