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Light-Regulated Changes in Abundance and Polyribosome Association of Ferredoxin mRNA Are Dependent on Photosynthesis

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In transgenic tobacco plants containing a pea ferredoxin transcribed region (*Fed-1*) driven by the cauliflower mosaic virus 35S promoter (P_{35S}), light acts at a post-transcriptional level to control the abundance of *Fed-1* mRNA in green leaves. To determine whether the light signal for this response involves photosynthesis, we treated transgenic seedlings with or without 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of photosynthetic electron transport. DCMU prevented the normal light response by blocking reaccumulation of *Fed-1* transcripts when dark-adapted green plants were returned to the light. In contrast, reaccumulation of light-harvesting complex B (*Lhcb*) transcripts was unaffected by DCMU treatment. Because *Fed-1* light regulation requires translation, we also examined polyribosome profiles. We found that *Fed-1* transcripts accumulated on polyribosomes in the light but were found primarily in nonpolyribosomal fractions in dark-adapted plants or in illuminated plants exposed to lower than normal light intensity or treated with DCMU. Surprisingly, although *Lhcb* mRNA abundance was not affected by DCMU, its polyribosomal loading pattern was altered in much the same way as was that of *Fed-1* mRNA. In contrast, DCMU had no effect on either the abundance or the polyribosome profiles of endogenous histone H1 or transgenic P_{35S}::*CAT* transcripts. Thus, our results are consistent with the hypothesis that a process coupled to photosynthesis affects the polyribosome loading of a subset of cytoplasmic mRNAs.

INTRODUCTION

mRNA for ferredoxin I (*Fed-1*), a nuclear-encoded component of photosystem I, accumulates more rapidly in the cytoplasm of light-treated pea seedlings than do other light-regulated transcripts (Kaufman et al., 1986; Sagar et al., 1988). Experiments with different portions of the *Fed-1* gene in transgenic tobacco leaves revealed that the mRNA abundance is light regulated even when the gene is transcribed from the constitutive cauliflower mosaic virus (CaMV) 35S promoter (Elliott et al., 1989). Transcriptional run-on assays suggest that the effect is post-transcriptional (Dickey et al., 1992). The *Fed-1* transcript can be deleted to include only the 5′ untranslated region (UTR) and one-third of the *Fed-1* coding region while still remaining light responsive. This short segment (228 bp) of the *Fed-1* transcript is referred to as the internal light regulatory element (iLRE). The iLRE confers light responsiveness to light-insensitive reporter gene transcripts when it is fused upstream of the reporter gene (Dickey et al., 1992).

Light regulation mediated by the *Fed-1* iLRE is more robust in reilluminated green plants than in etiolated seedlings (Gallo-Meagher et al., 1992). In contrast, the *Fed-1* promoter is minimally light responsive in green plants but more responsive in etiolated seedlings transferred to light. Together, these data suggest that transcriptional initiation is more important for *Fed-1* expression during the initial response of etiolated plants to light, whereas post-transcriptional regulation becomes more important once the plant develops functional chloroplasts (Gallo-Meagher et al., 1992).

Light regulation of the pea *Fed-1* transcript requires an open reading frame. When the *Fed-1* initiation codon is mutated to either a missense or a nonsense codon, light regulation of the *Fed-1* transcript is abolished (Dickey et al., 1994). These results suggest a link between the process of translation and iLRE-mediated effects on accumulation of the *Fed-1* message. Translation affects the stability of several other plant transcripts (reviewed in Gallie, 1993; Sullivan and Green, 1993). However, the relationship between light and translational control of mRNA stability is less clear. For example, in the chloroplasts of tobacco (Staub and Maliga, 1994) and barley (Gamble and Mullet, 1989) and in the cytoplasm of Amaranth (Berry et al., 1986, 1988, 1990), light affects the translation of several mRNAs without causing corresponding changes in their mRNA abundance. However, a correlation between light effects on translation and mRNA stability has been observed in Chlamydomonas chloroplasts (Yohn et al., 1996). *psbA*, the chloroplast-encoded photosystem II reaction center protein D1, has a decreased

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association with polyribosomes in the dark, suggesting a reduction in translational initiation. This loss of association with polyribosomes correlates with reduced mRNA stability and an overall decrease in protein production (Yohn et al., 1996).

Both translation and mRNA stability are thought to be enhanced by nuclear-encoded translational activators that bind the 5′ UTRs of several chloroplast-encoded RNAs (Jensen et al., 1986; Kuchka et al., 1988; Rochaix et al., 1989; Danon and Mayfield, 1991; Girard-Bascou et al., 1992). Light affects the translation of chloroplastic *psbA* RNA by altering photosynthetic electron transport and thus the redox state of the chloroplast. The change in redox state also results in the modification of a translational activator of *psbA* by altering the ability of the activator to bind the 5′ UTR of the *psbA* RNA in vitro (Danon and Mayfield, 1994). These data led Danon and Mayfield to suggest that the redox state of the chloroplast, in particular the redox state of thioredoxin, may be a signal for the translational activator binding to the 5′ UTR of *psbA* in Chlamydomonas chloroplasts.

Redox state changes conditioned by changes in photosynthetic electron transport signal a variety of responses in chloroplasts of algae and higher plants. These responses range from transcriptional initiation to post-translational modifications (Allen, 1992; Pearson et al., 1993; reviewed in Garcia-Olmedo et al., 1994; C.A. Allen et al., 1995; J.F. Allen et al., 1995; Levings and Siedow, 1995). An effect of the redox state on the expression of light-harvesting complex B (*Lhcb*, formerly known as *Cab*), a nuclear-encoded gene, has recently been described in the green algae *Dunaliella tertiolecta* (Escoubas et al., 1995; Maxwell et al., 1995). In this system, the transcription rate of *Lhcb* increases in low light and decreases in high light. One effect that light intensity changes can have is that they alter the average redox state of components of the chloroplast electron transport chain.

Inhibitors of chloroplastic electron transport can also be used to alter chloroplastic redox state. The inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) blocks the reduction of plastoquinone (Trebst, 1980) and keeps the plastoquinone pool oxidized. The addition of DCMU to *D. tertiolecta* in high light increases *Lhcb* transcription rates, suggesting that oxidized plastoquinol signals the increase (Escoubas et al., 1995). Conversely, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) blocks the oxidation of plastoquinol (Trebst, 1980) and keeps the plastoquinol pool reduced. DBMIB decreases *Lhcb* transcription rates under low-intensity light, suggesting that reduced plastoquinol signals the decrease (Escoubas et al., 1995). In addition, temperature can be used to control the excitation pressure on photosystem II in *D. salina*. Excitation pressure changes affect the redox poise of the quinone pool and result in changes in *Lhcb* mRNA abundance (Maxwell et al., 1995). At least in *Dunaliella* spp, therefore, there seems to be a mechanism for specific sensing of the redox state of plastoquinone in the chloroplast and a signaling pathway that leads to changes in the nuclear transcription of *Lhcb*. Taken together, the results in Chlamydomonas and *Dunaliella* spp suggest that redox sensing by both thioredoxin and plastoquinone can affect chloroplast and nuclear gene expression, respectively.

In this study, we present evidence that the nuclear-encoded *Fed-1* mRNA is loaded on polyribosomes in the light but not in the dark. In addition, the signal for increased polyribosome loading is sensitive to an alteration in photosynthetic electron transport. Finally, we suggest that *Fed-1* message abundance is increased under conditions that stimulate its translation.

RESULTS

A Photosynthesis Inhibitor Prevents *Fed-1* mRNA Accumulation but Not *Lhcb* mRNA Accumulation

Previous work has shown that the abundance of pea *Fed-1* mRNA in transgenic tobacco is four- to fivefold higher in reilluminated plants than in dark-adapted plants, even when mRNA is transcribed from the constitutive CaMV 35S promoter (P_{35S}). Although absolute levels of *Fed-1* mRNA have been shown to vary among transgenic tobacco lines, the light/dark ratio is consistent among lines (Dickey et al., 1992). Preliminary data suggested that very high fluences of light were necessary for the accumulation of *Fed-1* mRNA (data not shown). This result suggested that photosynthesis, rather than a photoreceptor such as phytochrome or cryptochrome, is required for increased *Fed-1* mRNA levels in response to light.

To test this hypothesis, we used DCMU to inhibit photosynthetic electron transport in established transgenic lines containing the constructs shown in Figure 1. Three-week-old seedlings were grown on 1.25% agar plates and typically had roots growing across the surface of the agar. These roots were treated with either 1 mM DCMU in 1% EtOH or 1% EtOH alone by layering the appropriate solution over the agar. After 3 hr in the light to allow DCMU uptake by transpiration, both DCMU-treated and control plants were put into darkness for 40 hr. Plants then either were illuminated for 2 or 6 hr or were left in darkness for an additional 2 hr. As shown in Figure 2, control plants accumulated *Fed-1* mRNA with a light/dark ratio of 2.0 ± 0.7 after 2 hr in the light and 4.0 ± 1.4 after 6 hr in the light. In contrast, DCMU-treated plants failed to accumulate the *Fed-1* transcript in the light, with a light/dark ratio of 0.7 ± 0.2 after 2 hr and 0.8 ± 0.2 after 6 hr in the light (Figure 2).

To determine whether the *Fed-1* sequence elements controlling the DCMU-sensitive mRNA accumulation are localized within the *Fed-1* iLRE (see Introduction), we tested transgenic seedlings containing *Fed-1* iLRE fused to the firefly luciferase (*LUC*) gene, shown as P_{35S}::*Fed-1* iLRE::*LUC* in Figure 1. Control plants had a light/dark ratio of 4.4 ± 1.7 af-

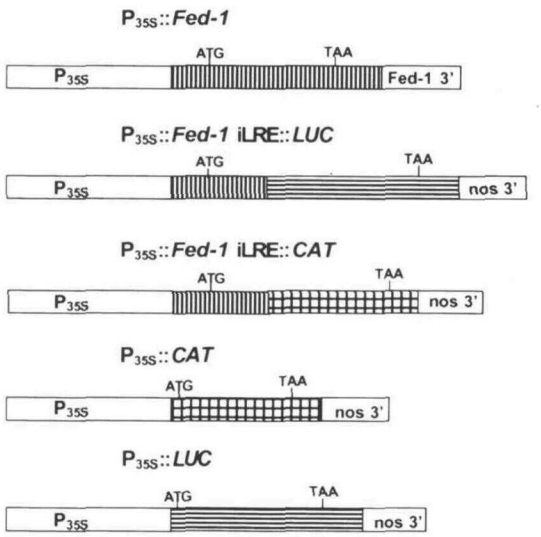

Figure 1. Transgene Constructs Used.

Constructs were made as described in Methods. All transgenes were driven by the CaMV 35S promoter (P_{35S}) and terminated by the nopaline synthase (nos) 3′ terminator, except for P_{35S}::*Fed-1*, which was terminated with the pea *Fed-1* 3′ terminator. The *Fed-1* transcribed sequence is represented by vertical stripes, *LUC* coding sequences by horizontal stripes, and *CAT* sequences by cross-hatches. The start and stop codons are indicated by ATG and TAA, respectively.

ter 2 hr of reillumination and 4.0 ± 0.3 after 6 hr of reillumination (Figure 2). As with the complete *Fed-1* transcript, *Fed-1* iLRE::*LUC* mRNA accumulation was inhibited by DCMU, exhibiting a light/dark ratio of 1.1 ± 0.3 after 2 hr of reillumination and 0.9 ± 0.1 after 6 hr of reillumination. In addition, we found that DCMU inhibited the light-regulated accumulation of *Fed-1* iLRE fused to chloramphenicol acetyltransferase (*CAT*) mRNA shown as P_{35S}::*Fed-1* iLRE::*CAT* in Figure 1 (data not shown). In contrast, the control P_{35S}::*LUC* transcript in tobacco is not light regulated (Figure 2), and as expected, treatment with DCMU had no discernible effect on the P_{35S}::*LUC* transcript levels (Figure 2). Thus, the *Fed-1* iLRE is both necessary and sufficient for DCMU-sensitive light regulation.

We also asked whether light-regulated *Lhcb* mRNA accumulation would be affected by DCMU. Blots identical to those used to analyze mRNA from the *Fed-1* transgene were hybridized with the *Lhcb* antisense riboprobe. The abundance of tobacco *Lhcb* mRNA was not altered by DCMU treatment. As shown in Figure 2, control plants had *Lhcb* light/dark ratios of 9.9 ± 4.4 after 2 hr of reillumination and 10.5 ± 3.4 after 6 hr of reillumination, whereas DCMU-treated plants had light/dark ratios of 9.6 ± 2.9 after 2 hr of reillumination and 9.4 ± 6.3 after 6 hr of reillumination (Figure 2). Thus, DCMU specifically affects the accumulation of P_{35S}::*Fed-1* mRNA.

DCMU Alters Polyribosome Loading of *Fed-1* and *Lhcb* mRNA in the Light

Because translation has been implicated in the light response of the *Fed-1* transcript, we tested whether the polyribosome loading of *Fed-1* mRNA differed in dark-adapted and reilluminated plants. Extracts from dark-adapted and reilluminated plants containing the P_{35S}::*Fed-1* transgene were fractionated on sucrose gradients designed to resolve polyribosomal and nonpolyribosomal ribonucleoprotein particles. Figure 3 shows the extent of polyribosome loading for

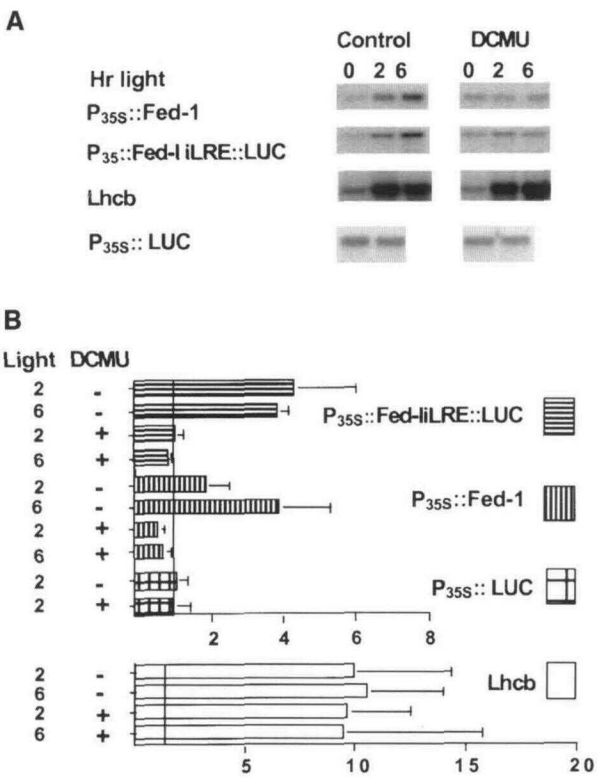

Figure 2. DCMU Inhibition of *Fed-1* mRNA Accumulation.

(A) Ten to 15 control or DCMU-treated (1 mM) transgenic plants from a transgenic line containing P_{35S}::*Fed-1*, P_{35S}::*Fed-1* iLRE::*LUC*, or P_{35S}::*LUC* were reilluminated for 0, 2, or 6 hr after a 40-hr dark adaptation. Total RNA (5 μg) was separated in each lane of an agarose gel, blotted, and hybridized with ^{32}P-labeled *Fed-1*, *LUC*, and *LUC* probes, respectively. Duplicate blots were hybridized with the ^{32}P-labeled antisense *Lhcb* probe to detect the endogenous *Lhcb* transcript.

(B) Quantitation of DCMU effects on *Lhcb* and *Fed-1* mRNA abundance is shown. RNA samples similar to those in **(A)** were probed as described, and the resulting hybridization signals were quantitated by using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The mean light/dark ratios for each reillumination time (2 or 6 hr of light) and DCMU treatment were plotted as a histogram; $n = 3$ ±SD.

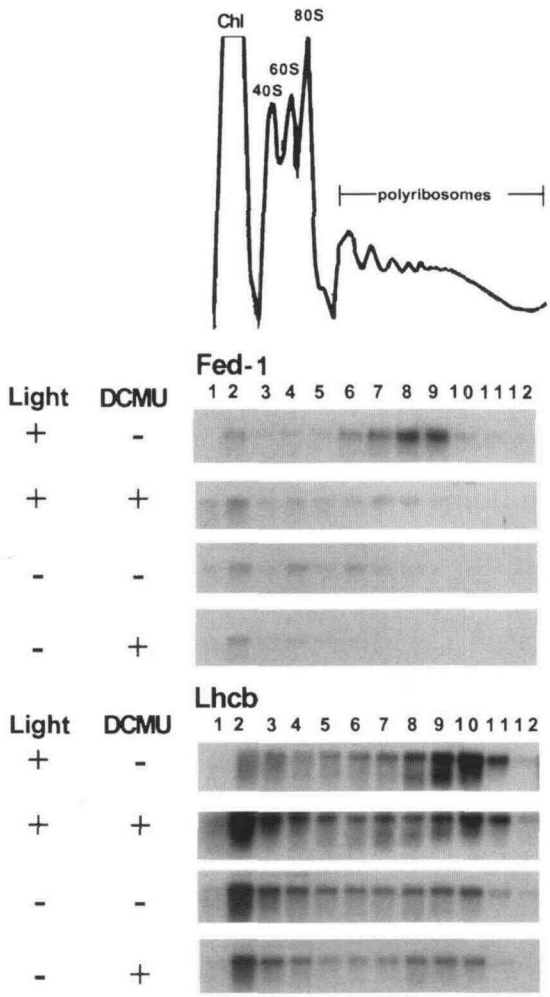

Figure 3. Polyribosome Association of P_{35S}::*Fed-1* Transgenic mRNA and Endogenous *Lhcb* mRNA.

RNAs from each fraction of a sucrose gradient were resolved by gel electrophoresis, blotted, and probed with antisense ^{32}P-labeled RNA to either *Fed-1* or *Lhcb*. Plants were treated with 1 mM DCMU and 1% EtOH (+DCMU) or 1% EtOH (−DCMU) and dark adapted for 40 hr followed by either 2 hr of reillumination (+Light) or an additional 2 hr of darkness (−Light). UV light tracing at the top was obtained from an illuminated sample. Chl indicates a UV light absorbance peak resulting from the chlorophyll layer at the top of the gradient; 40S, 60S, and 80S refer to small and large ribosomal subunits and monoribosomes, respectively. The UV light tracing is positioned to indicate the positions in the gradient of the various fractions analyzed on the RNA gel blots. Amounts of hybridizing RNA cannot be quantitatively compared between samples because of variations in the amount of tissue used and the efficiency of grinding.

Fed-1 mRNA by RNA gel blot hybridization of fractions from each gradient. A normalized comparison of *Fed-1* polyribosome loading patterns in light-treated control and DCMU-treated plants is presented in Figure 4A. These data show that 69% of the *Fed-1* transcript is found in the polyribosome fraction (gradient fractions 6 to 12) in extracts from plants reilluminated for 2 hr. In contrast, only 15% of the *Fed-1* transcript in dark-adapted plants was loaded on polyribosomes. Plants treated with DCMU and reilluminated for 2 hr had only 27% of their *Fed-1* RNA loaded onto polyribosomes. This is significantly less than the 69% of the *Fed-1* message loaded on polyribosomes in control reilluminated plants. Even more dramatically, the more heavily loaded polyribosome fractions (8 and 9) contained 35% of the total

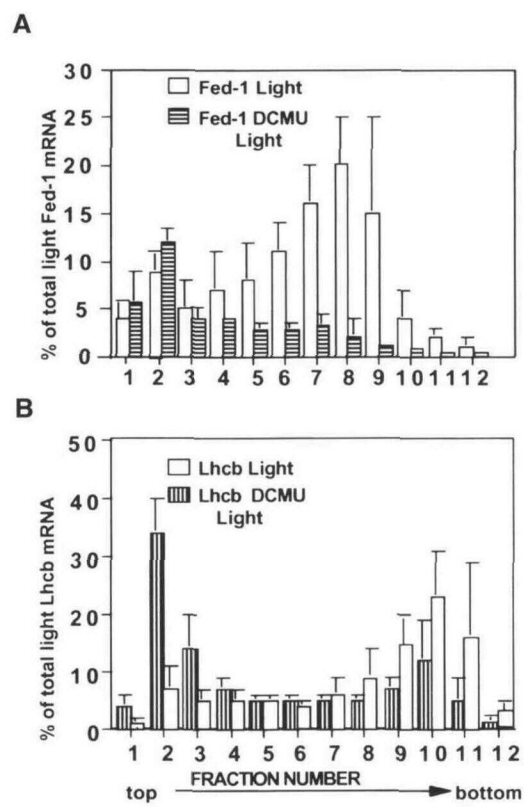

Figure 4. Quantitation of Polyribosome Association of P_{35S}::*Fed-1* Transgenic mRNA and Endogenous *Lhcb* mRNA.

(A) and **(B)** show PhosphorImager analysis of polyribosome profiles.
(A) RNA gel blots used to hybridize with the *Fed-1* probe.
(B) RNA gel blots used to hybridize with the *Lhcb* probe.
The gel blots were used to estimate the percentage of the total hybridization signal in each gradient fraction ($n = 3 \pm$SD). The data presented are for reilluminated *Fed-1* transgenic plants treated with or without DCMU. For control (Light) samples, the signal in each fraction is expressed as a percentage of the total signal in the gradient. Data from DCMU-treated samples were further normalized for the reduction of the total *Fed-1* mRNA abundance as measured (Figure 2).

Fed-1 message in the control light-treated plants, whereas only 8% of the transcripts present in DCMU-treated plants were in these fractions. Thus, the ribosome loading of the *Fed-1* message in the light is strongly affected by DCMU treatment, and DCMU treatment produces loading patterns similar to those of dark-adapted plants. To determine whether the *Fed-1* iLRE is sufficient to confer DCMU-sensitive polyribosome loading, we tested the P_{35S}::*Fed-1* iLRE::*CAT* construct and found a DCMU effect similar to that for full-length *Fed-1* mRNA (data not shown).

Because *Lhcb* mRNA accumulation is light regulated but the abundance of this mRNA does not change in DCMU-treated plants, we tested whether the polyribosome loading of *Lhcb* was altered by DCMU treatment. In reilluminated plants, 76% of the *Lhcb* message was loaded onto polyribosomes, whereas in dark-adapted plants only 33% of the remaining message was found in these fractions (Figure 3). This reduction was linked to a corresponding rise in the percentage of nonpolyribosomal *Lhcb* message. A normalized comparison of *Lhcb* polyribosome loading patterns in light-treated control plants and DCMU-treated plants is presented in Figure 4B. Gradient fraction 2 contained 7% of the total *Lhcb* mRNA in the light versus 32% in the dark. Surprisingly, even though *Lhcb* mRNA abundance was not affected by DCMU treatment, the *Lhcb* polyribosome fractionation profile for DCMU reilluminated plants resembled that for dark-adapted control plants, with only 34% loaded onto polyribosomes and 34% remaining in the non-polyribosome-associated fraction 2. Thus, DCMU blocks light effects on polyribosome loading for both *Fed-1* and *Lhcb* messages but affects accumulation of only the *Fed-1* transcript.

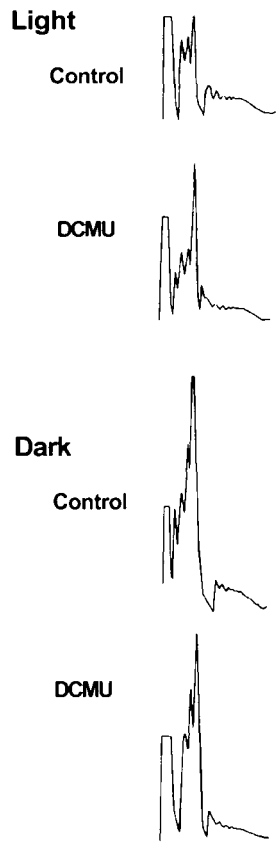

Figure 5. DCMU Effects on Total Polyribosome Profiles.

Transgenic P_{35S}::*Fed-1* tobacco seedlings were treated with 1 mM DCMU and 1% EtOH (DCMU) or 1% EtOH (Control), dark adapted for 40 hr, and either reilluminated for 2 hr (Light) or kept in darkness for an additional 2 hr (Dark). Shown are 254-nm UV light tracings of the sucrose gradients containing extracts from these plants. The direction of the sedimentation is from left to right.

Inhibition of Polyribosome Association by DCMU Is Limited to Specific Transcripts

Total polyribosome profiles were monitored by UV absorption during the gradient fractionation process. Figure 5 shows that in the light, the polyribosome/monoribosome ratio (area under the curve in the polyribosomal region relative to area under the 80S monoribosome peak) is greater than the polyribosome/monoribosome ratio in dark-adapted plants. These results suggest that overall translational activity is increased in the light. DCMU-treated plants in both the light and the dark showed a decrease in the polyribosome/monoribosome ratio similar to that seen after dark adaptation, suggesting a drastic effect of photosynthetic electron transport on overall translational efficiency.

Given these effects on overall translational activity, it was important to ask whether the DCMU effect is a general phenomenon or whether it is specific to certain transcripts. Therefore, we hybridized blots identical to those of Figure 3 with a probe for histone H1 mRNA. Figure 6 shows that DCMU treatment did not change the distribution of histone H1 mRNA in the gradient: histone H1 mRNA was localized primarily in the nonpolyribosomal fractions in both the DCMU-treated and control plants. We also tested whether *CAT* RNA from transgenic P_{35S}::*CAT* plants showed an alteration in polyribosome loading in the presence of DCMU. This mRNA was extensively ribosome associated, being found mainly in heavy polyribosomes (fractions 9 and 10). However, polyribosome loading of *CAT* mRNA was the same in plants reilluminated in both the presence and the absence of DCMU (Figure 6). Thus, DCMU does not affect the polyribosome distribution of two light-insensitive transcripts, one heavily loaded with ribosomes and the other one not.

Altering the Rate of Photosynthesis Affects *Fed-1* mRNA Accumulation and Polyribosome Loading

Given the dramatic effects of DCMU treatment on light-induced polyribosome loading of *Fed-1* mRNA, we asked

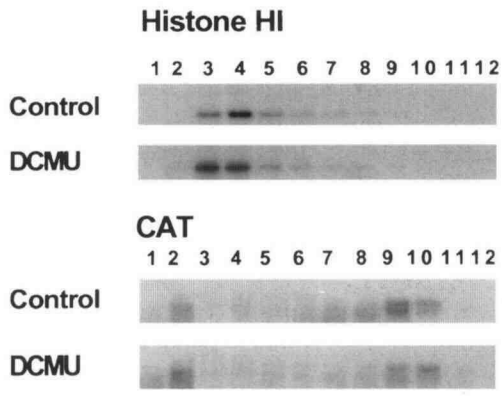

Figure 6. Polyribosome Association of Endogenous Histone H1 and Transgenic *CAT* mRNAs.

RNA from sucrose gradient fractions was resolved by gel electrophoresis, blotted, and probed with either histone H1 or *CAT* antisense ^{32}P-labeled RNA. Plants were treated with 1 mM DCMU and 1% EtOH (DCMU) or 1% EtOH (Control), dark adapted for 40 hr, and reilluminated for 2 hr. Numbers indicate the gradient fraction number as in Figure 3.

whether the degree of photosynthetic inhibition by DCMU correlates with the degree of inhibition of *Fed-1* mRNA polyribosome loading and *Fed-1* mRNA accumulation. We treated tobacco seedlings containing the P_{35S}::*Fed-1* transgene with various concentrations of DCMU and allowed uptake for 2 hr in the light. After dark adaptation, these plants were reilluminated for 4 hr. Plants from the same Petri plate were divided into samples for total RNA extraction, polyribosome extraction, and oxygen evolution analysis. As shown in Figure 7, increasing concentrations of DCMU caused simultaneous decreases in photosynthetic O_2 evolution, *Fed-1* mRNA polyribosome association, and *Fed-1* mRNA accumulation. It should be noted that the concentration of DCMU within the plant is likely to be considerably less than the concentration applied to the roots on the agar, because the system relies upon transpiration for uptake of the chemical. No concentration of DCMU tested inhibited photosynthesis completely, and even plants retaining 74% of the control levels of photosynthesis still had an appreciable inhibition of polyribosome association.

To examine further the correlation between photosynthesis and *Fed-1* mRNA regulation, we altered the rate of photosynthesis by changing the light intensity that we had used to reilluminate dark-adapted plants. Tobacco seedlings containing the P_{35S}::*Fed-1* transgene were grown under 120 μmol m^{-2} sec^{-1} light and then were dark-adapted for 40 hr, followed by a 4-hr reillumination at differing light intensities. The results shown in Figure 8 demonstrate that *Fed-1* polyribosome loading and *Fed-1* mRNA accumulation were saturated at 120 μmol m^{-2} sec^{-1}, whereas the rate of O_2 evolution still increased between 120 and 240 μmol m^{-2} sec^{-1}. Thus, *Fed-1* light regulation is altered by a moderate inhibition of photosynthesis, and the *Fed-1* mRNA response to light saturates before photosynthesis saturates.

DISCUSSION

The light and dark regulation of the pea *Fed-1* transcript in transgenic tobacco plants is controlled post-transcriptionally (Dickey et al., 1992). We have shown that *Fed-1* is preferentially loaded onto polyribosomes in the light, whereas in the dark most *Fed-1* transcripts are in the nonpolyribosomal fractions. Furthermore, the addition of the photosynthetic electron transport inhibitor DCMU or the lowering of light intensity prevented a light-induced increase in *Fed-1* transcript abundance and polyribosome loading. These results suggest that the light effect may be mediated by photosynthesis.

Photosynthetic electron transport might influence translation in the cytosol by a variety of mechanisms. For example, translation factors are known to be sensitive to pH changes (Vayda et al., 1995), and it is well known that illumination of leaves, which drives thylakoid electron transport and proton pumping into the thylakoid lumen, results in alkalization of both the stroma and the cytosol (Yin et al., 1990b). It has been suggested that export of assimilates from the chloroplast may somehow mediate the alkalization of the cytosol (and acidification of the vacuole) that was observed by Yin et al. (1990a). Translation factors are also known to be directly affected by phosphorylation (Garciabarrio et al., 1995), and it has been suggested that this control may be regulated by the redox state of the plastoquinone pool in the thylakoid membrane. In Dunaliella, Escoubas et al. (1995) postulated that *Lhcb* transcription is repressed by a cytosolic repressor factor that is phosphorylated by a chloroplast protein kinase coupled to plastoquinone redox. The relationship between *Fed-1* polyribosome association and photosynthesis exhibits a threshold effect rather than a simple linear relationship (Figures 7 and 8). Thus, it would be interesting to determine whether the threshold effect observed could be mediated by a sensor such as plastoquinone or thioredoxin.

Phosphorylation or pH changes, or both, could be involved in regulation of *Fed-1* expression. In both cases, changes in the cytosol may be greatest in the cytosol immediately surrounding the plastids. Thus, a specific effect on chloroplast-related mRNA translation might be anticipated if these transcripts are preferentially localized in the vicinity of chloroplasts, as has been reported recently (Marrison and Leech, 1994; Marrison et al., 1996). Consistent with this notion, translational initiation factor eIF-4F may associate with the cytoskeleton in plants (Bokros et al., 1995), and regulated subcellular localization of RNA has been observed in Drosophila (Gavis and Lehmann, 1994) and mammalian cells (Veyrune et al., 1996). Translation of *Fed-1*, *Lhcb*, and perhaps other chloroplast-related mRNAs thus could be susceptible to a localized modification of a factor in the vicinity

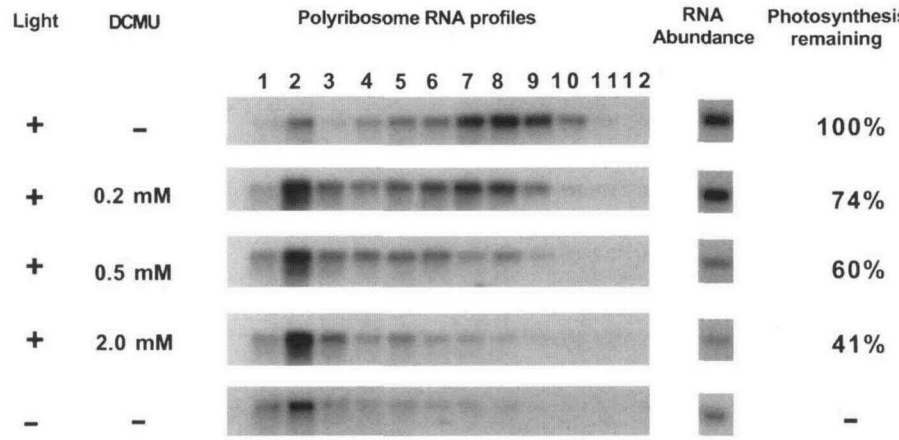

Figure 7. Polyribosome Association, *Fed-1* mRNA Accumulation, and Photosynthetic Inhibition as a Function of Increasing DCMU Concentration.

Transgenic P_{35S}::*Fed-1* tobacco plants treated with the indicated concentrations of DCMU were dark adapted for 40 hr and then reexposed to light for 4 hr (+Light) or kept in darkness for an additional 4 hr (−Light). From a single Petri plate, 10 plants were harvested into liquid nitrogen, homogenized, and then split in half for polyribosome fractionation and total RNA preparation. The remaining plants were used for measurement of oxygen evolution as an indication of photosynthetic capacity. All measurements were made at a white light fluence rate of 120 μmol m^{-2} sec^{-1}. Oxygen evolution is reported as a percentage of O_2 produced by an equal quantity (fresh weight) of untreated wild-type plants. Actual levels of O_2 evolved in micromoles per hour per gram of tissue (fresh weight) are 118, 87, 70, and 48 for 0, 200, 500, and 2000 μM DCMU, respectively. RNAs from each fraction of a sucrose gradient and from 5 μg of a total RNA preparation were resolved by gel electrophoresis, blotted, and probed with antisense ^{32}P-labeled RNA to *Fed-1*.

of the chloroplast. Our data might also be explained if *Fed-1* and *Lhcb* mRNAs were unable to compete as efficiently for translational initiation factors in darkness as they do in the light. In contrast, we have observed that the polyribosomal distributions of *CAT* and histone H1 mRNAs are not affected by DCMU treatment in the light. Future work is required to determine the mechanism by which changes in photosynthetic activity are reflected in the translation of cytoplasmic mRNAs.

It is a general trend that the levels of the *Fed-1* transcript in gradient fraction 2 are very similar in the light and the dark and that the reduction in total transcript abundance appears to reflect mainly a loss of transcript from the polyribosomal fractions (see Figure 4A). Because our fractionation procedure does not include a preliminary polyribosomal pelleting step, our gradients include both polyribosomal and nonpolyribosomal material. Thus, the transcripts we see in the lighter fractions may be either nonpolyribosomal ribonucleoprotein

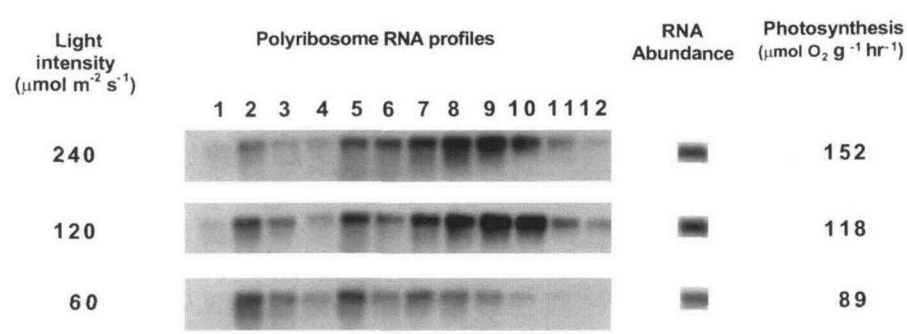

Figure 8. Polyribosome Association, *Fed-1* mRNA Accumulation, and Photosynthetic Activity as a Function of Light Intensity.

Transgenic P_{35S}::*Fed-1* tobacco plants were dark adapted for 40 hr and then reexposed to light for 4 hr at the indicated fluence rate. From a single Petri plate, 10 plants were harvested into liquid nitrogen, homogenized, and then split in half for polyribosome fractionation and total RNA preparation. The remaining plants were used to measure oxygen evolution. Oxygen evolution is reported as micromoles per hour per gram of plant tissue (fresh weight). RNAs from each fraction of a sucrose gradient and from 5 μg of a total RNA preparation were resolved by gel electrophoresis, blotted, and probed with antisense ^{32}P-labeled RNA to *Fed-1*.

particles or free mRNA. The presence of similar levels of ribosome-free mRNA in plants treated in light or dark, with or without DCMU, is consistent with the expectation that the CaMV 35S promoter will support continued transcription under all of these conditions.

Our *Fed-1* data alone do not permit us to distinguish between the effects of photosynthesis on translational initiation and elongation. To illustrate, we consider initiation or elongation effects separately, although we recognize that both processes might be affected simultaneously. Reduced initiation on a stable message would lead to a loss of polyribosome-associated message, and the message would accumulate to high levels in the nonpolyribosomal fractions. Alternatively, a reduced elongation rate on a stable mRNA would lead to the accumulation of ribosomes, shifting the message to even heavier polysomal fractions. Our data are not consistent with either of these simple models, because we see a loss of mRNA from the polyribosomes without a corresponding increase in nonpolyribosomal mRNA. However, our results can be reconciled with the initiation model if *Fed-1* mRNA is subject to rapid degradation primarily when dissociated from polysomes, or with the elongation model if degradation occurs primarily on polysomes when ribosome movement is stalled or slowed.

Because *Lhcb* mRNA abundance does not change in the presence of DCMU (Figure 2A), changes in its distribution on polyribosome gradients permit a clearer distinction between changes in translational initiation and elongation. In the light, DCMU treatment shifts a portion of the total *Lhcb* transcript to the nonpolyribosomal fraction 2 (Figure 3A). These results suggest that DCMU treatment results in an inhibition of translational initiation and that *Lhcb* mRNA is stable when dissociated from ribosomes. Supporting this idea, the *Fed-1* sequence can be modified so that its abundance no longer decreases in the dark, and in plants containing such constructs, darkness leads to accumulation of *Fed-1* mRNA in the nonpolyribosomal fractions (L.F. Dickey, manuscript submitted). Thus, we think it likely that one effect of DCMU is to inhibit translational initiation of a subset of transcripts, including *Fed-1* and *Lhcb.*

The accumulation of light-regulated transcripts other than *Fed-1* may also be affected by photosynthesis. Transcripts of the pea plastocyanin gene display many of the same characteristics displayed by ferredoxin mRNA (J. Gray, personal communication). Intriguingly, the nonphotosynthetic Chlamydomonas mutant *Cen* transcribes but is unable to accumulate at least five nuclear-encoded transcripts encoding photosynthesis proteins, suggesting that mutation in *Cen* is within a gene necessary for the stabilization of several photosynthetic messages. Included among these are *psaD* and *psaF*, the plastocyanin and ferredoxin docking proteins, respectively (Hahn et al., 1996). We suggest that for moderately stable mRNAs, such as *Fed-1*, light regulation of mRNA turnover in series with translation rates would lead to a multiplicative effect on protein production (E. Hansen, manuscript in preparation).

METHODS

Constructs and Transgenic Plants

The P_{35S}::*Fed-1* message construct has been described (Elliott et al., 1989). The P_{35S}::*LUC* construct was made by inserting the BglII-SacI fragment containing the firefly luciferase gene from pMON8796 (Monsanto Chemical Co., St. Louis, MO) into the BamHI-SacI site of pBI121. The in-frame fusion of P_{35S}::*Fed-1*::*LUC* was made via a three-way ligation using the vector fragment of HindIII-SacI–digested pBI121, a 228-bp HindIII-BglII–digested *Fed-1* internal light regulatory element (iLRE) fragment, and the *LUC* gene amplified by polymerase chain reaction. A 5′ sense primer was used to create an in-frame BglII fusion to the 3′ end of the *Fed-1* iLRE, and the 3′ antisense primer contained a SacI site for fusion to the pBI121 vector backbone. The P_{35S}::*CAT* construct was made by inserting the *CAT* gene from pCN4 (Pharmacia Biotechnology) into pBluescribe M13− (Stratagene, La Jolla, CA) to gain an SstI site at the 3′ end of the gene. The *CAT* gene, flanked by BamHI and SstI sites, was then used to replace the *GUS* gene in pBI121. P_{35S}::*Fed-1*::*CAT* (FC20) was made as described by Dickey et al. (1994).

Constructs were transferred into *Agrobacterium tumefaciens* LBA4404 by triparental mating. Plant transformation and regeneration were as previously described (Dickey et al., 1992). Seeds were collected from primary transformants for further analysis.

Plant Growth Conditions

Transgenic tobacco seeds were surface sterilized in a 10% solution of commercial bleach (0.525% sodium hypochlorite) and germinated on Murashige and Skoog mineral salts (Gibco BRL, Gaithersburg, MD) containing 50 μg/mL kanamycin and 1.25% phytagar (Fisher Scientific Co. Allied Corp., Pittsburgh, PA) for 1 week. Seedlings were transplanted to a density of 10 seedlings per 100-mm plate containing Murashige and Skoog medium with 1.25% phytagar. Seedlings were grown for 3 weeks in a growth chamber set on a 12-hr light/dark cycle with a mixture of fluorescent and incandescent lights giving a light intensity of ~120 μmol m^{-2} sec^{-1} between 380 and 780 nm. For plants treated with or without 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), 2 mL of 1 mM DCMU (or the concentration indicated in Figure 7) in 1% EtOH or 2 mL of 1% EtOH, respectively, was layered onto each plate, covering the roots growing on the agar surface. The plants then received an additional 3 hr of light before being transferred to darkness for 40 hr. After dark adaptation, the plants were either reilluminated at 120 μmol m^{-2} sec^{-1} (or at the light intensity indicated in Figure 8) for the amount of time stated in the legends to Figures 2, 3, and 5 to 8 or allowed to remain in the dark for an equivalent length of time. Leaves from all 10 to 15 plants on a plate were pooled and frozen in liquid nitrogen. Total RNA was isolated as described previously (Kaufman et al., 1985), or polyribosomal RNA was isolated as described below.

Polyribosomal RNA Fractionation

Sucrose gradients (15 to 60%) were created by layering 7 mL of 15% sucrose on top of 7 mL of 60% sucrose in Sorvall 17-mL polyallomer tubes (DuPont, Wilmington, DE). Stoppers were inserted into the tubes, which were then placed on their sides for 5.5 hr for maximal diffusion. Tubes were then turned upright. The resulting gradients

were stable for a week (Davies and Abe, 1995). Approximately 0.25 g of frozen leaf tissue was ground in liquid nitrogen with a mortar and pestle in 2 mL of U buffer (200 mM Tris-HCl, pH 8.5, 50 mM potassium chloride, 25 mM magnesium chloride, 2 mM EGTA, 100 μg/mL heparin, 2% polyoxyethylene, and 1% deoxycholic acid; Davies and Abe, 1995). Homogenates were centrifuged at 13,000*g* in a microcentrifuge for 15 min at 4°C. Supernatant (750 μL) was loaded onto 15 to 60% sucrose gradient and spun in a Sorvall AH629 swinging bucket rotor at 126,000*g* for 3.5 hr at 4°C.

Twelve 1-mL fractions were collected with a density gradient fractionator (model 185; Isco Inc., Lincoln, NE). Each fraction was dripped directly into 1 mL of phenol-chloroform, 25 μL of 10% SDS, 20 μL of 0.5 M EDTA, and 5 μL of 100 mM aurintricarboxylic acid. The resulting mixture was immediately vortexed and placed on ice. After centrifuging for 10 min at 16,000*g* in a microcentrifuge at 4°C, 500 μL of the aqueous fraction was precipitated in EtOH/NaOAc overnight at −70°C. Precipitates were collected by microcentrifugation (at 16,000*g* for 30 min at 4°C), the pellet was resuspended in 22 μL of 100 μM aurintricarboxylic acid, and 11 μL of each sample was glyoxylated. One-half of the glyoxylated mixture was loaded onto a 1% agarose gel buffered with sodium phosphate, pH 7.0, as described below.

RNA Gel Blot Hybridization

RNA gel botting was done as previously described (Elliott et al., 1989). Samples were separated by gel electrophoresis in an apparatus with circulating buffer and were blotted to GeneScreen (DuPont–New England Nuclear). Membranes were prehybridized in hybridization buffer (Frances et al., 1992) for at least 15 min at 67°C. Gene-specific riboprobes for pea histone H1 (PsHIb; Gantt and Key, 1987), *CAT* and *LUC* (Dickey et al., 1992), pea *Fed-1* (Dickey et al., 1994), and pea *Lhcb* (AB96; Frances et al., 1992) were synthesized with the Promega (Madison, WI) in vitro RNA transcription system, the appropriate polymerase, and ^{32}P-UTP. The radiolabeled probe was purified on a G25-150 Sephadex column and added to prehybridized membrane for hybridization overnight at 67°C. Blots were washed twice for 15 min at room temperature in 2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) and 0.1% SDS and then washed twice for 15 min at 67°C in 0.5 × SSC and 0.1% SDS. Blots were wrapped in Saran wrap and exposed to Kodak XAR film for 3 to 72 hr at −70°C with intensifying screens.

Photosynthetic Measurements

Oxygen consumption by whole intact leaves was first measured in the dark in the presence of saturating CO_2. Oxygen evolution was measured under 120 μmol m^{-2} sec^{-1} growth light conditions in a leaf disc oxygen electrode (Hansatech Ltd., Norfolk, UK). Light intensity was varied by using neutral density filters.

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