Sucrose Control of Phytochrome A Signaling in Arabidopsis

Paul P. Dijkwel,*b,1 Casper Huijser,a Peter J. Weisbeek,a Nam-Hai Chua,b and Sjef C. M. Smeekens*a,2

aDepartment of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands
bLaboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399

The expression of the Arabidopsis plastocyanin (PC) gene is developmentally controlled and regulated by light. During seedling development, PC gene expression is transiently induced, and this induction can be repressed by sucrose. In transgenic seedlings carrying a PC promoter–luciferase fusion gene, the luciferase-induced in vivo luminescence was similarly repressed by sucrose. From a mutagenized population of such transgenic seedlings, we selected for mutant seedlings that displayed a high luminescence level when grown on a medium with 3% sucrose. This screening of mutants resulted in the isolation of several sucrose-uncoupled (sun) mutants showing reduced repression of luminescence by sucrose. Analysis of the sun mutants revealed that the accumulation of PC and chlorophyll a/b binding protein (CAB) mRNA was also sucrose uncoupled, although the extent of uncoupling varied. The effect of sucrose on far-red light high-irradiance responses was studied in wild-type, sun1, sun6, and sun7 seedlings. In wild-type seedlings, sucrose repressed the far-red light-induced cotyledon opening and inhibition of hypocotyl elongation. sun7 seedlings showed reduced repression of these responses. Sucrose also repressed the far-red light-induced block of greening in wild-type seedlings, and both sun6 and sun7 were affected in this response. The results provide evidence for a close interaction between sucrose and light signaling pathways. Moreover, the sun6 and sun7 mutants genetically identify separate branches of phytochrome A–dependent signal transduction pathways.

INTRODUCTION

Light plays an important role in plant development. At an early stage of seedling development, the availability of light determines the morphology of dicotyledonous plants. Seedlings germinated in the dark have elongated hypocotyls and yellowish closed cotyledons with an apical hook. By contrast, seedlings germinated in the light have green expanded cotyledons and short hypocotyls. Molecularly, light induces the expression of many nuclear-encoded photosynthesis genes, such as CAB (encoding chlorophyll a/b binding proteins), RBCS (encoding the small subunit of ribulose-1,5-bisphosphate), and PC (encoding plastocyanin; reviewed in Thompson and White, 1991; Vorst et al., 1993). The perception of light is mediated by several light receptors that absorb light of different wavelengths. Red/far-red light (R/FR)–absorbing phytochromes and blue light/UV-A and UV-B receptors have been identified (Kendrick and Kronenberg, 1994). In Arabidopsis, the phytochrome gene family consists of at least five genes named PHYA, PHYB, PHYC, PHYD, and PHYE (Sharrock and Quail, 1989; Clack et al., 1994). The presence of multiple phytochromes suggests that each phytochrome may have a discrete function, and analysis of mutants defective in a specific phytochrome confirms this idea (Nagatani et al., 1991, 1993; Somers et al., 1991; Parks and Quail, 1993; Reed et al., 1993; Whitelam et al., 1993).

Recently, it has been shown that for phytochrome-induced CAB gene expression and chloroplast development, G protein activation is required, and this activates two distinct signaling pathways (Neuhaus et al., 1993; Bowler et al., 1994a, 1994b). One pathway requires calcium release and induces CAB gene expression and the development of immature chloroplasts that lack the photosynthetic protein complexes photosystem I and cytochrome b6f. Chloroplasts containing all of the protein complexes required for photosynthesis develop only when both calcium and a cGMP-dependent pathway are activated.

One well-studied type of phytochrome responses are the FR high-irradiance responses (FR-HIRs). For these responses, phyA is essential (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993). Characteristics of the FR-HIR are cotyledon opening and a strong inhibition of hypocotyl elongation. The magnitude of these responses depends on the wavelength, fluence rate, and duration of
illumination (Mancinelli, 1994). For chlorophyll biosynthesis, light of a shorter wavelength than FR is necessary; therefore, chlorophyll does not accumulate under continuous FR (FRc), and the seedlings remain yellowish. Another FR response has been described recently for both tomato and Arabidopsis seedlings (Van Tuinen et al., 1995; Barnes et al., 1996). It was found that continuous irradiation with FR can prevent subsequent greening by a continuous white light (Wc) treatment. Barnes et al. (1996) showed that the block of greening occurs during the FR illumination period and is correlated with the accumulation of aberrant vesicles in the plastids and a decrease of NADPH/protoclorophyllide oxidoreductase (POR) proteins and PORα and PORβ gene expression. The onset of the FR block of greening may be a combination of PORβ gene expression declining to levels that are insufficient to allow fresh POR synthesis to occur at the start of Wc illumination and an induction of vesicle formation within the plastid (Barnes et al., 1996).

Next to its signaling function, light also serves as the energy source for photoassimilatory processes. During photosynthesis, carbohydrates are synthesized, and many studies have suggested that photosynthesis is negatively regulated by sugars. Feedback inhibition of photosynthesis by sugars occurs at the level of electron transport and enzyme activity (reviewed in Woodrow and Berry, 1988; Portis, 1992) and through repression of nuclear genes that encode proteins involved in photosynthesis. Metabolic repression of CAB and/or RBGS gene expression has been found in photomixotrophic cultures and protoplasts of rapeseed (Harter et al., 1993), autotrophic cell cultures of Chenopodium (Krapp et al., 1993), and intact Arabidopsis, potato, tobacco, and tomato plants (Cheng et al., 1992; Krapp et al., 1993; Van Oosten et al., 1994). By using a transient expression system for maize protoplasts, it has been shown that the activity of seven maize photosynthetic gene promoters is repressed by sugars (Sheen, 1990; Jang and Sheen, 1994). Sugars are also involved in the regulation of many other genes (reviewed in Sheen, 1994; Thomas et al., 1995). For example, sugars repressed two genes that encode glyoxylate cycle enzymes in a cucumber cell culture (Graham et al., 1994), whereas sugars induced the expression of Arabidopsis chalcone synthase (CHS; Tsukaya et al., 1991) and nitrate reductase genes (Cheng et al., 1992) and soybean and Arabidopsis genes encoding storage proteins (Sadka et al., 1994; Berger et al., 1995).

Despite the evidence that carbohydrates regulate gene expression, not much is known about sugar-sensing and signal transduction processes that lead to the regulation of gene expression. It has been proposed that metabolic factors related to high carbohydrate content rather than the carbohydrate concentration itself are the signal for repression of gene expression (Krapp et al., 1993; Graham et al., 1994; Jang and Sheen, 1994; Sadka et al., 1994). Graham et al. (1994) and Jang and Sheen (1994) provided evidence that hexokinase may act as a key sensor and signal transmitter for sugar-repressed genes. This suggests similarities with glucose repression in yeast in which glucose repression was tightly linked with hexokinase photosystem II activity (Ma et al., 1989; Rose et al., 1991). It has been proposed that sugar-induced genes are activated in part by the accumulation of sugar phosphates and the concomitant reduction of cellular phosphate levels (Sacka et al., 1994). Here, protein phosphatases may also be involved (Takeda et al., 1994). However, it is unclear whether the same signal transduction processes result in repression of photosynthetic and other genes and induction of sugar-inducible genes. Also, little is known about the integration of metabolic signaling and light signal transduction processes.

A useful way to study signal transduction pathways molecularly is to isolate mutants defective in separate steps of the pathways and to clone and analyze the genes involved. The model plant Arabidopsis has been an excellent tool for the identification and isolation of such genes. For example, many mutants have been identified that are defective in light-dependent development. Mutants have been isolated that develop dark-grown characteristics in the light (Koomneel et al., 1980; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993) or light-grown characteristics in the dark (Chory et al., 1989b, 1991; Deng et al., 1991; Wei and Deng, 1992; Cabrera y Poch et al., 1993; Hou et al., 1993). The analysis of these mutants has contributed to the understanding of light-regulated seedling development. It should be emphasized that these mutants were isolated on the basis of their morphology. Such an approach seems less feasible for the isolation of mutants that are defective in metabolic regulation of gene expression, because such mutants may not show a morphological phenotype. In those instances, other screening methods can be more effective.

The expression of the Arabidopsis PC gene has been studied in some detail (Vorst et al., 1988, 1993; Flescher et al., 1994, 1996; Dijkwel et al., 1996). The expression of this gene can be induced by phytochrome through modulation of its promoter activity (P.P. Dijkwel, P.J. Welsbeek, and S.C.M. Smekens, unpublished results). Recently, it was shown that PC gene expression can be repressed by sucrose in young Arabidopsis seedlings (Dijkwel et al., 1996). Also, seedlings harboring a PC promoter–luciferase (PC–LUC) gene fusion showed similar repression of LUC activity; therefore, this repression is transcriptionally controlled. In PC–LUC plants, it is possible to measure PC promoter activity in vivo by counting the LUC-induced luminescence with sensitive video-imaging equipment (Millar et al., 1992a, 1992b). This nondestructive method for measuring reporter gene activity permits the identification and further growth of mutants with an altered PC gene expression pattern.

Using LUC as an in vivo marker for screening, we have identified Arabidopsis mutants that are defective in sucrose repression of PC gene expression. Further analysis revealed that these mutants were also defective in sucrose repression of CAB and RBGS genes. FR-HIRs were repressed by sucrose in the wild type but were uncoupled from sucrose repression in a subset of the mutants, suggesting that there is a close interaction between metabolic repression and light signaling.
RESULTS

Isolation and Genetic Analysis of sucrose-uncoupled Mutants

Sucrose represses the developmentally controlled induction of PC gene expression in light- and dark-grown Arabidopsis seedlings. Similarly, LUC activity in 4-day-old seedlings of transgenic plant lines harboring PC-LUC can be repressed by sucrose (Dijkwel et al., 1996). To obtain more insight into the signal transduction pathways that regulate sucrose-dependent repression of PC gene expression, we designed a screen to isolate mutants altered in this process. When PC-LUC plants are sprayed with luciferin, the substrate of LUC, in vivo LUC activity can be measured by counting luminescence with sensitive video-imaging equipment (Millar et al., 1992). This allows the nondestructive screening of hundreds of seedlings simultaneously. Table 1 shows that in vivo luminescence of PC-LUC seedlings was repressed eightfold by sucrose after 4 days of etiolation. Based on this difference, a mutant screen was performed to isolate mutants that were insensitive to sucrose repression of PC gene expression. Therefore, mutagenized seedlings were grown in the dark for 4 days on a 3% sucrose medium, and seedlings that showed a markedly higher luminescence than wild-type PC-LUC seedlings were selected.

Approximately 50,000 seeds of PC-LUC plant line pc1A2.7 (wild-type PC-LUC; Dijkwel et al., 1996) were treated with ethylmethane sulfonate (EMS), as described in Methods. M2-mutagenized seed was collected from 49 independent batches of M1 plants. The mutagenized seeds were sown on a medium containing 3% sucrose. After 4 days of growth in darkness, the in vivo luminescence of the seedlings was measured. Seedlings that had high luminescence levels under these growth conditions may be defective in sucrose repression of PC gene expression. Therefore, seedlings with a markedly higher luminescence than background were isolated and transferred to soil for seed production. Approximately 70,000 seeds were screened and resulted in the isolation of 17 sucrose-uncoupled (sun) mutants. These mutants showed reduced sucrose repression of LUC-induced luminescence after 4 days of growth in the dark (Table 1).

From the 17 sun mutants isolated, four mutants were selected for which the sucrose-uncoupled phenotype was particularly pronounced (Table 1). sun6 mutants show luminescence levels similar to wild-type PC-LUC levels when grown on a medium without sucrose, whereas the sunl and sun7 mutants had elevated luminescence levels on a medium without sucrose.

The sun mutants were backcrossed to the wild-type PC-LUC plant line. In the F2 population, the segregation of the sun mutations was analyzed (Table 2). The segregation of all of the sun mutations did not differ significantly from a 3:1 (wild-type PC-LUC/mutant) ratio. This indicates that these sun mutations segregate as a single nuclear recessive trait.

The four selected sun mutants were crossed with each other to determine whether these mutants are allelic (Table 3). Two alleles of sunl were found; however, the sunl, sun6, and sun7 mutations fall into different complementation groups. Additional alleles may be found in the other sun mutants.

Sucrose-Uncoupled mRNA Accumulation

The sun mutants could represent mutations affecting only the expression of the PC-LUC transgene. In the case of a true sucrose-sensing or signaling mutant, one would also expect that the expression of the endogenous PC gene would no longer be repressed by sucrose. Therefore, RNA was isolated from the wild-type PC-LUC and sun seedlings to determine whether PC gene expression was also uncoupled for sucrose repression. The seedlings were grown in darkness for 3 days on media with and without 3% sucrose, and RNA was isolated. The steady state RNA levels of PC and several additional genes were analyzed (Figure 1). No PC mRNA was detected in wild-type PC-LUC seedlings grown on a medium with 3% sucrose, in agreement with previous results (Dijkwel et al., 1996). All of the mutants

<table>
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<th>Table 1. LUC-induced Luminescence Phenotype of sun Mutants</th>
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<td>Mutant</td>
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<tr>
<td></td>
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<tr>
<td>Wild-type PC-LUC</td>
</tr>
<tr>
<td>sunl-1</td>
</tr>
<tr>
<td>sun1-2</td>
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<tr>
<td>sun6</td>
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<td>sun7</td>
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aLUC-induced luminescence was measured from the wild-type PC-LUC transgenic plant line and several sun mutants. The seedlings were grown for 4 days in darkness on media or without 3% sucrose. In vivo luminescence was measured as described in Methods.

<table>
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<th>Table 2. Segregation of sun Mutationsa</th>
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<tr>
<td>Mutant</td>
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<tr>
<td>--------</td>
</tr>
<tr>
<td>sunl-1</td>
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<tr>
<td>sun1-2</td>
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<td>sun6</td>
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a The sun mutants were crossed to the wild-type PC-LUC plant line, and the F2 generation of the crosses was plated on a medium with 3% sucrose. LUC-induced luminescence was measured after 4 days of seedling growth in darkness, as described in Methods.
b The $\chi^2$ is given for the ratio of 3:1 (wild type/mutant).
c Not significant at P = 0.05.
The sun mutants were backcrossed sun1-2, sun6, and sun7 mutants were backcrossed twice to wild-type PC–LUC plants (n = 12) were grown for up to 8 weeks on soil under long-day conditions (16 hr of light and 8 hr of dark; Figure 2), and bolting time was measured. Wild-type PC–LUC plants bolted after 37 ± 4 (sd) days, and sun6 (30 ± 4 days) and sun7 (41 ± 5 days) plants did not show a significantly different bolting time. However, sun1-2 plants bolted after only 21 ± 2 days. In addition, the sun1-2 mutant had more primary and secondary flower buds, and the rosette leaves were smaller and more yellowish. The sun1-2 mutant also showed reduced fertility, because of a much reduced number of seeds per silique. sun7 plants were much smaller as a result of both shorter petioles and smaller leaves, and the rosette leaves displayed enhanced senescence. In addition, sun7, like sun1-2, showed much reduced fertility. The sun6 mutant was indistinguishable from the wild type under these growth conditions.

**Phenotype of sun Mutants**

The sun1-2, sun6, and sun7 mutants were backcrossed twice to wild-type PC–LUC plants, and the phenotypes that cosegregated with the sucrose-uncoupled luminescence phenotype were determined. The phenotype of the backcrossed sun mutants did not differ markedly from the phenotype of the primary sun mutants (results not shown). The

<table>
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<th>Cross</th>
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<th>Mutant</th>
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<tr>
<td>sun1-1/sun1-1 × sun1-2/sun1-1</td>
<td>38</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
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<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>sun6/sun6 × sun7/sun7</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>sun7/sun7 × sun1-2/sun1-2</td>
<td>23</td>
<td>23</td>
<td>0</td>
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</table>

*a* The sun mutants were crossed, and the F1 generation of the crosses was sown on a medium with 3% sucrose. LUC-induced luminescence was measured after 4 days of seedling growth in the dark.

tested accumulated PC mRNA in the presence of sucrose, although the extent of PC mRNA accumulation varied among the mutants.

Similar to PC, expression of CAB and RBCS genes is also developmentally induced in etiolated seedlings (Brusslan and Tobin, 1982; Dijkwel et al., 1996). Therefore, it was interesting to determine whether these genes were also repressed by sucrose and uncoupled for repression in the sun mutants. CAB and RBCS mRNA accumulation was analyzed and, similar to PC gene expression, was repressed by sucrose in the wild type (Figure 1; for RBCS, results not shown). Similarly, the sun mutants were sucrose uncoupled for accumulation of CAB and RBCS mRNA. Thus, the sucrose repression system affects other nuclear-encoded photosynthetic genes as well. In addition to repression of gene expression, sugars also induce gene expression (Tsukaya et al., 1991; Sadka et al., 1994; Takeda et al., 1994). The CHS gene, which is involved in anthocyanin biosynthesis, is such a gene; its expression can be induced by light (Kubasek et al., 1992) and also by sugars (Tsukaya et al., 1991). In 3-day-old dark-grown seedlings, CHS was induced by sucrose in wild-type PC–LUC seedlings (Figure 1). Under these growth conditions, CHS was more strongly induced by sucrose in the sun mutants.

In summary, the selected sun mutants are uncoupled for sucrose repression of PC, CAB, and RBCS mRNA accumulation. Therefore, the SUN genes are most likely involved in sucrose sensing or signaling.

**Sucrose Represses FR Responses in Wild-Type Seedlings**

Because PC gene expression is both light and sucrose regulated, it was interesting to determine whether mutants that are defective in sucrose repression of PC gene expression also show an altered response to light. It was noted previously that sucrose represses the inhibition of hypocotyl elongation in FRc (Whitelam et al., 1993). Therefore, wild-type PC–LUC seedlings were grown in FRc (Figure 3). Wild-type PC–LUC seedlings grown on a medium without sucrose for 1 day in the dark followed by 4 days FRc exhibited yellowish, opened cotyledons and short hypocotyls (Figure 1).
Figure 2. Phenotypes of Eight-Week-Old sun Mutants.

Wild type PC-LUC (first plant), sun1-2 (second plant), sun6 (third plant), and sun7 (fourth plant) plants were grown for 8 weeks in long-day conditions (16 hr of light and 8 hr of dark) on soil and photographed.

3). These phenotypes are characteristics for FR-HIRs (Mancinelli, 1994). Interestingly, when wild-type PC-LUC seedlings were exposed to the same light conditions on a 3% sucrose medium, ~30% of the seedlings still had closed cotyledons and the seedlings had much longer hypocotyls (Figures 3 and 4).

The extent of FR-HIRs depends on the wavelength, the fluence rate, and the duration of illumination (Mancinelli, 1994). To examine more closely the effect of sucrose on FR responses, a fluence rate-response curve was determined for wild-type PC-LUC seedlings (Figure 4). The wild-type PC-LUC seedlings were grown in the dark and at six different fluence rates of FRc, ranging from 0.008 to 29.7 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \). Three different responses of the seedlings to FRc and sucrose were analyzed. Figure 4A shows the percentage of wild-type PC-LUC seedlings that had opened cotyledons after 5 days of growth in darkness or 1 day in darkness followed by 4 days in FRc. When grown in the dark or at low fluence rates of FRc (up to 0.047 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \)), all of the wild-type PC-LUC seedlings had closed cotyledons. When grown on a medium without sucrose, ~30% of the wild-type PC-LUC seedlings had open cotyledons at 0.31 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \). When grown on a 3% sucrose medium, cotyledon opening was repressed, and only at a fluence rate of 1.8 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \) did ~70% of the seedlings have open cotyledons. At this fluence rate, all seedlings grown on a medium without sucrose exhibited opened cotyledons. Thus, sucrose repressed cotyledon opening in a fluence rate-dependent manner.

In addition, the effect of sucrose on hypocotyl elongation was measured (Figure 4B). When grown in the dark, the wild-type PC-LUC seedlings had elongated hypocotyls. On sucrose plates, the wild-type PC-LUC seedlings had shorter hypocotyls, as previously observed (Dijkwel et al., 1996). When grown in FRc on a medium without sucrose, hypocotyl elongation of wild-type PC-LUC seedlings was not markedly inhibited up to a fluence rate of 0.31 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \). The most drastic decrease in hypocotyl length occurred between a fluence rate of 0.31 and 9.2 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \), and an additional increase of the fluence rate to 29.7 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \) did not result in a further shortening. Inhibition of hypocotyl elongation was repressed in seedlings that were grown on plates with sucrose. Here, the most drastic decrease in hypocotyl length occurred between a fluence rate of 1.8 and 29.7 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \). These fluence rates are

Figure 3. Phenotypes of sun Mutants after FRc Treatment.

Wild-type PC-LUC (WT) and sun seedlings were grown on a medium without (0%) or with (3%) 3% sucrose for 1 day in the dark, followed by 4 days in FRc at a fluence rate of 1.8 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \) (for details, see Methods). For each treatment, representative seedlings were selected and photographed. Both wild-type PC-LUC seedlings grown on a 3% sucrose medium have opened cotyledons, whereas the sun1 and sun6 mutants each have one seedling with closed and one with opened cotyledons. The apparent pigmentation of the hypocotyls of sun1-2 and sun6 seedlings is a photographic artifact. Bar = 2 mm.
The Plant Cell

A

Wild-type PC-LUC

0%

3%

sun1-2

sun6

sun7

Percentage of seedlings with opened cotyledons

Fluence rate (μmol m⁻² sec⁻¹)

B

Wild-type PC-LUC

0%

3%

sun1-2

sun6

sun7

Fluence rate (μmol m⁻² sec⁻¹)

Figure 4. FRc Fluence Rate-Response Curve.

Wild-type PC-LUC, sun1-2, sun6, and sun7 seedlings were grown on a medium without (0%) or with (3%) 3% sucrose. The seedlings were grown for 1 day in the dark, followed by 4 days in the dark (0 μmol m⁻² sec⁻¹), or six different fluence rates of FRc, ranging from 0.008 to 29.7 μmol m⁻² sec⁻¹. 

(A) After FRc treatment, the percentage of seedlings with opened cotyledons was calculated. Cotyledon opening was scored as described in Methods.

(B) After FRc treatment, the hypocotyl lengths were measured. Error bars indicate the standard deviation of the mean.

higher compared with those for seedlings grown on a medium without sucrose.

Recently, a novel FR-induced response was described for both tomato and Arabidopsis seedlings (Van Tuinen et al., 1995; Barnes et al., 1996). FR can induce a block of greening when seedlings are transferred to Wc after FRc treatment. After 5 days of darkness or 1 day of darkness followed by 4 days in FRc, the wild-type PC-LUC seedlings were transferred to a medium without sucrose and Wc. After an additional 4 days in Wc, the percentage of seedlings that had green cotyledons was scored (Figure 5). Wild-type PC-LUC seedlings that were grown in the dark deetiolated and turned green upon subsequent exposure to Wc. The lowest FRc fluence rate used resulted in a block of greening of ~50% of the wild-type PC-LUC seedlings when grown on a medium without sucrose. At 1.8 μmol m⁻² sec⁻¹, none of the seedlings turned green, whereas higher fluence rates resulted again in more green seedlings. Sucrose repressed the block of greening efficiently: 100% of the wild-type PC-LUC seedlings turned green at all fluence rates except 1.8 μmol m⁻² sec⁻¹. At this fluence rate, most seedlings lost their ability to green after subsequent Wc treatment, even when grown on a 3% sucrose medium. All of these experiments were repeated once, and the results were similar. In conclusion, in the wild-type seedlings, sucrose represses three FR-
FR-Induced Cotyledon Opening and Inhibition of Hypocotyl Elongation Are Sucrose Uncoupled in sun7 Seedlings

In addition to the wild-type PC–LUC seedlings, the sun1-2, sun6, and sun7 mutants were grown in FRc (Figure 3). Here, sun7 seedlings differed strongly from the wild type only when grown on a 3% sucrose medium: all sun7 seedlings had opened cotyledons and had shorter hypocotyls than did wild-type PC–LUC seedlings (Figures 3 and 4).

To examine more closely the effect of sucrose on FRc-induced cotyledon opening and inhibition of hypocotyl elongation in the sun mutants, we determined a fluence rate–response curve for the sun1-2, sun6, and sun7 mutants (Figure 4). Both the mutants sun1-2 and sun6 did not respond in markedly different ways from the wild type with respect to cotyledon opening. Cotyledon opening, however, was sucrose uncoupled in sun7 seedlings (Figure 4A). Regardless of the presence of sucrose in the growth medium, some sun7 seedlings showed opened cotyledons at a fluence rate of 0.047 μmol m⁻² sec⁻¹. At this fluence rate, all wild-type PC–LUC seedlings had closed cotyledons, and this observation suggests that sun7 seedlings were more sensitive to FR.

In addition, hypocotyl elongation was measured (Figure 4B). When the sun seedlings were grown in the dark, hypocotyl elongation was not markedly inhibited by sucrose. This is in contrast with wild-type PC–LUC seedlings and suggests that the selected mutants are sucrose uncoupled for inhibition of hypocotyl elongation in the dark. In addition, the hypocotyls of sun7 seedlings were shorter than those of wild-type PC–LUC seedlings. Also, sun7 seedlings were different from wild-type PC–LUC seedlings under FRc growth conditions (Figure 4B). A fluence rate of 0.008 μmol m⁻² sec⁻¹ caused a decrease of the hypocotyl length in sun7 seedlings that were grown on a medium without sucrose. When subjected to this fluence rate, sun7 seedlings that were growing on a sucrose-containing medium had longer hypocotyls. However, the ratio of hypocotyl length on sucrose-containing media versus sucrose-free media remained approximately the same at all fluence rates. Moreover, irrespective of the presence of sucrose in the growth medium, the most drastic decrease in hypocotyl length occurred between a fluence rate of 0.31 and 9.2 μmol m⁻² sec⁻¹, and a further increase of the fluence rate to 29.7 μmol m⁻² sec⁻¹ did not result in a further reduction of hypocotyl length. Thus, in the sun7 mutant, sucrose has a reduced capacity to repress sucrose-mediated inhibition of hypocotyl elongation. All of these experiments were repeated, and similar results were obtained. Thus, the sun7 mutant is sucrose uncoupled for FRc-induced cotyledon opening and inhibition of hypocotyl elongation.

sun6 and sun7 Mutants Are Affected in FR-Induced Block of Greening

To determine the effect of the sun mutations on FR-induced block of greening, the sun mutants were transferred to agar media without sucrose and were given Wc after dark or FRc treatment. After 4 days in Wc, the percentage of seedlings that had green cotyledons was counted (Figure 5). sun1-2 seedlings did not respond in markedly different ways from wild-type PC–LUC seedlings. In contrast, sun6 seedlings were less able to deetiolate than the wild type when grown on a medium without sucrose. This suggests that the SUN6 gene may have a function in the deetiolation process. In addition, sun6 seedlings were sucrose uncoupled for the block of greening at low fluence rates, ranging from 0.008 to 1.8 μmol m⁻² sec⁻¹. In these instances, sucrose did not effectively repress the block of greening. Only at a fluence rate of 0.047 μmol m⁻² sec⁻¹ did sucrose have some repressive effect on the block of greening, however, not to a level similar to that of the wild-type PC–LUC. At higher fluence rates, sucrose repressed the FR-induced block of greening more effectively.

On a medium without sucrose, the sun7 mutants were more resistant to the FR-induced block of greening than were wild-type PC–LUC seedlings at all fluence rates tested. This resulted in a higher percentage of seedlings with green cotyledons after Wc treatment (Figure 5). When grown on a medium with 3% sucrose, during the FRc treatment, the block of greening was completely abolished, because all seedlings turned green, even at the most effective fluence rate of 1.8 μmol m⁻² sec⁻¹. Comparable results were obtained in a duplicate experiment.

In conclusion, the sun6 mutant is sucrose uncoupled for the block of greening up to a FR fluence rate of 1.8 μmol m⁻² sec⁻¹. The sun7 mutant shows a reduced sensitivity to FR-induced block of greening in the absence of sucrose, whereas sun7 seedlings are insensitive to the block in the presence of sucrose.

DISCUSSION

Isolation of sun Mutants by Using an in Vivo Selectable Marker

In light- or dark-grown Arabidopsis seedlings, PC gene expression can be repressed by sucrose (Dijkwel et al., 1996). LUC activity can be similarly repressed in wild-type PC–LUC seedlings. This repression is more efficient in dark-grown than in light-grown seedlings. Based on the in vivo expression of the LUC reporter gene, a genetic screen was designed to isolate mutants defective in sucrose repression of PC gene expression. For this purpose, EMS-mutagenized PC–LUC seedlings showing high luminescence levels on 3% sucrose media were isolated. In several of these mutants,
the LUC-induced luminescence was less sensitive to sucrose repression as compared with the wild-type PC-LUC and were called sun mutants (Table 1). The sun6 mutant had wild-type luminescence levels on media without sucrose, whereas the sun1 and sun7 mutants had increased luminescence levels on sucrose-free media. Interestingly, these latter sun mutants did not accumulate more PC mRNA when grown on media without sucrose as compared with the wild-type PC-LUC (Figure 1). One explanation for this observation is that the endogenous PC gene contains additional regulatory elements for the regulation of gene expression. These elements apparently limit accumulation of "excess" PC mRNA, possibly by mediating increased mRNA turnover. Such elements may be present in the transcribed region of the PC gene, which are absent in the PC-LUC construct. We cannot exclude, however, that the sun1 and sun7 mutations somehow affect LUC mRNA or LUC protein levels. Also, energy metabolism may be affected in these sun mutants, resulting in increased availability of cellular ATP, a substrate for LUC.

Our results show that the sun mutants are signal transduction pathway mutants and not mutations affecting only the PC promoter or other parts of the PC-LUC transgene. In addition to LUC-induced luminescence, PC, CAB (Figure 1), and RBCS (results not shown) mRNA accumulation was also insensitive to sucrose repression in the sun mutants. Thus, the signal transduction pathway leading to sucrose repression of PC gene expression also affects other nuclear-encoded photosynthesis genes. The SUN genes must therefore represent functions that are required for the regulation of groups of genes rather than individual genes.

Because CHS gene expression can be induced by sugars (Tsukaya et al., 1991), we measured CHS mRNA in dark-grown seedlings to determine the effect of the sun mutations on a sugar-inducible gene. The CHS gene was more strongly induced by sucrose in the sun mutants than in wild-type PC-LUC seedlings. This indicates that the signal transduction processes leading to sucrose repression of PC gene expression may be different from the signal transduction processes for induction of CHS gene expression by sucrose. This is in agreement with recent results (R.B. McGrath, Y. Wu, G. Neuhaus, and N.-H. Chua, manuscript submitted). By treatment of the tomato aurea mutant and a photomixotrophic soybean cell suspension culture with sucrose and pharmacological agents, it was shown that independent signal transduction pathways cause repression of CAB and induction of CHS gene expression. However, the sun mutations have an effect on CHS gene expression. One explanation for this observation is that there is some sort of crosstalk between the two signaling pathways. For example, a block of the signal flow in one pathway, caused by a sun mutation, may result in an increase of the signal flow in another pathway, resulting in the overexpression of CHS gene expression. It should be noted, however, that CHS gene expression can be induced by many other external stimuli, such as light, wounding, or stress (Choppell and Hahlbrock, 1984; Kubasek et al., 1992; Martin, 1993), and a mutation that affects light signal transduction processes or that causes additional stress may result in higher CHS mRNA levels. Also, CHS gene expression is expressed as part of the plant defense response (Choppell and Hahlbrock, 1984; Lamb et al., 1989). Herbers et al. (1996a) have provided evidence that sugars are involved in the induction of plant defense responses and that sun mutations that cause changes in soluble sugar levels may induce CHS gene expression as part of a defense response.

Genetic analyses revealed that the sun mutations segregate for a single nuclear recessive mutation. Two alleles were found for sun1. Other alleles of sun1, sun6, and sun7 may be found in the other 13 as yet uncharacterized sun mutants. In conclusion, the use of an in vivo selectable marker resulted in the isolation of at least three sun loci that are affected in signal transduction pathway processes that lead to sucrose repression of PC gene expression.

**Sucrose Repression of phyA Signal Transduction Pathways**

Arabidopsis seedlings exposed to FRc undergo partial photomorphogenesis. The seedlings have short hypocotyls and opened cotyledons. Chlorophyll does not accumulate, however, and the seedlings are yellowish. Recently, a novel FR-dependent response was described for both tomato and Arabidopsis seedlings (Van Tuinen et al., 1995; Barnes et al., 1996). It was found that seedlings that were treated with FRc lost the ability to turn green in subsequent WC treatment. Barnes et al. (1996) showed that this block of greening occurred during the FRc treatment and was repressed by sucrose. All of these FR-dependent responses are believed to be mediated by phyA (Mancinelli, 1994; Smith, 1995; Barnes et al., 1996).

We have studied the effect of sucrose on FR responses in detail at six fluence rates of FRc. In wild-type PC-LUC seedlings that were grown on a 3% sucrose medium, cotyledon opening was repressed, depending on the fluence rate (Figures 3 and 4A). In addition, wild-type PC-LUC seedlings had much longer hypocotyls when grown on plates with 3% sucrose (Figures 3 and 4B), which is in agreement with an observation made by Whetem et al. (1993). For similar FR responses, seedlings that were grown on a 3% sucrose medium required an approximately five times higher fluence rate than did seedlings grown on a medium without sucrose. The observation that sucrose represses FRc responses in a fluence rate-dependent manner suggests that sucrose represses the FR signal rather than the FR response.

The block of greening was studied by transferring the wild-type PC-LUC seedlings to WC after FRc treatment. Up to a fluence rate of 1.8 μmol m⁻² sec⁻¹, an increase in the fluence rate resulted in an increase in the block of greening. Higher fluence rates, however, resulted in a decrease of the block of greening. Thus, the highest fluence rate was not the
most effective one for the induction of block of greening. These results suggest that for FRc, one signal operates at low fluence rates and induces the block of greening, whereas a separate, high fluence rate-dependent signal can override this block, resulting in normal greening of the cotyledons. Sucrose was an efficient repressor of the block of greening (Figure 5), as has been found by Barnes et al. (1996). However, the repressing effect of sucrose was not complete. When wild-type PC-LUC seedlings were grown on 3% sucrose media at a fluence rate of 1.8 μmol m⁻² sec⁻¹, ~80% of the seedlings failed to green in subsequent Wc treatment. Thus, both on media with and without sucrose, the most effective fluence rate was 1.8 μmol m⁻² sec⁻¹. This suggests that sucrose represses the block of greening rather than the FR signal that causes block of greening. For example, sucrose may stimulate POR gene expression independent of light, thereby partially abolishing the repressive effect of FR on POR gene expression. In conclusion, sucrose represses phyA-dependent responses in wild-type PC-LUC seedlings.

Functions of the SUN6 and SUN7 Genes in Sucrose Repression of FR Signaling Pathways

Sucrose-mediated repression of cotyledon opening and inhibition of hypocotyl elongation are reduced in sun7 seedlings (Figures 3 and 4). These results provide evidence that the SUN7 gene product plays a role in the sucrose-dependent repression of cotyledon opening and inhibition of hypocotyl elongation in FR (Figure 6). However, independent of the FR fluence rate, hypocotyls of sun7 seedlings were longer on sucrose media than on media without sucrose. This suggests that for sun7 seedlings, sucrose represses inhibition of hypocotyl elongation also in a fluence rate-independent manner.

Sucrose did not efficiently repress the block of greening in sun6 seedlings up to a FR fluence rate of 1.8 μmol m⁻² sec⁻¹. This indicates that SUN6 plays a role in the sucrose-dependent repression of the FR-induced block of greening. Thus, SUN7 plays a role in the sucrose-dependent repression of cotyledon opening and inhibition of hypocotyl elongation, whereas SUN6 plays a role in the block of greening but not in cotyledon opening and inhibition of hypocotyl elongation. Therefore, these mutants genetically specify two separate branches of phyA-dependent pathways. This supports results obtained by Barnes et al. (1996). It was shown that both phy1 and hy5 (for far-red elongated hypocotyl and long hypocotyl, respectively) mutants develop long hypocotyls in FRc. Only phy1 seedlings, however, are resistant for the block of greening. Therefore, it was concluded that the signal that initiates the block of greening is transduced by a pathway in which PHY1 but not HY5 is a component. sun7 seedlings showed a reduced sensitivity to FR-induced block of greening on media with and without sucrose and shows that SUN7 plays a role in this response as well.

The results presented here are summarized in a model shown in Figure 6. In this model, FR induces three responses through the action of phyA. One branch of the pathway results in cotyledon opening and inhibition of hypocotyl elongation. A separate branch causes block of greening. These responses can be repressed by sucrose. SUN6 is involved in the sucrose-dependent repression of the block of greening, and SUN7 is involved in the sucrose-dependent repression of cotyledon opening and inhibition of hypocotyl elongation. In addition, SUN7 stimulates the FR-induced block of greening in either a sucrose-dependent or -independent way.

It is as yet unclear whether the above-mentioned repressive effects of sucrose on light signaling are specific for phyA. When Arabidopsis seedlings were germinated in a single fluence rate of W, R, or blue light, cotyledon opening was stimulated and hypocotyl elongation was inhibited (results not shown). When wild-type PC-LUC seedlings were grown on sucrose-containing media, some repressive effect of sucrose on inhibition of hypocotyl elongation was observed in all light qualities tested. sun7 seedlings germinated in these light qualities had shorter hypocotyls most markedly on 3% sucrose media (results not shown), indicating that sucrose may require the SUN7 gene in other light qualities as well for sucrose repression of the inhibition of hypocotyl elongation by light. The repressive effect of sucrose on FR signaling is fluence rate dependent, and it is likely that this is the same in other light qualities.

Metabolic Repression and Light Signaling

Metabolic repression of gene expression of light-regulated photosynthetic genes, such as CAB and RBCS genes, has been proposed to be a mechanism for the feedback inhibition of photosynthesis by carbohydrates (Sheen, 1990; Harter et al., 1993; Krapp et al., 1993; Jang and Sheen, 1994; Van Oosten et al., 1994). The analysis of several sun mutants provided evidence for a close interaction between metabolic repression and light
signaling in Arabidopsis seedlings. It is likely that in mature plants, a similar interaction occurs between light and metabolite signaling systems. Such a close interplay between two regulatory pathways would allow the plant to respond in an integrated manner to environmental changes. Although the function of the sun mutations was studied in seedlings, clearly the SUN genes also play a role in mature plants (Figure 2). The phenotype of the sun1 and sun7 mutants suggests an interaction between metabolic repression and light signaling in mature plants. sun1 plants showed reduced fertility and flowered 2 weeks earlier than did wild-type PC-LUC plants, and the leaves appeared more yellow when grown under long-day conditions (results not shown). A role for sugars was suggested in the stimulation of floral transitivity and flowered >2 weeks earlier than did wild-type plants, such a defect may cause an exaggerated light response, resulting in short petioles and reduced cell expansion. In addition, sun7 plants, like sun1 plants, produced much less seed per silique, which indicates that sugars may play a role during meiosis or seed maturation.

Under long-day conditions, sun6 mutants were indistinguishable from the wild type. Recent evidence, however, suggests that sun6 plants show a reduction in the feedback inhibition of photosynthesis by the sugar analog 2-deoxy glucose (J.-J. Van Oosten, A. Gerbaud, C. Huijser, P.P. Dijkwel, N.-H. Chua, and S.C.M. Smeekens, manuscript submitted). In conclusion, we have analyzed several signal transduction mutants that are defective in metabolic repression of photosynthetic gene expression. Our results suggest a close interaction between metabolic repression and light signaling processes. Determination of the exact nature of the sun mutants will provide more insight in sugar-sensing systems in plants and the interaction with light signaling.

METHODS

Plant Materials and Growth Conditions

The plastocyanin–luciferase (PC–LUC) construct was assembled and transferred into Arabidopsis thaliana C24, as described in Dijkwel et al. (1996). The PC–LUC transgenic plant line pc1A2.7 (wild-type PC–LUC) has been described elsewhere (Dijkwel et al., 1996). For the experiments, seeds were surface sterilized by washing for 5 min in a commercial bleach and rinsed four times with sterile MilliQ water (Millipore, Bedford, MA). Seeds were sown onto germination medium (Valvekens et al., 1998) without sucrose or with 3% (88 mM) sucrose in 0.1% (v/v) agarose. For the mutant screen, ~450 surface-sterilized seeds were transferred to 10 mL of MilliQ and sown onto a filter paper that was placed on a 15-cm-round plate containing germination medium with 3% sucrose. The water was then carefully removed, and the seeds were equally distributed to allow facile selection of putative mutants. For mRNA analysis, ~300 surface-sterilized seeds were transferred to 10 mL of MilliQ water and sown onto filter paper contained in a 15-cm-round plate. The plates containing the seeds were wrapped in aluminum foil, and the seeds were allowed to imbibe for 4 days at 4°C. After imbibition, the seeds were irradiated with 45 min of red light (R) or 1 hr of white light (W) to promote germination. (No difference was found between the two light treatments.) For in vivo luminescence measurements, the plates were transferred to a room with green safelight, and the seeds were sprayed with a 5-mM luciferin (Analytical Bioluminescence Laboratories, San Diego, CA) and 0.01% (v/v) Triton X-100 solution to decrease the stability of the LUC protein already present in the seeds (Millar et al., 1992b). The plates were wrapped in aluminum foil and stored in a light-tight box in a growth chamber at 22°C. Just before imaging of LUC-induced luminescence, the seedlings received an additional spray of luciferin solution. For experiments with light-grown seedlings, the seedlings were transferred to a growth chamber at 20 or 22°C and continuous W (Wc).

Mutagenesis

Approximately 50,000 seeds of the PC–LUC plant line pc1A2.7 were allowed to imbibe for 5 days at 4°C. The seeds were dried and subsequently transferred to 25 mL of MilliQ water supplemented with 31.5 μL of ethyl methanesulphonate (EMS; Sigma). The seeds were stored for 24 hr at 22°C in the dark. The EMS-treated M1 seeds were carefully washed with MilliQ water before being sown on soil in 49 separate containers. The seeds were transferred to W (16 hr of light and 8 hr of dark) for EMS-mutagenized M2 seed production. EMS-mutagenized M2 seed from each of the individual containers was harvested separately.

Mutant Selection

Putative mutants were selected after 4 days of growth in the dark. The selection of mutants was complicated by the difference in luminescence between the individual seedlings. Luminescence between
nonmutagenized individual wild-type PC-LUC seedlings differed by a factor of 10. This characteristic of the luminescence measurement may favor the selection of false-positive seedlings. Therefore, we selected as putative mutants only seedlings with more than an ~1.5-fold higher luminescence compared with the best-expressing wild-type PC-LUC seedlings. Because the sucrose-uncoupled (sun) mutants were isolated from independent EMS-MZ mutagenized M2 seed batches, they must represent individual mutational events in different M1 plants.

In Vivo Imaging of Luciferase-Induced Luminescence

Seeds were sown onto plates and treated as described above. The seedlings were sprayed with a luciferin solution (5 mM luciferin and 0.01% Triton X-100), and luminescence was imaged using an intensified camera (VIM) and an ARGUS-50 photon-counting image processor (Hamamatsu Photonics Systems, Bridgewater, NJ). Exposure times were 20 min for dark-grown and 5 min for light-grown seedlings. Luminescence levels were calculated using the ARGUS-50 image analysis software.

RNA Analysis

Seedlings were frozen in liquid nitrogen, and total RNA was isolated according to the method of Brusslan and Tobin (1992). Total RNA (7.5 μg) was electrophoresed and transferred to hybond N (Amer sham) filters, as described previously (see volume 10, pages 5 to 7, of Focus, published by Bethesda Research Laboratories). After hybridization in a hybridization buffer (Church and Gilbert, 1984) for 24 hr at 65°C, the filters were washed twice at room temperature in 0.1% (w/v) SDS and 0.5 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate) and twice at 55°C in the same buffer. The filters were subsequently exposed to x-ray films for autoradiography. Filters that were stripped by boiling briefly in a 1% SDS and 0.1 × SSC solution were used for rehybridization.

Far-Red Light Fluence Rate–Response Studies

For the far-red light (FR) fluence rate–response studies, a threshold box unit was used in combination with an interference filter of λmax 731 nm (Baird-Atomic, Bedford, MA; bandwidth at 50% of λmax at 11.2 nm), as described by Peters et al. (1992). The fluence rate–response studies were performed in duplicate. Figures 4 and 5 show the data of the first experiment. For each individual data point, 10 to 14 seedlings were used. In the duplicate experiment, the light intensity was ~10% lower for each fluence rate, due to aging of the lamp. Here, 15 to 20 seedlings were used for each data point, and the results obtained were comparable with those of the first experiment. For hypocotyl length measurements, the seedlings were spread out on a medium containing 0.7% agar and photographed; hypocotyl lengths were subsequently measured from enlarged photographs.

During etiolation, the cotyledons are fully aligned from base to tip, and we never observed cotyledon opening under dark conditions. Light-dependent opening of cotyledons was scored when the cotyledon tips had separated and were pointing away from each other. Thus, any degree of cotyledon opening was scored as such. For the greening experiments, the seedlings were transferred to plates containing 0.7% agar and Wc. The number of seedlings with green cotyledons was counted after an additional 4 days of growth. Cotyledons were defined as green when they had both a green color and were fully expanded. Cotyledons that failed to green were shrivelled and were white or transparent. Several seedlings with white cotyledons had green shoot meristems. During the experiments, we noted a difference between the Arabidopsis C24 and Landsberg erecta ecotype when grown on media without sucrose. When exposed to high fluence rates of continuous FR (FRc), a much greater percentage of wild-type PC-LUC (C24 ecotype) seedlings greened than did Landsberg erecta seedlings; for example, with the highest fluence rate, all wild-type PC-LUC seedlings greened, whereas ~10% of the Landsberg erecta seedlings greened.

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