A photosynthetic mutant of Arabidopsis thaliana, hcf5, was isolated by screening M2 seedlings for high chlorophyll fluorescence. Thylakoid morphology was strikingly abnormal, with large grana stacks and almost no stroma lamellae. Fluorescence induction kinetics, activity assays, and immunoblotting showed that photosystem II was absent. Polypeptides of the photosystem I complex, the transcript was present at very low levels, the pattern of transcripts suggests that the hcf5 locus may encode a product required for the correct expression of several chloroplast genes.

Thylakoid membrane biogenesis requires contributions from both nuclear and chloroplast genomes. Most of the genes for thylakoid proteins have been cloned and sequenced, but we still have little information about the many nuclear genes that are required for chloroplast development (Rochaix, 1992). It has been estimated that at least 200 nuclear genes are essential for thylakoid membrane biogenesis in Chlamydomonas reinhardtii (Rochaix and Erickson, 1988). At least 14 nuclear complementation classes are required for the correct splicing of just one chloroplast transcript, that of psaA (Choquet et al., 1988).

Most of the well-characterized nuclear mutations affecting chloroplast electron transport have the hcf mutant phenotype (Bennoun and Levine, 1967; Miles, 1980). Mutant cells emit high levels of red fluorescence when illuminated with blue light because the absorbed light energy cannot be employed to drive electron transport and to establish an electrochemical potential. Many photosynthetic mutants displaying the hcf phenotype have been isolated in the green alga Chlamydomonas sp. (Rochaix, 1992) and in several higher plants, particularly maize (Miles, 1994; Barkan et al., 1995) and barley (Simpson and von Wettstein, 1980). In both higher plants and Chlamydomonas sp. these mutants have played an important role in establishing the polypeptide composition of the major macromolecular complexes of the thylakoid membrane (Chua and Bennoun, 1975; Chua et al., 1975; Metz and Miles, 1982; Lemaire and Wollman, 1989). Some Chlamydomonas sp. hcf mutants are defective in the accumulation of a single chloroplast transcript (Rochaix, 1992), whereas several higher plant mutants are defective in one or more polycistronic transcripts (Barkan et al., 1986, 1994, 1995; Rochaix, 1992). Mutants defective in the translation of one (Rochaix, 1992) or many (Barkan, 1993) chloroplast messages have also been reported, suggesting the involvement of nuclear-encoded proteins in translation of certain mRNAs as well as in their processing and stabilization.

The crucifer Arabidopsis thaliana has been used extensively as a model system for higher plant genetics and molecular biology because of its small genome size, short generation time, and availability of mutants (Goodman et al., 1995). We have previously reported the isolation and characterization of the Arabidopsis mutant line hcf2, which has pleiotropic defects at the level of photosynthetic polypeptides and an over-accumulation of the petA transcript (Dinkins et al., 1994). A number of Arabidopsis hcf mutants with a variety of phenotypes have also been isolated by screening progeny of ethyl methanesulfonate-mutagenized seed (Dinkins, 1992; Meurer et al., 1996b), but in most cases the mutations have no effect on chloroplast transcripts. In this paper we describe one of the exceptions: the mutant hcf5, which is deficient in all photosynthetic electron transport complexes as well as in Rubisco. These biochemical defects are correlated with altered steady-state levels of several chloroplast transcripts.

MATERIALS AND METHODS

Plant Growth Conditions

Plants were grown in a controlled environment chamber under fluorescent lights (100-150 μE m⁻² s⁻¹, 23°C, 16 h of

Abbreviations: CF, coupling factor; CPII', CPII, trimer and oligomer forms of LHCl, the major chlorophyll a/b protein complex of PSII; CP29, minor chlorophyll a/b protein complex of PSII; hcf, high chlorophyll fluorescence.
light/8 h of dark) in a vermiculite:peat (3:1, v/v) mixture, watered from the bottom, and given 20-20-20 all-purpose fertilizer (Plant Products, Co., Ontario, Canada) at a rate of 0.8 g L⁻¹, as needed. To obtain mutant tissue for study, seed from heterozygous plants segregating for the hcf phenotype was surface-sterilized and sown on plates containing agar (0.8 g L⁻¹), Suc (30 g L⁻¹), and one-half Murashige and Skoog salts (Murashige and Skoog, 1962), supplemented with 100 mg L⁻¹ inositol, 1 mg L⁻¹ pyridoxine-HCl, 1 mg L⁻¹ nicotinic acid, and 10 mg L⁻¹ thiamine. Plates were placed in a growth chamber under full-spectrum fluorescent lights (Vita-light, General Electric; 25-40 μE m⁻² s⁻¹, 9 h of light/15 h of dark) at 23°C. Mutant seedlings were distinguished from their wild-type siblings by their abnormally high red fluorescence when illuminated by near-UV light, as described by Miles (1980). Young plants were then transferred to large-diameter (250-mm) plates to allow for leaf expansion. After approximately 4 weeks (at the onset of bolting), the aerial portions of the plants were harvested. All measurements on hcf plants were done in parallel on wild-type siblings growing on the same plates.

**Mutant Isolation and Genetic Analysis**

Seed of Arabidopsis thaliana (L.) Heynh. wild-type Columbia was mutagenized with ethyl methanesulfonate, and a large number of small M₁ bulk populations (5-10 plants) grown from this seed were screened for the hcf phenotype by plating a sample of surface-sterilized M₁ seed on Suc-supplemented plates (Dinkins et al., 1994). About 100 M₂ plants from each bulk that showed significant numbers of the hcf phenotype were grown, seed was collected individually from each plant, and a small sample was screened on agar. Additional details on Arabidopsis hcf mutant screening procedures will be published elsewhere (R.D. Dinkins, unpublished data). Mutant line hcf5 was derived from M₁ bulk no. 302 and found to be segregating in the M₂ generation at a ratio suggesting a single nuclear recessive. The line was subsequently self-pollinated to eliminate additional background phenotypes prior to the experiments presented here, which were performed on plants from the M₅ to M₆ generations. Because the hcf phenotype is seedling-lethal in soil, the line is maintained by screening for heterozygous plants each generation.

**Fluorescence Measurements**

In vivo fluorescence induction curves were obtained at room temperature on 2- to 3-week-old seedlings plated on 5% Suc-containing medium using the computer-aided fluorescence imaging apparatus described by Fenton and Crofts (1990). Measurements were made on individual cotyledons or first leaves. Similar results were obtained with a modulated fluorescence apparatus (PAM 101, Walz, Effeltrich, Germany) (Schreiber et al., 1986). Petri plates containing hcf plants and their wild-type siblings were dark-adapted for 5 min prior to illumination.

**EM**

EM was carried out on leaves of the mutants and their wild-type siblings grown on 5% Suc agar plates for 2 weeks. The leaf tissue was fixed in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 h and postfixed in 1% (w/v) osmium tetroxide in the same buffer for 1 h. The tissues were then dehydrated in a graded ethanol series, followed by propylene oxide, and then embedded in Spurr's resin. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined in an electron microscope (EM10, Zeiss).

**Thylakoid Membranes, Pigment Analysis, and PSII Activity**

Thylakoid membranes were isolated by grinding the leaves in 20 mM Tricine (pH 8.0), 10 mM NaCl, and 0.4 M Suc with a mortar and pestle. The homogenate was filtered through 125-μm bolting silk and centrifuged at 4340 g for 12 min. The pellet was washed once in the same buffer and suspended in 20 mM Tricine (pH 8.0), 150 mM NaCl, and 5 mM MgCl₂. Total chlorophyll per plant was determined by assaying the thylakoid membrane fraction and the material retained on the bolting silk by the method of Arnon (1949).

For HPLC analysis of pigments, 3- to 4-week-old hcf and wild-type plants were dark-adapted for 1 to 2 h, then leaves were harvested and ground to a fine powder in liquid nitrogen. The powder was dispersed in 80% v/v acetone buffered with 10 mM Hepes, pH 7.5. An equal volume of diethyl ether was added to the acetone extract and the acetone was removed with 3 to 4 volumes of 10% KCl. After extensive washing with water the organic phase was evaporated under nitrogen and the pigments were redissolved in ethanol and used immediately for HPLC analysis. Pigments were separated by reversed phase HPLC using a LiChrospher 100 RP-18 column (5 μm, 4 mm i.d. × 125 mm length) (Merck, Darmstadt, Germany) with a linear gradient starting with 88% organic solvent (75:15:5 acetonitrile:methanol:tetrahydrofuran, v/v/v) and ending with 100% organic solvent after 12 min at a flow rate of 2 mL min⁻¹. The pigments were detected at 445 nm using a Waters 994 photodiode array detector. Identification of the pigments was done by a comparison of their absorption spectra and retention behavior to those of purified pigments. Chlorophyll a (Sigma) was used as a calibration standard.

PSII activity was assayed spectrophotometrically by the reduction of 2,6-dichlorophenolindophenol monitored at 595 nm in a reaction mixture containing 5 μg mL⁻¹ of chlorophyll, 20 mM Mes buffer, pH 6.0, and 0.1 mM 2,6-dichlorophenolindophenol, with either water or 2 mM diphenylcarbazide as electron donors.

**Electrophoresis and Immunoblotting**

For separation of chlorophyll-protein complexes, thylakoid membrane samples corresponding to 25 μg of chlorophyll were pelleted, washed with 65 mM Tris-maleate (pH 7.0), and solubilized in 88 mM octyl-β-D-glucopyranoside at a detergent:chlorophyll ratio of 30:1 (v/v) (Camm and...
4°C in the dark on a 1.5-mm-thick, 10% polyacrylamide gel. Denaturing gels were run on 10% polyacrylamide gels containing 0.1 M Tricine in the cathode buffer, as described by Schägger and von Jagow (1987), and modified so that the final concentration of Tris (pH 8.25) in the gel was 1.0 M. Thylakoid membrane samples were pelleted and resuspended in 65 mM Tris-HCl (pH 6.8), 20 mM DTT, 10% ethylene glycol, and 2% SDS and heated at 65°C for 15 to 20 min prior to loading onto the gel. For immunoblotting, samples of mutant thylakoids containing 5 μg of chlorophyll were compared with a series of decreasing amounts of wild-type thylakoids. Electrophoresis was at 35 mA for 3 to 5 h, at 5 to 6 h at room temperature. Proteins were transferred onto nitrocellulose and visualized, as described by White and Green (1987). For immunodetection of the Rubisco large subunit, total soluble protein from equal fresh weights of normal and mutant tissue were compared.

The antisera used to determine the presence or absence of specific thylakoid polypeptides were prepared against barley CPI (PSI reaction center complex) and CPII, the major chlorophyll a/b light-harvesting complex associated with PSII (White and Green, 1987); wheat CF1 (Moase and Green, 1981); spinach CF1 subunits II and VI (Bengis and Nelson, 1975); Cyt f (gift of R. Malkin); PSI chlorophyll a-protein core complexes CP47 and CP43 of Chlamydomonas (gift of N.-H. Chua); PSI core complex D1 (gift of A. Eastman), Cyt b559 (gift of W. Cramer); and Rubisco large subunit (gift of P. Westhoff). Following staining with one antibody, the nitrocellulose filter was stripped overnight in a solution containing 0.1 M Gly (pH 2.2), 20 mM magnesium acetate, and 50 mM KC1 (Legocki and Verma, 1981) and rebotted up to three times with other antisera.

**RESULTS**

**Physical Characteristics and EM**

The mutant line containing hcf5 was isolated by screening the M2 progeny of ethyl methanesulphonate-mutagenized seeds for high chlorophyll (red) fluorescence under UV-A light (Dinkins et al., 1994). Because homoygous mutant plants (hcf/hcf) are seedling-lethal in soil (due to inability to support autotrophic growth), the line was maintained by selling individual plants and screening the seed for each generation to identify the heterozygotes. A red filter was used to verify the difference between chlorophyll fluorescence (with a maximum at approximately 685 nm) and the blue fluorescence emitted by other fluorescing phenotypes such as the Trp synthesis mutants (e.g. Bender and Fink, 1995). Under visible light mutant plants are lighter green than their wild-type siblings. All measurements reported here were from mutant plants and their wild-type siblings grown under sterile conditions on the same plates (Dinkins et al., 1994).

When the seedlings were still small, hcf and wild-type plants were transferred to fresh medium in large-diameter (250-mm) Petri plates to allow for leaf expansion. Table I shows that after 4 weeks the mutant plants were significantly smaller than their wild-type siblings in spite of being grown on 5% Suc. They had only 21% of the normal amount of chlorophyll per milligram fresh weight and the chlorophyll a/b ratio was significantly lower. Mutant plants occasionally formed flowers when grown in culture, but did not set seed.

Figure 1 shows that there are striking differences in chloroplast ultrastructure between hcf5 and wild-type siblings. The mutant thylakoid membrane system (Fig. 1B)
Table 1. Physical characteristics of hcf5 mutant and wild-type siblings

In each experiment, 40 to 80 hcf and wild-type plants were harvested at approximately 4 weeks after germination. Values are means ± sd (n = 3). Fresh weight refers to aerial portion of plant. Chlorophyll was determined by the method of Arnon (1949). PSII activity was measured as light-driven reduction of 2,6-dichlorophenol-indophenol (mmol mg⁻¹ chlorophyll h⁻¹) with 2 mm diphenyl carbazide as electron donor at 595 nm, pH 6.0.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Plant Fresh Wt</th>
<th>Chlorophyll per Fresh Wt</th>
<th>Chlorophyll a/b Ratio</th>
<th>PSII Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>hcf5</td>
<td>17.6 ± 7.7</td>
<td>0.22 ± 0.02</td>
<td>1.60 ± 0.08</td>
<td>0</td>
</tr>
<tr>
<td>Wild type</td>
<td>54.3 ± 4.1</td>
<td>1.04 ± 0.07</td>
<td>2.67 ± 0.17</td>
<td>147 ± 0.17</td>
</tr>
<tr>
<td>% (hcf5/wild type)</td>
<td>32</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fluorescence and Electron Transport

The room temperature fluorescence-induction curves of mutant hcf5 plants and their normal siblings are shown in Figure 2. To record the kinetics of the fluorescence rise, a computer-aided video fluorescence imaging system (Fenton and Crofts, 1990) was employed. When normal, dark-adapted plants are exposed to light, their fluorescence rapidly rises from a low initial fluorescence (F₀) to maximum fluorescence (Fₘ), then drops to a steady-state level due to reoxidation of PSII electron acceptors and the establishment of the transmembrane potential (Krause and Weis, 1991). The wild-type siblings on 5% Suc displayed typical normal fluorescence kinetics compared with plants in soil (compare with Artus and Somerville, 1988), indicating that the conditions on these plates do not adversely affect the development of the electron transport system. Plants with the hcf5 mutant phenotype, on the other hand, had a high initial fluorescence (F₀), and essentially no variable fluorescence (Fₘ-F₀). This characteristic pattern is typical of a blockage of electron flow.

Figure 1. Ultrastructure of typical wild-type (A) and hcf5 (B) chloroplasts. Bar = 1 μm.

Figure 2. Fluorescence induction kinetics recorded from wild-type (WT) and hcf5 (hcf) mutant leaves using the computer-aided fluorescence imaging apparatus described by Fenton and Crofts (1990). Traces shown are averages of emission curves from five (WT) and three (hcf5) plants.
through PSII (Chua and Bennoun, 1975; Miles, 1980); it was confirmed by in vitro assays of PSII activity in thylakoids (Table I). PSII electron transport activity was undetectable above the background level even using the artificial donor diphenylcarbazide.

Pigments and Chlorophyll-Protein Complexes

Table II shows that all the pigments normally present in wild-type plants were also present in the mutants, although in different proportions. The HPLC analyses confirm that the chlorophyll a/b ratio is significantly lower. When expressed on a fresh weight basis, β-carotene was the pigment most severely depleted in the mutant, with only 6% of the wild-type level (calculated from Tables I and II). The xanthophylls neoxanthin, violaxanthin, and lutein were at 34, 41, and 45% of wild-type levels, respectively, although they were enriched relative to chlorophyll a (Table II).

Electrophoresis on nondenaturing (“green”) gels was used to analyze PSII pigment-protein complexes in mutant and wild-type plants (Camm and Green, 1989). Figure 3 shows that bands corresponding to CP47 and CP43, the core chlorophyll a-binding proteins of PSII, were greatly reduced. The CPI band (PSI reaction center and internal antenna) was also depleted, but the chlorophyll a/b antenna complexes CP1, CPII, and CP29 appeared to be normal. These results are consistent with the depletion of β-carotene, which is found mainly in PSI and PSII core complexes, and suggest that most of the chlorophyll is associated with the light-harvesting complexes, particularly LHCCI, which has a low chlorophyll a/b ratio (Camm and Green, 1989).

Photosynthetic Proteins

Thylakoid polypeptides were completely denatured, separated by gel electrophoresis, and immunoblotted successively with several antibodies to determine the presence or absence of specific polypeptides. Figure 4 shows that two PSII proteins, the chlorophyll a-binding apoprotein of CP47 and the Cyt b559 polypeptide, were depleted, with the former being almost undetectable in the mutant. The apoproteins of CP43 and the reaction center polypeptide D1 were also present at very low levels (data not shown). Thus, it appears that all polypeptides associated with the PSII core are missing or severely reduced. This was not unexpected, as it has been previously reported that mutations affecting any component of PSII have a pleiotropic effect, leading to the loss of all PSII-associated polypeptides (Metz and Miles, 1982; Jensen et al., 1986; Rochaix and Erickson, 1988).

The mutation affected other photosynthetic polypeptides as well. Cyt f was less than 10% of the control, and the α

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Chlorophyll-protein complexes of hcf5 mutant (hcf) and wild-type (Wt) thylakoids resolved on nondenaturing “green gel” (unstained). Each lane is loaded with thylakoid membranes containing 25 μg of total chlorophyll, solubilized with 88 mM octyl glucoside.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Immunoblots of photosynthetic membrane proteins. Washed thylakoids (5 μg of chlorophyll) of mutant (hcf5/hcf5) plants were compared with wild-type (Wt) thylakoids corresponding to a fraction of the mutant chlorophyll, i.e. 7.5, 5.0, 2.5, 1.0, and 0.5 μg of chlorophyll, respectively. Samples were solubilized in 2% SDS, separated on SDS-PAGE, transferred to nitrocellulose, and immunostained with antisera to CF1, Cyt f (petA protein), CP47 (psbB protein), CF1, and Cyt b559.
and β subunits of CF, which are not resolved from each other on this gel system, were 10 to 20% of the control (Fig. 4). Because equal amounts of chlorophyll were loaded in mutant and control lanes and the mutant is depleted in core chlorophyll a complexes, the other thylakoid polypeptides should have been overrepresented in the mutant lanes if they were unaffected by the mutation. PSI polypeptides were also at significantly lower levels in the mutant (Fig. 5). The polypeptides of CPI, the PSI reaction center, were almost undetectable, and the PSI subunit II polypeptide PsaD was about 10% of normal levels. The PSI “Subunit VI” antiserum (Bengis and Nelson, 1975) reacts with three polypeptides in wild-type thylakoids, but these were below the limit of detection in the mutant. Thylakoid membrane proteins are not the only photosynthetic proteins affected in this mutant; immunoblotting of the chloroplast soluble fraction showed that the large subunit of Rubisco was present at less than 6% of normal levels on a fresh weight basis (Fig. 5).

**Steady-State RNA Levels**

To determine if any of the observed differences in the hcf5 mutant could be due to an effect of the mutation on chloroplast transcripts, total leaf RNA was isolated and probed with specific nuclear and ctDNA sequences. After hybridization to one or more probes, filters were stripped and hybridized with a chloroplast rDNA probe to verify that differences were not due to a general decrease in chloroplast RNA in the mutant. Figure 6 shows that rbcL message was only 1 to 3% of wild-type levels, expressed as a fraction of total RNA. In contrast, the psbA message appeared to be present at normal levels in hcf5 plants.

Like other plants, Arabidopsis has a polycistronic psbB-psbH-petB-petD transcript that undergoes a complex series of processing events (Tanaka et al., 1987; Barkan, 1988; Westhoff and Herrmann, 1988). Figure 7 shows RNA blots...
from mutant and wild-type siblings hybridized with the probes shown as double-headed arrows in the map. The pattern of bands seen with wild-type Arabidopsis RNA was very similar to that of tobacco mRNA run on the same gel (Tanaka et al., 1987; data not shown).

The most striking changes in the hcf5 RNAs were the almost complete absence of the monocistronic psbH transcript at 0.3 kb and the marked decrease of all other transcripts containing psbH. This included bands at 3.2, 2.8, and 2.4 kb that hybridized with both psbB and psbH probes, as well as the three large transcripts of 6.6, 5.6, and 4.6 kb that hybridized with all four probes (Fig. 7). By analogy with other plants, the latter probably represent the complete polycistronic transcript (6.6 kb) and polycistronic transcripts from which one or two introns have been spliced out (Tanaka et al., 1987; Barkan, 1988; Westhoff and Herrmann, 1988). The steady-state levels of all three species were decreased in the hcf5 plants. Although there were some changes in intensity of the smaller bands detected only by the psbB probe, they were not as marked. The psbB probe terminates before the 3' end of the gene, so it would not detect any monocistronic transcripts originating from ycf8 (also known as orf31, orf38, or psbT), the gene for a small PSII protein that lies downstream of and is cotranscribed with psbB (Hird et al., 1991; Monod et al., 1992).

The petB and petD probes hybridized with bands of 3.2, 2.9, 2.4, 1.8, and 1.5 kb. The 3.2-kb band was increased in the mutant, in contrast to the 3.2-kb band detected with the psbB and psbH probes. It also hybridized to a probe specific for the petD intron (data not shown). The 2.9-kb band just below it did not hybridize with the intron-specific probe. This band was missing in the mutant but appeared to be replaced with a band of similar intensity migrating slightly faster. The rather diffuse band at 1.8 kb was also missing in the mutant, as was a 1.1-kb band that hybridized only with the petB probe. However, the 0.8-kb band, which presumably represents the final petB transcript, was unchanged. The mutation had little effect on bands hybridizing only with the petD probe.

Not all chloroplast transcripts were affected. In addition to psbA, only minor differences were found with probes for psaB, petA, psaD, and atpA (data not shown). Nuclear genes lhcB1 and lhcA4 (encoding polypeptides of the peripheral light-harvesting complexes of PSII and PSI, respectively), psaD (encoding an essential polypeptide of PSI), and rbcS appeared to be expressed at wild-type levels (data not shown).

**DISCUSSION**

In this paper we describe the isolation and characterization of the A. thaliana high chlorophyll fluorescence mutant hcf5. The kinetics of fluorescence induction as well as the absence of PSI activity showed that PSI is severely affected, and immunoblotting confirmed that all PSI polypeptides are either lacking or at very low levels compared to wild type. However, the mutation appears to have a pleiotropic effect on other components of the electron transport system. Polypeptides of PSI, the Cyt f-b complex, and CF, were also depleted or absent (Fig. 4). In contrast, the chlorophyll a/b antenna complexes LHCI and CP29 appeared normal on non-denaturing gels (Fig. 3), and transcripts of the nuclear genes encoding an LHCII polypeptide (Lhcb1) and a PSI LHCI polypeptide (Lhca4) were present in normal amounts in the mutant. This shows that the pleiotropic nature of the mutant is not due to an underlying defect in chlorophyll synthesis.

Besides high fluorescence, the most striking phenotypic trait was the drastic alteration of thylakoid ultrastructure (Fig. 1). Almost all the thylakoids were appressed, although a few single thylakoids were observed around the periphery of the thylakoid stack. The chloroplast morphology of hcf5 most closely resembles the barley PSII mutants at the viridis-ε locus, but there are also some barley PSI mutants with diminished amounts of stroma thylakoids, e.g. *viridis-h* (Simpson and von Wettstein, 1980). Although the ultrastructural defects of the barley mutants cannot be related to specific photosynthetic defects (Simpson and von Wettstein, 1980), the high degree of thylakoid appression in hcf5 is probably due to the severe depletion of both photosystems and the consequent predominance of LHCCI in the thylakoid membrane, because inter-thylakoid interaction of LHCCI is known to be one of the major factors in thylakoid adhesion (Staehelin, 1987).

The hcf5 mutant is unique in having decreased levels of all psbB operon transcripts containing psbH, no detectable psbH monocistronic transcript, and extremely low levels of rbcL transcript. In addition, there is an overaccumulation of the 3.2-kb petB-petD transcript and a decrease in two of the smaller transcripts containing petB, although the petB monocistronic transcript appears to be present in normal amounts. These defects suggest that the normal Hcf5 gene function is involved in both the processing and the stability of selected chloroplast transcripts. Because these processes have been shown to be mediated in vitro by proteins interacting with the 3' end of the mRNA (Schuster and Gruissem, 1991; Hayes et al., 1996), RNA blots from hcf5 and normal siblings were probed with Arabidopsis genes for two of these proteins, 28RNP (Schuster and Gruissem, 1991; S. Abrahamson, personal communication) and 33RBP (Cheng et al., 1994). No differences in transcript abundance were detected (S. Abrahamson, personal communication; A. DeLisle, personal communication). Other proteins binding to the 5' end of chloroplast transcripts have been implicated in translational initiation and stability in *Chlamydomonas* (Nickelsen et al., 1994; Zerges and Rocheaix, 1994; Mayfield et al., 1995; Yohn et al., 1996). If hcf5 were defective in a protein required for the translatability of a number of chloroplast messages while affecting the stability of only certain ones, it could account for the pleiotropic effects of this mutation on PSI, PSII, and the Cyt complex.

The lowered levels of the rbcL transcript could be an indirect effect of a decrease in translation, because it has been shown that in spinach this mRNA is highly sensitive to changes in chloroplast translation and processing (Klaff and Gruissem, 1991; Schuster and Gruissem, 1991). Several maize mutants with general defects in chloroplast protein
synthesis due to decreased association of mRNAs with ribosomes have lowered amounts of rbcL message (Barkan, 1993). It has been suggested that this message, but not other chloroplast messages, was destabilized when not associated with ribosomes (Barkan, 1993). If hcf5s were such a mutation, it would explain the low levels of rbcL message but not the depletion of psbH-containing transcripts or what appears to be a splicing or endonucleolytic process affecting petB-petD transcripts.

Most of the hcf mutants isolated in higher plants are missing or depleted in one of the macromolecular complexes of the photosynthetic membrane but do not show any effect at the level of chloroplast mRNA (Barkan et al., 1995). Only three mutants of maize, hcf38, crp1, and crp2 (Barkan et al., 1986, 1994), appear to be deficient in the accumulation of chloroplast transcripts. The hcf38 mutant lacks most of the psbH containing products of the psbB polycistronic operon and has lower levels of petA, atpB/E, and psaA transcripts, but has normal levels of the rbcL message. The crp1 mutation appears to affect the accumulation of the monocistronic petB and petD products and the translatability of petA and petD, but not petB messages (Barkan et al., 1994). While our paper was in preparation, another Arabidopsis mutant, hcf109, which is defective in the psbB operon transcripts, was reported (Meurer et al., 1996a). In hcf109 the transcripts containing psbB rather than psbH were depleted, and petB and petD were unaffected. In addition, three other polycistronic operons (psbD/C, ndhF, and ndhH) showed selective depletion of certain transcripts. All of the higher plant mutants are therefore different from each other and are similar only in that the mutations all affect polycistronic transcripts and all have pleiotropic effects. In no case do the alterations in chloroplast transcripts explain all the downstream effects on the pigment analyses.

Further exploration of these complex mutant phenotypes should be greatly aided by the isolation of T-DNA or transposon tagged alleles (Goodman et al., 1995).

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