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Coordination of Plastid and Light Signaling Pathways upon Development of *Arabidopsis* Leaves under Various Photoperiods

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ABSTRACT Plants synchronize their cellular and physiological functions according to the photoperiod (the length of the light period) in the cycle of 24 h. Photoperiod adjusts several traits in the plant life cycle, including flowering and senescence in annuals and seasonal growth cessation in perennials. Photoperiodic development is controlled by the coordinated action of photoreceptors and the circadian clock. During the past 10 years, remarkable progress has been made in understanding the molecular mechanism of the circadian clock, especially with regard to the transition of *Arabidopsis* from the vegetative growth to the reproductive phase. Besides flowering photoperiod also modifies plant photosynthetic structures and traits. Light signals controlling biogenesis of chloroplasts and development of leaf photosynthetic structures are perceived both by photoreceptors and in chloroplasts. In this review, we provide evidence suggesting that the photoperiodic development of *Arabidopsis* leaves mimics the acclimation of plant to various light intensities. Furthermore, the chloroplast-to-nucleus retrograde signals that adjust acclimation to light intensity are proposed to contribute also to the signaling pathways that control photoperiodic acclimation of leaves.

Key words: acclimation; chloroplast biology; circadian clock; leaf/vegetative development; light signaling; photomorphogenesis; plastid signaling.

INTRODUCTION

Plant development is controlled by numerous external factors that coordinate the timing of developmental and adaptive processes to meet the requirements of the environment. The quantity and quality of light and the length of the diurnal light period in a day cycle of 24 h (photoperiod) together with the temperature and availability of nutrients adjust the morphology and extent of plant growth as well as the timing of the annual developmental phases in nature. From these variables, the day length is the most reliable indicator for the annual season because of its high predictability. Strict response to photoperiod is critical for perennial overwintering plants in temperate latitudes to adjust their yearly development with favorable growth conditions and to initiate bud formation and growth cessation before the cold season. Length of photoperiod is important also for annual plant species in adjusting the transitions of developmental phases from juvenile to vegetative and from vegetative to reproductive phase during their lifecycle.

Plant photoperiodic responses are classified into three categories: short-day (SD) responses, in which the response occurs in photoperiod shorter than the critical photoperiod; long-day (LD) responses, in which the response occurs in photoperiod longer than the critical photoperiod; and day-neutral (DN) responses. Plants showing the response under distinct photoperiod are called SD, LD, or DN plants, respectively. Obligate SD or LD plant species show the response only under inducing photoperiod. In facultative SD or LD plants, the response is promoted by a short or long photoperiod, respectively, but it can be induced also by other photoperiods. In the latter case, the intensity of the response is weaker and/or the initiation of the response is delayed. Timing of flowering is one of the few photoperiodic responses that have been minutely characterized at the molecular level (recent reviews, see Turck et al., 2008; Imaizumi, 2010). Recently, however, growing interest has been paid to the initiation of bud dormancy and the

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cessation of growth in trees (Jimenez et al., 2010; Kozarewa et al., 2010; Olsen, 2010). Molecular dissection of the initiation, transition, and development of the photoperiodic responses is crucial, since the photoperiod contributes to the control of several scientifically and economically important plants traits, including leaf morphology, vegetative production, seed production, stress tolerance, and dormancy.

Light is the major environmental factor that adjusts the photosynthetic traits in plant species. Light signaling pathways associated with de-etiolation of seedlings and with acclimation to light intensity have been actively studied in plants (Nagy and Schafer, 2002; Sullivan and Deng, 2003; Jung and Chory, 2010), while photoperiodic adjustments in chloroplast structure and function are less well characterized. Recently, it was proposed that the photoreceptor-dependent signaling pathways interact with chloroplast retrograde signaling pathways by either promoting or antagonizing each other, depending on the processes dissected (Ruckle et al., 2007; Ruckle and Larkin, 2009). Here, we review the photosynthetic traits and structures controlled by the length of the photoperiod. Specific focus is put on chloroplast biogenesis and plastid-derived signals in the control of light intensity-dependent and photoperiodic growth in *Arabidopsis*.

ACCLIMATION OF *ARABIDOPSIS* ACCORDING TO THE LENGTH OF THE PHOTOPERIOD

Arabidopsis Col-0 is a facultative LD plant, in which photoperiods longer than 12 h (LD) accelerate flowering by several weeks in comparison with photoperiods shorter than 12 h (SD). SD distinctly extends the vegetative phase of *Arabidopsis* and delays senescence. The number of leaves in mature rosette is on average 40% higher in SD than in LD plants (Cookson et al., 2007). This is opposite to perennial deciduous trees, in which the SD promotes leaf senescence (Zhao et al., 2009) that is related to the growth cessation at the end of the growing season.

The ability of *Arabidopsis* to react to the daily light rhythm increases growth, whereas incorrect matching of endogenous rhythms with environmental rhythms reduces plant fitness. For example, the extension of external light–dark cycle of 24 to 28 h (14-h L/14-h D) reduced the areal biomass production by ~50% (Dodd et al., 2005). Likewise, the growth of the short- and long-circadian period mutants with altered endogenous clock periods was promoted by the external day–night cycle corresponding to their own endogenous circadian rhythms (Dodd et al., 2005). Furthermore, the arrhythmic plants overexpressing the molecular oscillation component CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) of the circadian clock and grown under a normal 24-h cycle had distinctly lower net CO_2 assimilation and biomass production than wild-type plants (Dodd et al., 2005). Adjustment of the growth with the external light–dark cycle is partially attained by circadian-clock-dependent control of global gene expression. Indeed, 5.5–15.4% of *Arabidopsis* genes have been estimated to be regulated by the circadian clock (Covington et al., 2008).

Length of the photoperiod has a distinct influence on biomass production, leaf and cell structure, and on the ultrastructure of chloroplasts. In general, *Arabidopsis* plants grown under SD or LD photoperiod with similar light intensity show both structural and photosynthetic characteristics typical of shade or sun plants, respectively (Figure 1; Lepistö et al., 2009). Like sun plants (Walters and Horton, 1995; Lake et al., 2001), LD-grown plants and plants grown under continuous light have thicker leaves, long-shaped palisade cells, high stomatal index in leaf epidermis, and smaller grana stacks in chloroplasts when compared to SD-grown plants (Figure 1; Lepistö et al., 2009). For example, growth of *Arabidopsis* in LD substantially increased the stomatal index (the ratio of the number of stomata to the total number of epidermal cells) about 40% as compared with SD plants (Lepistö et al., 2009). Furthermore, the net CO_2 assimilation per rosette area (measured at ambient CO_2 and saturating light intensity) is about 20% higher in LD-leaves, whereas the mitochondrial respiration rate is only 50% of that measured in SD-leaves (Lepistö et al., 2009). LD-leaves also have a higher chlorophyll (Chl) content per leaf area due to thicker leaves compared to SD-leaves and the Chl a/b ratio in LD-leaves is similar to plants grown at medium or high light (Walters and Horton, 1995; Lepistö et al., 2009).

Substantial increase in Chl a/b ratio of LD-leaves implies photoperiodical changes in the composition of the light-harvesting complexes of the thylakoid membranes. The grana stacks are smaller in chloroplasts of LD-leaves in comparison to SD-leaves (Figure 1). Accordingly, the amount of the major trimeric chlorophyll a/b-binding proteins of the Photosystem II (PSII) antenna (LHCII) in thylakoid membranes is declined in LD-leaves as compared to SD-leaves (Lepistö et al., 2009; Victor et al., 2010). The relative proportion of the representative subunits of PSII, PSI, and cytochrome bf complexes did not, however, differ significantly in SD- and LD-leaves (A. Lepistö, E. Pakula, E. Rintamäki, unpublished results), neither did the maximal electron transport rates estimated for the SD- and LD-grown plants (Lepistö et al., 2009).

METABOLIC AND TRANSCRIPTOMIC MODIFICATIONS IN *ARABIDOPSIS* LEAVES GROWN UNDER SD AND LD PHOTOPERIODS

Anatomical and photosynthetic traits of leaves indicate that the acclimation of *Arabidopsis* to SD and LD photoperiods mimics the responses detected in leaves acclimated to low light and medium/high light, respectively. The question is whether the photoperiodic development is controlled by the same signaling network mediating the light-intensity-dependent acclimation of plants. Redox signals that arise from chloroplasts play a major role in the development of high-light structures in leaves (Nott et al., 2006; Piippo et al., 2006; Pfannschmidt et al., 2009). Short-term transfer of *Arabidopsis* to high light enhances the production of reactive oxygen species that has been suggested to initiate high-light acclimation (Vanderauwera

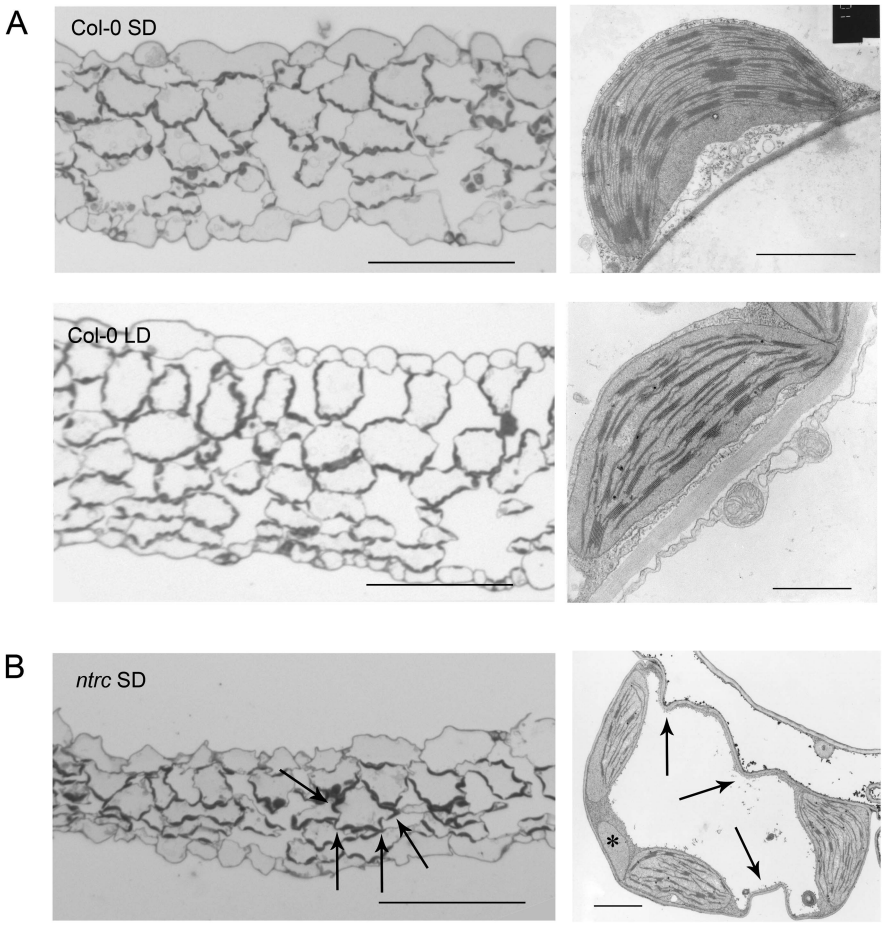

Figure 1. Light Micrographs of Leaf Cross-Sections and Electron Micrographs of Chloroplasts in Col-0 **(A)** and *ntrc* **(B)**.
Plants were grown under short day (SD) for 4 weeks and under long day (LD) for 3 weeks. Arrows indicate the irregular shape of the *ntrc* cells. * indicates a plastid-like organelle in *ntrc* cell. Scale bars: 100 μm for light micrographs and 2 μm for electron micrographs.

et al., 2005; Muhlenbock et al., 2008; Foyer and Noctor, 2009). In the course of high-light acclimation, elevated ROS production is compensated for by induction of antioxidant systems in leaves (Mittler et al., 2004), which in turn prevents the oxidation of leaf cells. In *Arabidopsis* acclimated to LD photoperiods, no substantial amounts of superoxide or H_2O_2 were found to accumulate in illuminated leaves (Figure 2). Furthermore, the growth in the LD photoperiod was shown to modify only slightly the antioxidant levels in *Arabidopsis* leaves. Catalase activity has been reported to rise in LD-grown leaves in comparison to SD-leaves, whereas the steady-state contents and the oxidation level of ascorbate and glutathione were not markedly different in SD- and LD-leaves (Queval et al., 2007). These reports suggest that the production and detoxification of ROS are balanced in plants acclimated to LD.

In contrast to LD conditions, H_2O_2 accumulated in *Arabidopsis* leaves upon acclimation to SD photoperiod (Figure 2). Chloroplasts may contribute to increased accumulation of ROS in SD-leaves, since the thylakoid membranes isolated from SD-acclimated *Arabidopsis* (A. Lepistö, E. Pakula, J. Toivola, A. Krieger-Liszkay, F. Vignols, E. Rintamäki, unpublished results) and tobacco leaves (Michelet and Krieger- Liszkay, 2011) produced more ROS than thylakoids isolated from LD-acclimated plants. Furthermore, the abundance of photorespiratory enzymes, except peroxisomal catalase, increased in SD-acclimated plants (Victor et al., 2010). This suggests an elevation in peroxisomal H_2O_2 production in leaves as well. Accordingly, acclimation to SD conditions has been shown to result in increased expression of H_2O_2 marker genes (Queval et al., 2007). The growth in SD conditions also promotes the ascorbate metabolism in leaves. The abundances of the enzymes related to ascorbate biosynthesis, monodehydroascorbate reductase, and dehydroascorbate reductase were three to fourfold higher in SD-acclimated shoot tips of grapevine in comparison to plants acclimated to LD (Victor et al., 2010). Regardless of the changes in antioxidant components, higher accumulation of ROS in illuminated SD-leaves (Figure 2) suggests that ROS production is controlled in SD-leaves instead of complete elimination of oxidants. The elevated oxidative state of SD-cells likely operates as a control loop in adjusting the redox-controlled metabolism to the photoperiod during growth.

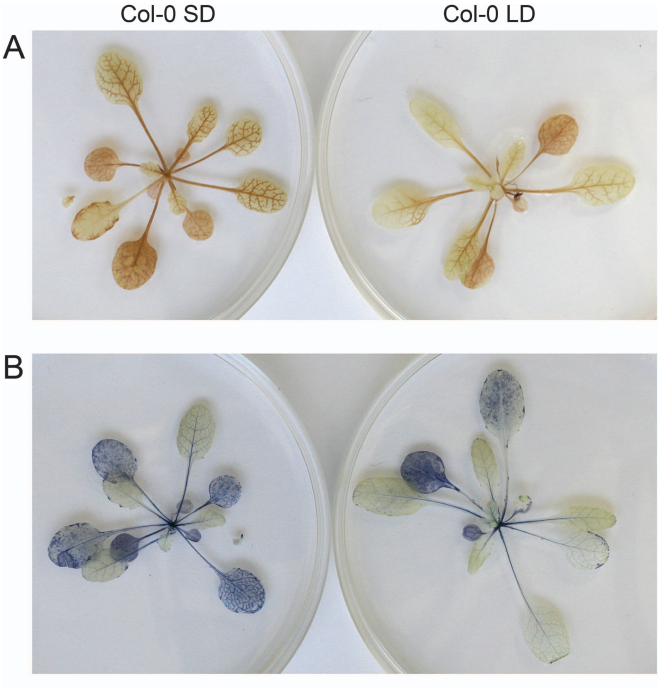

Figure 2. Accumulation of H_2O_2 **(A)** and Superoxide **(B)** in Col-0 Leaves Grown under SD or LD Conditions.

Accumulation of H_2O_2 and superoxide was detected using DAB (diaminobenzidine; Sigma-Aldrich) and NBT (nitroblue tetrazolium; Sigma-Aldrich) substrates, respectively. Rosettes were excised at the end of the light period, and incubated on Petri dishes containing 0.1 mg ml^{-1} solution of DAB (pH 3.8) or a 5 mg ml^{-1} solution of NBT overnight in darkness. In the subsequent morning, the dishes were transferred to growth light (130 μmol photons m^{-2} s^{-1} at 20°C) for 1 h and, thereafter, the rosettes were incubated in ethanol until chlorophyll was bleached.

Length of the photoperiod modifies not only the ROS metabolism, but also the sugar metabolism of leaves. Control of metabolism and growth by photoperiod has been tested by transferring 21-day-old *Arabidopsis* plants to various light–dark regimes with 2–12 h of light in 24-h cycles and by analyzing the growth rate of rosettes, the metabolites (sugars, amino acids, organic acids) and metabolic enzymes in leaves 3 weeks after the transfer (Gibon et al., 2009). In this experiment, the highest positive correlation was found between the growth rate of rosettes and the degradation rate of starch in the dark. Growth under the SD photoperiod increased the synthesis rate of starch in the light period, whereas the degradation rate of starch in the dark period was strongly decreased in comparison to LD (Lu et al., 2005; Gibon et al., 2009). The molecular mechanism controlling the transient formation of starch under various light–dark regimes is not known, but several mechanisms including feedback inhibition from carbohydrate metabolism, redox regulation, and transcriptional control of chloroplast enzymes have been proposed (Zeeman et al., 2007). Accumulation of sucrose and maltose in night correlated positively with the starch degradation rate (Lu et al., 2005), suggesting that the feedback inhibition from end products may not be a primary cause for slow starch degradation rate in SD-leaves. The expression of the genes encoding starch-metabolizing enzymes is under light-dependent circadian control (Lu et al., 2005). However, the degradation rate of starch declined/increased already in the first night after a change of photoperiod from LD to SD and vice versa, respectively (Lu et al., 2005), suggesting that, if the transcriptional control is involved in the regulation of starch breakdown, the signal should preferably come directly from chloroplast to nucleus rather than from the external input.

Redox regulation of enzymes in starch metabolism likely is a key mechanism that controls the differential starch turnover in plants acclimated to SD and LD photoperiods. ADP-glucose pyrophosphorylase (AGPase) is a key enzyme in starch synthesis that controls the flux from photosynthates to starch. AGPase is a heterotetrameric enzyme that consists of large and small subunits, and is redox-activated in light by thioredoxin that reduces the disulphide bridge between small subunits (Hendriks et al., 2003). Also, the enzymes involved in starch degradation, glucan, water dikinase (GWD), dual specificity protein phosphatase (DSP4), and β-amylase 1 (BAM1) have been shown to be under redox control (Mikkelsen et al., 2005; Sokolov et al., 2006; Sparla et al., 2006). Prior to degradation by amylases, starch granules are reversibly phosphorylated by GWD and DSP4 (Zeeman et al., 2007). This reversible phosphorylation is proposed to disrupt the crystalline structure of amylopectin and mutant analyses have shown that both enzymes are necessary for efficient remobilization of starch in *Arabidopsis* (Yu et al., 2001; Ritte et al., 2002; Zeeman et al., 2010). All these enzymes are reported to be regulated by thioredoxins (Hendriks et al., 2003; Mikkelsen et al., 2005; Sokolov et al., 2006), pointing to the importance of the thioredoxin system in the regulation of starch metabolism. Besides controlling enzyme activities, thioredoxins are involved in ROS scavenging (Mittler et al., 2004). Thus, the elevated accumulation of ROS in illuminated SD-leaves (Figure 2) may impact on the activity of the enzymes in starch metabolism by challenging the thioredoxin systems in chloroplast. Photosynthetic carbon fixation is feedback-regulated by starch metabolism (Stettler et al., 2009). It is thus likely that the redox-dependent regulation of starch metabolism adjusts the rate of photosynthetic carbon fixation with the growth potential of SD-acclimated *Arabidopsis*.

As reviewed in the previous chapters, particularly short photoperiods induce structural and metabolic changes in *Arabidopsis* leaves. Global transcript profiling approaches have been used to reveal the specific gene clusters related to the acclimation of *Arabidopsis* to the SD photoperiod and to the maintenance of the metabolic state in the SD photoperiod (Queval et al., 2007; Tables 1 and 2). When *Arabidopsis* plants grown for 2 weeks under a 12-h/12-h photoperiod were transferred to SD, the majority of genes differentially expressed in leaves in the second day after the transfer were up-regulated (Cluster 1 genes, Table 1). These Cluster 1 genes are postulated to be important for acclimation of *Arabidopsis* to the SD photoperiod, based both on the

Table 1. Differentially Expressed Genes (Cluster 1) in *Arabidopsis* Leaves after Transfer from 12L/12D Rhythm to Short-Day Conditions.

AGI code	Fold change	Description	Location	Biological process
AT3G27060*	7,12	ATTSO2		Cell cycle
AT1G28160	3,69	Member of the ERF subfamily B-1 of ERF/AP2 transcription factor family	Nucleus	Transcription
AT2G40350*	3,67	Member of the DREB subfamily A-2 of ERF/AP2 transcription factor family		Regulation of transcription
AT4G30650*	3,23	Unknown protein		
AT1G69190	2,96	Bifunctional cytosolic hydroxymethyldihydropterin pyrophosphokinase/dihydropteroate synthase (HPPK/DHPS)	Cytosol	Tetrahydrofolate biosynthesis
AT1G28520	2,92	VASCULAR PLANT ONE ZINC FINGER PROTEIN		
AT2G15970*	2,82	*ARABIDOPSIS THALIANA* COLD-REGULATED413 PLASMA MEMBRANE 1	Plasma membrane, vacuole	
AT1G53290	2,79	Galactosyltransferase family protein		Protein glycosylation
AT2G24330	2,74	Unknown protein		
AT2G21660*	2,70	COLD, CIRCADIAN RHYTHM, AND RNA BINDING 2 (CCR2) (AtGRP7)		Circadian rhythm
AT3G13500	2,64	Unknown protein		
AT5G15960↑	2,62	Cold and ABA inducible protein kin1		
AT2G30720	2,61	Thioesterase family protein		
AT2G24290	2,38	Na+- and K+-sensitive 1		
AT3G26470	2,37	Unknown protein		
AT1G13930*	2,33	Involved in response to salt stress		
AT2G35733	2,33	Unknown protein		
AT2G47070	2,32	SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 1		
AT2G42070	2,31	*ARABIDOPSIS THALIANA* NUDIX HYDROLASE HOMOLOG 23	Chloroplast	
AT3G26740↓*	2,26	CCR-LIKE	Chloroplast	Circadian clock
AT1G10760*	2,13	STARCH EXCESS1, starch degradation	Chloroplast	Carbohydrate metabolism
AT2G40840*	2,12	DISPROPORTIONATING ENZYME 2	Cytosol	Carbohydrate metabolism
AT1G20440↑*	2,10	COLD-REGULATED 47 Dehydrin		
AT4G11600*	2,08	GLUTATHIONE PEROXIDASE 6	Chloroplast, mitochondria, cytosol	Oxidative stress defense
AT4G26820	2,06	Unknown protein		
AT1G05170↑	2,04	Galactosyltransferase family protein		Protein glycosylation
AT1G52870	2,04	Peroxisomal membrane protein-related		
AT3G18080*	2,04	B-S GLUCOSIDASE 44	Cell wall	Carbohydrate metabolism
AT5G62350*	2,04	Invertase/pectin methylesterase inhibitor family protein		
AT5G01370	2,03	ALC-INTERACTING PROTEIN1	Nucleus	
AT1G20620*	2,01	CATALASE 3	Mitochondrion, peroxisome	Hydrogen peroxide catabolic processes
AT5G13930↑	0,35	CHALCONE SYNTHASE	ER	Flavonoid biosynthesis

Arabidopsis was grown for 2 weeks in 12-h photoperiod and then transferred to 8-h SD photoperiod for 2 d. Gene expression is indicated as a ratio of transcript level in leaves transferred to SD in comparison to leaves before the transfer. The fold-change values are means of three independent biological replicates. ↑↓, genes induced or repressed by high-light treatment, respectively (accession AT-00246 in Genevestigator database, Kleine et al., 2007). * Genes repressed in *Arabidopsis* shoot apex after transfer of 5-week-old plant from SD to LD photoperiod (see text for details). Rosette leaves were harvested from plants grown under 100 μmol photons m^{-2} s^{-1} at 20°C under 12L/12D for 2 weeks and thereafter transferred to SD (8L/16D) for 2 d. Total RNA was isolated with Trizol reagent and labeled by the aminoallyl method with Cy3 or Cy5 fluorescent dyes. RNA isolation, cDNA synthesis, labeling, hybridization, and the data analysis were performed as described in Lepistö et al. (2009). Genes up-regulated more than twofold or down-regulated more than 0.5-fold with $P < 0.10$ are shown in the table.

biological process assigned to the differentially expressed gene and on the previous microarray analyses (the Genevestigator database of 6100 ATH1 experiments, www.genevestigator.com/gv/index.jsp; Hruz et al., 2008). Stimulated Cluster 1 genes include a gene associated with the cell cycle as well as genes involved in the regulation of transcription and circadian rhythm. Furthermore, ~50% of Cluster 1 genes were moderately or strongly repressed in *Arabidopsis* shoot apex after

Table 2. Differentially Expressed Genes (Cluster 1) in SD-Grown *Arabidopsis* Leaves in Comparison to LD-Grown Leaves.

AGI code	Fold change	Description	Location	Biological process
AT4G27440↓	16,71	PROTOCHLOROPHYLLIDE OXIDOREDUCTASE B	Chloroplast	Chlorophyll biosynthesis
AT2G24330	5,14	Unknown protein		
AT3G52610	5,04	Unknown protein		
AT1G28520	5,01	VASCULAR PLANT ONE ZINC FINGER PROTEIN (VOZ1)		
AT2G42070	4,83	*ARABIDOPSIS THALIANA* NUDIX HYDROLASE HOMOLOG 23	Chloroplast	
AT4G26820	4,43	Unknown protein		
AT5G03350↓	4,18	Legume lectin family protein		
AT1G53290	4,06	Galactosyltransferase family protein		Protein glycosylation
AT5G02160↓	3,81	Unknown protein	Chloroplast	
AT1G12090	3,57	EXTENSIN-LIKE PROTEIN		Lipid transport
AT2G15970	3,41	COLD REGULATED 413 PLASMA MEMBRANE 1		
AT2G26830	3,38	EMBRYO DEFECTIVE 1187		
AT2G16030	3,22	Methyltransferase		
AT1G33850	3,13	40S ribosomal protein S15		Translation
AT1G28160	3,13	Member of the ERF subfamily B-1 of ERF/AP2 transcription factor family	Nucleus	Transcription
AT3G15000	3,10	DAG (differentiation and greening)-like	Mitochondria	
AT2G44930	3,04	Unknown protein		
AT2G20420	2,98	Succinyl-CoA ligase	Mitochondrion	
AT1G49500↓	2,90	Unknown protein		
AT5G50890	2,86	Unknown protein		
AT5G62350	2,84	Invertase/pectin methylesterase inhibitor family protein		
AT1G73770	2,79	Unknown protein		
AT4G01210	2,69	Glycosyltransferase family protein		
AT3G08010	2,63	ATAB2	Chloroplast	Biogenesis of Photosystem I and II
AT2G30720	2,47	Thioesterase family protein		
AT1G48920	2,43	NUCLEOLIN LIKE 1	Nucleolus	rRNA processing
AT2G45170↓	2,40	AUTOPHAGY 8E		Autophagy
AT2G26135	2,36	Zinc finger family protein		
AT4G14230	2,29	Unknown protein		
AT1G20620	2,25	CATALASE 3	Mitochondrion, peroxisome	Hydrogen peroxide catabolic processes
AT5G01370	2,22	ALC-INTERACTING PROTEIN1	Nucleus	
AT1G20020	2,13	LEAF FNR 2	Chloroplast	Photosynthesis
AT3G15800	2,07	Glycosyl hydrolase family 17 protein		Carbohydrate metabolism
AT5G58250	2,06	Unknown protein		
AT5G45300	2,04	BETA-AMYLASE 2		Carbohydrate metabolism
AT5G14200	0,48	ISOPROPYLMALATE DEHYDROGENASE 1		Leucine biosynthesis
AT3G07440	0,48	Unknown protein		
AT2G15020	0,47	Unknown protein		
AT4G13770	0,45	CYTOCHROME P450 83A1		Glucosinolate biosynthetic process
AT5G04140	0,43	FERREDOXIN-DEPENDENT GLUTAMATE SYNTHASE 1	Chloroplast, mitochondrion, apoplast	Photorespiration
AT2G38170	0,43	ATCAX1, RARE COLD INDUCIBLE 4	Vacuole	Cellular manganese and zink ion homeostasis

Table 2. Continued

AGI code	Fold change	Description	Location	Biological process
AT2G38230	0,42	PYRIDOXINE BIOSYNTHESIS 1.1	Cytosol, chloroplast	Vitamin biosynthesis
AT3G22890	0,42	ATP SULFURYLASE 1	Chloroplast	Sulfate assimilation
AT1G64500	0,33	Glutaredoxin family protein		Cell redox homeostasis
AT1G23130	0,33	Polyketide cyclase/dehydrase and lipid transport superfamily protein		
AT1G67865	0,33	Unknown protein		
AT2G21970	0,32	STRESS ENHANCED PROTEIN 2, chlorophyll a/b-binding protein	Chloroplast	Photosynthesis
AT4G35090	0,32	CATALASE 2	Peroxisome	Photorespiration
AT1G37130	0,29	*ARABIDOPSIS* NITRATE REDUCTASE 2	Plasma membrane, vacuole	Nitrate assimilation
AT3G09390	0,28	*ARABIDOPSIS THALIANA* METALLOTHIONEIN-1		Cellular copper ion homeostasis

The fold-change values are means of three independent biological replicates. ↓ Genes repressed by high-light treatment (accession AT-00246 in Genevestigator database; Kleine et al., 2007).
Rosette leaves were harvested from plants grown under 100 μmol photons m^{-2} s^{-1} at 20°C under SD for 4 weeks and under LD for 3 weeks. Total RNA was isolated with Trizol reagent and labeled by the aminoallyl method with Cy3 or Cy5 fluorescent dyes. RNA isolation, cDNA synthesis, labeling, hybridization, and the data analysis were performed as described in Lepistö et al. (2009). Genes up-regulated more than twofold or down-regulated more than 0.5-fold with $P < 0.10$ are shown in the table.

transfer of 5-week-old plants from the SD to LD photoperiod promoting flowering (accession number AT-00326 in Genevestigator database; Balasubramanian et al., 2006). This indicates that the Cluster 1 genes are important in maintenance of the vegetative phase of the shoot apex in SD conditions. Although the SD-grown leaves structurally and functionally resemble the leaves acclimated to low light intensity (Figure 1; Lepistö et al., 2009), only five genes differentially expressed after transfer of plants from 12L/12D rhythm to SD respond to light intensity (Table 1). From these genes, *COLD, CIRCADIAN RHYTHM, AND RNA BINDING2 -LIKE* gene (*CCR-LIKE*) is an interesting one, since its expression is controlled by circadian clock, photoperiod, and light intensity (Table 1). The expression of CCR-*LIKE* is stimulated in leaves transferred to short photoperiod, whereas the gene is repressed in shoot apex after transfer of plants to long photoperiod and also in leaves exposed to high light. *CCR-LIKE* shows homology to *CCR2* gene that is also up-regulated in *Arabidopsis* leaves under the SD photoperiod (Table 1). CCR2 controls the stability of its own and other target transcripts (Staiger et al., 2003), while *CCR-LIKE* gene encodes chloroplast-localized protein with unknown function. Despite the accumulation of ROS in SD-leaves, the Cluster 1 genes do not respond to treatment of leaves with H_2O_2 (accession number AT-00185 in Genevestigator database), suggesting that the expression of these genes is not primarily controlled by H_2O_2 signaling cascade. Therefore, the enhanced accumulation of H_2O_2 in SD-acclimated plants is likely not a factor that induces acclimation to the SD photoperiod.

The transcript profiling of plants shifted to the SD photoperiod did not highlight any distinct metabolic pathway (Table 1). The genes involved in sugar and starch metabolisms were induced, which is likely linked with the modification of the diurnal cycle of starch metabolism in SD-plants. The key enzyme in flavonoid biosynthesis, *CHALCONE SYNTHASE*, was strongly repressed after transfer to the SD photoperiod, being in accordance with the low accumulation of anthocyanins in SD-grown *Arabidopsis* leaves (Lepistö et al., 2009).

Comparison of transcript levels in SD- and LD-acclimated leaves did not reveal either any drastic differences in the expression of genes involved in primary metabolism or stress responses (Table 2; Queval et al., 2007). The majority of differentially expressed genes were activated under SD conditions in comparison to LD-grown leaves (Table 2). Thirty-four percent of the Cluster 1 genes (Table 1) were also differentially expressed in leaves grown in SD conditions (Table 2). Repressed genes in SD include genes connected to nitrogen (*NITRATE REDUCTASE 2* and *FERREDOXIN-DEPENDENT GLUTAMATE SYNTHASE 1*) and sulfate assimilation (*ATP SULFURYLASE 3*), implying reduced growth capacity of SD-grown *Arabidopsis*. Also, some distinct genes related to cellular redox control (*CATALASE 2*, *THIOREDOXIN H3*, *METALLOTHIONEIN-1*, *GLUTAREDOXIN*) were repressed in SD-acclimated plants. *CATALASE 2* encodes a peroxisomal isoform of catalase that detoxifies H_2O_2 produced in photorespiration. Interestingly, the abundances of other photorespiratory enzymes except CATALASE2 were higher in SD-acclimated plants (Victor et al., 2010). This indicates that photorespiration is enhanced in SD-leaves, while the scavenging machinery in peroxisome is likely down-regulated. This provides further evidence for the hypothesis that elevated production of ROS in SD-leaves is an inductive regulatory mechanism that controls metabolism in SD-acclimated leaves and not a consequence of oxidative stress.

Acclimation of plants to low light increases the light-harvesting capacity in chloroplasts, especially in Photosystem II (Walters and Horton, 1995). Acclimation of *Arabidopsis* to SD induced identical modifications in thylakoid LHCII complexes as acclimation to low light intensity (Figure 1; Lepistö

et al., 2009). The high accumulation of *PROTOCHLOROPHYLLIDE OXIDOREDUCTASE B* (*PORB*) transcripts in SD-grown leaves is likely associated with the tendency of the SD photoperiod to maintain high light-harvesting capacity compared to LD-grown plants (Table 2). POR catalyzes the light-dependent reaction of chlorophyll biosynthesis and this enzyme is encoded by three genes in *Arabidopsis* (*PORA*, *PORB*, *PORC*) (Reinbothe et al., 1996). From POR genes, both *PORB* and *PORC* are expressed in *Arabidopsis* rosette leaves under rhythmic growth conditions (Matsumoto et al., 2004), whereas only *PORB* gene is repressed by high-light treatment (accession AT-00246 in Genevestigator database; Kleine et al., 2007). The other genes of chlorophyll biosynthesis were not up-regulated in SD-grown *Arabidopsis* (Table 2). This discrepancy may be due to the lower response of the other chlorophyll biosynthesis genes to changes in light intensity (accession AT-00246 in Genevestigator database; Kleine et al., 2007). Higher level of PORB transcript in SD-grown leaves in comparison to LD-leaves is likely related to a tendency to maintain higher LHCII capacity in SD-chloroplasts in comparison to LD-chloroplast.

LIGHT SIGNALING PATHWAYS CONTROLLING THE DEVELOPMENT OF PHOTOSYNTHETIC TRAITS

Light controls the entire plant lifecycle from the germination of seeds to the production of the new generation (Sullivan and Deng, 2003). The structural and functional characterization of photosynthetic traits in *Arabidopsis* leaves acclimated to various photoperiods indicates that the photoperiod-induced modifications in leaf anatomy, photosynthetic parameters, and ultrastructure of chloroplasts mimic the changes observed in leaves acclimated to different light quantities (Lepistö et al., 2009). Thereby, the question is how the light-intensity-dependent and photoperiod-dependent signaling pathways are interacting with each other upon leaf development. Light is directly perceived by blue (cryptochromes CRY, phototropins, and zeitlupe ZTL) and red (phytochromes PHY) light receptors that, in turn, activate the complex signaling networks inducing a high number of light responses in plants, including photomorphogenetic and photoperiodic development. Besides light receptors, chloroplasts also mediate light-induced signals that control the biogenesis of chloroplast and acclimation of plants to light intensity.

Molecular Bases of Plant Circadian Clock

The photoperiodic signaling pathway has mainly been dissected in the transition from vegetative phase to flowering phase, whereas less attention has been paid to the photoperiodic control of the vegetative development. The regulatory pathway leading to induction of flowering in *Arabidopsis* under LD comprises extremely complex networks of multiple functionally redundant regulators within a circadian clock (for recent comprehensive reviews, see Turck et al., 2008; Harmer, 2009; Imaizumi, 2010; Song et al., 2010). In brief, the ability to respond to photoperiod requires the mechanism to measure the day length via the action of circadian clock. Under conditions promoting flowering, light receptors entrain the circadian clock to a 24-h cycle. The light signaling pathway that resets the clock is still not clear but light induces the expression of the genes within a clock, *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), and *PSEUDO RESPONSE REGULATORs* (*PRRs*) (Harmer, 2009). These genes are proposed to act in the clock transcriptional feedback loops together with *TIMING OF CAB EXPRESSION 1* (*TOC1*) and other clock genes (Imaizumi, 2010; Song et al., 2010). The feedback loops control the interaction of clock components, ZTL and GIGANTEA (GI), which, in turn, are involved in the regulation of CONSTANS (CO) expression, a master clock-dependent transcription regulator (reviewed by Imaizumi, 2010). Furthermore, light also affects posttranscriptional regulation of CO protein by red-light-dependent (PHYB) destabilization and far-red-light (PHYA) and blue-light-induced (CRY2) stabilization of CO protein (Valverde et al., 2004; Jang et al., 2008; Liu et al., 2008). Accordingly, CO protein accumulates only at the end of the LD photoperiod. CO protein promotes flowering by inducing the expression of a floral integrator gene *FLOWERING LOCUS T* (*FT*). The photoperiod is perceived in leaf vascular tissues, in which the CO and FT proteins accumulate only under favorable photoperiod. FT protein is transported to shoot apex to promote induction of genes inducing flower development (Corbesier et al., 2007; Turck et al., 2008). Importantly, this simplified summary depicts only the main streams of photoperiodic regulatory systems in flowering. Besides the interaction with clock components, light signaling has multiple independent targets in the regulatory photoperiodic networks. Furthermore, circadian clock outputs also control the light signaling input to the clock (Harmer, 2009). Many known clock genes have also a discrete role in light signaling (see the references in Harmer, 2009), indicating the intimate relationship between the clock and light signaling in plants. Recently demonstrated epigenetic control of flowering further inserts the complexity of the photoperiodic regulatory network in *Arabidopsis* (Jiang et al., 2008; He, 2009; Jackson, 2009).

Tuberization in potatoes as well as bud formation and growth cessation in trees are photoperiodic responses that have been less distinctly dissected at the molecular level compared to induction of flowering. Nevertheless, SD-induced tuberization in potato and dormancy in trees seem to recruit molecular components identical to those involved in the induction of flowering in *Arabidopsis*, namely CO and FT orthologs in potato and *Populus* (reviewed by Lagercrantz, 2009; Olsen, 2010). For example, CO–FT regulon controls the active shoot elongation of *Populus* under the LD photoperiod (Bohlenius et al., 2006; Olsen, 2010), suggesting the existence of a general mechanism involving FT as a final target in various photoperiodic signaling networks in different plant species.

Light Signaling Pathways

A number of comprehensive reviews on chloroplast biogenesis, signaling networks of light receptors, and chloroplast-

to-nucleus retrograde signaling pathways have recently been published (Bae and Choi, 2008; Larkin and Ruckle, 2008; Pogson et al., 2008; Woodson and Chory, 2008; Kleine et al., 2009; Inaba, 2010; Jung and Chory, 2010). Here, only an overview on these signaling pathways is presented. Light is a primary environmental cue that controls the biogenesis of chloroplasts in angiosperm species. In dark-germinated seedlings, proplastids differentiate into etioplasts, in which a substantial number of photosynthetic proteins are already present, including POR enzyme, protease complexes, ATPase, Rubisco, Cyt b6f, and individual subunits of photosystems (Kanervo et al., 2008). Upon light treatment of the etiolated seedlings, a large number of photosynthetic proteins accumulate rapidly in 24 h (Kanervo et al., 2008). The development of etioplast to chloroplast is triggered by light by two primary mechanisms. First, the phytochromes and cryptochromes induce a removal of the repressor molecules from nucleus, such as CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and PHYTOCHROME-INTERACTING FACTORs (PIFs), that maintain plant scotomorphogenic development in darkness (Bae and Choi, 2008; Bu et al., 2011). These repressors of photomorphogenesis prevent the accumulation of the positive transcription factors of light-induced genes by triggering their proteolytic degradation in the 26S proteasome. After removal of the repressors from the nucleus, the positive transcription factors, including HY 5 (LONG HYPOCOTYL 5), LAF1 (LONG AFTER FAR-RED LIGHT 1), HFR1 (LONG HYPOCOTYL IN FARRED 1), and GOLDEN2-LIKEs (GLKs) (Bae and Choi, 2008; Waters et al., 2009), accumulate, which, in turn, activate the expression of photosynthesis-associated nuclear genes (PhaNGs). Second, in angiosperms, the chlorophyll synthesis depends on light (Reinbothe et al., 1996). The reduction of protochlorophyllide to chlorophyllide is energized by photons absorbed by protochlorophyllide bound to the POR enzyme.

Besides photoreceptor-mediated pathways, the retrograde signals from chloroplast to nucleus have also been shown to modify the expression of PhaNGs. The transcription of PhaNGs is down-regulated if the biogenesis of chloroplast is restrained or the chloroplast function is severely defective (Pogson et al., 2008; Woodson and Chory, 2008; Inaba, 2010). To dissect the nature of the retrograde signals, a genetic screen for *gun* mutants (*genomes uncoupled*) was employed in *Arabidopsis* (Mochizuki et al., 2001). The isolated *gun* mutants had a higher number of PhaNGs transcripts in seedlings treated with plastid-bleaching-inducing herbicide, norflurazon compared to wild-type line, indicating a weakened repression signal from chloroplast to nucleus. All but one (*gun1*) *gun* line had mutations in genes encoding the enzymes of the tetrapyrrole pathway that produces chlorophyll, heme, and the chromophore of phytochromes in chloroplasts (Nott et al., 2006). Mg-protoporphyrin, a first intermediate of the chlorophyll branch of the tetrapyrrole pathway was identified as a promising signaling component (Strand et al., 2003). However, the re-analyses of the accumulation of chlorophyll intermediates in *gun* mutants have challenged the hypothesis of Mg-protoporphyrin as a repressing signal for PhaNGs transcription (Mochizuki et al., 2008; Moulin et al., 2008). On the other hand, it has been reported that the incubation of *Chlamydomonas* cells in darkness with Mg-protoporphyrin or hemin activated a set of light-responsive nuclear genes (Vasileuskaya et al., 2004; von Gromoff et al., 2006, 2008). Furthermore, a recent report by Woodson et al. (2011) shows that transgenic plants overexpressing plastid *FERROCHELATASE 1* (*FC1*) have a *gun* phenotype in the presence of norfluranzon. FC1 catalyzes heme synthesis in chloroplast. According to the authors' conclusion, heme that is exported from chloroplast may be used as a signal to control PhaNG expression in nucleus via an unknown mechanism. Finally, the intermediates of chlorophyll biosynthesis were also recently demonstrated to act as a positive plastidial signal in the regulation of the nuclear DNA replication in unicellular red alga and in synchronized plant suspension culture during the cell division (Kobayashi et al., 2009, 2011). These examples suggest that tetrapyrrole intermediates can, indeed, initiate the signal from chloroplast to nucleus.

Besides scoto/photomorphogentic differentiation of plants, the development of the leaf photosynthetic structures depends on the light intensity in the plant habitat. Plants adjust the leaf and cell morphology as well as the molecular composition and the number of chloroplasts to the incident light conditions to optimize the absorption and conversion of solar energy to biomass. This acclimation includes the modulation of the stoichiometry of photosystems and the light-harvesting antenna size in thylakoids, changes in the number of stromal enzymes, and the induction of a complex set of antioxidant systems in high light (Walters and Horton, 1995; Vanderauwera et al., 2005; Bartoli et al., 2006; Li et al., 2009). It has been suggested that the photoreceptors do not play a major role in the acclimation of photosynthesis to light intensity (Walters et al., 1999). Instead, a number of studies point to the contribution of chloroplast-to-nucleus retrograde signals in the light intensity-dependent modification of the chloroplast ultrastructure (Pfannschmidt et al., 1999; Pursiheimo et al., 2001; Piippo et al., 2006; Muhlenbock et al., 2008; Foyer and Noctor, 2009). Vivid debate has been raised on the origin of the chloroplast signals in the light acclimation process. The altered redox state of the photosynthetic electron transport chain (PET) is essential for the initiation of acclimatory processes, whereas both the redox state of the plastoquinone pool (Pfannschmidt et al., 1999) and the acceptor side of PSI (Piippo et al., 2006) have been proposed to be the primary source of a PET signal. In the latter case, both the reactive oxygen species (Muhlenbock et al., 2008) and the thylakoid-bound STN7 kinase (Pursiheimo et al., 2001; Pesaresi et al., 2007), the activity of which is controlled both by PET and thioredoxin (Vener et al., 1997; Rintamäki et al., 2000), are conceivable signaling candidates.

Only a few downstream components involved in the chloroplast-to-nucleus retrograde signaling have been identified so far. In contrast to the other *gun* mutants, *gun1* did not exhibit lesions in tetrapyrrole metabolism. *GUN1* encodes a chloroplast

pentatricopeptide repeat-containing protein (Koussevitzky et al., 2007) that is proposed to act as a switchboard mediating the signal inside the chloroplast from tetrapyrrole intermediates, from chloroplast translation machinery (Koussevitzky et al., 2007; Woodson and Chory, 2008; Cottage et al., 2010), and probably also from the redox state of PET (Inaba, 2010; Sun et al., 2011) to an unknown component. A recent paper reported on the identification of a highly promising component that mediates the signal from chloroplast to nucleus. Sun et al. (2011) demonstrated that a chloroplast signal triggered a proteolytic cleavage of an envelope-bound plant homeodomain transcription factor PTM. The N-terminal fragment of PTM was transmitted to the nucleus, where it activated the expression of *ABI4*, an AP2-type transcription factor that has been previously shown to act downstream from GUN1 in the plastid-derived signaling pathway (Koussevitzky et al., 2007). Demonstration that PTM indeed acts downstream of GUN1 would significantly further elucidate the plastid-to-nucleus signaling pathway in plant cells.

ABI4 represses the expression of PhaNGs by binding to the CCAC motif upstream of light-responsive genes (Koussevitzky et al., 2007). Two positive transcription factors (GLK1 and GLK2) are essential for proper biogenesis of chloroplasts and influence the acclimation of plant to light intensity (Waters et al., 2009). GLKs preferably induce genes encoding enzymes of the tetrapyrrole pathway and nuclear encoded photosystem components (Waters et al., 2009). GLKs may act as a shared component of both photoreceptor-dependent and plastid signal-dependent signaling, since the expression of *GLK*s is regulated by PhyA and PhyB (Tepperman et al., 2006), while GLK2 has been shown to be sensitive also to plastid-derived signals (Waters et al., 2009).

COORDINATION OF LIGHT INTENSITY-DEPENDENT, PHOTOPERIODIC, AND CHLOROPLAST SIGNALING PATHWAYS IN THE DIFFERENTIATION AND ENVIRONMENTAL ACCLIMATION OF LEAVES

Both light receptors and chloroplast signals contribute to the control of leaf acclimation to light quantity, and an interaction between these signaling pathways has recently been proposed (Ruckle et al., 2007; Ruckle and Larkin, 2009). In this review, we have demonstrated that shortening of the photoperiod also alters the photosynthetic structures resembling the acclimation to low light. An interesting question is how closely the different signaling pathways are interconnected in guiding of leaf differentiation under various light regimes: the quantity, quality, and duration of light per day. Today, only fragments of the interconnected light signaling networks are known. Shading experiments have demonstrated that the light-intensity-dependent development of leaf anatomy is controlled by a systemic signal from mature leaves to developing leaves (Lake et al., 2001; Yano and Terashima, 2001), whereas chloroplasts differentiate according to local signal perceived in the developing leaves (Yano and Terashima, 2001). Accordingly, high-light-illuminated developing leaves have shade-type leaf anatomy with sun-type chloroplasts, if the mature leaves were shaded during differentiation of the young leaves. Thus, the signal determining the chloroplast ultrastructure may be perceived locally in chloroplasts of developing leaves, while the light-intensity-dependent systemic signaling arising from mature leaves resembles the mobile signal that controls photoperiodic flowering in *Arabidopsis*. This unidentified systemic signal may contribute both to light-intensity and photoperiod-dependent pathways.

Mutation in Chloroplast Proteins Alters the Plant Developmental Program by Modifying Chloroplast-to-Nucleus Retrograde Signaling

Arabidopsis mutants with defects in genes encoding chloroplast components show mutant phenotype only under specific environmental conditions (Yu et al., 2007; Kim et al., 2008; Sirpiö et al., 2008; Lepistö et al., 2009; Rosso et al., 2009; Tikkanen et al., 2010). The *flu* mutant is an elegant example of the case in which the phenotype can be caused by the activation of signaling cascade by chloroplast signal and not directly by the physiochemical effects of a compound accumulating in mutant plants. The *flu* mutant is defective in feedback control of chlorophyll biosynthesis and accumulates protochlorophyllide in darkness (Meskauskiene et al., 2001). The *flu* mutant is viable under continuous light, but, if the light-germinated *flu* seedlings are transferred to the dark, a subsequent illumination of seedlings induces the production of $^1O_2$ by protochlorophyllide and results in photobleaching of the plant (Kim et al., 2008). Under these conditions, the photobleaching is not directly due to the oxidative damage caused by $^1O_2$. Instead, $^1O_2$ initiates chloroplast-to-nucleus signaling that activates the suicidal program in *flu* seedlings (Kim et al., 2008). This plastid-initiated and $^1O_2$-mediated cell death is controlled by two chloroplast proteins, EXECUTER 1 and 2 (EX1, EX2), mutations of which in *flu* background totally suppress *flu* phenotype in dark/light transition of *ex1 ex2 flu* seedlings (Wagner et al., 2004; Kim et al., 2008). Nevertheless, the conditions causing high accumulation of protochlorophyllide in the dark and drastic production of $^1O_2$ upon subsequent light period induce oxidative damage both in *flu* and in *ex1 ex2 flu* seedlings, indicating that the output responses to $^1O_2$ (signaling or damage) depend on the concentration of the effector produced in the cell.

Mutations in genes encoding chloroplast components also modify the morphogenetic development of leaves, especially the differentiation of mesophyll cells (Figure 1; Knappe et al., 2003; Hricova et al., 2006). Perturbation of leaf differentiation may be due to the lack or deficient function of a mutated chloroplast protein. Alternatively, if the chloroplast-generated signals interfere with other signaling networks, the signal from malfunctional chloroplast may impact on the developmental processes. A variegated mutant *chlorophyll a/b-binding protein underexpressed 1* (*cue1*) is an example of a signal from

malfunctional chloroplast that interferes with the developmental processes in the *Arabidopsis* leaf (Knappe et al., 2003). The chloroplasts in bundle sheath cells have unique redox, hormonal, and carbon metabolism (especially shikimate pathway) (recent review by Kangasjärvi et al., 2009), suggesting that bundle sheath cell chloroplasts likely have a minor role in the photosynthetic yield of leaves and, instead, they receive environmental signals to control the development of young leaves. The *cue1* is deficient in plastidic phosphoenolpuryvate (PEP) phosphate translocator 1 (PPT1) that provides PEP to shikimate pathway. The *cue1* has abnormal mesophyll cells with undeveloped chloroplast and green paraveinal region with properly developed chloroplast (Streatfield et al., 1999; Knappe et al., 2003). Thereby, it is surprising that PPT1 is not present in wild-type mesophyll cell chloroplasts, since the *PPT1* gene is mainly expressed in parenchyma cells of vascular tissues (Knappe et al., 2003). It was proposed that the signal generated in plastids of vascular tissue is crucial for proper differentiation of mesophyll cells and for biogenesis of chloroplasts in the interveinal mesophyll region of leaves (Figure 3A).

Disturbed energy balance may be a major cause of the developmental disorders in mutants with dysfunctional chloroplasts. For example, mutant alleles of the *SCABRAS3* gene encoding the nuclear-encoded plastid RNA polymerase showed roundish vegetative leaves with lateral teeth and protruding leaf laminae and severely impaired differentiation of mesophyll cells (Hricova et al., 2006). The authors suggested that proliferation of mesophyll cells and chloroplast biogenesis are coordinated during leaf development, which may be controlled by the energy signaling network. Recently, a central integrator of transcription networks linking the plant stress, energy, and developmental signaling was identified (Baena-Gonzalez and Sheen, 2008). The KIN10/11 protein kinases were shown to have a pivotal role in controlling energy balance, growth, and survival of *Arabidopsis*. Since chloroplasts are essential for plant energy homeostasis, the chloroplast-generated signals are obvious factors contributing to the transcription networks controlled by KIN10/11.

Interference of chloroplast-generated signals with other signaling networks in plants raises a question about the homogeneity of the signals coming from the chloroplasts. In plant cells, all chloroplasts are autonomous with regard to biogenesis and function and they communicate with the nucleus independently from each other (Yu et al., 2007). Besides the variegated-type mutants, in which each cell line has either functional or undifferentiated chloroplasts, mutations in the nuclear-encoded chloroplast proteins can generate photosynthetic cells with heterogeneous plastids (Aseeva et al., 2007; Nakanishi et al., 2009). For example, both wild-type chloroplasts and irregularly differentiated plastids were detected in a single cell of the knockout lines of a regulatory protein, CHLOROPLAST NADPH-THIOREDOXIN REDUCTASE (NTRC) (Figure 1; Lepistö et al., 2009). The presence of heterogeneous plastids in mesophyll cells of the *ntrc* knockout mutants was accompanied by the irregularly shaped palisade mesophyll cells (Figure 1). Heterogeneous chloroplast population in an *ntrc* cell may send contradictory signals to the nucleus, thereby confusing the nuclear-controlled developmental processes. The ultrastructure of chloroplasts as well as the leaf phenotype of the moderate *vipp1* knock-down mutant (VESICLE INDUCING PLASTID PROTEIN 1; see Aseeva et al., 2007) substantially resembles that of the *ntrc* line. VIPP1 has been suggested to be essential to the formation of thylakoid membrane lipid bilayers (Kroll et al., 2001; Westphal et al., 2001). Interestingly, the dose of VIPP1 protein in leaves affects the differentiation of chloroplasts; *vipp1* knock-down mutants with only 20% VIPP1 left in the leaves had undifferentiated chloroplasts, whereas, in the mutants with 40% VIPP1, both functional chloroplasts and undifferentiated plastids are present in a single cell (Aseeva et al., 2007). The variation in chloroplast differentiation stage in a single cell of the *vipp1* knock-down and *ntrc* knockout mutants suggests that (1) a threshold amount of the certain activity missing in these mutants is needed for the proper differentiation of chloroplasts and (2) the nuclear-encoded resources are not equally distributed to every chloroplast in a single cell. Deterioration of the morphological development and acclimation capacity of leaf cells detected in *ntrc* (Lepistö et al., 2009) and other pale green mutants of chloroplast proteins (Yu et al., 2007) suggests that the contradictory signals from chloroplasts with different functional status interfere with the nuclear-controlled developmental processes.

Case Studies

Below, we describe two case studies indicating how light intensity-dependent, photoperiodic, and chloroplast signaling pathways act in the developmental process (stomatal development) or control a biosynthesis of cellular component (anthocyanin biosynthesis).

Case Study 1: Stomatal Development Controlled by Environmental Cues

Operation of stomata is closely associated with the photosynthetic performance of leaves. Stomata restrict excess loss of water from plants but simultaneously they allow sufficient supply of CO_2 to photosynthesis. This trade-off situation is controlled by short-term regulation of stomatal aperture in leaves and by long-term regulation of the number of stomata in leaf epidermis. A complex regulatory network consisting of basic-helix-loop-helix (bHLH) transcription factors and negative regulators controls the differentiation and distribution of stomata in *Arabidopsis* epidermis (Bergmann and Sack, 2007; Casson and Gray, 2008; Serna, 2009). The negative regulators (e.g. TOO MANY MOUTHS, YODA) control the density of stomata in leaf epidermis by preventing the development of adjacent protodermal cells to guard cells (Bergmann and Sack, 2007). The number of stomata in leaf blade is modulated by environmental variables, such as light intensity, photoperiod, and CO_2 level. High light, long photoperiod, and low CO_2 increase the

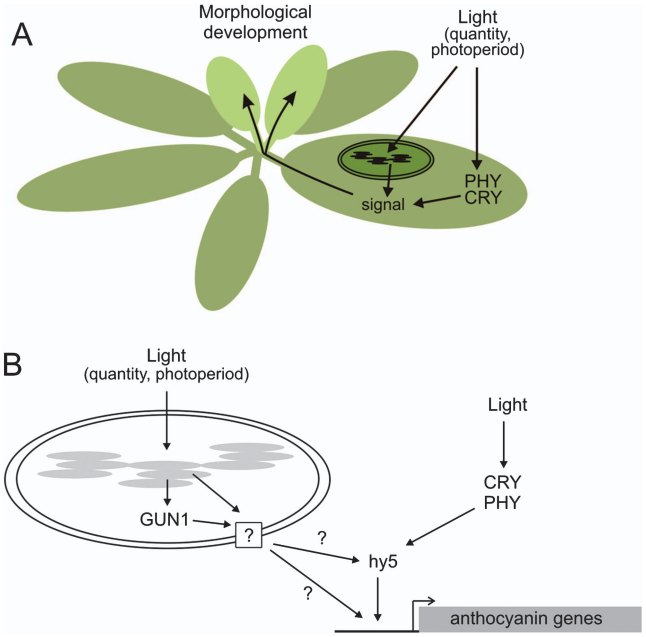

Figure 3. Diagrams Depicting the Proposed Mechanisms How Light, Perceived by Chloroplasts and Photoreceptors, Is Mediated to Signals that Interfere with the Morphological Development of Plant and with the Regulation of Anthocyanin Gene Expression.

(A) Light perceived by chloroplasts and photoreceptors in mature leaves generates a systemic signal that is crucial for proper morphological development of young leaves.

(B) Transfer of plants to an altered photoperiod modifies the redox homeostasis in chloroplasts. Signal directly from PET or mediated by GUN1 is transferred to cytosol by an unknown mechanism. This chloroplast-derived signal may control the expression of anthocyanin genes independently or via the components of the light receptor signaling pathway.

stomatal index in leaves, while low light, short photoperiod, and high CO_2 have an opposing effect (Lake et al., 2001; Casson et al., 2009; Lepistö et al., 2009). As in the acclimation of leaf anatomy to light intensity, the environmental signal to stomatal development is perceived by the mature leaves and transported to developing leaves by an unknown mechanism (Lake et al., 2001). Photoreceptors control the stomatal development by COP1-dependent signaling (Kang et al., 2009). Casson et al. (2009) showed that light intensity-dependent distribution of stomata in epidermis relied on PhyB and PIF4 transcription factor. The question is, what are the downstream components controlled by a systemic signal? The membrane-bound negative regulators, SUBTILISIN-LIKE PROTEASE 1 (SDD1) and EPIDERMAL PATTERING FACTORs (EPF) 1 and 2, control the stomatal index in leaves by repressing the differentiation of guard cells in epidermis (Bergmann and Sack, 2007; Casson and Gray, 2008). Accordingly, mutations in *SDD1* or *EPF1* increased the stomatal index in leaf epidermis in comparison to wild-type plants (Berger and Altmann, 2000; Hara et al., 2007). Coupe et al. (2006) also reported that shading of the mature leaves induced the expression of *SDD1* in non-shaded young leaves, in which the differentiation of guard cells is reduced. Thereby, the expression of *SDD1* may be a potential target of light-intensity-dependent systemic signal in *Arabidopsis* (Figure 3A).

Besides photoreceptors, chloroplasts in mature leaves likely mediate the light-dependent signal to expression of the negative regulators of stomatal development, *SDD1*, *EPF1*, and *EPF2*. Mutations in PhaNGs frequently modify the ability of a leaf to correctly respond to environmental changes, which likely is due to the misleading signals from malfunctional chloroplast to nuclear gene expression. Accordingly, a slightly lower stomatal index and substantially increased stomatal density were detected in the *ntrc* lines than in wild-type *Arabidopsis* acclimated to short photoperiod that may be due to the detected repression of *SDD1* and *EPF1* expression in the *ntrc* line (Lepistö et al., 2009). We hypothesize that, as in low light, acclimation of plant to short photoperiod enhances the expression of the negative regulators *SDD1*, *EPF1*, and *EPF2* in developing leaves, resulting in the reduced differentiation of guard cells in epidermis and lower value of stomatal index in SD-leaves (Lepistö et al., 2009). In *ntrc* lines, the signals from malfunctional chloroplasts restrain the full activation of negative regulators of stomatal differentiation in SD-grown plants that consequently allows more meristemoid mother cells to develop to guard cells in leaf primordium (Figure 3A).

Case Study 2: Biosynthesis of Anthocyanins in *Arabidopsis* Leaves

Anthocyanins are pigmented flavonoids that are synthesized as a response to various environmental cues including light, temperature, water deficiency, herbivores, and pathogens. They are proposed to protect plant leaves from photodamage induced by high light or altered light conditions (Jaakola and Hohtola, 2010). Accordingly, different qualities of light (white, red, blue, far red, UVA, and UVB) and high light activate the expression of the anthocyanin genes (Vanderauwera et al., 2005; Shin et al., 2007; Cominelli et al., 2008; Jaakola and Hohtola, 2010). Production of anthocyanins is frequently used as a visible marker in the studies of light-induced signaling pathways. Expression of anthocyanin biosynthetic genes is controlled by MYB- and basic helix-loop-helix-related (bHLH) transcription factors (Vom Endt et al., 2002). These interacting transcription factors form a complex regulatory network that both positively and negatively controls the anthocyanin biosynthesis genes (Allan et al., 2008; Cominelli et al., 2008; Dubos et al., 2008).

Light-dependent environmental factors diversely modulate the balance and the number of the regulatory complexes induced by MYB and bHLH transcription factors (Dubos et al., 2008). The photoreceptor-dependent signaling components PIF3 and HY5 have been reported to act as positive regulators of anthocyanin biosynthesis (Shin et al., 2007). Furthermore, high light strongly activates the expression of anthocyanin biosynthesis genes (Page et al., 2011). Accordingly, a high increase in the anthocyanin accumulation was observed also in sweet potato grown under LD photoperiod as compared

to growth under SD photoperiod (Carvalho et al., 2010), supporting the conclusion that long photoperiod mimics the high-light conditions in plant acclimation. Accordingly, a massive repression of the biosynthetic and regulatory anthocyanin genes is detected in SD-grown *Arabidopsis* if compared to LD-grown plants (Table 3).

In photoreceptor mutants and in mutants deficient in the light signaling component, anthocyanin genes are not induced with high-light treatment (Table 3), indicating that the photoreceptors mediate the high-light signals to anthocyanin genes. However, if the expression of anthocyanin genes in high-light-exposed *cry1* and *hy5* mutants is compared with the growth-light-illuminated *cry1* and *hy5* mutants, a substantial activation of the anthocyanin genes is still detected (Table 3). Thereby, other signaling pathways also likely contribute to the regulation of anthocyanin genes. Manipulation of the activity of the photosynthetic electron transfer chain (PET) has demonstrated that the reduction of PET induced and the oxidation of PET reduced the accumulation of anthocyanins in *Lemna gibba* (Akhtar et al., 2010). Furthermore, the *ntrc* mutants with heterogeneous chloroplasts (Figure 1) accumulate a significantly lower amount of anthocyanin than wild-type *Arabidopsis* (Lepistö et al., 2009), suggesting a chloroplast-originated signal in the regulation of anthocyanin biosynthesis. Accordingly, the genes of the anthocyanin biosynthesis are strongly repressed in illuminated *gun1 gun5* double mutant (Table 3). Furthermore, anthocyanins were nearly absent in *gun1 cry1* double mutants illuminated with high light, whereas about 40% of anthocyanins were present in the single *cry1* mutant (Ruckle and Larkin, 2009). Low accumulation of anthocyanin was also detected in *gun1* mutants under conditions that stimulated anthocyanin synthesis in wild-type plants (Ruckle and Larkin, 2009; Cottage et al., 2010). Thereby, GUN1 likely mediates a positive chloroplast signal to nuclear anthocyanin genes (Ruckle and Larkin, 2009; Figure 3B).

Chloroplast signal has been suggested to rely on the reactive oxygen species, but, in the case of light-dependent regulation of anthocyanin biosynthesis, ROS likely plays a minor role. The expression of anthocyanin genes did not change significantly in *flu* mutants producing $^1O_2$ in chloroplast (Table 3). The external treatment of plants with H_2O_2 induced a slight repression of anthocyanin gene expression (Table 3), whereas the induction of anthocyanin genes by high light was delayed in H_2O_2-accumulating *cat2* mutant deficient in peroxisomal catalase activity (Vanderauwera et al., 2005). These experiments indicate that the accumulation of ROS in cells has an opposite effect on the expression of anthocyanin genes than high light. Thereby, a response of anthocyanin biosynthesis to high light is mediated by the signaling pathway not related to ROS signaling.

Table 3. Stimulation/Repression of Genes Encoding Anthocyanin Biosynthetic Enzymes in Col-0 and Mutant Lines Treated with Different Light Quantity, Quality and Photoperiod, and with Hydrogen Peroxide.

Experimental set–up[a]	Control[a]	Expression of anthocyanin genes[b]	Material[c]	Accession number in Genevestigator[d]
Col-0 L/white light	Col-0 Dark	++	Seedlings grown in light/dark	AT-00002
Col-0/blue light	Col-0 Dark	+++	7–day-old seedlings, GL	AT-00246
Col-0/HL/3 h	Col-0 GL	+++	7–day-old seedlings, GL	AT-00246
cry1 /HL/3 h	Col-0 HL/3 h	—	7–day-old seedlings, GL	AT-00246
hy5/HL/3 h	Col-0 HL/3 h	——	7–day-old seedlings, GL	AT-00246
cry1/HL/3 h	*cry1* GL	++	7–day-old seedlings, GL	AT-00246
hy5/HL/3 h	*hy5* GL	++	7–day-old seedlings, GL	AT-00246
gun1gun5/white light	Col-0/white light	—	Seedlings with cotyledons fully open	AT-00083
Col-0/SD	Col-0/LD	——	Rosettes with eight leaves	AT-00214
flu/light	Col-0	NC	Adult rosette leaves	AT-00287
Col-0/10 mM H_2O_2	Col-0 Water	—	Hypocotyl and cotyledon emergence	AT-00185

Expression of 23 genes encoding enzymes in flavonoid biosynthetic pathway (Vanderauwera et al., 2005) was analyzed using Genevestigator database of *Arabidopsis* ATH1 22k mircoarray experiments. The experiments tested are indicated by the accession number in Genevestigator

a Description of treated and control plants used in the experiments. GL, growth light 100 μmol photons m^{-2} s^{-1}; HL, high light 1000 μmol photons m^{-2} s^{-1}; SD and LD, plants grown under short and long photoperiods, respectively.

b Stimulation or repression of the expression of the gene cluster is indicated as follows: the majority of the tested genes are up-regulated under experimental set-up: ++, the transcript ratio of the treated sample to the control sample is on average 1.5–2.5; +++, the transcript ratio of the treated sample to the control sample is on average >2.5. The majority of the tested genes are repressed under experimental set-up: –, the transcript ratio of the treated sample to the control sample is on average 0.5–0.8; —, the transcript ratio of the treated sample to the control sample is on average 0.2–0.5; ——, the transcript ratio of the treated sample to the control sample is on average < 0.3. NC, no changes.

c Plant growth condition and age of plants used in the experiments.

d Publications or contributors indicated in data depository: AT-00002, M. Alvarez; AT-00246, Kleine et al., 2007; AT-00083, A. McCormac; AT-00214, Wigge et al., 2005; AT-00287, Lee et al., 2007; AT-00185, R. Mittler, R. Mittler, H. Townsend, Z. Emmerson, B. Schildknecht.

We hypothesize that photoperiodic signal to anthocyanin biosynthesis mimics the high-light signaling and can be mediated by chloroplast components (Figure 3B). Transfer to shorter/longer photoperiod than a plant has experienced previously modifies the redox homeostasis of chloroplasts probably by modification of PET. GUN1 acts downstream of PET and mediates the signal to cytoplasm by an unknown mechanism. The chloroplast-derived signal may control the expression of anthocyanin genes independently or interfere with the components of the light signaling pathway.

ACCESSION NUMBERS

Array design and data from this article have been deposited at ArrayExpress under accession number E-MEXP-3331.

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