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, the duration of daylength 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; Christie, 2007; Li and Yang, 2007; Demarsy and Fankhauser, 2009; Jenkins, 2009; Franklin and Quail, 2010). The genome of the model plant Arabidopsis (Arabidopsis thaliana) encodes for five different phytochromes (phyA–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 receptors (Jenkins, 2009). Together, these photoreceptors control the timing and extent of many developmental transitions, including seed germination, seedling deetiolation, 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; Franklin and Quail, 2010; Mathews, 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. Further-
more, 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 (cR) and shade avoidance responses (Casal et al., 2003; Franklin and Quail, 2010; Mathews, 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, 2004, 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 (Lin and Todo, 2005; Christie, 2007; Li and Yang, 2007; Demarsy and Fankhauser, 2009). 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 cR, whereas phototropins play only subsidiary roles (Ohgishi et al., 2004). Furthermore, it is known that phyA can also function as a blue light photoreceptor (Casal and Mazzella, 1998; Neff and Chory, 1998; Poppe et al., 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, 2004, 2006; Wang et al., 2002; Ulm et al., 2004; Jiao et al., 2005). 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 deetiolation. Therefore, 4-d-old, dark-grown ecotype Columbia-0 (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 light (cFR) was applied to induce phyA-dependent HIR responses, whereas cR was used to stimulate all phytochrome photoreceptors, including the light-stable forms (Supplemental Fig. S1). Furthermore, another group of seedlings were treated with continuous blue light (cB) to stimulate photoreceptors that function in this part of the spectrum. Other seedlings were treated with a single, short pulse of red light (pR), UV-A light (pUV-A), or UV-A/B light (pUV-A/B) before transfer back to darkness (Supplemental 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 Affymetrix 25K microarrays, which cover nearly the complete genome of Arabidopsis.

This 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 subsets 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 deetiolation.

## RESULTS

### Selection of Light-Regulated Genes

To reveal gene expression patterns at the initial light response during seedling deetiolation, 4-d-old, dark-grown wild-type Arabidopsis seedlings were treated with cFR, cR, or cB or received pR, pUV-A, or pUV-A/B before transfer back to darkness. Samples from deetiolated plants and the corresponding dark controls were taken 45 min and 4 h 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 (eight light treatments × two time points × three 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 (Supplemental Fig. S2).

To select light-regulated genes in the data sets, expression values of light-treated samples were compared with those of the corresponding dark controls. Transcript levels were regarded as light regulated if they exhibited, first, a 2-fold or greater up-regulation or down-regulation of signal intensities and, second, a statistically significant difference in 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 genes (3% of genes on the chip) and 2,447 genes (11% of genes on the chip) were regarded as...
being light regulated at 45 min and 4 h, respectively (Supplemental Tables S1 and S2). A higher number of genes exhibited significantly increased transcript levels rather than reduced levels both at 45 min (556 up versus 228 down) and 4 h (1,405 up versus 1,042 down).

The Influence of Different Light Treatments on the Number of Light-Regulated Genes Significantly Changes during the Course of the Deetiolation 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. 1, A and 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, and pR; Fig. 1B).

The transcript accumulation patterns completely changed at 4 h. Now, the highest number of transcripts became up-regulated and down-regulated under cFR, followed by cB (Fig. 1, C and 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 with 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 function; (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...
the 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. 1, B and 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 overrepresented 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 with 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 with other light treatments (Supplemental 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. 1, C and D). This finding is most probably related to strong alterations of general metabolism that are to be expected during deetiolation 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; Supplemental Fig. S3, A and 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, and 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; Supplemental Fig. 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. 1, C and 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; Supplemental Fig. S3, A and B).

Treatments with cR and pUV-A/B resulted in very complex effects on gene expression patterns at 4 h. Compared with 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; Supplemental Fig. S3, A and B). Irradiation with cR for 4 h also triggered down-regulation of transcript levels from a high number of hormone-related genes compared with results obtained at 45 min, whereas the effect of pUV-A/B seems to level out upon transfer to darkness (Fig. 1; Supplemental Fig. 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; Supplemental Fig. S3), indicating that pR and pUV-A have a rapid but transient effect on transcriptional regulation during deetiolation 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; Supplemental 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; Supplemental 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. 2, 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 with the different light treatments at 45 min (Fig. 2A; Supplemental Table S3). Correlation coefficients were high for comparisons of expression values between the different light treatments at 45 min (Fig. 2A; Supplemental 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, and cB) more greatly alters gene expression than stimulation of the phytochrome system (cFR, cR, and 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. 2; Supplemental Table S3), indicative of the establishment of light-specific patterns of gene regulation. Samples irradiated with cR exhibited slightly reduced correlation coefficients compared with all other light treatments, including pR, at 4 h (Fig. 2B; Supple-
mental 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 with the transient effect induced by the pulse treatment.

Compared with 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; Supplemental 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 with the corresponding dark control and between the two pulse treatments (Fig. 2B; Supplemental Table S3). These data again indicate that the influence of pR and pUV-A light-regulated gene expression levels out upon transfer to darkness for an extended time.

Compared with other light treatments, the lowest correlation coefficients were obtained for samples treated with cFR and cB for 4 h (Fig. 2B; Supplemental Table S3). In contrast, a very high correlation ($r \approx 0.97$; Supplemental 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, first, cFR and cB coregulate a common set of genes during later stages of Arabidopsis seedling deetiolation and, second, 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 with those of all the remaining light treatments. The procedure was repeated for each light quality and for each time point using Venn diagram analyses (Supplemental 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 Expressionist 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 from 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 with dark controls, these analyses only took into account whether the expression level differs from the dark expression level but do not include comparisons with the expression levels obtained with other light treatments. Therefore, as a third criterion for a
light-specific effect, we tested whether signal intensities of proposed light-specific genes exhibit a statistically significant difference (multiple ANOVAs) from 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; Supplemental 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; Supplemental 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; Supplemental Figs. S4 and S5). Of the genes down-regulated by cFR at 4 h, only 11 out of 106 (10.3%) exhibited significantly reduced transcript levels compared with other light treatments (multiple ANOVAs). Among the remaining 95 genes, 28 genes already exhibited significantly increased transcript levels under cB compared with other light treatments. Thus, they seem to belong to a group of genes that becomes down-regulated by cFR and cB (group 8down; see Fig. 3 below) rather than 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 with other light treatments (multiple ANOVAs). 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 levels 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 criterion of a 2-fold or greater up-regulation or down-regulation of signal intensities compared with dark controls. Finally, from the remaining six genes, three genes produced only very low signal intensities close to the detection level of microarray analysis and three 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 Coregulated 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 coregulated 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 1up) or decrease (group 1down) in transcript accumulation at 45 min, a permanent increase (group 2up) or decrease (group 2down) of transcript levels at 45 min and 4 h, or a late increase (group 3up) or decrease (group 3down) at 4 h (Fig. 3, A–C; Supplemental Fig. S6, A–C; Supplemental Tables S4 and S5).

| Light Treatment | No. (m) of Specifically Regulated Genes | m/n录用 | FDR录用 | Significant No. above FDR录用 | No. of Genes with Significantly Altered Transcript Levels录用 |
|-----------------|---------------------------------------|---------|--------|-------------------------------|-------------------------------------------------------------|
| Up-regulated genes |                                       |         |        |                               |                                                             |
| cFR             | 0                                     | 0       | 0.06 (0.05) | No                        | –                                                         |
| pR              | 0                                     | 0       | 0.06 (0.07) | No                        | –                                                         |
| cR              | 1                                     | 0.002   | 0.05 (0.04) | No                        | –                                                         |
| cB              | 10                                    | 0.018   | 0.04 (0.03) | No                        | –                                                         |
| pUV-A           | 47                                    | 0.085   | 0.05 (0.07) | Yes                       | 14                                                        |
| pUV-A/B         | 209                                   | 0.38    | 0.06 (0.10) | Yes                       | 177                                                       |
| Down-regulated genes |                                      |         |        |                               |                                                             |
| cFR             | 1                                     | 0.004   | 0.04 (0.05) | No                        | –                                                         |
| pR              | 4                                     | 0.018   | 0.05 (0.07) | No                        | –                                                         |
| cR              | 1                                     | 0.004   | 0.04 (0.04) | No                        | –                                                         |
| cB              | 38                                    | 0.169   | 0.05 (0.03) | Yes                       | 11                                                        |
| pUV-A           | 9                                     | 0.040   | 0.05 (0.07) | No                        | –                                                         |
| pUV-A/B         | 47                                    | 0.208   | 0.05 (0.10) | Yes                       | 13                                                        |

录用Venn diagram analyses were used to compare lists of genes that exhibited significantly altered transcript levels upon specific light treatments compared with dark controls.录用^n录用 = total number of up-regulated (n录用 = 556) or down-regulated (n录用 = 228) genes.录用^FDR录用 were estimated according to Benjamini and Hochberg (1995) or by calculating the ratio of genes that exhibited artificially altered transcript levels in the three biological replicates of the given light treatment (values in parentheses).录用^Numbers录用 were regarded as significantly different if the number of regulated genes exceeded both estimates of FDR.录用^Signal intensities of a single gene under the given light quality were compared with signal intensities obtained for all other light treatments (one-way ANOVA; comparison versus single control; Holm-Sidak test; P < 0.05).
Another group (group 4up) contains genes that specifically increased at 45 min in response to pUV-A (Fig. 4D; Supplemental Tables S4 and S5). Several genes were identified that became specifically down-regulated by cB at 45 min (group 4down; Supplemental Fig. S6D; Supplemental 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 5down) and one additional group of early up-regulated genes that can be separated into two subgroups (group 5aup and group 5bup). The group of early down-regulated genes (group 5down) shows a rapid decline in transcript levels irrespective of the applied light treatment (Supplemental Fig. S6E; Supplemental Table S5). Group 5down transcript levels also remained low 4 h after irradiation with cFR, cB, and cR, whereas pulse treatments (pR, pUV-A, and pUV-A/B) did not cause a response that lasted until the later time point. The two subgroups of early up-regulated genes (group 5aup and group 5bup) 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. 3, E and F; Supplemental Table S4). Transcript levels in subgroup 5aup were high at 45 min but dropped at 4 h (Fig. 3E). In contrast, the second subgroup of early up-regulated genes (group 5bup) 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 toward the applied light treatments. Group 6up and group 6down include genes that become up-regulated or down-regulated by all light treatments, although cFR, cB, and cR were often more effective compared with pulse treatments (Fig. 3F; Supplemental Fig. S6E; Supplemental Tables S4 and S5). Many transcripts belonging to group 6up encode for components of PSI and PSII and the corresponding antenna complexes that are necessary to perform photosynthesis (Supplemental Table S4). Groups 7up and 7down 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; Supplemental Fig. S6F; Supplemental Tables S4 and S5). Groups 8up and 8down consist of genes that become solely up-regulated or down-regulated by cFR and cB (Fig. 4H; Supplemental Fig. S6G; Supplemental Tables S4 and S5).

Establishment of Marker Genes

To verify our classification into different coregulated groups and to characterize marker genes for additional studies, we performed quantitative real-time PCR (qRT-PCR) analyses with a subset of light-up-regulated genes. Specific oligonucleotides were designed to determine transcript levels of PHYTOCHROME KINASE SUBSTRATE1 (PKS1), ARABIDOPSIS PSEUDO-RESPONSE REGULATOR5 (APRR5), and LONG HYPCOTYL5 (HY5), which represent group 5up, CHLOROPHYLL A/B-BINDING PROTEIN4 (CAB4),
DEFECTIVE IN INDUCED RESISTANCE1 (DIR1), and AT5G48490 were selected to represent group 6 up. CHALCONE SYNTHASE (CHS) represents genes of group 7 up, whereas AT5G42760 belongs to group 8 up (Supplemental 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 Cooperate 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 deetiolation, most obviously for early light-regulated genes that belong to group 5up. 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; Supplemental Fig. S6). To explain the observed lack of differences between the different light treatments in early light responses, it might be hypothesized that a 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 (Casal and Mazzella, 1998; Neff and Chory, 1998; Poppe et al., 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 toward cFR with respect to PKS1, APRR5, and HY5 transcript accumulation at 45 min, whereas responses toward 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 toward 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. These data clearly indicate 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 deetiolation in Arabidopsis.

Results from microarray experiments with phot1 phot2 and cry1 cry2 double mutants indicated that cryptochromes 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.

**PhyA and Cryptochrome Photoreceptors Are Involved in the Regulation of Gene Expression under cR and cB at 4 h**

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 the expression of marker genes for groups 5up, 6up, 7up, and 8up. The lack of phyA clearly abolished far-red light-

![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-d-old, dark-grown, wild-type (black bars) and phyA-211 (gray bars) seedlings that were either kept in darkness or were treated with cFR, cB, cR, 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 means of at least four 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. Error bars indicate se.](image-url)
induced transcript accumulation of all tested marker genes (Fig. 4B). In contrast to the strong diminishment of cFR-regulated transcript accumulation, responses toward cR, pR, and pUV-A/B remained relatively unaltered or became enhanced in phyA-211. These data indicate 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 8up (genes exclusively up-regulated by cFR and cB). The loss of both cryptochromes did not alter blue light-induced transcript accumulation of AT5G42760 (group 8up) or CAB4, a marker gene of group 6up (very high sensitivity toward all applied light treatments). A reduction in transcript levels was only detected for CHS and HY5, marker genes for groups 5up and 7up (genes that exhibit a strong increase in transcript levels under cFR, cB, and pUV-A/B but do not respond to cR and pR treatments). Taken together, these data indicate 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 deetiolation. 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-d-old, etiolated Arabidopsis seedlings that were either pulse irradiated or continuously irradiated for 45 min and 4 h. At 45 min, transcripts of approximately 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 (approximately 1,000), cR (approximately 600), and cB (approximately 900) at 4 h (Fig. 1). These results are in good agreement with published data obtained with full-genome chips for early or late light-regulated genes in etiolated seedlings that were either pulse irradiated or continuously irradiated for 45 min and 4 h.

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The number of light-regulated genes at 4 h is comparable to the results obtained for seedlings that had 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

Figure 5. Analyses of transcript accumulation patterns of marker genes in the cry1-304 cry2-1 double mutant under cB. Bar charts show fold induction values for transcript levels of selected marker genes in 4-d-old, dark-grown, wild-type (black bars), phyA-211 (light gray bars), and cry1-304 cry2-1 (dark gray bars) seedlings that were either kept in darkness or treated with cB for 45 min or 4 h. Relative expression levels were calculated as described in Figure 4. All data represent means of at least four 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. Error bars indicate sd.
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 d after germination, the data give 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; Supplemental Fig. S3), similar to former studies (Tepperman et al., 2001, 2004, 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 deetiolation (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; Supplemental Fig. 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 a 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 coregulated by more than one light quality (Fig. 3; Supplemental Fig. S6). A major exception to this rule was seen with pUV-A/B treatment, which induces specific subsets of genes at 45 min and at 4 h that did not respond to any other light treatment, including pUV-A (Tables I and II; Supplemental Fig. S4). The specificity of the UV-A/B response indicates that the corresponding genes seem to be dispensable for the shift from skotomorphogenesis to 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 cutoff filters to eliminate UV-C light together with short wavelengths of UV-B light, 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 light.

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; Supplemental 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 8th marker genes, blue light still caused clear alterations in the expression of most marker genes in the phyA knockout mutant, which indicates that other photoreceptors are involved in the regulation of gene expression toward this light quality and can compensate for phyA function (Figs. 4 and 5). Thus, regulatory similarities between cFR and cB might rather be caused by the convergence of light signaling cascades at the level of or downstream of phytochrome and blue light photoreceptors.

Several additional findings confirm the coaction 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...
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; Supplemental 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 5up and group 5down became up- or down-regulated to a similar extent by all applied light qualities at 45 min, including pR and pUV-A (Fig. 3; Supplemental Fig. 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 dominant effect of phyA causes the observed absence of light-specific effects. This phytochrome accumulates to very high levels in darkness and triggers very-low-fluence responses that become induced by even weak light inputs by nearly all light qualities (Casal et al., 2003; Franklin and Quail, 2010; Mathews, 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, 2004, 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 (Figs. 4 and 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 with 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 time points (Fig. 3; Supplemental Fig. 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 (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam 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 with 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; Supplemental Fig. 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 treatment, the observed difference most probably cannot be attributed to the stimulation of a different set of photoreceptors. Because the magnitude of HIRs depends 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 provide 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 light. This finding indicates that the proposed UV-B photoreceptor 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 phyA and 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 deetiolation. 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 to 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 adaptation 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 Col-0 Arabidopsis (Arabidopsis thaliana) seeds (18 mg) were surface sterilized and sown on filter paper placed upon Murashige and Skoog agar plates without sugar. Plates were stratified for 48 h in the dark (8°C), after which germination was induced by 2 h of cR (39 μmol m\(^{-2}\) s\(^{-1}\)). Seedlings were grown in darkness for an additional 94 h at 22°C before the onset of light treatments. Modified Prado 500-W universal projectors (Leitz) were used as light sources together with Xenophot long-life lamps (Osrarn) for irradiations with far-red (cFR), red (cR, pR), and blue (cB) light. Far-red light was obtained by passing the light beam through a 715-nm DAL interference filter (λ\(_{\text{max}}\) = 715 nm, half-bandwidth = 15 nm; Schott), and blue light was obtained by passing the light beam through a 453-nm DAL interference filter (λ\(_{\text{max}}\) = 453 nm, half-bandwidth = 18 nm; Schott). All red light treatments were performed with a KG65 double glass filter (λ\(_{\text{max}}\) = 650 nm, half-bandwidth = 15 nm; Balzers). Fluence rates for cFR, cR, and cB were adjusted to 10 μmol m\(^{-2}\) s\(^{-1}\), which induces a saturating response with respect to hypocotyl elongation in the 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 TL 40W/12 UV fluorescent tubes (λ\(_{\text{max}}\) = 310 nm, half-bandwidth = 40 nm; Philips) were used as a light source together with quartz cutoff filters to perform pulse treatments with UV-A (pUV-A) and UV-A/B (pUV-A/B) light. Light was filtered through a WG307 cutoff filter (3 mm; half-maximal transmission, 307 mm; Schott) to obtain UV-A/B light (7 W m\(^{-2}\)) and through a WG327 cutoff filter (3 mm; half-maximal transmission, 327 mm; Schott) 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 Supplemental Figure 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 (pre-dominantly hypocotyls and cotyledons) were cut from the paper using an electric hair clipper (Braun 5280; Braun). Individual samples were collected in two 1.5-mL tubes filled with seven glass beads of 1.7 to 2 mm (Roth) and shaken two times for 10 s in a Silamat S5 shaker (Ivoclar Vivadent). Total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen). Ground material was extracted using buffer RLT (RNeasy lysis buffer with guanidine thiocyanate). The two samples of an individual sample were passed individually through a QIAshredder spin column and reunified afterward. All further manipulations were performed as described by the manufacturer.

Microarray Analyses

Hybridization of the Affymetrix 25K microarrays (ATH1 GeneChip) was performed according to the manufacturer’s protocol (http://www.affymetrix.com). Biotin-labeled RNA was synthesized by in vitro transcription using the Enzo Bioarray RNA labeling kit (Enzo Diagnostics). Hybridizations and extraction of raw data were performed at the Nottingham Arabidopsis Stock Centre (United Kingdom) as part of the AGenExpress project, an international cooperation project for genome-wide expression profiling of Arabidopsis wild-type Col-0 coordinated by the German Arabidopsis Functional Genomics Network and including contributions from Germany, supported by the Deutsche Forschungsgemeinschaft, as well as substantial contributions by RIKEN (Japan), the National Science Foundation (United States; via funding of The Arabidopsis Information Resource and the 2010 Program), the Biotechnology and Biological Sciences Research Council (United Kingdom; 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://jps.weigelworld.org/expressv/expressv.jsp), Genevestigator (https://www.genevestigator.com/gv/index.jsp), and the Arabidopsis eFP Browser (bcc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi).

Data Analyses

Raw data were processed using the 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 were condensed to gene expression values using the Affymetrix statistical algorithm (MASS). Absolute data files were generated and exported to Genedata Analyst for further analyses using Genevestigator Expressionist software. Analyses included selection of light-regulated genes by normalization to the corresponding dark controls, calculation of FDRs according to Benjamini and Hochberg (1995), correlation analyses, and creation of Venn diagrams. As a further estimate for FDR (Tables I and II), all three biological replicates for a specific light treatment were compared pairwise with each other, and the proportions of transcripts that exhibited significant differences in transcript levels (greater than 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 three pairwise comparisons of otherwise identical light treatments. One-way ANOVA was done using the SigmaStat 9.0 software tool.

qRT-PCR

Arabidopsis total RNA was treated with DNaseI according to the manufacturer’s specifications (Qiagen). SuperScript III Reverse Transcriptase (Invitrogen) was used with a dT\(_{20}\) oligomer for cDNA synthesis according to the manufacturer’s instructions. qRT-PCR was carried out in 96-well format using a 7300 Real-Time PCR System (Applied Biosystems) and TaqMan probes (Applied Biosystems). qRT-PCR was performed using the Absolute QPCR Rox Mix Kit following the manufacturer’s instructions (Thermo Scientific). TaqMan probes and primer pairs for each marker gene (Supplemental 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 cycle of threshold (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 four 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 plots showing the distribution of signal intensities for all analyzed probes (approximately 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, signaling, 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 subgroups 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 pairwise 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 qRT-PCR.

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