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Analysis of Hydrogen Peroxide–independent Expression of the High-light–inducible *ELIP2* Gene with the Aid of the *ELIP2* Promoter–Luciferase Fusion¶

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ABSTRACT

Intense and excessive light triggers the evolution of reactive oxygen species in chloroplasts, and these have the potential to cause damage. However, plants are able to respond to light stress and protect the chloroplasts by various means, including transcriptional regulation at the nucleus. Activation of light stress–responsive genes is mediated *via* hydrogen peroxide–dependent and –independent pathways. In this study, we characterized the Early-Light–Inducible Protein 2 (*ELIP2*) promoter–luciferase gene fusion (*ELIP2::LUC*), which responds only to the hydrogen peroxide–independent pathway. Our results show that *ELIP2::LUC* is expressed under nonstressful conditions in green tissue containing juvenile and developing chloroplasts. Upon light stress, expression was activated in leaves with mature as well as developing chloroplasts. In contrast to another high-light–inducible gene, *APX2*, which responds to the hydrogen peroxide–dependent pathway, the activation of *ELIP2::LUC* was cell autonomous. The activation was suppressed by application of 3-(3,4)-dichlorophenyl-1,1-dimethylurea, an inhibitor of the reduction of plastoquinone, whereas 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, an inhibitor of the oxidation of plastoquinone, gave the contrasting effect, which may suggest that the redox state of the plastoquinone plays an important role in triggering the hydrogen peroxide–independent light stress signaling.

INTRODUCTION

For plants, sunlight is the one and only source of energy for photosynthesis and thus is indispensable for the growth of plants. However, an intense and unmanageable amount of light (high light) spells danger for the plant. Under high-light conditions, electrons leak from excited chlorophylls as well as from the photosynthetic electron transport system (ETS), resulting in the generation of oxygen and lipid radicals. These damage proteins, lipids, pigments, DNA and all other components of the chloroplast (1). However, higher plants have developed several strategies to protect the chloroplasts from high light. These include reduction of antenna size, downregulation of Photosystem II, photorespiration, development of radical scavengers and induction of nonphotochemical quenching to reduce the excitation energy transferred from the antenna to the reaction centers (1–3). Some of these high-light responses are controlled at the level of gene expression.

Several nuclear genes have been reported to be activated by high light, including Early-Light–Inducible Protein (*ELIP*) (4), genes encoding active oxygen scavengers (5), actin, LEA, metallothionein (6), a putative transcription factor (7) and others with unknown functions (6,8). Recently, microarray analysis has revealed about 100 high-light–inducible genes of *Arabidopsis* (9,10). The *Arabidopsis APX2* gene that encodes cytosolic ascorbate peroxidase has been most frequently used to study the molecular mechanism of high-light activation of nuclear gene expression. The high-light activation of *APX2* is mediated by hydrogen peroxide, which accumulates through excess light-dependent generation of oxygen radicals (11). Furthermore, it has been shown that the high-light signal for activation of *APX2* is mediated from cell to cell (11). In addition, reduction of the plastoquinone pool has been suggested to be necessary for activation of *APX2*. However, many things need to be revealed to understand the whole signaling pathway from high-light perception to gene expression, including the relationship between reduction of the plastoquinone pool and generation of hydrogen peroxide, its perception and the downstream events after hydrogen peroxide accumulation. Furthermore, it is not known if all the high-light–regulated genes are controlled by the same mechanism as *APX2*.

The *ELIP* was first identified from the pea as a transcript that was rapidly induced by light during greening (12). Subsequent analyses revealed that *ELIP* belongs to the *CAB* superfamily and associates with Photosystem II at the thylakoid membrane (4,13). Because its expression is induced by light stress in many species, it has been speculated that ELIP is an antistress component for the

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Abbreviations: APX2::LUC, APX2 promoter–luciferase fusion; CCD, charge-coupled device; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4)-dichlorophenyl-1,1-dimethylurea; *ELIP,* early-light–inducible protein; *ELIP2::LUC, ELIP2* promoter–luciferase fusion; ETS, electron transport system; mRNA, messenger RNA; RNase, ribonuclease; RT-PCR, reverse transcriptase–polymerse chain reaction.

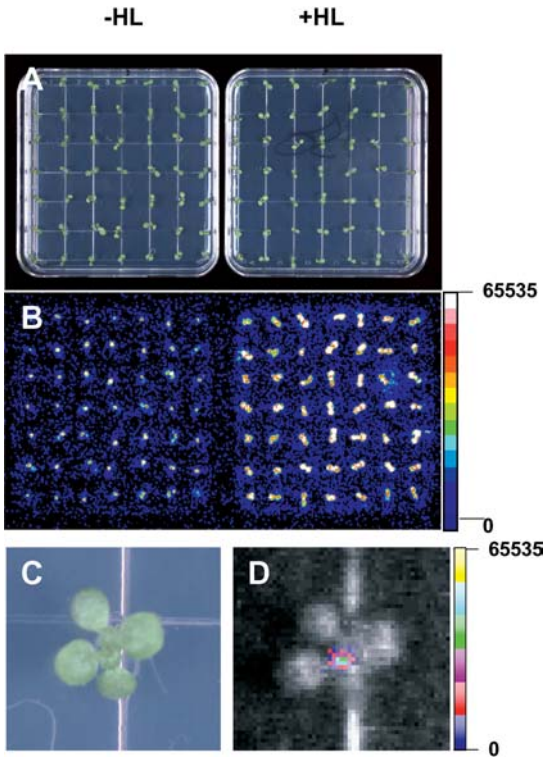

Figure 1. Observation of *ELIP2::LUC* activation by high light. A and B: Induction of luciferase activity by high light in *ELIP2::LUC* transgenic plants observed with a high-performance CCD camera. A: Photograph of 8 day old transgenic seedlings. B: Pseudocolor image of bioluminescence by luciferase activity. Left, seedlings treated without high light (–HL). Right, seedlings treated with high light (+HL, 150 W m^{-2} for 3 h). The color scale on the right shows the luminescence intensity from dark blue (lowest) to white (highest). C: Photograph of a 2 week old seedling grown under low light (6 W m^{-2}). D: The corresponding bioluminescence image representing luciferase activity. The picture in (D) is a synthetic image created by overwriting the bioluminescence image (color) onto the black-and-white image of the seedling. Both images in (D) were captured by the same CCD camera. Color bar indicates the scale of the color.

protection of Photosystem II, although the biochemical function of ELIP remains to be elucidated (4).

Arabidopsis has two *ELIP* genes, *ELIP1* (MIPS protein code At3g22840) (14) and *ELIP2* (At4g14690) (15,16). Both genes are activated by light stress (15), but their differential dependence on HY5 was also observed in expression during deetiolation (16). Recent analyses of the *Arabidopsis ELIP2* gene have revealed that its expression is also activated by high-light treatment, and the activation is mediated by hydrogen peroxide (15,17). Furthermore, using an *ELIP2* promoter–luciferase fusion (*ELIP2::LUC*), it was shown that transcriptional activation induced by high light driven by the *ELIP2* promoter was achieved independent of hydrogen peroxide (17). Therefore, high-light induction of *ELIP2* gene expression is achieved by both hydrogen peroxide–dependent and –independent signaling pathways.

To investigate the newly identified hydrogen peroxide–independent pathway for the high-light response, we analyzed expression profiles of *ELIP2::LUC*, which responds only to the hydrogen peroxide–independent pathway. After comparison with gene expression under the control of the *APX2* promoter, we reveal that the hydrogen peroxide–dependent and –independent pathways have distinct characteristics in addition to some common features.

MATERIALS AND METHODS

Plant material and light treatment. Preparation of transgenic *Arabidopsis* containing *ELIP2::LUC* (17), *APX2* promoter–luciferase fusion (*APX2::LUC*) (11), *35S::LUC* and plastocyanin promoter–luciferase fusion (*PC::LUC*) (18) is described elsewhere. Seeds of wild-type and transgenic *Arabidopsis* were surface sterilized, sown on GM medium supplemented with 0.8% Bactoagar (Difco, Detroit, MI) and 1% sucrose, cold treated for 2–4 days and grown under low-light conditions (6 W m^{-2}, equivalent to *ca* 30 μmol m^{-2} s^{-1}) at 22°C (17). Eight day-old seedlings were subjected to high-light treatment with a fluence rate of 150 W m^{-2} (equivalent to *ca* 800 μmol m^{-2} s^{-1}) for 3 h, as described previously (17). For the experiments shown in Fig. 5, leaves grown in a greenhouse were detached, kept on GM medium for 1 day under low light (6 W m^{-2}) at 22°C and locally irradiated with high light (150 W m^{-2}) for 3 h at 22°C with the aid of stainless sheets cut to remove either a straight slit or a Y-shaped slit. For 3-(3,4)-dichlorophenyl-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) treatments, 2 mL of the inhibitor solutions containing 1.8% (vol/vol) ethanol were sprayed per circular culture dish (10 cm diameter) 3 h before high-light treatments and sprayed again just before high-light treatment only for the DBMIB treatment (19). For controls, the corresponding amount of the ethanol solution without DCMU or DBMIB was sprayed.

Luciferase assay. *In vitro* luciferase assays and *in vivo* assays using a high-performance charge-coupled device (CCD) camera were performed as described previously (17). For *in vivo* temporal analysis as shown in Fig. 3, seeds were sown in 96-cell black plates containing GM medium supplemented with 0.8% Bactoagar, 1% sucrose and 1 m*M* luciferin. They were cold treated for 2–4 days. The plates were sealed with clear adhesive sheets that had a tiny hole for each well and put into an automated scintillation counter (Topcount, Packard Japan, Tokyo, Japan). The plates were kept in the dark assay chamber for 5 min before starting the counting to allow the delayed chlorophyll fluorescence to fade. An assay was done for a week at 22°C under dark or light (6 W m^{-2}) conditions. The data from an assay were logged into a text file and subsequently analyzed using Excel software (Microsoft Japan, Tokyo, Japan). Typically, an assay for a week was composed of about 400 data points.

RNA analysis. Eight day-old seedlings were treated with or without high light for 3 h, and the aerial part of the seedlings was harvested for RNA extraction (20). The *ELIP2* messenger RNA (mRNA) was detected by quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) according to a previous report (17). As a negative control, a RNA prep was treated with ribonuclease (RNase) A for 6 h at 37°C and used as a template for RT-PCR.

RESULTS

Expression of *ELIP2::LUC* under nonstressful conditions

It has been reported that *ELIP* expression of higher plants is regulated at the level of protein degradation (21,22) as well as transcription (17). The possibility of posttranscriptional regulation has also been suggested (17). Use of a promoter–reporter gene fusion is a way to distinguish between transcriptional and post-transcriptional regulations. In this report, we focused on the transcriptional regulation of *ELIP2* with the aid of an established transgenic *Arabidopsis* line containing *ELIP2::LUC* (17).

Expression of *Arabidopsis ELIP2* is induced by high-light treatment (15,17) as is expression of the chimeric *ELIP2::LUC* (17). Using the gene fusion, activation can be observed visually with a high-performance CCD camera. Figure 1B (right) shows that high light activates *ELIP2::LUC* expression and that the response was observed in whole cotyledons. Quantitative *in vitro* luciferase assays revealed that the *ELIP2::LUC* was activated approximately 300-fold by a 3 h treatment of high light (150 W m^{-2}) (Table 1). The *ELIP2::LUC* expression was highly specific to photosynthetic organs (*i.e*. cotyledons and leaves; data not shown), which is consistent with the role of the *ELIP* products as antistress

Table 1. Response of *ELIP2::LUC* to high-light stress*

Treatment	*35S::LUC*	*ELIP2::LUC*
−HL	45.82 + 27.29	28.28 + 11.23
+HL	54.55 + 27.27	8719.32 + 1910.35

**35S::LUC* (18) and *ELIP2::LUC* seedlings grown under low light (6 W m^{-2}) were treated with low light (–HL, 6 W m^{-2}) or high light (+HL, 150 W m^{-2}) for 3 h before *in vitro* luciferase assays were carried out. Averages and standard deviations of luciferase activity (cpm) per milligram of protein are shown. The background activity of the nontransgenic extract was around 1 to 5 cpm mg^{-1} protein under our experimental conditions.

components physically associated with Photosystem II (23). Under low-light conditions, *ELIP2::LUC* expression is two orders of magnitude lower than the induced level, although some level of expression was observed (Table 1; Fig. 1B, left). A close inspection of the expressed tissue, as shown in Fig. 1D, revealed that *ELIP2::LUC* expression was limited to the area around the shoot apical meristem, which contains juvenile chloroplasts under development. This observation is similar to the case of the barley *ELIP* gene, which is expressed at the basal segment of leaves that contains the youngest tissue in the monocotyledonous leaf (24).

Consistent with *ELIP2::LUC* expression, analysis of endogenous *ELIP2* mRNA by RT-PCR, as shown in Fig. 2, also detected the transcript under nonstressful conditions, whereas it was not detected in RNase-treated controls or no-RNA controls. Quantitative analysis revealed that the expression level under noninduced conditions was 3.7% of the induced level. These data may suggest that the light stress response constitutively occurs in tissue containing developing chloroplasts.

Although high-light response of the endogenous *ELIP2* gene determined at the transcript level showed 27-fold induction (Fig. 2), the induction of *ELIP2::LUC* analyzed by the reporter activity was much higher (Table 1). Although response in the enzymatic reporter activity is reflected not only by the amount of the reporter transcript, and thus they could have quantitative differences, the major reason of the observed difference is not known.

Transient expression of *ELIP2::LUC* during greening

To further investigate the relationship between *ELIP2::LUC* expression and chloroplast development, we analyzed *ELIP2::LUC* expression during chloroplast development. During seedling development under constant light conditions, proplastids within the cotyledons develop into green chloroplasts that have the ability to perform photosynthesis (25). *In vivo ELIP2::LUC* expression during this process was monitored with the aid of an automated scintillation counter. As shown in Fig. 3A, *ELIP2::LUC* expression was transiently induced at 2–3 days after the start of growth (germination). A *PC::LUC* (18) was also induced during seedling development, but the expression level was maintained at a high level after induction. Interestingly, the expression of *ELIP2::LUC* preceded the induction of *PC::LUC*. Because plastocyanin is a component of the photosynthetic ETS, the earlier induction of *ELIP2::LUC* may suggest that antistress components including *ELIP* are prepared before construction of the photosynthetic ETS.

When dark-grown etiolated seedlings are illuminated, etioplasts synchronously start development and turn into chloroplasts (25). During this greening process, *ELIP2::LUC* expression was

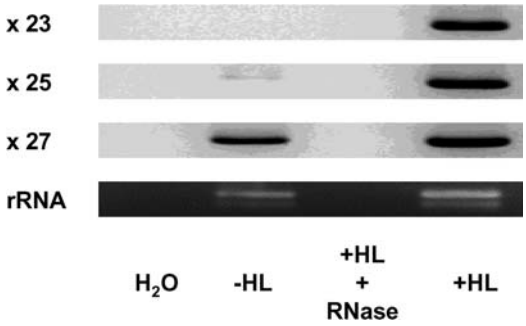

Figure 2. Expression of the internal *ELIP2* transcript under nonstressful conditions. Fluorescence image of *ELIP2* product in RT-PCR after 23, 24 and 27 cycles. Equal amount of total RNA prepared from the aerial part of 8 day old seedlings was subjected to RT-PCR. H_2O, a control without any RNA samples; –HL, RT-PCR product from untreated seedlings; +HL, RT-PCR product from high-light–treated seedlings; and +HL + RNase, RT-PCR product of RNase-treated sample from high-light–treated seedlings. Ribosomal RNA by ethidium bromide staining of the template RNA after electrophoresis.

investigated, and it was found that *ELIP2::LUC* was transiently activated during greening (Fig. 3B). The length of activation was 32 h from the start of illumination to the first trough of the expression level. Although transient induction was reproducibly observed with similar peak height and overall length of the induced period (*ca* 35 h), the shape of the peak varied somewhat from experiment to experiment for unknown reasons (data not shown). In contrast to the downregulation after activation of *ELIP2::LUC*, *PC::LUC* expression was kept high for days after light-shift (Fig. 3B). Transient expression of barley *ELIP* was also reported (26); however, downregulation after activation appears to be slower in the case of the *Arabidopsis ELIP2::LUC*. Thus, *ELIP2::LUC* was found to be expressed in tissue containing developing chloroplasts under nonstressful conditions.

Cell-autonomous stimulation of *ELIP2::LUC*

An *Arabidopsis* cytosolic ascorbate peroxidase gene, *APX2*, is activated by high light (5), and the response has been shown to be mediated by hydrogen peroxide, which accumulates during high-light treatment and the resultant evolution of active oxygen species (11). The *APX2::LUC* construct responds to high-light treatment as well as to hydrogen peroxide. The high-light signal to *APX2::LUC* has been demonstrated to be transduced beyond the high-light–treated cells, indicating intercellular and systemic signaling for *APX2::LUC* activation (11).

To examine whether *ELIP2::LUC*, which responds to the hydrogen peroxide–independent pathway, is also regulated by intercellular signaling, we examined the effect of local irradiation of high light on *ELIP2::LUC* expression. As shown in Fig. 4A, a detached leaf was covered by a stainless sheet with a slit cut out, and the leaf was irradiated with high light (150 W m^{-2}). As a result, only the area exposed by the slit was treated with high light (Fig. 4A, lighter area). Figure 4B shows the response of *ELIP2::LUC*. As can be seen, *ELIP2::LUC* activation was restricted only to the irradiated area, and no activation was observed beyond the area. Figure 4C shows the response of *APX2::LUC*. Consistent with a previous report (11), activation of *APX2::LUC* was observed not only at the irradiated area but also at the base of the leaf that had not been irradiated (arrow), showing intercellular induction of

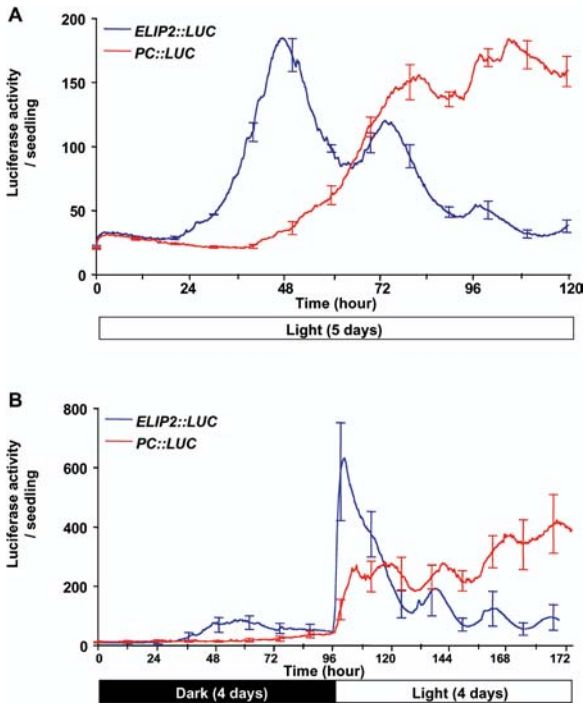

Figure 3. Transient expression of *ELIP2::LUC* expression during chloroplast development. Real time monitoring of *ELIP2::LUC* expression was performed with the aid of an automatic scintillation counter. As a control, a plastocyanin promoter fusion, *PC::LUC* (18), was also monitored. Luciferase activity (cps) of individual seedlings was calculated from *ca* 20 seedlings. A: Seeds were allowed to germinate and develop into dicotyledonous seedlings under continuous light (6 W m^{-2}). B: Seeds were allowed to germinate and grow in the dark for 96 h before inducing greening of the resultant etiolated seedlings by illumination with continuous low light (6 W m^{-2}).

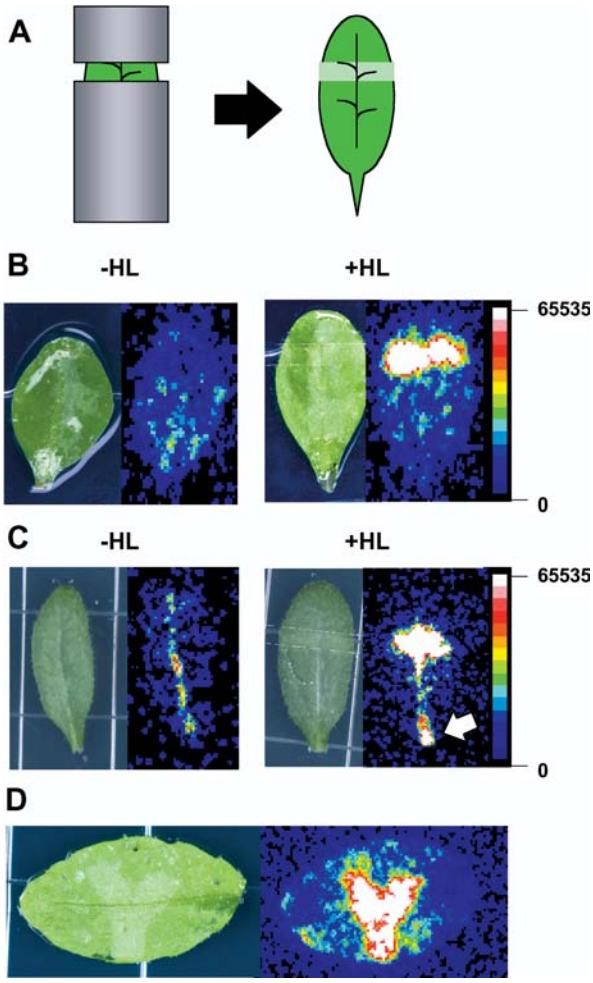

Figure 4. Cell-autonomous activation of *ELIP2::LUC* expression by high light. A: Illustration of the procedure for local irradiation experiments. Leaves containing either *ELIP2::LUC* or *APX2::LUC* were exposed to high light (150 W m^{-2}) for 3 h while covered by a stainless plate with a slit. As a result only the area corresponding to the slit was locally irradiated with high light. Photographs of the leaves and the luciferase activity observed by a high-performance cooled CCD camera are shown in (B–D). The leaves were treated with (+HL) or without (–HL) high light. B: Response of *ELIP2::LUC* transgenic leaf. C: Response of *APX2::LUC* transgenic leaf. Dotted lines in (B) and (C) indicate the area illuminated with high light. The arrow indicates activated area of *APX2::LUC* caused by cell-to-cell signaling. D: Response of *ELIP2::LUC* by Y-shaped irradiation.

APX2::LUC. In Fig. 4D, high light was given to *ELIP2::LUC* in the shape of a "Y." As in the previous experiment, *ELIP2::LUC* showed the cell-autonomous nature of gene activation, and in this case no intercellular signaling was observed in either the proximodistal direction or the mediolateral orientation. Thus, high-light signaling to *ELIP2::LUC*, which represents the hydrogen peroxide–independent pathway, has been shown to be cell autonomous, in contrast to that of *APX2::LUC*, which corresponds to the hydrogen peroxide–dependent pathway.

Role of the plastoquinone pool for activation of *ELIP2::LUC*

Irradiation of leaves with high light causes the production of superoxide radicals from the photosynthetic ETS (1). The superoxide radicals are enzymatically converted to hydrogen peroxide and oxygen, and accumulation of the hydrogen peroxide mediates signal transduction to the *APX2* promoter (11). The activation of *APX2* is suppressed by DCMU, an inhibitor of reduction of the Q_B site of the Photosystem II, and activated by DBMIB, an inhibotor of the cytochrome *b*–cytochrome *f* complex (27), suggesting that the redox state of the plastoquinone plays an important role in the activation of *APX2* (11).

In the case of the hydrogen peroxide–independent pathway, the early events that trigger the signaling are not known. To elucidate them, we examined the effect of the inhibitors of the ETS on the activation of *ELIP2::LUC*. As shown in Fig. 5A, high-light activation of *ELIP2::LUC* was inhibited by DCMU, in accordance with the increased concentration of DCMU, whereas expression of a control *35S::LUC* was not affected by DCMU. On the other hand, DBMIB did not suppress high-light activation of *ELIP2::LUC*, but enhancement depending on the concentration was observed in the absence of the HL treatment, which was not observed in the case of *35S::LUC* (Fig. 5B). Therefore, suppression of high-light signaling to *ELIP2::LUC* by DCMU should not be the result of blocking the whole ETS, and this reciprocal effect suggests that reduction of the plastoquinone pool is indispensable for activation of the *ELIP2::LUC* expression. Because *35S::LUC* expression was not affected by the inhibitors (Fig. 5A,B), the DCMU-specific suppression of *ELIP2::LUC* expression was not the result of inhibition of the specific activity of the luciferase reporter but was specific to the *ELIP2* promoter, consistent with the above interpretation.

The DCMU-specific effect is not restricted to the inhibition of reduction of the plastoquinone pool, but production of hydrogen peroxide has also been reported to be inhibited by DCMU and, depending on the concentration, enhanced by DBMIB (28). However, because *ELIP2::LUC* did not respond to hydrogen peroxide (17), this secondary effect is negligible. Therefore, these analyses may suggest that activation of high-light signaling through the hydrogen peroxide–independent pathway requires reduction of the plastoquinone pool, as suggested for activation of *APX2* (11).

DISCUSSION

High-light signaling and plastid signaling

The high-light response requires corresponding photoreceptors for recognition of the high light. Previous studies indicate that blue light is the most effective for eliciting the response of the pea *ELIP* (21). Therefore, a blue-light receptor like Cryptochrome or Phototropin might be the high-light receptor (4). Another possibility is that the light reaction at the thylakoid membrane might trigger high-light signaling. In this case, light-harvesting proteins containing chlorophylls and carotenoids are considered to be "photoreceptors." Taking into account the following observations, it is suggested that the latter is the case for the high-light response of *ELIP2::LUC*. First, photooxidation of chlorophylls by inhibition of carotenoid biosynthesis by norflurazon activated *ELIP2::LUC* expression (17). The activation by norflurazon was inhibited by okadaic acid, which also suppressed the high-light response (17). This suggests that the signaling pathways to *ELIP2::LUC* for high-light and norflurazon treatments are the same. Second, the high-light response of *ELIP2::LUC* was inhibited by DCMU, which blocks Photosystem II (Fig. 5). Taken together, we propose that high-light signaling to *ELIP2::LUC* is classified as one of "plastid signaling," which is a hypothetical signaling pathway from the chloroplast to the nucleus (29). Our proposal is further supported by the finding that *ELIP2::LUC* is activated by targetitoxin, an inhibitor of the plastidic transcription (17).

There are many reports of plastid signaling in a variety of experimental systems. In higher plants, the plastid signal has been reported to conduct downregulation of several nuclear-encoded photosynthetic genes by malfunction or developmental arrest of the chloroplast. Several causes of this have been reported, including deletion of chloroplast DNA, defect in the plastidic ribosome or metabolism, depletion of carotenoids, inhibition of plastidic transcription and translation and also inhibition of the redox state within the photosynthetic ETS (19,29–32). Because developmental arrest of the chloroplast does not always trigger a plastid signal (33), some restricted aspects of the chloroplast development could be suggested to be critical for the initiation of the signal. Monocellular algae also show this type of signaling. The most well-characterized case is the positive control of a nuclear HSP70 gene of *Chlamydomonas* in response to chloroplast development. Its light induction has been revealed to be mediated by light-dependent synthesis of chlorophyll species within the chloroplast (34,35). Because of the various types of responses mentioned above, it would be reasonable to assume that multiple systems are categorized as plastid signals. In any case, the molecular machineries for signal transduction from inside the chloroplast to the nucleus have not been characterized, although hypothetical machineries for the signal transduction are discussed by Sussek and Chory (30).

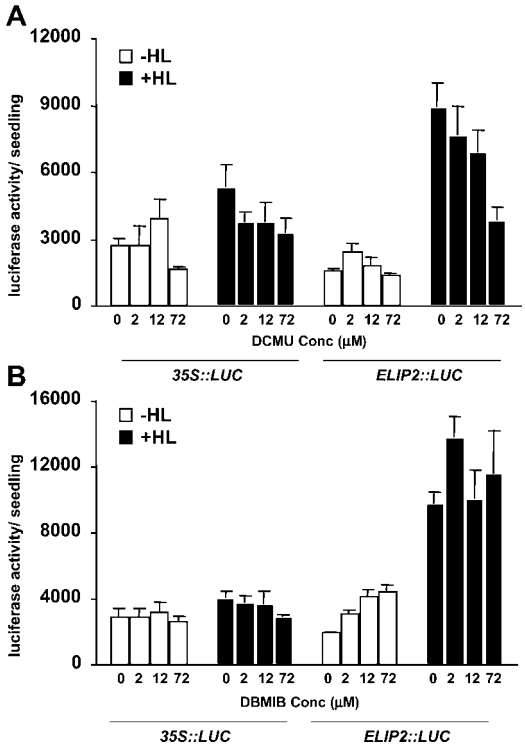

Figure 5. Role of photosynthetic electron transport in high-light signaling to *ELIP2::LUC*. Seedlings were treated under low-light conditions (6 W m^{-2}) for 8 days, sprayed with DCMU or DBMIB at the indicated concentrations (–HL) and exposed to high light (+HL) for 3 h (black bar). Sprayed amount was 2 mL each per circular culture dish (10 cm diameter). For a control, a transgenic line containing *LUC* driven by the constitutive 35S promoter (*35S::LUC*) was assayed in parallel. Averages and standard deviations of luciferase activities (relative light units) are shown. A: Response to DCMU. B: Response to DBMIB.

Expression of *ELIP2::LUC* and juvenile chloroplasts

Our analysis revealed that the high-light–inducible *ELIP2::LUC* is expressed under nonstressful conditions around the shoot apical meristem and is transiently activated during greening. These findings raise a possibility that high-light signaling is constitutively activated during the early stage of chloroplast development. There are two possibilities for these expression profiles. One possibility is that light stress occurs constantly in juvenile and developing chloroplasts even under low-light conditions, and *ELIP2* responds to the stress signal. Because chloroplasts at the early stage of greening do not contain the complete photosynthetic ETS and thus the two photosystems are not well connected there, as reported in the case of barley greening (36), it might be reasonable to assume the occurrence of light stress at this stage. During barley greening, transient evolution of hydrogen peroxide has been reported (36), which is a symptom of light stress. Another possibility is that expression of *ELIP2* during chloroplast development is developmentally programmed, regardless of the actual condition of the chloroplasts. Support for this idea comes from the finding that transient expression during seedling development was observed not only in the light (Fig. 3A) but also in the dark (Fig. 3B, expression

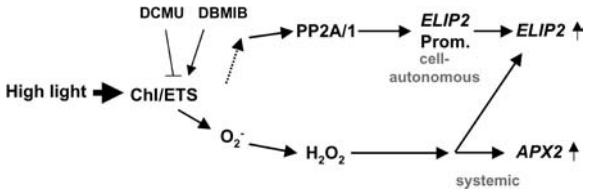

Figure 6. Summary of the hydrogen peroxide–dependent and –independent pathways for high-light signaling. PP2A/1 means protein phosphatase Type 2A or 1 (17). *ELIP2* Prom. means behavior of *ELIP2::LUC* in this figure.

at Days 2 and 3), when seedlings should be free from light stress. However, in light of the current available information, it is difficult to be conclusive; thus, further investigation is necessary to understand how *ELIP2* expression is regulated under low-light conditions.

Harari-Steinberg *et al*. (16) reported the expression of *ELIP1* and *ELIP2* during the early phase of *Arabidopsis* greening. According to their work, expression of *ELIP1* and *ELIP2* of etiolated seedlings was activated by red, far-red and blue light, and analysis with the aid of photoreceptor mutants revealed that the activation was mediated by *PHYA*/*PHYB* and some unidentified blue-light receptor that is different from *CRY1, CRY2* or *NPH1*. Therefore, whether the blue-light response of etiolated seedlings is of the same signaling pathway as high-light response is still an open question.

Hydrogen peroxide–dependent and –independent pathways

Pioneering work by Karpinski *et al*. (1999) revealed that high-light signaling to *Arabidopsis APX2* is mediated by hydrogen peroxide. The characteristics of the signaling were investigated, and it was suggested that reduction of the plastoquinone pool is necessary for the response (11). Subsequently, a hydrogen peroxide–independent pathway has been revealed with the aid of *ELIP2::LUC* transgenic *Arabidopsis* (17). Therefore, the question arises whether a prerequisite of the latter pathway is also a reduction of the plastoquinone pool.

Our results presented in this report agree with the notion that this reduction is necessary. Assuming a critical role of plastoquinone in the activation of the signaling, the presence of a monitoring system might be suggested for the redox state of the plastoquinone pool that initiates the high-light signaling. Another possibility is that a reduced plastoquinone pool causes some kind of stress, and this might trigger the signaling. Because methyl viologen–mediated superoxide production did not activate *ELIP2::LUC* in our preliminary experiments (M. Kimura and Y. Y. Yamamoto, unpublished), superoxide does not appear to be involved in the triggering. Further study is necessary to elucidate how the light stress is recognized and the corresponding signaling is triggered. Figure 6 summarizes the characteristics of the two signaling pathways.

Although the two signaling pathways share this feature, a difference was also found. The hydrogen peroxide–mediated signaling is transduced systemically, whereas the independent pathway is cell autonomous (Fig. 4). These different behaviors conform the idea of distinct signal transduction of the two pathways, as illustrated in Fig. 6, and might provide their differentiated physiological significance.

In summary, the hydrogen peroxide–dependent and –independent pathways are suggested to have shared characteristics at the very early steps, evidenced by sensitivity to DCMU and DBMIB, but at the later steps dependence on hydrogen peroxide and intercellular characteristics are different.

Cross talk with other signals

Hydrogen peroxide is not only involved in the light stress signaling. It is a mediator of UV-B (37), ozone (38), wounding (39) and also pathogen infection (40). These abiotic and biotic stresses might activate a common group of genes that are required for "general" stress responses. Recently, several genes that respond to multiple stresses have been identified (41). In addition, systematic microarray analysis revealed general antistress factors that respond to high light, drought, salt and cold stresses (Kimura *et al.,* unpublished). Assuming cross talk with other stress signals through hydrogen peroxide, though this assumption does not have any experimental support now, the hydrogen peroxide–dependent light stress signaling might activate the general antistress components, and the hydrogen peroxide–independent signaling leads to light stress–specific responses. The systemic nature of the former pathway would have physiological significance, taking this hypothetical broad antistress role into consideration.

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