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Research category: SYSTEM BIOLOGY, MOLECULAR BIOLOGY, AND GENE REGULATION
Genome-wide analysis of light-dependent transcript accumulation patterns during early stages of Arabidopsis seedling de- etiolation

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This research was supported by the DFG-funded AtGenExpress project, by the Biotechnology and Biological Sciences Research Council (UK) via funding of the GARNET initiative, by the *Arabidopsis* Functional Genomic Network program of the Deutsche Forschungsgemeinschaft (KR 2020/1-3 and 1-4) to TK, the DFG grant “Analysis of phytochrome A-dependent light signaling in *Arabidopsis thaliana*” (KR2020/2-3) to FP and TK, and a grant of the Freiburg Institute for Advanced Studies (FRIAS), School of Life Sciences to TK.

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Light is among the most important exogenous factors that regulate plant development. To sense light quality, intensity, direction and duration, plants have evolved multiple photoreceptors that enable detection of photons from the UV-B to the far-red spectrum. To study the effect of different light qualities on early gene expression, dark-grown Arabidopsis seedlings were either irradiated with continuous far-red, red or blue light or received pulses of red, UV-A or UV-A/B light. The expression profiles of seedlings harvested at 45 min and 4 h were determined on a full genome level and compared to the profiles of dark controls. Data was used to identify light-regulated genes and to group these genes according to their light responses. While most of the genes were regulated by more than one light quality, a considerable number of UV-B-specific gene expression responses were obtained. An extraordinarily high similarity in gene expression patterns was obtained for samples that perceived continuous irradiation with either far-red or blue light for 4 h. Mutant analyses hint that this coincidence is caused by a convergence of the signaling cascades that regulate gene expression downstream of cryptochrome blue light photoreceptors and phytochrome A. Whereas many early light-regulated genes exhibited uniform responses to all applied light treatments, highly divergent expression patterns developed at 4 h. This data clearly indicates that light signaling during early de-etiolation undergoes a switch from a rapid, but unspecific, response mode to regulatory systems that measure the spectral composition and duration of incident light.
Light is one of the most important external factors essential for the survival of plants in their natural environment. The major source of energy for plant photosynthesis is the red through blue spectrum of the sun, for which plants must compete. Because far-red light is not absorbed by photosynthetically active tissues, this light quality is used to detect the presence of photosynthetically active competitors. Light is also a source of stress, because high light intensities and UV-B light can cause damage to cells. Additionally, duration of day length is used to gain information about hostile conditions for plant development in ecosystems with distinct seasonal changes (Sullivan and Deng, 2003; Chen et al., 2004; Franklin et al., 2005; Jenkins, 2009; Franklin and Quail, 2010).

To sense light quality, intensity, direction and duration, higher plants have evolved with many families of photoreceptors that cumulatively absorb photons from the UV-B to the far-red spectrum of sunlight (Franklin et al., 2005; Li and Yang, 2007; Christie, 2007; Demarsy and Fankhauser, 2009; Jenkins, 2009; Franklin and Quail, 2010). The genome of the model plant Arabidopsis thaliana (Arabidopsis) encodes for five different phytochromes (phyA to phyE), two cryptochromes (cry1 and cry2), two phototropins (phot1 and phot2), and three zeitlupe-like proteins. Physiological and molecular approaches give additional hints for the existence of UV-B receptor(s) (Jenkins, 2009). Together, these photoreceptors control the timing and extent of many developmental transitions, including seed germination, seedling de-etiolation, phototropism, shade avoidance responses, circadian rhythms and flowering time (Chen et al., 2004; Franklin et al., 2005; Franklin and Quail, 2010).

Perception of red and far-red light is mediated by phytochromes (Casal et al., 2003; Mathews, 2010; Franklin and Quail, 2010). The light labile phyA accumulates to very high levels in darkness, which enables sensing of the extremely low amounts of light that trigger so-called very-low-fluence responses (VLFRs). Furthermore, phyA controls high-irradiance responses (HIRs) under strong, continuous far-red light. The more light stable phytochromes, phyB to phyE, predominantly regulate classical red/far-red reversible responses, such as responses toward strong continuous red light and shade avoidance responses (Casal et al., 2003; Mathews, 2010; Franklin and Quail, 2010). Microarray studies with phyA, phyB, and phyA phyB double mutants showed that cR-induced expression of early response genes is dominated by phyA, whereas cR-imposed repression of early response genes is also controlled by phyC.
phyD or phyE (Tepperman et al., 2001; Tepperman et al., 2004; Tepperman et al., 2006).

Blue and UV-A light are sensed by a multitude of photoreceptors in higher plants that belong to completely different protein families, including Zeitlupe-like proteins; phot1 and phot2, mediating movement processes; and cry1 and cry2 controlling long-term adaptations of plant development, circadian rhythms and transition to flowering (Christie, 2007; Demarsy and Fankhauser, 2009; Lin and Todo, 2005; Li and Yang, 2007). Comparison of transcript accumulation patterns of cry1 cry2 phot1 phot2 quadruple mutants and the corresponding triple mutants demonstrated that cryptochromes are key regulators of early blue-light-induced gene expression in seedlings grown under continuous red light, whereas phototropins play only subsidiary roles (Ogishi et al. 2004). Furthermore, it is known that phyA can also function as a blue light photoreceptor (Casal and Mazzella, 1998; Poppe et al., 1998; Neff and Chory, 1998).

Microarrays can analyze gene expression on a nearly full-genome scale. Because light causes dramatic morphological and physiological changes during seedling development of higher plants, the switch between skotomorphogenesis and photomorphogenesis has been used to study light-regulated changes in gene expression using different mutants, light qualities, sampling times, and light treatments (Ma et al., 2001; Tepperman et al., 2001; Wang et al., 2002; Tepperman et al., 2004; Ulm et al., 2004; Jiao et al., 2005; Tepperman et al., 2006). In contrast to already published results, the aim of this study was the determination of early light-dependent gene expression patterns in wild type seedlings under different light qualities at the onset of de-etiolation. Therefore, 4-day-old, dark-grown Col-0 wild-type seedlings received different irradiation programs and were harvested 45 min or 4 h after exposure to light. Light treatments were adjusted to stimulate specific light responses mediated by the different photoreceptor systems in plants. Continuous far-red (cFR) light was applied to induce phyA-dependent HIR responses, whereas continuous red light (cR) was used to stimulate all phytochrome photoreceptors, including the light stable forms (Fig. S1). Furthermore, another group of seedlings were treated with cB to stimulate photoreceptors that function in this part of the spectrum. Other seedlings were treated with a single, short pulse of either red-light (pR), UV-A light (pUV-A) or UV-A/B light (pUV-A/B) before transfer back to darkness (Fig. S1). Samples treated with pR were included in order to gather
information about genes that react very sensitively to phytochrome-induced light signaling. UV-A and UV-A/B pulse treatments were included in order to study the function of the proposed UV-B receptor. RNA samples were hybridized with Affimetrix 25K microarrays, which cover nearly the complete genome of Arabidopsis.

The current investigation of light-specific transcript accumulation patterns revealed that most light-regulated genes become up- or down-regulated by more than one light quality. A major exception was UV-B, which is able to regulate specific sub- sets of genes. Microarray data and results obtained with marker genes in photoreceptor mutants indicate that phyA and cryptochrome signaling converge to regulate blue-light-dependent gene expression. Furthermore, our results clearly indicate that the mode of light signaling changes during early phases of seedling de- etiolation.

RESULTS

Selection of light-regulated genes

To reveal gene expression patterns at the initial light response during seedling de-etiolation, 4-day-old, dark-grown wild-type Arabidopsis seedlings were treated with either cFR, cR, cB or received pR, pUV-A or pUV-A/B before transfer back to darkness. Samples from de-etiolated plants and the corresponding dark controls were taken 45 minutes and 4 hours after the onset of light treatment. Light treatments were always started exactly 96 h after the start of the initial red-light treatment used to synchronize germination induction, in order to avoid interferences with inputs from the circadian clock. Total RNA isolations from two biological samples were pooled for microarray analyses. Three replicates (representing six biological samples) were hybridized for each light treatment and the corresponding dark controls. The signal intensities of the 48 arrays (8 light treatments x 2 time points x 3 replicates) were consistent across the three replicates for each light treatment as well as across all samples. No microarrays were obvious outliers in terms of median or distribution of signal intensities (Fig. S2).
To select light-regulated genes in the datasets, expression values of light-treated samples were compared to those of the corresponding dark controls. Transcript levels were regarded as light-regulated if they exhibited, first, a two-fold or greater up-regulation or down-regulation of signal intensities and, second, a statistically significant difference to expression values in the corresponding dark control (t-test: \( p < 0.05 \)) adjusted for a false discovery rate (FDR) of \( q < 0.05 \) (Benjamini and Hochberg, 1995). In total, transcripts of 784 (3% of genes on chip) and 2,447 (11% of genes on chip) genes were regarded as being light-regulated at 45 min and 4 hours, respectively (Table S1, Table S2). A higher number of genes exhibited significantly increased transcript levels rather than reduced levels both at 45 min (556 up vs. 228 down) and 4 h (1405 up vs. 1042 down).

The influence of different light treatments on the number of light-regulated genes significantly changes during the course of the de-etiolation process

Pulse-treatments with UV-A/B and UV-A light enhanced transcript levels of the highest numbers of genes at 45 min, whereas irradiations with cFR, cR, cB and pR induced fewer genes (Fig. 1A). In contrast, the largest number of transcripts became down-regulated under cB at 45 min (Fig. 1A, B). Nevertheless, at 45 min pUV-A and pUV-A/B still reduced the transcript levels of a higher number of genes at 45 min than the light qualities stimulating the phytochrome system (cFR, cR, pR; Fig. 1B).

The transcript accumulation patterns completely changes at 4 h. Now, the highest number of transcripts became up-regulated and down-regulated under cFR, followed by cB (Fig. 1C, D). Treatments with cR and pUV-A/B were less effective, but still caused the induction and the repression of a higher number of genes compared to pR and pUV-A. (Fig. 1). The strong effects of cFR, cR, and cB indicate that continuous irradiation triggers signaling cascades regulating high irradiance responses at 4 h.
Categorization of light-responsive genes

Genes with significantly altered transcript levels under the different light treatments were classified into six different categories: (1) transcription factors, (2) hormone biosynthesis, (3) genes related to other signaling processes (protein kinases, protein phosphatases, ubiquitin ligases), (4) plastid genes, (5) a miscellaneous group with genes encoding for metabolic enzymes and for proteins related to plant defense or stress responses, and (6) genes with unknown function. The relative proportion of unknown genes with altered transcript levels remained more or less constant between all treatment groups (Fig. 1).

The development of chloroplasts from etioplast progenitors is one of the most important events during transition from skotomorphogenesis to photomorphogenesis. Only a limited number of plastid-related genes were up-regulated at the early time point (Fig. 1A), and plastid-related genes were nearly completely missing in the group of down-regulated genes at both analyzed time points (Fig. 1B, D). The onset of chloroplast development is reflected in the microarray data by the high number of transcripts of plastid-related genes that become up-regulated 4 h after the start of the light treatments. A high proportion of plastid-related genes exhibited a high sensitivity toward light, because this functional class is clearly over-represented in the group of genes that became up-regulated upon pR or pUV-A at 4 h (Fig. 1C).

Pulses of UV-A/B and UV-A light caused strong changes in a larger number of miscellaneous genes compared to other light qualities at 45 min. More detailed analysis revealed that pUV-A/B and pUV-A did not induce a higher proportion of stress-related genes in the miscellaneous group compared to other light treatments (Table S1). The strongest effects on miscellaneous gene expression were detected under cFR and cB at 4 h, whereas cR light treatment was slightly less efficient (Fig. 1C, D). This finding is most probably related to strong alterations of general metabolism that are to be expected during de-etiolation and chloroplast development under prolonged irradiation.

About 40% of early light-regulated transcripts encode for factors related to transcriptional control, signaling and hormone function (Fig. 1). Pulses of UV-A and UV-A/B light were most effective in the up-regulation of genes related to transcriptional regulation and general signaling at 45 min (Fig. 1; S3A, B). Irradiation with cB, pUV-A and pUV-A/B reduced the highest number of transcripts of genes...
related to both signaling and transcriptional regulation compared with light qualities stimulating the phytochrome system (cFR, cR, pR). Hormone-related genes mainly became down-regulated under all applied light conditions at 45 min, with the cB, pUV-A and pUV-A/B treatments being the most effective (Fig. 1; S3C).

At 4 h, genes encoding for factors involved in transcriptional regulation, hormone function and other signaling processes became a smaller proportion of light-regulated transcripts (Fig. 1C, D), but not necessarily decreased in the absolute number of genes that exhibit light-induced changes in transcript levels. Irradiation with cFR and cB altered the expression of a number of genes related to transcriptional regulation and signaling and triggered the down-regulation of a higher number of hormone-related genes at 4 h (Fig. 1; S3A, B).

Treatments with cR and pUV-A/B resulted in very complex effects on gene expression patterns at 4 h. Compared to results obtained at 45 min, a lower number of transcription- and signaling-related genes exhibited increased transcript levels, whereas the number of down-regulated genes was enhanced (Fig. 1; S3A, B). Irradiation with cR for 4 h also triggered down-regulation of transcript levels from a high number of hormone-related genes compared to results obtained at 45 min, whereas the effect of pUV-A/B seems to level out upon transfer to darkness (Fig. 1; S3C). Treatments with pR and pUV-A had no or only a very weak effect on transcript accumulation of transcription-, hormone- and signaling-related genes at 4 h (Fig. 1; S3), indicating that pR and pUV-A have a rapid but transient effect on transcriptional regulation during de-etiolation and that continuous light treatments are necessary to cause strong alterations to signaling processes, transcriptional regulation, and hormone function.

The different light treatments induced a common set of early light-regulated genes

Correlation analyses between the three different replicates for each light program were performed for those genes found to be light regulated (Fig. 2A; Table S3). In contrast to the comparison of absolute numbers of genes that become up- or down-regulated by the different light treatments, correlation analyses also include differences in expression levels. High correlation coefficients (r >0.95; Table S3)
were obtained for the three replicates within the different treatment groups at the two analyzed time points, which indicate high reproducibility of the data obtained by the microarray analyses (Fig. 2A, B; quadrates in the diagonal).

Low correlation coefficients, indicative of strong differences in transcript accumulation patterns, were only obtained when comparing signal intensities of dark controls to the different light treatments at 45 min (Fig. 2A; Table S3). Correlation coefficients were high for comparisons of expression values between the different light treatments at 45 min (Fig. 2A; Table S3), indicating that the applied light qualities regulate the expression of a common set of early light-responsive genes. Small differences in correlation coefficients only became visible when comparing samples that received pUV-A/B, pUV-A or cB with those that were treated with cFR, cR or pR. This finding again indicates that stimulation of the blue light and UV-B light receptors (pUV-A/B, pUV-A, cB) more greatly alters gene expression than stimulation of the phytochrome system (cFR, cR, pR) at 45 min.

Light-specific transcript accumulation patterns became highly variable at 4 h

Correlation analyses exhibited more complex patterns at 4 h (Fig. 2A, B; Table S3), indicative of the establishment of light-specific patterns of gene regulation. Samples irradiated with cR exhibited slightly reduced correlation coefficients to all other light treatments, including pR, at 4 h (Fig. 2B; Table S3). The reduced correlation to pR indicates that cR leads to more permanent alterations in the transcript accumulation pattern, indicative of the establishment of HIRs, compared to the transient effect induced by the pulse treatment.

Compared to the other light treatments, correlation analyses revealed a relatively high degree of variation between individual replicates of pUV-A/B-treated samples at 4 h (Fig. 2B; Table S3). Correlation coefficients were low between most pUV-A/B replicates and samples that perceived cFR, cB, cR and pR. Differences were less clear between pUV-A- and pUV-A/B-treated samples, because only a few replicates exhibited clearly reduced correlation coefficients. Taken together, correlation analyses again indicate that UV-B light exerts a specific effect on light-regulated gene expression at 4 h.
There were very small differences in gene expression patterns when comparing samples treated with pR and pUV-A both to the corresponding dark control and between the two pulse treatments. (Fig. 2B; Table S3). This data again indicates that the influence of pR and pUV-A light-regulated gene expression levels out upon transfer to darkness for an extended time.

Compared to other light treatments, the lowest correlation coefficients were obtained for samples treated with cFR and cB for 4 h (Fig. 2B; Table S3). In contrast, a very high correlation ($r \geq 0.97$; Table S3) was detected for transcripts that become up- and down-regulated by cFR and cB at 4 h (Fig. 2B). Comparably high values were only detected within the three biological replicates of individual light treatments and between the 4-h pR and pUV-A samples. These results clearly indicate that, firstly, cFR and cB co-regulate a common set of genes during later stages of Arabidopsis seedling de-etiolation and that, secondly, the effect of these two light qualities on gene expression differs from other light treatments.

UV-B light regulates specific groups of genes that do not respond to other light qualities

To identify potential groups of genes that respond to one specific light treatment, transcripts regulated by more than one light quality were eliminated by comparing one set of up- and down-regulated transcripts to those of all the remaining light treatments. The procedure was repeated for each light quality and for each time point using Venn diagram analyses (Fig. S4). The quantities of specific genes identified by this approach are given in Tables I and II.

To verify the existence of light quality-specific genes, additional statistical testing was performed using three different approaches. First, FDRs were calculated using the Genedata Experessionist software (Benjamini and Hochberg, 1995). Second, FDRs were estimated from comparisons of microarray signals obtained for the three independent replicates of the different light treatments. The number of specifically up- or down-regulated genes was only regarded as significantly different to background if its proportion value $m/n$ ($m$ = number of specifically up- or down-regulated genes at 45 min or 4 h; $n$ = total number of up- or down-regulated genes at 45 min or 4 h) exceeded both estimations of the FDR (Tables I and II). Because Venn diagram
analyses were performed on the light-regulated genes that exhibited a 2-fold or greater change compared to dark controls, these analyses only took into account whether the expression level differs from dark expression level, but does not include comparisons to the expression level obtained with other light treatments. Therefore, as a third criteria for a light-specific effect, we tested whether signal intensities of proposed light-specific genes exhibit a statistically significant difference (multiple ANOVA) to signal intensities obtained with all other light treatments.

At 45 min, statistical analyses verified the existence of groups of genes that became specifically up- and down-regulated by pUV-A/B, down-regulated under cB and up-regulated by pUV-A (Table I; Fig. S4). In contrast, no significant responses were detected for light treatments that stimulate phytochrome photoreceptors (cFR, cR and pR) at 45 min (Table I; Fig. S5). At 4 h, only pUV-A/B and cFR were able to up- or down-regulate transcript levels of a specific set of genes (Table II; Fig. S4, S5). Of the genes down-regulated by cFR at 4 h, only 11 out of 106 (10.3%) exhibited significantly reduced transcript levels compared to other light treatments (Table II). Additional analyses revealed that the remaining 11 genes also exhibited significantly altered signal intensities under cB compared to other light treatments. Thus, they seem to belong to a group of genes that becomes down-regulated by cFR and cB (group $^\text{down}$; see below) rather then by cFR alone. Among the 186 genes that were identified as being specifically up-regulated by cFR at 4 h, 107 genes (57.5%) did not show significant differences in transcript levels compared to other light treatments (multiple ANOVA). Among the 79 remaining genes, 28 genes already exhibited significantly increased transcript levels at 45 min irrespective of the applied light treatment. Furthermore, 45 of the 79 genes also accumulated significantly increased transcript level either under cB and pUV-A/B or under cB alone at 4 h. The genes were excluded from Venn diagram analyses because they did not completely fulfill the criteria of a two-fold or greater up-regulation or down-regulation of signal intensities compared to dark controls. Finally, from the remaining 6 genes, 3 genes produced only very low signal intensities close to the detection level of microarray analysis and 3 genes (At1g65560, At5g54280, and At4g12970) reached signal intensities between 30 and 60 under cFR. According to these observations, it remains highly questionable whether a group of genes exists that becomes specifically up-regulated by cFR alone.
Classification of groups of co-regulated genes

During our search for genes that are specifically regulated by a given light treatment, we realized that most of the transcripts become up- or down-regulated by a combination of different light treatments. To identify groups of co-regulated genes, K-means clustering analysis was performed with the set of light-regulated transcripts. Six groups of genes could be identified that specifically responded to pUV-A/B. These groups exhibited an early transient increase (group 1\textsuperscript{up}) or decrease (group 1\textsuperscript{down}) in transcript accumulation at 45 min, a permanent increase (group 2\textsuperscript{up}) or decrease (group 2\textsuperscript{down}) of transcript levels at 45 min and 4 h, or a late increase (group 3\textsuperscript{up}) or decrease (group 3\textsuperscript{down}) at 4 h (Figs. 3A to 3C; S6A to S6C; Table S4, S5).

Another group (group 4\textsuperscript{up}) contains genes that specifically increased at 45 min in response to pUV-A (Fig. 4D; Table S4, S5). Several genes were identified that became specifically down-regulated by cB at 45 min (group 4\textsuperscript{down}; Fig. S6D; Table S5). These two groups might reflect specific responses mediated by blue-light photoreceptors.

According to clustering analyses, we propose the existence of one additional group of early down-regulated genes (group 5\textsuperscript{down}) and one additional group of early up-regulated genes that can be separated into 2 sub-groups (group 5a\textsuperscript{up} and group 5b\textsuperscript{up}). The group of early down-regulated genes (group 5\textsuperscript{down}) shows a rapid decline in transcript levels irrespective of the applied light treatment (Fig. S6E; Table S5). Group 5\textsuperscript{down} transcript levels also remained low 4 h after irradiation with cFR, cB and cR, whereas pulse treatments (pR, pUV-A, pUV-A/B) did not cause a response that lasted until the later time point. The two sub-groups of early up-regulated genes (group 5a\textsuperscript{up} and group 5b\textsuperscript{up}) displayed a strong increase in transcript levels under all applied light treatments at 45 min but differed with respect to light effects at 4 h (Fig. 3E, F; Table S4). Transcript levels in sub-group 5a\textsuperscript{up} were high at 45 min, but dropped at 4 h (Fig. 3E). In contrast, the second sub-group of early up-regulated genes (group 5b\textsuperscript{up}) still accumulated high amounts of transcripts upon irradiation with cFR, cB and pUV-A/B at 4 h (Fig. 3F).

An analysis of transcripts that were significantly altered only at 4 h but that did not exclusively respond to pUV-A/B detected three different groups. Groups of late light-regulated genes were separated based on differences in responses towards...
the applied light treatments. Group $6^{\text{up}}$ and group $6^{\text{down}}$ include genes that become up-regulated or down-regulated by all light treatments, although cFR, cB and cR were often more effective compared to pulse treatments (Fig. 3F; S6E; Table S4, S5). Many transcripts belonging to group $6^{\text{up}}$ encode for components of photosystems I and II and the corresponding antenna complexes that are necessary to perform photosynthesis (Table S4). Groups $7^{\text{up}}$ and $7^{\text{down}}$ include transcripts that strongly respond to cFR, cB and pulses of UV-A/B, whereas all other light treatments did not cause clear alterations in transcript levels (Fig. 4G; S6F; Table S4, S5). Groups $8^{\text{up}}$ and $8^{\text{down}}$ consist of genes that become solely up-regulated or down-regulated by cFR and cB (Fig. 4H; S6G; Tabel S4, S5).

**Establishment of marker genes**

To verify our classification into different co-regulated groups and to characterize marker genes for additional studies, we performed quantitative real-time polymerase chain reaction (qRT-PCR) analyses with a sub-set of light-up-regulated genes. Specific oligonucleotides were designed to determine transcript levels of $\text{PKS1 (PHYTOCHROME KINASE SUBSTRATE 1)}$, $\text{APRR5 (ARABIDOPSIS PSEUDO-RESPONSE REGULATOR 5)}$ and $\text{HY5 (LONG HYPOTYL 5)}$, which represent group $5^{\text{up}}$. $\text{CAB4 (CHLOROPHYLL-A/B-BINDING PROTEIN 4)}$, $\text{DIR1 (DEFECTIVE IN INDUCED RESISTANCE 1)}$ and $\text{AT5G48490}$ were selected to represent group $6^{\text{up}}$. $\text{CHS (CHALCONE SYNTHASE)}$ represents genes of group $7^{\text{up}}$, whereas $\text{AT5G42760}$ belongs to group $8^{\text{up}}$ (Fig. S7). Etiolated Col-0 wild-type seedlings were subjected to the same light treatments that had been used for microarray analyses, and total RNA was extracted at 45 min and 4 h. There were some differences in relative expression levels between microarray analyses and qRT-PCR results, but they did not change the overall light-dependent transcript accumulation patterns.
Different photoreceptors co-operate to regulate early light-induced gene expression

Comparison of transcript accumulation patterns at 45 min and 4 h indicates that light signaling is modulated during the course of seedling de-etiolation, most obviously for early light-regulated genes that belong to group 5^up. Whereas all applied light treatments were more or less equally effective in the regulation of transcripts in these groups at 45 min, strong differences were detected for the different light treatments at 4 h (Fig. 3; Fig. S6). To explain the observed lack of differences between the different light treatments in early light responses, it might be hypothesized that one single photoreceptor predominantly regulates early light signaling of genes in group 5^up. The best candidate for such a dominating photoreceptor is phyA, because this phytochrome accumulates to very high levels in etiolated seedlings and because physiological analyses with wild type and mutant plants clearly demonstrated that phyA can also function as a blue light receptor (Neff and Chory, 1998; Poppe et al., 1998; Casal and Mazzella, 1998).

Transcript levels of marker genes for group 5^up were determined by qRT-PCR in phyA-211 deletion mutants under the different light treatments. The lack of phyA caused a reduced response towards cFR with respect to PKS1, APRR5 and HY5 transcript accumulation at 45 min, whereas responses towards cB remained unaltered (Fig. 4A). Transcript levels of PKS1 and APRR5 also remained unaltered in phyA-211 seedlings that perceived cR and pR. In contrast, the deletion of phyA seemed to cause a slight increase in sensitivity towards pUV-A and pUV-A/B, even though interpretation is difficult because of the high variance of qRT-PCR data. Slightly different results were obtained for HY5 transcripts (Fig. 4A). Etiolated phyA-211 seedlings exhibited reduced induction of HY5 under cR and upon pR treatment, whereas responses toward pUV-A and pUV-A/B remained unaltered. This data clearly indicates that phyA is the only light receptor that senses far-red light in etiolated seedlings. Nevertheless, its function is partially or completely dispensable for the detection of red, blue, UV-A and UV-B light during early phases of seedling de-etiolation in Arabidopsis.

Results from microarray experiments with phot1 phot2 and cry1 cry2 double mutants indicated that crytochromes are mainly involved in the regulation of gene expression under cB, whereas phototropins are responsible for the control of
movement processes and cell elongation under cB (Ohgishi et al., 2004). Therefore, blue light responses of early light-induced genes were measured in cry1-304 cry2-1 double mutants. The lack of both cryptochrome photoreceptors did not alter transcript levels of PKS1, APRR5 and HY5 marker genes under cB at 45 min (Fig. 5A). This finding demonstrates that cry1 and cry2 light receptors are dispensable for blue-light-induced transcript accumulation of early light-induced marker genes. It also shows that blue light is efficiently sensed by other photoreceptors present in etiolated seedlings.

**Phytochrome A and cryptochrome photoreceptors are involved in the regulation of gene expression under continuous far-red and blue-light at 4 hours**

Etiolated phyA-211 and wild-type seedlings were treated with different light programs while cry1-304 cry2-1 double mutants were treated with cB, harvested 4 h after the onset of light, and analyzed for expression of marker genes for groups 5\textsuperscript{up}, 6\textsuperscript{up}, 7\textsuperscript{up} and 8\textsuperscript{up}. The lack of phyA clearly abolished far-red-light-induced transcript accumulation of all tested marker genes (Fig. 4B). In contrast to the strong diminishment of cFR-regulated transcript accumulation, responses towards cR, pR and pUV-A/B remained relatively unaltered or became enhanced in phyA-211. This data indicates that phyA is dispensable for the detection of red light and UV-B light at 4 h.

Analyses with the phyA-211 and cry1-304 cry2-1 loss-of-function mutants revealed a very complex pattern for cB-regulated transcript accumulation at 4 h (Fig. 5B). The loss of phyA caused at least a weak reduction in transcript levels of all tested marker genes, but did not fully abolish blue light responses. The reduction was most severe with AT5G42760, which represents group 8\textsuperscript{up} (genes exclusively up-regulated by cFR and cB). The loss of both cryptochromes did not alter blue-light-induced transcript accumulation of AT5G42760 (group 8\textsuperscript{up}) or CAB4, a marker gene of group 6\textsuperscript{up} (very high sensitivity towards all applied light treatments). A reduction in transcript levels was only detected for CHS and HY5, marker genes for groups 5\textsuperscript{up} and 7\textsuperscript{up} (genes that exhibit a strong increase in transcript levels under cFR, cB and pUV-A/B but do not respond towards cR and pR treatments). Taken together, these data indicates that phyA is involved in blue-light-regulated gene expression at 4 h, but that
it is not the only photoreceptor responsible for the detection of this part of the sunlight spectrum during early phases of seedling de-etiolation. The weak effect on transcript accumulation in *cry1-304 cry2-1* double mutants indicates that cryptochrome function is completely or at least partially dispensable for blue-light-dependent expression of marker genes in etiolated seedlings at 4 h.

**DISCUSSION**

**Light alters the expression of a high number of genes at the onset of photomorphogenesis**

Plants evolved with different photoreceptors that sense the light spectrum from the UV-B to the far-red range of the sun spectrum. These photoreceptors cooperate to control plant photomorphogenesis. In order to gain insight into the initial steps in light-induced gene expression patterns under different light qualities, microarray analyses were performed using total RNA samples from 4-day-old, etiolated Arabidopsis seedlings that were either pulse-irradiated or continuously irradiated for 45 min and 4 h.

At 45 min, transcripts of ~250 genes exhibited significant changes upon blue, red and far-red light pulse treatments, whereas a much higher number of genes exhibited significantly increased or reduced transcript levels under cFR (~1000), cR (~600) and cB (~900) at 4 h (Fig. 1). These results are in good agreement to published data obtained with full-genome chips for early or late light-regulated genes in etiolated seedlings that were irradiated with cR or cFR (Tepperman et al., 2006).

The number of light-regulated genes at 4 h is comparable to the results obtained for seedlings that have been grown under continuous white light, cB, cR and cFR for 7 d (Jiao et al., 2005), which reported fewer genes regulated by cFR. This discrepancy most probably does not depend on the differences in light sources or light intensities, because saturating photon fluence rates were used for both studies, but might rather depend on the duration of light treatments. Because Jiao et al. (2005) irradiated seedlings for 7 days after germination, the data gives insight into differences in transcript accumulation patterns between photomorphogenic and skotomorphogenic development under the different light qualities, while our experiments were designed to analyze early responses after the onset of light.
Light causes ongoing alterations in gene expression patterns of signaling-related genes during early stages of photomorphogenesis

A high proportion of early light-induced genes encodes for signaling components and transcription factors (Fig. 1; S3), similar to former studies (Tepperman et al., 2001; Tepperman et al., 2004; Tepperman et al., 2006). Several transcription factors are responsible for strong alterations in light responses, and the rapid up- or down-regulation of this group of genes further indicates that transcriptional cascades trigger downstream genes during the course of seedling de-etiolation (Quail, 2002; Jiao et al., 2007). Alterations in signaling and transcription factor transcripts at 4 h might be indicative of an adaptation toward different light qualities, since clear differences in the number of light-regulated genes became evident between the different applied light treatments.

Light-regulated transcript accumulation patterns of hormone-related genes differed from those of signaling-related genes and genes involved in transcriptional regulation. Hormone-related genes mainly became down-regulated under cFR, cB and cR at 4 h (Fig. 1; S3), comparable to Folta et al. (2003). This finding might reflect a general switch in regulatory requirements between skotomorphogenesis, during which development is controlled by endogenous factors like plant hormones and photomorphogenesis, during which light becomes more important and hormone-related genes might be shut down.

UV-B light has an unique role in the regulation of photomorphogenesis

Searching for light-specific responses revealed that most of the applied light treatments induced or repressed an overlapping set of genes and that most genes were co-regulated by more than one light quality (Fig. 3; S6). A major exception to this rule was seen with pUV-A/B treatment, which induces specific sub-sets of genes at 45 min and at 4 h that did not respond to any other light treatment, including pUV-A (Table I, II; Fig. S4). The specificity of the UV-A/B response indicates that the corresponding genes seem to be dispensable for the shift from skoto-
photomorphogenesis under light conditions that do not include UV-B light. Genes specifically regulated by UV-B might only be important for the plant’s rapid adaptation to the proposed destructive effect of this light quality.

The observed differences between pUV-A/B and the other light treatments might either be caused by regulatory events downstream of the proposed UV-B photoreceptor(s) or by a deleterious effect of this light quality on plant tissues that does not occur upon exposure to far-red, red, blue and UV-A light. Several lines of evidence favor the first hypothesis. First, experimental conditions were adapted to minimize damaging responses by UV-B (Ulm et al., 2004). Light emitted by the UV lamps was filtered by 305-nm cut-off filters to eliminate UV-C together with short wavelengths of UV-B, and irradiation time was limited to 5 min. Second, similar low doses of pUV-A/B did not induce visible damage or necroses in seedlings (Ulm et al., 2004; Oravecz et al., 2006; Favory et al., 2009). Finally, the set of pUV-A/B-specific genes was not enriched with genes that are commonly up-regulated under severe stresses like wounding, drought, salt stress or high doses of UV-C.

**Signaling cascades downstream of phyA and blue light photoreceptors seem to converge to regulate alterations in gene expression patterns**

Another interesting result of our data analyses is the similarity of transcript accumulation patterns between cFR- and cB-treated seedlings, especially at 4 h (Fig. 3; Table S3). Correlation coefficients reached levels exceeding 0.97, and further classification of transcript accumulation patterns revealed that cFR-regulated genes normally exhibited comparable effects on gene expression under cB. The only exception was a small number of genes that became specifically down-regulated by cB at 45 min.

An explanation for the observed correlation of gene expression patterns between cFR and cB is a dominant function of phyA in the regulation of not only cFR but also cB responses in etiolated seedlings, which are known to accumulate high levels of the photoreceptor. Determination of transcript levels of different marker genes in phyA-211 and cry1 cry2 double mutants confirmed former studies, which demonstrated that phyA can function as a blue light receptor (Casal and Mazzella, 1998; Neff and Chory, 1998; Poppe et al., 1998). However, with the exception of the
group 8\textsuperscript{up} marker genes, blue light still caused clear alterations in the expression of most marker genes in the \textit{phyA} knock-out mutant, which indicates that other photoreceptors are involved in the regulation of gene expression toward this light quality and can compensate for \textit{phyA} function (Fig. 4, 5). Thus, regulatory similarities between cFR and cB might rather be caused by convergence of light signaling cascades at the level of or downstream of phytochrome and blue light photoreceptors.

Several additional findings confirm co-action of cryptochromes and phytochromes in light signaling. Results obtained with a quintuple mutant lacking all phytochromes demonstrated that Arabidopsis is able to survive when plants are kept in blue light (Sellaro et al., 2009), indicating that cryptochromes can replace phytochrome function. Detailed studies with the promoter of a chalcone synthase gene from mustard demonstrated that a minimal promoter element is able to mediate light responses towards cFR, cB and UV-light. Thus, downstream signaling cascades seem to use identical promoter elements to trigger light-dependent gene expression (Kaiser et al., 1995). Finally, it has been reported that a direct interaction between both signaling cascades at the level of the photoreceptor molecules exists and that cry1 and cry2 are targets of a phyA-related kinase activity (Ahmad et al., 1998).

The mode of light signaling seems to change during early stages of the deetiolation process

Comparison of light dependent expression patterns at 45 min and 4 h gives clear hints for a switch in the mode of light regulation during the course of the deetiolation process. Correlation analyses revealed very high degrees of similarity between transcript accumulation patterns obtained with the different light treatments at 45 min (Fig. 2A; Table S3). No or only minor differences were obtained comparing the number of light-regulated genes upon pR and pUV-A treatments on the one hand and cFR, cB, and cR on the other (Fig. 1). With the exception of genes specifically responding to pUV-A/B and the small number of genes that became specifically down-regulated by cB and up-regulated by pUV-A, most of the early light regulated genes from group 5\textsuperscript{up} and group 5\textsuperscript{down} became up- or down-regulated to a similar extent by all applied light qualities at 45 min, including pR and pUV-A (Fig. 3; S6). The paucity of early light-specific responses indicates that these genes are mainly
responsible for a rapid, general response to light irrespective of the applied light quality.

One might think that the dominate effect of phyA causes the observed absence of light-specific effects. This phytochrome accumulates to very high levels in darkness and triggers VLFR responses that become induced by even weak light inputs by nearly all light qualities (Casal et al., 2003; Mathews, 2010; Franklin and Quail, 2010). Furthermore, microarray analyses using phyA and phyB mutants indicated that phyA plays a dominant role during early red and far-red light signaling (Tepperman et al., 2001; Tepperman et al., 2004; Tepperman et al., 2006). Nevertheless, transcript accumulation analyses in phyA-211 show that the lack of the photoreceptor leads to a reduction of responses to cFR, but does not completely abolish responses toward other light treatments at 45 min (Fig. 4, 5). These results demonstrate that the absence of light-specific effects during early light responses cannot be ascribed to a dominant effect of phyA in early light signaling.

In contrast, analyses of transcript accumulation patterns exhibited a much higher degree of divergence in responses toward the different applied light treatments at 4 h. Compared to results obtained at 45 min, clear differences were obtained in the number of genes that became up- or down-regulated under the different applied light treatments (Fig. 1). Correlation analyses also revealed strong differences between the different light treatments at 4 h (Fig. 2B). Light-regulated genes could be classified into groups according to their light-specific transcript accumulation patterns at the later point of time (Fig. 3; S6). Taken together, these results indicate that etiolated seedlings start to develop more diverse light-specific responses during the course of light treatments in the first hours of irradiation.

The observed alteration in light responses between 45 min and 4 h likely accompanies a switch toward high irradiance response modes of light signaling (Whitelam et al., 1993; Parks and Quail, 1993; Nagatani et al., 1993). While pulse treatments regulated at least the same number of genes as cFR, cB, and cR at 45 min, pulse effects seem to level out compared to other light treatments at 4 h (Fig. 1). All groups of light-regulated genes, except group 6, needed cFR, cB, cR or pUV-A/B to reach full light responses at 4 h (Fig. 3; S6). Correlation analyses revealed a high similarity in transcript accumulation patterns between dark controls and samples treated either with pR or pUV-A at 4 h (Fig. 2B). Because pR and pUV-A should be able to stimulate phytochrome and cryptochrome photoreceptors similar to cFR, cR or
cB treatments, the observed difference most probably can not be attributed to the stimulation of a different set of photoreceptors. Because the magnitude of HIRs depend on photon fluence rates and continuing irradiation, the switch in light responses between 45 min and 4 h would indicate an adaptation of signaling processes toward the measurement of light intensities during the course of irradiation in etiolated seedlings.

CONCLUSION

Our data provides clear evidence that UV-B light regulates the expression of specific sets of genes that do not respond to other light qualities, including UV-A. This finding indicates that the proposed UV-B photoreceptor(s) exclusively triggers the expression of genes that might help the plant to adapt to this deleterious light quality and plays a unique role independent of the other photoreceptors. Data analyses exhibited an extraordinarily high similarity of transcript accumulation patterns of seedlings irradiated with cFR and cB for 4 h. This finding, together with analyses of transcript accumulation patterns of marker genes in \textit{phyA} and \textit{cry1 cry2} mutants, hints that phyA is a potent blue light photoreceptor and that signaling cascades downstream of both phyA and blue light photoreceptors seem to converge to control comparable sets of genes. Our analyses further indicate that the mode of light signaling changes during de-etiolation. A high number of early light-regulated genes seem to follow an all-or-none mode of transcriptional regulation independent of the applied light treatment. This ‘light is on’ response might be necessary to enable rapid, but unspecific, responses on the level of gene expression as a first and fast adaptation at the onset of light. At 4 h, light-induced expression patterns became more divergent and pR and pUV-A treatments were no longer sufficient to induce full alterations in gene expression. This switch toward the high irradiance mode of light response might reflect a light-driven adaption in light signaling, which would help plants to adapt to the spectral composition, duration, and intensity of the incident light at later developmental stages.
MATERIALS AND METHODS

Growth conditions and light treatments

Wild-type (ecotype Columbia-0) *Arabidopsis* seeds (18 mg) were surface sterilized and sown on filter paper placed upon MS-Agar plates without sugar. Plates were stratified for 48 hours in the dark (8 °C), after which germination was induced by two hours of continuous red light (39 µmol m^{-2} s^{-1}). Seedlings were grown in darkness for additional 94 h at 22 °C before the onset of light treatments. Modified Leitz Prado 500-W universal projectors (Leitz, Wetzlar, Germany) were used as light sources together with Osram Xenophot longlife lamps (Osram, Berlin, Germany) for irradiations with far-red (cFR), red (cR, pR) and blue light (cB). Far-red light was obtained by passing the light beam through a 715 nm DAL interference filter (λ_{max} = 715 nm; half-bandwidth = 15 nm; Schott, Mainz, Germany), and blue light was obtained by passing the light beam through a 453 nm DAL interference filter (λ_{max} = 453 nm; half-bandwidth = 18 nm; Schott, Mainz, Germany). All red light treatments were performed with a KG65 double glass filter (λ_{max} = 650 nm; half-bandwidth = 15 nm; Balzers, Liechtenstein). Fluence rates for cFR, cR and cB irradiation were adjusted to 10 µmol m^{-2} s^{-1}, which induces a saturating response with respect to hypocotyl elongation in wild-type. For pR treatments etiolated seedlings were exposed to red light (50 µmol m^{-2} s^{-1}) for 1 min before transfer back to darkness. Six Philips TL 40W/12 UV fluorescent tubes (λ_{max} = 310 nm, half-bandwidth = 40 nm; Philips, Hamburg, Germany) were used as a light source together with quartz cut-off filters to perform pulse treatments with UV-A (pUV-A) and UV-A/B light (pUV-A/B). Light was filtered through a WG307 cut-off filter (3 mm, half-maximal transmission: 307 nm; Schott, Mainz, Germany) to obtain UV-A/B light (7 W m^{-2}) and through a WG327 cut-off filter (3 mm; half-maximal transmission: 327 nm; Schott, Mainz, Germany) to obtain UV-A light (4 W m^{-2}). For pUV-A and pUV-A/B treatments, etiolated seedlings were exposed to light for 5 min before transfer back to darkness. Emission spectra of all light sources are given in Fig. S1.
RNA isolation

Filter papers with seedlings on top were removed from agar and immediately frozen in liquid nitrogen. The upper parts of frozen seedlings (predominantly hypocotyls and cotyledons) were cut from the paper using an electric hair-clipper (Braun 5280, Braun, Germany). Individual samples were collected in two 1.5 ml tubes filled with seven glass beads of 1.7-2 mm (Roth, Karlsruhe, Germany) and stored at -70 °C. To grind the material, tubes were frozen in liquid nitrogen and shaken two times for 10 seconds in a Silamat S5 shaker (Ivoclar Vivadent, Ellwangen, Germany). Ground material was extracted using Buffer RLT. The two samples of an individual sample were passed individually through a QIAshredder spin column and re-unified afterwards. All further manipulations were performed as described by the manufacturer.

Microarray analyses

Hybridization of the Affymetrix 25K microarrays (ATH1 Genechip, Affymetrix) was performed according to manufacturer’s protocol (Santa Clara, CA, USA, and http://arabidopsis.org/servlets/TairObject?type=protocol&id=501713121). Biotin-labeled RNA was synthesized by in vitro transcription using Enzo Bioarray RNA labeling kit (Enzo Diagnostics, Farmingdale, NY, USA). Hybridizations and extraction of raw data were performed at Nottingham Arabidopsis Stock Centre (UK) as part of the AtGenExpress project, an international cooperation project for genome-wide expression profiling of Arabidopsis wild type Col-0 coordinated by the German Arabidopsis Functional Genomics Network (AFGN), and includes contributions from Germany, supported by DFG, as well as substantial contributions by RIKEN (Japan), NSF (USA; via funding of TAIR and the 2010 program), BBSRC (UK; via funding of the GARNET initiative), and the Max Planck Society. Data were connected to several open-access bioinformatics tools such as AtGenExpress Visualization Tool (http://jsp.weigelworld.org/expviz/expviz.jsp), Genevestigator...
Raw data were processed using Genedata Refiner Array according to the following protocol: Summary statistics were executed for the imported raw data and diagnostics on control genes was conducted via control gene statistics; defective areas on the chip were detected and quality classification was performed for each chip; data was condensed to gene expression values using the Affymetrix statistical algorithm (MAS5). ABS data files were generated and exported to Genedata Analyst for further analyses using Genedata Expressionist software (Genedata, Basel, Switzerland). Analyses included selection of light-regulated genes by normalization to the corresponding dark controls, calculation of False Discovery Rates (FDR) according to Benjamini and Hochberg (1995), correlation analyses, and creation of Venn diagrams. As a further estimate for FDR (Tables I, II), all three biological replicates for a specific light treatment were compared pair wise to each other, and the proportions of transcripts that exhibited significant differences in transcript levels (> 2-fold change; t-test: \( p < 0.05 \)) were calculated. The FDR was estimated as the mean of the calculated relative proportion of differentially regulated transcripts from all 3 pair wise comparisons of otherwise identical light treatments. One Way ANOVA was done using the SigmaStat 9.0 software tool.

**Quantitative Real Time PCR**

*Arabidopsis* total RNA was treated with DNaseI according to the manufacturer's specifications (Qiagen, Hilden, Germany). Superscript III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany) was used with a dT\(_{20}\)-Oligomer for cDNA synthesis according to the manufacturer’s instructions. Quantitative RT–PCR was carried out in 96-well format using a 7300 Real-Time PCR System and TaqMan probes (Applied Biosystems, USA). PCR reactions were performed using the ABsolute QPCR Rox Mix Kit following the manufacturer's instructions...
(ThermoScientific, Hamburg, Germany). TaqMan probes and primer pairs for each marker gene (Table S5) were designed using Primer Express Version 3.0 (Applied Biosystems). qGene software (http://www.gene-quantification.info) was used to calculate optimized standard curves and optimal CT-values from raw data for the different samples. CT-values from individual marker genes were further normalized to CT-values obtained for the constitutively expressed ACTIN1 transcripts, which served as an endogenous control for the efficiency of qRT-PCR. All results presented were based on 4 biological replicates that were measured in triplicate.

Supplemental Data

The following materials are available in the online version of this article:

Supplemental Figure S1. Spectral composition of light sources used for the experiments.

Supplemental Figure S2. Box blots showing the distribution of signal intensities for all analyzed probes (~24,000) among the different microarrays hybridized for this study.

Supplemental Figure S3. Visual summary of the number of up- and down-regulated genes that are related to transcriptional regulation, signalling and hormone function.

Supplemental Figure S4. Specificity and interference of light-regulated genes in response to the different applied light treatments.

Supplemental Figure S5. Specificity and interference of light-regulated genes in response to light treatments that stimulate specific sub-groups of photoreceptors.

Supplemental Figure S6. Classification of down-regulated genes according to their responses toward different light treatments after 45 min and 4 h.
Supplemental Figure S7. Comparison of microarray data and qRT-PCR measurements for representative marker genes.

Supplemental Table S1. List of genes that exhibit significantly altered transcript levels at 45 min.

Supplemental Table S2. List of genes that exhibit significantly altered transcript levels at 4 h.

Supplemental Table S3. Correlation coefficients for pair wise comparisons of signal intensities of light-regulated genes in the different replicates.

Supplemental Table S4. List of up-regulated genes classified according to their responses toward different light treatments after 45 min and 4 h.

Supplemental Table S5. List of down-regulated genes classified according to their responses toward different light treatments after 45 min and 4 h.

Supplemental Table S6. Oligonucleotides used for quantitative real-time PCR.

ACKNOWLEDGEMENTS

We thank Stefan Kircher for his critical scientific comments and Anita K. Snyder for her helpful comments on the manuscript.


*Curr Biol* 19: 1216-1220


FIGURE LEGENDS

**Figure 1.** Numbers of light-regulated genes in different functional categories at 45 min and 4 h. The figure shows the number of genes that exhibited significant increase or decrease in transcript levels compared to the corresponding dark controls. 4-day-old, dark-grown, wild-type *Arabidopsis* seedlings were treated with continuous far-red light (cFR), continuous red light (cR), continuous blue light (cB) or received one saturating red light pulse (pR), UV-A pulse (pUV-A) or UV-A / UV-B pulse (pUV-A/B). Samples were taken 45 minutes and 4 h after the onset of light treatment, together with the corresponding dark controls. Genes showing significantly altered transcript levels were sorted into different functional categories according to their gene ontology (GO) annotation. A, Number and functional classification of genes that exhibit significantly increased transcript levels at 45 min. B, Number and functional classification of genes that exhibit significantly reduced transcript levels at 45 min. C, Number and functional classification of genes that exhibit significantly increased transcript levels at 4 h. D, Number and functional classification of genes that exhibit significantly reduced transcript levels at 4 h.

**Figure 2.** Multiple correlation analysis of transcript accumulation patterns obtained under the different light treatments. Correlation analyses were restricted to microarray data of genes that exhibited a significant change in transcript levels under any of the applied light treatments. Correlation coefficients were calculated by pair wise comparison of microarray data of the 3 replicates that have been hybridized for each light treatment and the dark controls. Magenta color indicates high and green indicates low correlation coefficients. A, Correlation analyses using data sets of light-regulated genes obtained from RNA samples isolated 45 min after the onset of light treatments. B, Correlation analyses using data sets of light-regulated genes obtained for RNA samples isolated 4 h after the onset of light treatments. Light treatments: D, dark control; cFR, continuous far-red light; cB, continuous blue light; cR, continuous red light; pR, red-light-pulse; pUV-A, pulse of UV-A light; pUV-A/B, pulse of UV-A and UV-B light.
Figure 3. Classification of up-regulated genes according to their responses to different light treatments at 45 min and 4 h. Genes were selected based on similar responses to the different applied light qualities at 45 min and 4 h. The signal intensities are shown for a representative number of genes in each group. A, Group 1\textsuperscript{up}; increased transcript levels solely upon pUV-A/B treatment at 45 min. B, Group 2\textsuperscript{up}; increased transcripts levels upon pUV-A/B treatment at 45 min and 4 h. C, Group 3\textsuperscript{up}; up-regulated upon pUV-A/B treatment at 4 h. D, Group 4\textsuperscript{up}; up-regulated upon pUV-A treatment at 45 min. E, Group 5a\textsuperscript{up}; increased transcripts levels under all applied light treatments at 45 min, but did not show a clear light response at 4 h. E, Group 5b\textsuperscript{up}; up-regulated under all applied light treatments at 45 min and still exhibited increased transcript levels under cFR, cB and pUV-A/B at 4 h. F, Group 6\textsuperscript{up}; increased transcript levels under all applied light treatments at 4 h. G, Group 7\textsuperscript{up}; increased transcript levels under cFR, cB and upon pUV-A/B treatment at 4 h. H, Group 8\textsuperscript{up}; up-regulated only under cFR and cB at 4 h of irradiation. D, dark control.

Figure 4. Analyses of light-specific transcript accumulation patterns of marker genes in the \textit{phyA-211} mutant. Bar charts show fold-induction values for transcript levels of selected marker genes in 4-day-old, dark-grown, wild-type (black bars) and \textit{phyA-211} (grey bars) seedlings that were either kept in darkness (D) or were treated with cFR, cB, cR and pR, pUV-A or pUV-A/B. Results of qRT-PCR experiments from qGene software were first normalized according to the constitutively expressed \textit{ACTIN1} (\textit{AT2G37620}) gene. Normalized expression values were than used to calculate fold induction with respect to the corresponding dark controls. All data represent the mean of at least 4 independent biological replicates. A, Fold induction values for marker genes obtained from samples that were harvested 45 min after the onset of light treatments. B, Fold induction values for marker genes obtained from samples that were harvested 4 h after the onset of light treatments. Bars, standard errors of the mean.

Figure 5. Analyses of transcript accumulation patterns of marker genes in the \textit{cry1-304 cry2-1} double mutant under continuous blue light. Bar charts show fold-induction values for transcript levels of selected marker genes in 4-day-old, dark-grown, wild-
type (black bars), phyA-211 (light grey bars) and cry1-304 cry2-1 (dark grey bars) seedlings that were either kept in darkness or treated with cB light for 45 min or 4 h. Relative expression levels were calculated as described in Figure 4. All data represent the mean of at least 4 independent biological replicates. A, Fold induction values for marker genes after 45 min of blue light irradiation. B, Fold induction values for marker genes after 4 h of blue light irradiation. Bars, standard errors of the mean.
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<th>m / n b</th>
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<th>Significant number above FDRd</th>
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*Venn diagram analyses were used to compare lists of genes that exhibited significantly altered transcript levels upon specific light treatments compared to dark controls; n = total number of up-regulated (n = 556) or down-regulated genes (n = 228); FDRs were estimated according to Benjamini & Hochberg (1995) or by calculating the ratio of genes that exhibited artificially altered transcript levels in the 3 biological replicates of the given light treatment (values in brackets); numbers were regarded as significantly different if the number of regulated genes exceeded both estimates of FDR; signal intensities of a single gene under the given light quality were compared to signal intensities obtained for all other light treatments (one way ANOVA; comparison versus single control; Holm-Sidak test; p <0.05).
Table II: Light-specific responses at 4 h

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<th>Significant number above FDRᵈ</th>
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<td>0.05 (0.01)</td>
<td>no</td>
<td>-</td>
</tr>
<tr>
<td>cR</td>
<td>15</td>
<td>0.014</td>
<td>0.05 (0.02)</td>
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<tr>
<td>cB</td>
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<td>0.037</td>
<td>0.05 (0.03)</td>
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<tr>
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<td>0.05 (0.09)</td>
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<tr>
<td>pUV-A/B</td>
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<td>0.176</td>
<td>0.04 (0.12)</td>
<td>yes</td>
<td>92</td>
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</tbody>
</table>

ᵃVenn diagram analyses were used to compare lists of genes that exhibited significantly altered transcript levels upon specific light treatments compared to dark controls; ᵇn = total number of up-regulated (n = 1405) or down-regulated genes (n = 1042); ᵇFDRs were estimated according to Benjamini & Hochberg (1995) or by calculating the ratio of genes that exhibited artificially altered transcript levels in the 3 biological replicates of the given light treatment (values in brackets); ᵇnumbers were regarded as significantly different if the number of regulated genes exceeded both estimates of FDR; ᵇsignal intensities of a single gene under the given light quality were compared to signal intensities obtained for all other light treatments (one way ANOVA; comparison versus single control; Holm-Sidak test; p <0.05).
Figure 1. Numbers of light-regulated genes in different functional categories at 45 min and 4 h. The figure shows the number of genes that exhibited significant increase or decrease in transcript levels compared to the corresponding dark controls. 4-day-old, dark-grown, wild-type Arabidopsis seedlings were treated with continuous far-red light (cFR), continuous red light (cR), continuous blue light (cB) or received one saturating red light pulse (pR), UV-A pulse (pUV-A) or UV-A / UV-B pulse (pUV-A/B). Samples were taken 45 minutes and 4 h after the onset of light treatment, together with the corresponding dark controls. Genes showing significantly altered transcript levels were sorted into different functional categories according to their gene ontology (GO) annotation. A, Number and functional classification of genes that exhibit significantly increased transcript levels at 45 min. B, Number and functional classification of genes that exhibit significantly reduced transcript levels at 45 min. C, Number and functional classification of genes that exhibit significantly increased transcript levels at 4 h. D, Number and functional classification of genes that exhibit significantly reduced transcript levels at 4 h.
Figure 2. Multiple correlation analysis of transcript accumulation patterns obtained under the different light treatments. Correlation analyses were restricted to microarray data of genes that exhibited a significant change in transcript levels under any of the applied light treatments. Correlation coefficients were calculated by pair wise comparison of microarray data of the 3 replicates that have been hybridized for each light treatment and the dark controls. Magenta color indicates high and green indicates low correlation coefficients. A, Correlation analyses using data sets of light-regulated genes obtained from RNA samples isolated 45 min after the onset of light treatments. B, Correlation analyses using data sets of light-regulated genes obtained for RNA samples isolated 4 h after the onset of light treatments. Light treatments: D, dark control; cFR, continuous far-red light; cB, continuous blue light; cR, continuous red light; pR, red-light-pulse; pUV-A, pulse of UV-A light; pUV-A/B, pulse of UV-A and UV-B light.
Figure 3. Classification of up-regulated genes according to their responses to different light treatments at 45 min and 4 h. Genes were selected based on similar responses to the different applied light qualities at 45 min and 4 h. The signal intensities are shown for a representative number of genes in each group. A, Group 1\textsuperscript{up}; increased transcript levels solely upon pUV-A/B treatment at 45 min. B, Group 2\textsuperscript{up}; increased transcripts levels upon pUV-A/B treatment at 45 min and 4 h. C, Group 3\textsuperscript{up}; up-regulated upon pUV-A/B treatment at 4 h. D, Group 4\textsuperscript{up}; up-regulated upon pUV-A treatment at 45 min. E, Group 5a\textsuperscript{up}; increased transcripts levels under all applied light treatments at 45 min, but did not show a clear light response at 4 h. E, Group 5b\textsuperscript{up}; up-regulated under all applied light treatments at 45 min and still exhibited increased transcript levels under cFR, cB and pUV-A/B at 4 h. F, Group 6\textsuperscript{up}; increased transcript levels under all applied light treatments at 4 h. G, Group 7\textsuperscript{up}; increased transcript levels under cFR, cB and upon pUV-A/B treatment at 4 h. H, Group 8\textsuperscript{up}; up-regulated only under cFR and cB at 4 h of irradiation. D, dark control.
Figure 4. Analyses of light-specific transcript accumulation patterns of marker genes in the phyA-211 mutant. Bar charts show fold-induction values for transcript levels of selected marker genes in 4-day-old, dark-grown, wild-type (black bars) and phyA-211 (grey bars) seedlings that were either kept in darkness (D) or were treated with cFR, cB, cR and pR, pUV-A or pUV-A/B. Results of qRT-PCR experiments from qGene software were first normalized according to the constitutively expressed ACTIN1 (AT2G37620) gene. Normalized expression values were than used to calculate fold induction with respect to the corresponding dark controls. All data represent the mean of at least 4 independent biological replicates. A, Fold induction values for marker genes obtained from samples that were harvested 45 min after the onset of light treatments. B, Fold induction values for marker genes obtained from samples that were harvested 4 h after the onset of light treatments. Bars, standard errors of the mean.
Figure 5. Analyses of transcript accumulation patterns of marker genes in the cry1-304 cry2-1 double mutant under continuous blue light. Bar charts show fold induction values for transcript levels of selected marker genes in 4-day-old dark-grown wild type (black bars), phyA-211 (light grey bars) and cry1-304 cry2-1 (dark grey bars) seedlings that were either kept in darkness or treated with cB blue light for 45 min or 4 h. Relative expression levels were calculated as described in Figure 4. All data represent at least 4 independent biological replicates. A, Fold induction values for marker genes after 45 min of blue light irradiation. B, Fold induction values for marker genes after 4 h of blue light irradiation. Bars, standard errors of the mean.