Plastid redox state and sugars: Interactive regulators of nuclear-encoded photosynthetic gene expression

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Feedback regulation of photosynthesis by carbon metabolites has long been recognized, but the underlying cellular mechanisms that control this process remain unclear. By using an *Arabidopsis* cell culture, we show that a block in photosynthetic electron flux prevents the increase in transcript levels of chlorophyll a/b-binding protein and the small subunit of Rubisco that typically occurs when intracellular sugar levels are depleted. In contrast, the expression of the nitrate reductase gene, which is induced by sugars, is not affected. These findings were confirmed *in planta* by using *Arabidopsis* carrying the firefly luciferase reporter gene fused to the plastocyanin and chlorophyll a/b-binding protein 2 gene promoters. Transcription from both promoters increases on carbohydrate depletion. Blocking photosynthetic electron transport with 3-(3′, 4′-dichlorophenyl)-1,1′-dimethylurea prevents this increase in transcription. We conclude that plastid-derived redox signaling can override the sugar-regulated expression of nuclear-encoded photosynthetic genes. In the sugar-response mutant, *sucrose uncoupled 6* (*sun6*), plastocyanin-firefly luciferase transcription actually increases in response to exogenous sucrose rather than decreasing as in the wild type. Interestingly, plastid-derived redox signals do not influence this defective pattern of sugar-regulated gene expression in the *sun6* mutant. A model, which invokes a positive inducer originating from the photosynthetic electron transport chain, is proposed to explain the nature of the plastid-derived signal.

Plant cells are capable of a remarkable diversity of metabolic processes. Which processes operate at any moment in time is largely determined by environmental cues, but how these signals are perceived and transduced into appropriate metabolic responses remains unclear. For example, processes that produce soluble sugars (photosynthesis, starch degradation, gluconeogenesis, etc.) must balance those utilizing them (respiration, growth processes, etc.). A similar, although somewhat simpler, situation occurs in yeast, and it is now generally accepted that hexokinase senses soluble sugar levels in the cytoplasm and exerts transcriptional control on cell carbon metabolism. However, despite much effort, the details on how hexokinase regulates transcription in yeast is unclear. Evidence has also been provided that hexokinase senses hexose sugars in plant cells and generates a signal that results in decreased transcription of nuclear-encoded photosynthetic and glyoxylate cycle genes (1–5). Other studies have provided evidence for a hexokinase-independent sensing mechanism for hexose sugars as well as a separate mechanism involving sucrose transport across the plasma membrane (6–8).

Recent work performed on isolated chloroplasts of higher plants (9) has suggested that plastoquinone (PQ) redox status regulates the transcription of genes localized in the plastid genome coding for the core proteins of photosystems (PS) I and II (*psaA/B* and *psbA*, respectively). Similar experiments with cyanobacteria have also implicated a role for photosynthetic electron transport (PET) in regulating the expression of core PS I and II proteins (10). More surprisingly, the plastid redox state has also been implicated, albeit indirectly, in the regulation of various nuclear-encoded genes associated with PET (11–13), photomorphogenesis (14), and antioxidant levels (15).

We were interested in further exploring a possible role for plastid redox signaling on nuclear-encoded gene expression, and establishing whether this and the sugar-sensing mechanism operate on the same genes through a common signaling pathway. By using both cell cultures and transgenic plants carrying promoter-reporter gene fusions, we show that the same nuclear-encoded photosynthetic genes respond to both sugars and plastid-derived redox signals and that the plastid-derived signal can override the sugar response. Furthermore, we show that photosynthetic gene expression in the *Arabidopsis sucrose uncoupled 6* (*sun6*) sugar response mutant (insensitive to the phytohormone abscisic acid), rather than being repressed, is stimulated by addition of sucrose, and unresponsive to the plastid redox status.

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Materials and Methods

Cell Suspension Culture Growth Conditions. Cell suspension cultures were initiated by May and Leaver (16). Cultures were grown as described (17), except that Murashige–Skoog medium with minimal organics was used.

Starvation Time Course. Suspension cultures in the early exponential phase (3 day old) were washed and resuspended to the original cell density in medium without sugar. Aliquots of 8 ml were transferred into 25-cm^3 tissue culture flasks and put back into the growth room but shaken at 80 rpm. Samples were taken both before and after the wash, and at the times indicated. After harvesting, all samples were washed with distilled water and either used immediately or pelleted and stored at −20°C until required.

Sugar Assays. Frozen tissue was extracted with 80% ethanol and sugar levels measured by enzyme assays as described (18).

Chlorophyll Assays. Chlorophyll was extracted with minor adjustments for cell culture and assayed as described (19).

RNA Analysis. Total RNA was isolated based on the method described (20). RNA blotting onto nylon membrane was followed by hybridization as reported (21). Quantification was

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Abbreviations: *CAB,* chlorophyll a/b-binding protein; *RBCS,* small subunit of Rubisco; *NR,* nitrate reductase; PET, photosynthetic electron transport; DCMU, 3-(3′, 4′-dichlorophenyl)-1,1′-dimethylurea; PQ, plastoquinone; *PC,* plastocyanin; *LUC,* firefly luciferase; PS, photosystem; PAR, photosynthetically active radiation; *sun,* sucrose uncoupled; *abi,* abscisic acid insensitive.

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performed by using a Fuji phosphoimager. Probes used were the *Arabidopsis* chlorophyll a/b-binding protein gene 2 (*CAB2*) (22), an expressed sequence tag for the small subunit of Rubisco (*RBCS*) (GenBank accession no. T04228), an expressed sequence tag for nitrate reductase (*NR*) (GenBank accession no.T88297), a genomic clone of *Arabidopsis* chalcone synthase (23), and as constitutive control, an ADP-ribosylation factor clone (24).

Culture Treatments. Three-day-old cultures were washed as described above. Controls were resuspended in medium with 3% (wt/vol) sucrose. Where appropriate, 3-(3′, 4′-dichlorophenyl)-1,1′-dimethylurea (DCMU) in ethanol was added at time 0 to a final concentration of 10 μM; controls were treated with ethanol (0.1%). Aliquots (8 ml) were incubated for 24 h under the conditions given earlier.

Respiration and Photosynthesis Measurements. After a 24-h incubation, samples were diluted to a cell density of 5 ± 0.5 mg fresh weight/ml with cell culture medium with or without 3% (wt/vol) sucrose. Sodium bicarbonate, freshly buffered to pH 5.8, was added to a concentration of 3 mM to avoid limitation of photosynthesis by CO_2 availability. Respiration and photosynthesis rates were measured by using a Hansatech (King's Lynn, U.K.) oxygen electrode thermostated at 22 ± 0.05°C. Where appropriate, samples were irradiated with a 50-watt quartz-halogen dichroic lamp (Osram, Berlin) attenuated with Balzers (Vaduz, Lichtenstein) neutral density filters to provide an intensity of 20 μmol of photons/m^2/s photosynthetically active radiation (PAR).

Measurement of PQ Redox State. Culture samples were prepared as described above. Samples were placed in a stirred thermostated (22°C) cuvette located in a Perkin–Elmer LS-50 luminometer operating in the prompt fluorescence time base mode. Measurements were made as described (25) with the following modifications (excitation, 440 nm; emission, 685 nm; and 2.5-nm slit width delivering 0.5 μmol/m^2/s onto the cuvette face). Actinic light intensities were attenuated with Balzers neutral density filters and measured by using a Li-Cor (Lincoln, NE) LI-190SB quantum sensor. Samples were exposed to the measuring beam for a minimum of 2 min and the resulting F_0 fluorescence level (PQ pool fully oxidized) determined. Cells were then exposed to 20 μmol/m^2/s PAR (unless indicated otherwise) for 5 min and the new steady-state level F_S determined (PQ redox state partially reduced and poised by conditions). Finally, DCMU was added (10 μM final concentration) and the resulting steady-state level F_{max} measured (PQ pool fully reduced). The redox state of the PQ pool was then determined as $(F_S - F_0)/(F_{max} - F_0)$ (26).

Luminescence Measurements. Plants were grown in soil at 22°C in a greenhouse with a 16-h light/8-h dark cycle. Rosette stage plants were placed in the dark for 24 h to allow leaf starch breakdown. Leaves were then detached from submerged plants or 4-mm diameter leaf discs removed. Leaf tissue was then floated on half-strength Murashige–Skoog medium with either 3% (wt/vol) sucrose or 1.6% (wt/vol) mannitol as an osmotic control. Where appropriate, 10 μM DCMU was added to the incubation media. Samples were prepared by using a modified method of Millar (27). Leaf tissue was prepared with luciferin and images acquired and quantified after 20 min by using a photon-counting camera (Photek, St. Leonards on Sea, U.K.) and IFS32 software (4 C, Maidenhead, U.K. and Photek).

Results

Carbohydrate Regulation. Fig. 1 presents data on the effects of carbohydrate starvation (i.e., removal of sucrose) on *Arabidopsis thaliana* cell suspension cultures. Intracellular levels of glucose, fructose, and sucrose began to decline after 2 h of starvation, and reached a basal minimal level by 24 h (Fig. 1*A*). Starch levels remained low at all times (data not shown). Four hours after removal of sucrose, a significant increase ($P < 0.05$) in total chlorophyll levels was observed, suggesting the induction of genes associated with the photosynthetic apparatus (Fig. 1*B*). No concomitant increase in the chlorophyll a/b ratio was detected, indicating the synthesis of both the chlorophyll a-containing core complexes and the peripheral light harvesting chlorophyll a/b complexes (Fig. 1*B*). RNA blot analysis on samples starved for

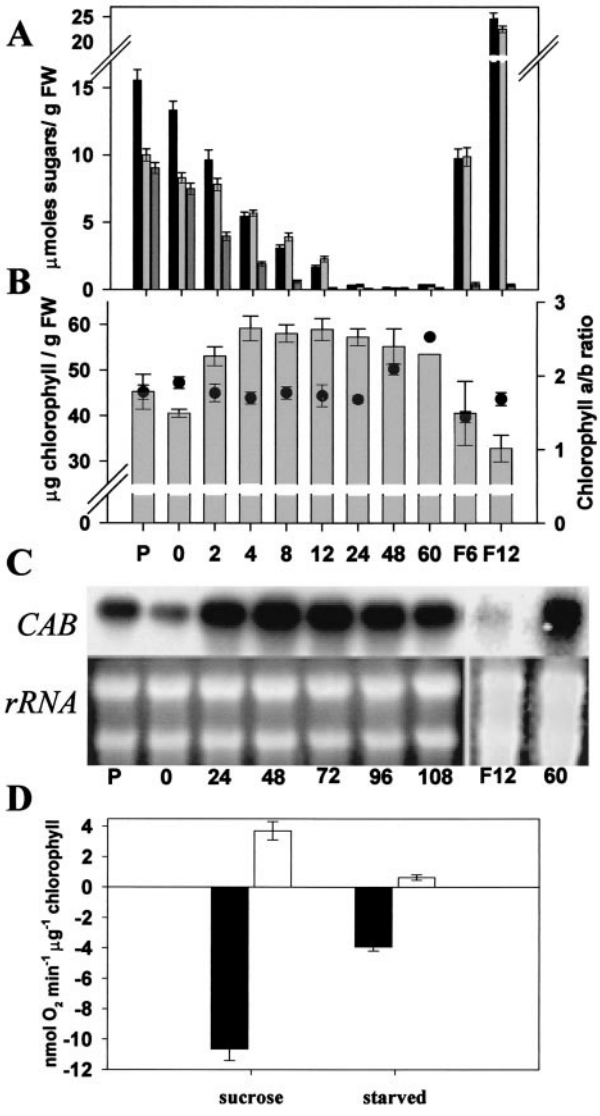

Fig. 1. Response of suspension culture cells to starvation. Three-day-old *A. thaliana* suspension culture was washed and resuspended in medium with or without 3% (wt/vol) sucrose as described in the text. Prewash and time 0 samples were taken in addition to those over the indicated time course. After starvation, where indicated, sucrose was added to a final concentration of 3% (wt/vol). All values are the average and standard errors of the given number of replicates. P, prewash; 0, postwash; all numbers represent hours of starvation; and F6 and F12 cells were starved for 48 h and then refed sucrose for 6 or 12 h, respectively, before harvesting. (*A*) Intracellular glucose (■); fructose (□); and sucrose (▩) levels during starvation; $n = 3$ independent samples. (*B*) Total chlorophyll (□) and chlorophyll *a*/*b* ratios (●); $n = 3$ independent samples. (*C*) RNA blot analysis on 7 μg of total mRNA by using the *CAB2* gene as probe. (*D*) Respiration (black bars) and photosynthesis (white bars) rates after 24 h of sucrose or starvation treatment; $n = 3$.

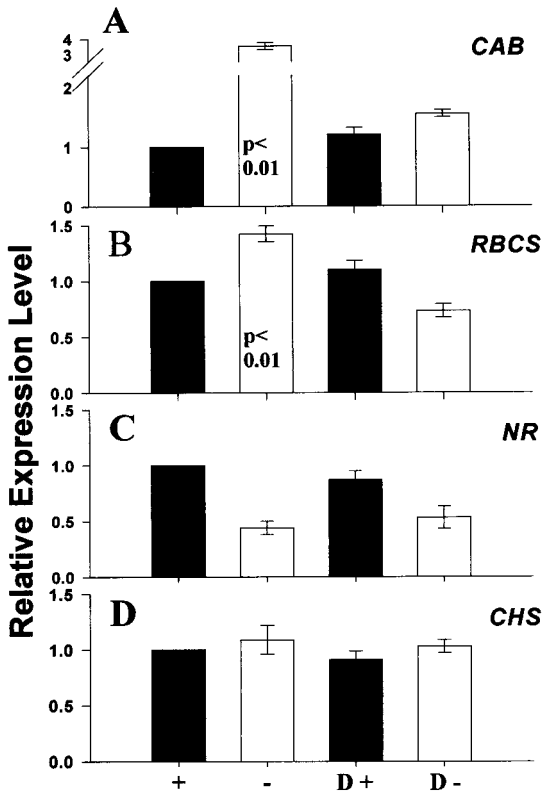

Fig. 2. DCMU prevents the starvation-induced increase in transcript levels of nuclear-encoded photosynthetic genes. Samples were prepared as described in Fig. 1. DCMU in ethanol was added at time 0 to a final concentration of 10 μM; controls were treated with ethanol (0.1%). Samples were removed after 24 h and RNA extracted for RNA blot analysis. Autoradiographs of the resulting RNA blots were scanned and signal intensity quantified; the values presented are the averages and standard errors from six independent experiments; the values have been normalized on the constitutively expressed gene (*ARF*), and are presented relative to the expression level in sucrose-treated cells. An analysis of variance test was performed on the data and significant probability levels given on the graph. (*A*) *CAB*; (*B*) *RBCS*; (*C*) *NR*; (*D*) chalcone synthase, *CHS*; (−) and (+), absence and presence of 3% (wt/vol) sucrose; and D, presence of 10 μM DCMU.

different periods of time suggest that *CAB* transcript levels are inversely correlated with intracellular sugar concentrations, being suppressed when sugar levels are high (pre- and postwash), and increasing only when sugar levels began to decline (>24 h). Furthermore, refeeding cells with sucrose after 48 h of starvation resulted in a strong decrease in the *CAB* transcript (Fig. 1*C*) and chlorophyll (Fig. 1*B*) levels, and concomitant increase in intracellular sugars (Fig. 1*A*). Both starved and sucrose-replete cells showed a significant capacity for photosynthesis and respiration, indicating functional electron transport chains (Fig. 1*D*). These results are consistent with the findings from other plant systems which show that expression of nuclear-encoded photosynthetic genes is inversely correlated with intracellular soluble sugar levels (6, 28, 29).

Plastid-Derived Redox Signal. RNA blot analyses on other nuclear-encoded genes suggests that the response reported above is specific for the transcript levels of photosynthetic genes (Fig. 2). After 24 h of starvation, *CAB* (Fig. 2*A*) and *RBCS* (Fig. 2*B*) transcript levels increased significantly ($P < 0.01$) over those of sucrose-fed cells. In contrast, the corresponding levels for *NR* showed a positive correlation with intracellular sugars (Fig. 2*C*), whereas those of chalcone synthase were insensitive to sugar

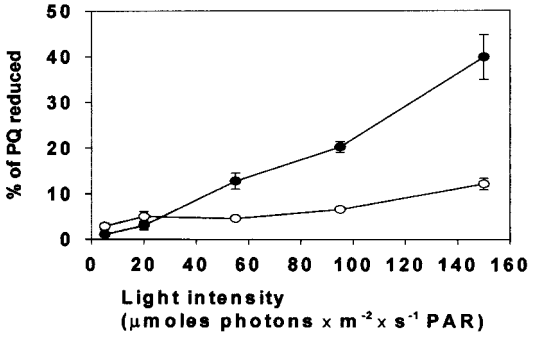

Fig. 3. The effect of light on PQ redox state in starved and sucrose-replete cells. Three-day-old cultures were prepared as described in Fig. 1 and *Materials and Methods*. Chlorophyll fluorescence emanating from PS II was measured over the range of indicated light intensities (5–155 μmol of photons/m^2/s PAR) and the redox state of PQ was calculated as $(F_S - F_0)/(F_{max} - F_0)$ (see *Materials and Methods* for details). Each value represents the average and SEM of $n = 3$ independent samples. (○) 3% (wt/vol) sucrose; and (●) starved for 24 h.

status (Fig. 2*D*). Addition of DCMU, an inhibitor of PET, to samples abolished the starvation-induced increase in both *CAB* and *RBCS* transcript levels (Fig. 2 *A* and *B*), but had no effect on *NR* transcript abundance (Fig. 2*C*). DCMU addition had no effect on either the respiration rate, or the rates at which sugars were depleted on starvation (data not shown). These results suggest that the normal starvation-induced increase in the transcript levels of nuclear-encoded photosynthetic genes requires PET and is modulated through a plastid-derived signal.

The redox status of the PQ pool has been widely implicated as a regulatory signal controlling chloroplast activity (30, 31) and the induction of chloroplast-encoded photosynthetic genes (9). To determine whether the effects reported above are mediated through a similar mechanism, the PQ redox state was monitored by using chlorophyll fluorescence. With both starved and sucrose-fed cells, increasing light intensity resulted in a progressive reduction of the steady-state PQ pool redox potential. However, at the experimental growth conditions of 20 μmol of photons/m^2/s PAR, the PQ pool redox state of both starved and sucrose-fed cells were not significantly different, and remained almost fully oxidized (Fig. 3). In the DCMU-treated cells reported above, the PQ pool would also have been fully oxidized because PQ reduction (by PS II) but not its oxidation (by PS I) would have been blocked.

The evidence presented above demonstrates that high intracellular sugars repress the abundance of nuclear-encoded photosynthetic gene transcripts through the action of an uncharacterized cytosolic signaling mechanism that may involve hexokinase. However, the removal of the sugar-repression signal alone does not result in increased transcript levels for these genes; the experiments with DCMU clearly indicate that an additional signal is required that depends on PET.

Transcriptional Control. Transgenic *Arabidopsis* lines [*CAB2-LUC* (27) and *PC-LUC* (32)] carrying constructs of the luciferase reporter gene (*LUC*) fused behind the endogenous *CAB2* and the plastocyanin gene (*PC*) promoters were used to test this effect *in planta*. Fig. 4*A* presents the results from a typical experiment on detached leaves from the *PC-LUC* line. Luminescence was low in the presence of sugar (+) and strongly increased in starved samples (−). However, luminescence remained low when leaves were starved for 24 h in the presence of DCMU (D−). Similar results were obtained with detached leaves from *CAB2-LUC* lines (images not presented). To quantify the luminescence, experiments were performed on leaf discs

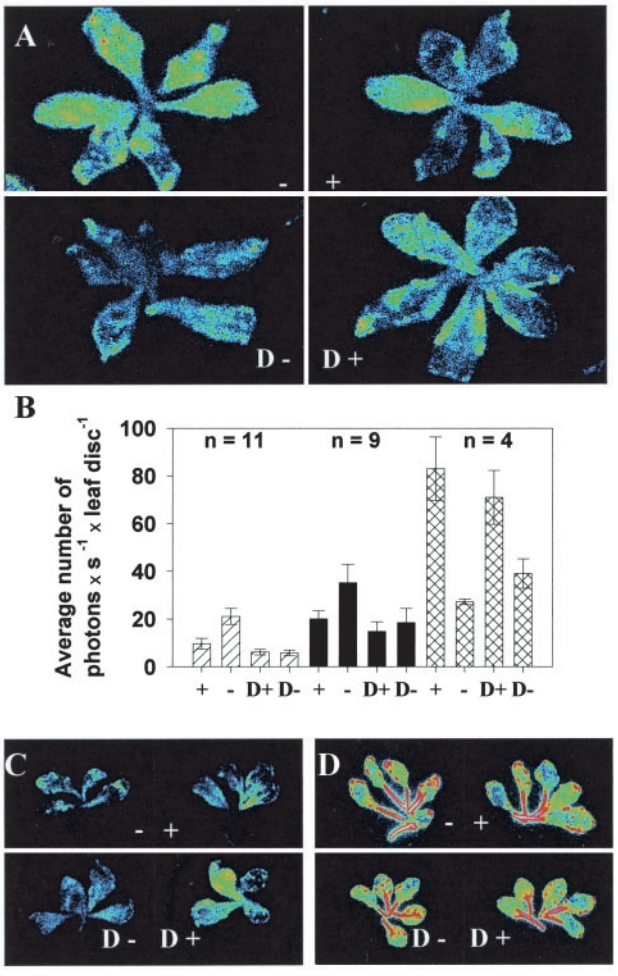

Fig. 4. DCMU prevents induction of photosynthetic genes in transgenic plants, but not in the sugar response mutant *sun6*. Luminescence from detached *A. thaliana* leaves carrying the *PC-LUC* (*A*); *PC-LUC* in *sun6* background (*C*); and *CaMV35S-LUC* (*D*) constructs. Detached leaves were harvested and treated as described in *Materials and Methods*. Pseudocolors represent luminescence intensity with low (dark blue) and very high (red). (*B*) Quantified luminescence of 4-mm diameter leaf disks from *CAB2-LUC* (diagonal shading); *PC-LUC* wild type (solid shading); and *sun6* (hatched shading) plants. Values represent the averages and SEM of the number of photons emitted per leaf disk/s. Treatment codes: (−) and (+), absence and presence of 3% (wt/vol) sucrose for 24 h; and D, presence of 10 μM DCMU for 24 h before measurement.

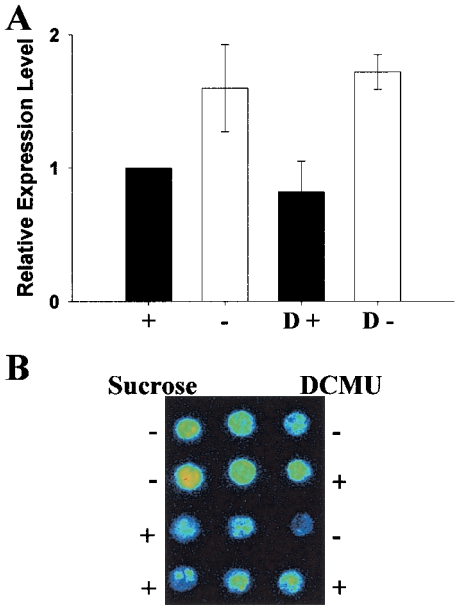

Fig. 5. DCMU does not repress photosynthetic genes of the nucleus. Cell cultures and leaf disks from the *PC-LUC* lines were prepared as described in *Materials and Methods* and Figs. 2 and 4 with the exception that DCMU was added after samples had been starved for 24 h. (*A*) *CAB* transcript levels were determined 3 h after DCMU addition by RNA blot analysis as described in Fig. 2. The averages and SEM are shown ($n = 3$); (−) and (+), absence and presence of 3% (wt/vol) sucrose; D, presence of 10 μM DCMU. (*B*) Pseudocolor luminescence images of *PC-LUC* leaf disks 4 h after DCMU addition (blue, low luminescence; red, high luminescence).

from the *CAB2-LUC* (diagonal shading) and *PC-LUC* (solid shading) lines (Fig. 4*B*). The results from the detached leaves and leaf discs are entirely consistent with those obtained from the cell suspension cultures (Figs. 1 and 2).

Fig. 4*C* presents detached leaf images from a similar experiment by using the *Arabidopsis* mutant *sun6*, also carrying the *PC-LUC* reporter gene construct. Quantified data from leaf discs of the *sun6* mutant is presented in Fig. 4*B* (hatched shading). *Sun6* was isolated as a line in which sugar failed to repress *PC* expression (32), and it has been shown recently that *sun6* is allelic to *abscisic acid insensitive* (*abi4*) because of a disruption of an *APETELLA 2*-like transcription factor (33). In sharp contrast to the wild-type lines, sugar produced a 3-fold increase in luciferase activity over the levels in starved tissues. Our results suggest that, rather than being repressed by sugars, PC transcription is actually increased in the rosette leaves of the *sun6* mutant. Furthermore, blocking PET with DCMU does not affect the sucrose-dependent increase of PC transcription in the *sun6* mutant. Therefore, it can be concluded that a lesion in the *sun6/abi4* gene, which disrupts the sugar repression response in young seedlings, causes sugar hypersensitivity in fully expanded leaves. This hypersensitive response is not influenced by the plastid-derived signal.

In vivo luciferase activity is dependent on endogenous ATP. To ensure that the effects of sugar and DCMU seen in the *CAB2-LUC* and *PC-LUC* lines did not arise as a consequence of altered ATP levels, experiments were carried out on a transgenic line carrying the constitutively expressed *CaMV35S-LUC* construct. Neither sugar nor DCMU affect luciferase activity in this line (Fig. 4*D*; quantified data not presented).

The requirement for PET for the induction of nuclear-encoded photosynthetic gene transcription was uncovered by DCMU addition at the time of sugar removal from transcriptionally repressed samples. To investigate the effects of PET on transcriptionally active samples, DCMU was added to starved cell cultures (Fig. 5*A*) and starved *PC-LUC* leaf discs (Fig. 5*B*). In both cases, DCMU had no effect on gene expression. Longer treatments of cell cultures and leaf discs with DCMU gave similar results (data not shown).

To summarize, the experiments on the effects of sugar and DCMU on *CAB2* and *PC* expression *in planta* support the conclusion drawn from RNA analysis on cell suspension cultures that a chloroplast-derived signal modulates the derepression of nuclear-encoded photosynthetic genes. However, this same signal does not have an effect on *CAB* and *PC* genes once they are derepressed.

Discussion

Plastids are the site of photosynthesis and numerous other essential metabolic processes in higher plants. To perform these tasks, both plastid- and nuclear-encoded proteins are required, but to function optimally, plastid metabolism must be tightly

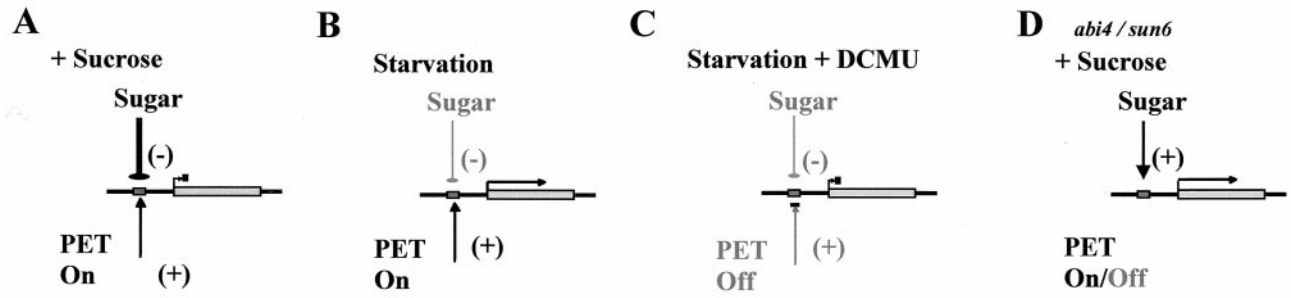

Fig. 6. Model showing control of nuclear gene transcription by cytosolic sugar status and photosynthetic electron transport. PET gives rise to a positive signal that enhances nuclear gene transcription. However, the low light levels used in this study (20 μmol photons/m^2/s PAR) evoke only a moderately weak signal, and in the presence of the strong antagonistic repression arising from exogenous sugars, transcription is suppressed (*A*). Starvation in the light results in the removal of the sugar-dependent repression allowing the chloroplast-derived positive regulator to induce transcription (*B*). However, derepression in starved cells is blocked if PET is inhibited (*C*). In the *sun6* mutant, sugars induce photosynthetic gene expression irrespective of the status of PET (*D*).

integrated with that of the whole cell. Within the plastid, a complex array of mechanisms operating at the level of transcription and posttranslation have been identified (34, 35) including redox regulation of both C-3 cycle enzymes (36–38) as well as transcript abundance (9) and translation (39) of the plastid-encoded genes for the photosynthetic reaction centers. Whereas the redox state of the photosynthetic electron transport chain has been shown to regulate the expression of plastid-encoded photosynthetic genes (9), there have been no convincing reports to date of a plastid-derived redox signal operating to regulate nuclear-encoded photosynthetic genes in higher plants. In contrast, a large volume of literature has accumulated in support of a sugar-sensing mechanism. Here, high levels of intracellular sugars are thought to represses the expression of the nuclear-encoded photosynthetic genes *CAB, PC*, and *RBCS*, and high rates of transcription occur only when sugar levels fall below some threshold level. We set out to establish whether any plastid-derived signals influence nuclear-encoded photosynthetic gene expression, and if so, whether they operate through a completely separate signaling mechanism, or by interaction with the sugar-sensing signaling mechanism.

Our experiments with cell cultures clearly demonstrate that intracellular sugar levels are inversely correlated with *CAB* and *RBCS* transcript levels. However, low endogenous sugar levels alone do not result in increased transcript levels; the experiments with DCMU demonstrate that a plastid-derived signal which is generated by PET is also required. It is well established that DCMU is a specific inhibitor blocking electron flow beyond the Q_A-binding site in PS II. No other sites of DCMU action in plant cells are documented, and our results confirm that DCMU does not affect dark respiration rates or the sugar status of cells during starvation. The luminescence experiments with the *PC-LUC* and *CAB2-LUC Arabidopsis* lines gave results that are entirely consistent with those from the cell suspension cultures, and allow us to conclude that the sugar-sensing and chloroplast-derived signals modulate the rates of gene transcription rather than transcript stability.

RNA blot analysis on starved and sucrose-fed suspension cell cultures suggest that the *NR* gene is responsive to sugars (Fig. 2), but unlike *CAB* and *RBCS*, shows a positive correlation with sugar levels and is not affected by DCMU. These results demonstrate the specificity of the DCMU effect on photosynthetic genes that are typically repressed by sugars.

What redox component in the chloroplast evokes nuclear-encoded gene transcription? The observation that in our experiments PQ remained almost fully oxidized in both starved (inducing) and sucrose-replete (suppressing) conditions (Fig. 3) suggests that the PQ redox state is not involved. Furthermore, because the oxidation/reduction of PQ is the rate-limiting step in PET, we conclude all components of the PET chain were oxidized, regardless of sugar status, and therefore none of these give rise to the redox signal affecting nuclear gene transcription. Our experiments using inhibitors of PET support this view. With DCMU, all components downstream of its site of action (i.e., distal of the Q_B-binding site), including PQ, would have been fully oxidized, whereas those upstream (proximal of Q_B), would have been fully reduced. It is difficult to reconcile the observed gene expression patterns in DCMU-treated samples (always suppressed, reduced proximal/oxidized distal components) and untreated samples (induced by starvation, fully oxidized PET) with the concept that the redox status of a PET chain component gives rise to nuclear gene transcription. Extensive experiments to confirm this by using the inhibitor 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, which blocks downstream of PQ, were inconclusive because of the labile nature of 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone in our experimental conditions. Taken together, we conclude that PET is required to evoke the redox signal mechanism, but that this probably operates through a stromal redox component. Possible candidates include thioredoxin or glutathione (15). However, it is conceivable that subtle changes (within the 0–10% reduced range) in the redox state of a PET chain component are sufficient to trigger nuclear gene transcription. Careful and detailed analysis of electron flux through the components of the photosynthetic electron transport chain, including cyclic electron transport, by using a range of light intensities should resolve this issue.

A model may be proposed to account for our observations on the dependence of nuclear-encoded photosynthetic gene expression on PET. This model relies on the generation of a positive regulator (inducer) by active PET and is summarized in Fig. 6. The positive regulator model predicts that under the experimental conditions used here (20 μmol photons/m^2/s PAR), a moderately weak inductive signal arises from PET, but the strong antagonistic suppression arising from exogenous sugars prevents transcription (Fig. 6*A*). Starvation in the light results in the removal of the sugar-dependent suppression allowing the chloroplast-derived positive regulator to induce transcription (Fig. 6*B*). However, transcription in starved cells is blocked if PET is inhibited (Fig. 6*C*). From this model, it follows that altering either the sugar status of the cell or the rate of PET can set the balance between sugar-dependent gene suppression and redox-dependent gene transcription. The experiments described in this study do not completely rule out the possibility that inactive PET could generate a negative signal regulating photosynthetic gene expression in the nucleus, rather than the positive regulator model as proposed. However, evidence from *Arabidopsis genome uncoupled* mutants that exhibit nuclear-encoded photosynthetic gene expression in the absence of chloroplast development (40),

and from experiments with the inhibitors of carotenoid biosynthesis, Norflurazon and Amitrole (41), supports the positive regulator model.

Several recent reports have demonstrated the involvement of the plant hormone abscisic acid in sugar-mediated responses (33, 42, 43). By using the *Arabidopsis sun6* mutant, which is allelic to the abscisic acid-insensitive mutant *abi4*, we demonstrate that *PC* transcription is induced rather than repressed by sugars and PET has no effect (Figs. 4*C* and 6*D*). It is interesting to note that in *sun6*, *PC* transcription is behaving in a similar fashion to that of *NR* in that it is induced by sugars (Figs. 2*C* and 4*B*). In the case of *NR*, PET also had no effect on transcript abundance. It is remarkable that the sugar-repressible, PET-sensitive *PC* promoter is converted to being sugar inducible and PET insensitive by a mutation in the *ABI4* gene.

The model we have proposed provides plants with a flexible mechanism for tailoring cell metabolic processes to the appropriate tasks determined by the whole plant in its unique environment. Clearly, the presence of abundant light should trigger the synthesis of new photosynthetic apparatus. However, this is an appropriate response only when there is a requirement for energy or carbon fixation to meet either the immediate demands of the photosynthetic cell or the demands from sink tissues. When soluble sugar levels rise, for example, because of an impairment of phloem loading by either genetic or environmentally determined growth cessation, the synthesis of new photosynthetic apparatus will be curtailed until the demand for carbon increases. A major challenge for future research will be to elucidate how sugar-, PET-, and abscisic acid-derived regulatory mechanisms interact to optimize photosynthetic gene expression in response to demand.

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