From Genes to Photosynthesis in Arabidopsis thaliana

Dario Leister* and Anja Schneider†

*Abteilung für Pflanzenzüchtung und Ertragsphysiologie, Max-Planck-Institut für Züchtungsforschung, D-50829 Köln, Germany, and †Botanisches Institut der Universität zu Köln, D-50931 Köln, Germany

Although photosynthesis in higher plants is of cyanobacterial descent, it differs strikingly in organization and regulation from the prokaryotic process. Genomics, proteomics, and comparative genome analysis are now providing powerful new tools for the molecular dissection of photosynthesis in higher plants. Mutant screens and reverse genetics identify an increasing number of gene–function relationships that have a bearing on photosynthesis, revealing a marked interdependency between photosynthesis and other cellular processes. Photosynthesis-related functions are mostly located in the chloroplast, but can also be located in other compartments of the plant cell. The analysis by DNA-array hybridization of mRNA expression patterns both in the chloroplast and the nucleus, under various environmental conditions and/or in different genetic backgrounds that affect the function of the plastid, is rapidly improving our understanding of how photosynthesis is regulated, and it reveals that plastid-to-nucleus signaling plays a central role in its control.

**KEY WORDS:** Chloroplast, Expression profiling, Genomics, Mutant, Photosynthesis, Photosystem, Signaling.

I. Introduction

During photosynthesis in higher plants, photons are absorbed by light-harvesting complexes (LHCs) of the two photosystems PSI and PSII. Transfer of the absorbed energy to reaction centers leads to a series of electron transport steps that ultimately result in the oxidation of water and the synthesis of NADPH and ATP. The photosynthetic reactions take place in
the chloroplast, an organelle that descended from an ancestral cyanobacterial endosymbiont (Douglas, 1998). In addition to its role in photosynthesis, the chloroplast is involved in the synthesis of many essential cellular compounds, such as amino acids, fatty acids and lipids, plant hormones, nucleotides, vitamins, as well as secondary metabolites. The chloroplast also functions in the assimilation of nitrogen and sulfur. The organelle possesses its own genome (the plastome) and houses the machinery required for its expression, but most chloroplast proteins are now encoded in the nucleus and must be imported from the cytoplasm. Thus, photosynthesis takes place within a compartment rich in interdependent metabolic processes, which are subject to complex regulation in response to environmental fluctuations and changes in the developmental state of the organelle. Furthermore, these processes must be coordinated with the activities of the nuclear and mitochondrial genomes in the cell.

Research on photosynthesis has therefore greatly benefited from the sequencing of the genome of the flowering plant Arabidopsis thaliana (The Arabidopsis Genome Initiative, 2000), and the subsequent development and application of tools that permit large-scale, systematic investigations of gene function (Pesaresi et al., 2001a; Leister, 2003). As a result, a new picture is emerging of how photosynthesis operates in flowering plants, to what extent it differs from prokaryotic photosynthesis, and how it interacts with other plastid and nonplastid functions. Moreover, the fact that the process depends upon a variety of other plastid and nonplastid functions makes photosynthetic performance a suitable phenotypic indicator for the genetic dissection of photosynthesis-related cellular processes. Such an analysis will eventually enable us to describe the photosynthetic regulatory network and predict how alterations in specific components modulate the process. Ultimately, this should provide a rationale for the improvement of photosynthetic efficiency by molecular plant breeding.

II. Evolutionary Considerations

A. Origin of Plastids and Their Cyanobacterial Heritage

Chloroplasts arose through endosymbiosis from cyanobacteria. Contemporary cyanobacterial genomes encode several thousand proteins; numbers range from less than 2000 open reading frames (ORFs) (Prochlorococcus) to more than 7000 ORFs (Nostoc) (http://www.jgi.doe.gov/JGI_microbial/html/). The earliest estimate of the number of cyanobacterial genes present in a plant genome (Abdallah et al., 2000) relied on comparative BLAST analysis of the then partially sequenced genome of A. thaliana and
the first fully sequenced genome of a cyanobacterial species (Synechocystis). Around 1500 proteins of cyanobacterial origin were predicted to be encoded in the nucleus of Arabidopsis, about half of which were targeted to the chloroplast (Abdallah et al., 2000). Abdallah et al. (2000) predicted the existence of more than 1000 chloroplast proteins of noncyanobacterial descent. Other studies, based on phylogenetic analysis of the entire A. thaliana genome or samples of it, concluded that between 400 and 2200 Arabidopsis genes were derived from cyanobacteria (The Arabidopsis Genome Initiative, 2000; Rujan and Martin, 2001).

The most thorough analysis of the cyanobacterial heritage in the Arabidopsis nuclear genome to date involved comparisons with the genomes of three cyanobacterial species, 16 other reference prokaryotes, and budding yeast (Martin et al., 2002). Of the 24,990 Arabidopsis proteins considered, 9368 were sufficiently conserved for primary sequence comparison. Among these, 866 have only cyanobacterial homologues, and an additional set of 834 proteins group together with cyanobacterial proteins in phylogenetic trees. Assuming that Arabidopsis proteins originating from cyanobacteria do not preferentially belong to the 9368 most conserved ones, it can be concluded that 18% [(866 + 834)/9368] of the Arabidopsis genome (~4500 genes) derives from cyanobacterial progenitor genes. This estimate of 4500 genes does not imply that the ancestor of plastids donated all of these to the nucleus; possibly far fewer genes were transferred, which were subsequently amplified in the nucleus due to gene or genome duplications (The Arabidopsis Genome Initiative, 2000).

Contemporary plastids resemble their cyanobacterial relatives in several respects; they possess thylakoid membranes (Vothknecht and Westhoff, 2001) and 70 S-type ribosomes (Yamaguchi and Subramanian, 2000; Yamaguchi et al., 2000), and use similar cell division proteins (Osteryoung and McAndrew, 2001), light-dependent chlorophyll biosynthesis (Suzuki and Bauer, 1995), and the Sec-, Tat-, and SRP-types of protein targeting to thylakoids (Robinson et al., 2001). However, no more than half of the plant proteins that originated from cyanobacteria are targeted back to the plastid (Abdallah et al., 2000; Martin et al., 2002) (Fig. 1), implying that a large fraction of proteins of cyanobacterial descent have acquired roles in diverse nonplastid functions (Leister and Schneider, 2003). Conversely, a substantial number of proteins for which no links with cyanobacteria can be established are targeted to plastids (Abdallah et al., 2000; Leister, 2003). Such eukaryotic additions include novel (i.e., plant-specific) photosynthetic (Scheller et al., 2001) and ribosomal (Yamaguchi and Subramanian, 2000; Yamaguchi et al., 2000) proteins, and novel domains that have been added to otherwise cyanobacterially derived proteins. Well-studied plastid functions that appear to be completely “novel”—not derived from prokaryotes—include the machinery responsible for importing proteins across the plastid envelope (Jarvis and Soll, 2001), the “spontaneous” targeting of proteins to
FIG. 1 Genes of cyanobacterial origin and the subcellular targeting of their products in Arabidopsis. Chloroplasts bounded by double membranes, such as those of Arabidopsis, originate from a cyanobacterial-like endosymbiont. Because the identity of the endosymbiont is unknown, the three cyanobacterial species whose genome sequences are known (Synechocystis sp. PCC6803, Prochlorococcus marinus, and Nostoc punctiforme) were used to reconstruct protein phylogenies (Martin et al., 2002). The number and intracellular localization of cyanobacterium-derived gene products are as in Leister (2003), and the number of nuclear genes of cyanobacterial origin comes to about 4300. Black lines trace the origin of the 87 plastome and the 4300 nuclear genes of
thylakoid membranes (Robinson et al., 2001), and the light-harvesting antenna complexes (LHCs) associated with the cores of the photosystems (Montane and Kloppstech, 2000).

Chloroplast proteins are not only of dual evolutionary origin, they are also synthesized in two different cellular compartments, as mentioned above. The vast majority of plastid proteins are encoded in the nuclear genome, synthesized in the cytoplasm as precursors with N-terminal transit peptides on 80 S-type ribosomes, and imported into plastids by the Tic/Toc machinery (Jarvis and Soll, 2001). Only a relatively small number of plastid proteins (about 90 in A. thaliana) are encoded by the plastome and synthesized by 70 S-type ribosomes in the plastids themselves. The distribution of genes coding for plastid proteins into two genomes is the result of massive gene transfer from the genome of the endosymbiont to the nucleus, which has reduced the size of the organellar genome by well over an order of magnitude. Comparison of the plastomes sequenced so far reveals that approximately 40 plastid genes are common to all extant chloroplasts (Martin et al., 1998, 2002). Why have a separate genome and an energetically expensive expression apparatus been retained for the production of these relatively few plastid proteins? Conversely, what has prevented the transfer of these genes to the nucleus? Such questions have been addressed repeatedly (Douglas, 1998; Martin et al., 1998; McFadden, 1999; Race et al., 1999), and it appears that, for this set of genes, positive selection for transcription/translation within the organelle accounts for their failure to be successfully incorporated into the nuclear genome.

As a consequence of the coexistence of three genomes (including mitochondrial DNA) in different compartments of the plant cell, distinct modes of coordination and regulation of their expression, as well as mechanisms for information exchange between the three compartments, have had to be established to optimize the performance of plant cells and their organelles (Rodermel, 2001) (see also Section IV).

B. Evolution of the Photosynthetic Apparatus

1. General Features and Protein Targeting

At first, the photosynthetic apparatus of the flowering plant A. thaliana appears very similar to that of cyanobacteria. Closer inspection of the multi-protein complexes involved in the light-driven reactions reveals significant
differences with respect to both their composition and the amino acid sequence of specific photosynthetic proteins. The photosynthetic machinery of *A. thaliana* consists of chimeric multiprotein complexes, containing plastome- and nucleus-encoded subunits. The latter include proteins that are orthologous to cyanobacterial polypeptides and proteins that are found only in the plant lineage (summarized in Fig. 2).

**FIG. 2** Composition of the photosynthetic apparatus in the thylakoid membrane, summary of the evolutionary origin of its subunits (A), and available mutants (B). (A) Known structural data were used to illustrate the composition of multiprotein complexes, but the positions of subunits within the complexes are largely arbitrary. Plastome-encoded subunits are indicated in white. Nucleus-encoded proteins are depicted in gray or black (if the protein has no cyanobacterial ortholog). PC, plastocyanin; Fd, ferredoxin; FNR, ferredoxin-NADPH reductase; LHC, light-harvesting complex; PS, photosystem. (B) The same figure as in (A) is used to illustrate the availability of mutant alleles or transgenic lines with silenced genes for photosynthetic proteins. Plastome-encoded subunits are indicated in light-green or dark-green shading (if inactivation of the corresponding gene has been reported). Nucleus-encoded proteins are depicted in orange or red (if knockout or down-regulation of the protein has been achieved). Note that although mutants from several plant and algal species are considered, the composition of the photosynthetic apparatus refers only to *Arabidopsis*, which in this regard differs in some respects from algal species. YCF3 and YCF4 (not shown) are localized on the thylakoid membrane, but are not stably associated with a multiprotein complex.
The photosynthetic apparatus of higher plants is located in the thylakoids of chloroplasts. Thylakoid membranes are organized into stacked (grana) and unstacked (stromal lamellae) regions, and the various components of the photosynthetic machinery tend to distribute unequally in these different domains. Grana are PSII-rich, whereas PSI accumulates in stromal lamellae. The degree of membrane stacking can vary, depending on the quality and quantity of the incident light.

Because of the compartmentation of the plastid organelle, most of the proteins of the photosynthetic apparatus, and particularly those encoded in the nucleus and synthesized in the cytoplasm, have to cross several membranes, including the outer and inner chloroplast envelopes and the thylakoid membrane, before they reach their destination. Therefore, many of these proteins are synthesized as precursors that contain one or more pre-sequences: the so-called chloroplast transit peptide (cTP) is necessary for transport across the outer and inner envelopes, and several types of lumenal transit peptides (lTP) are needed for transport into or across the thylakoid membrane (Fig. 3).

Chloroplast precursor proteins are directed by the so-called “guidance complex” to the Tic/Toc translocon, a eukaryote-specific mechanism (Jarvis and Soll, 2001). After import into the chloroplast, the cTP is cleaved by the stromal processing peptidase. No additional transit peptides are required for the sorting of proteins to the inner envelope or the stroma. In contrast, at least four different pathways exist for uptake from the stroma into, or passage through, the thylakoid membrane in the chloroplasts of higher plants (Robinson et al., 2001). Three of these depend on a second N-terminal sequence, which is located directly C-terminal to the cTP and cleaved by the thylakoidal processing peptidase.

Substrates for the ATP-dependent Sec pathway contain a Sec-type thylakoid targeting signal, interact with the stromal SecA protein, and are translocated in an unfolded state through the Sec translocon. The ion gradient dependent twin-arginine translocation (Tat) pathway accomplishes the transport of fully folded precursors whose lumenal transit peptides have a characteristic twin-arginine motif preceding the hydrophobic core. The signal recognition pathway is thought to be reserved for integration of thylakoid membrane proteins such as the light-harvesting protein Lhcb1, and requires a stromal recognition particle (SRP), FtsY, Alb3, GTP, and a translocation apparatus. Interestingly, the information for targeting to the thylakoid is located within the mature Lhcb1 protein. The fourth, so-called “spontaneous,” pathway mediates the insertion of selected thylakoid membrane proteins and also requires the presence of a second cleavable signal peptide that is superficially similar to Sec-type signal peptides.

Recent findings support the existence of a fifth transport mechanism that depends on the flow of membrane elements via vesicular transport to build
up and sustain the lipid backbone of the thylakoid membrane system (Kroll et al., 2001). Of the five mechanisms of thylakoid targeting outlined above, the so-called “spontaneous” pathway operates only in plants (Robinson et al., 2001).
2. The Photosystems and Their Light-Harvesting Complexes

Whereas the chloroplast multiprotein complexes PSII, PSI, the cytochrome \( b_6/f \) complex, and the plastid ATPase descend from cyanobacterial progenitors, the light-harvesting complexes of the two photosystems, LHCII and LHCI, are eukaryotic inventions. The eukaryotic light-harvesting complexes, LHCI and LHCII, serve to channel light energy to the photosystems, and respond to the wavelength and intensity of incident light. Thus they control the allocation of light energy to the photosystems, and protect them from the inhibitory effects of intense radiation (photoinhibition) by dissipating excess energy. Monitoring of changes in chlorophyll fluorescence can be exploited in screens for mutants affected in many aspects of photosynthetic function (see below).

LHCs have replaced prokaryotic light-harvesting complexes such as the phycobilisomes, and are thought to descend from “small chlorophyll \( a/b \) binding (CAB) protein-like proteins” (SCP), which can still be found in contemporary cyanobacteria and are thought to be involved in tetrapyrrole biosynthesis (Xu et al., 2002).

PSII and PSI from cyanobacteria and plants are similar in their function and composition (He and Malkin, 1998). PSII is the site where electrons and oxygen are released from water, and plastoquinone is reduced to plastoquinol. PSII is the most complex multiprotein assembly found in the thylakoid membrane, containing at least 29 subunits in higher plants, whereas the cyanobacterial PSII has about 21 subunits. Most of the subunits are common to the PSII complexes in both divisions; the reaction center proteins D1 and D2, copies of cytochrome \( b_{559} \) (PSII-E and -F), CP43 and 47, and the 33-kDa protein (PSII-O) of the oxygen-evolving complex are invariably shared (Table I). In addition to PSII-O, the oxygen-evolving complex of higher plants contains PSII-P and -Q, both nucleus-encoded. In contrast, cyanobacterial PSII contains the subunits V (cytochrome \( c_{550} \)) and U, instead of PSII-P and -Q. The T subunit is found in the D1–D2 complex in both cyanobacteria and higher plants, whereas subunit W is specific to the latter.

PSI from plants, algae, and cyanobacteria mediates light-driven electron transport across the thylakoid membrane, from soluble lumenal to soluble stromal electron carriers. The oxidized PSI is reduced on the luminal site by plastocyanin or cytochrome \( c_6 \). Cyanobacterial PSI contains at least 11 subunits: PSI-A to -F and PSI-I to -M. In higher plants PSI possesses at least four further subunits, PSI-G to -O, whereas no homolog of the cyanobacterial PSI-M has yet been found (Scheller et al., 2001). Interestingly, a number of the plant PSI subunits have additional domains. For example, after cleavage of the transit peptide, plant PSI-D, -E, and -F still contain N-terminal extensions of 30 to 40 amino acid residues, relative to their cyanobacterial counterparts.
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\(^a\) MIPS accession numbers are from MIPS (www.mips.gsf.de) (continued)
**TABLE I** (continued)

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**LHCl and LHClI**

| Lhca1  | LHCI-720 | Lhca1 | At3g54890 |                           |
| Lhca2  | LHCI-680 | Lhca2 | At3g61470 | *Ganeteg et al., 2001*    |
| Lhca3  | LHCI-680 | Lhca3 | Atlg61520 | *Ganeteg et al., 2001*    |
| Lhca4  | LHCI-720 | Lhca4 | At3g47470 | *Zhang et al., 1997*      |
| Lhca5  |          | Lhca5 | Atlg45474 |                           |
| Lhca6  |          | Lhca6 | Atlg19150 |                           |
| Lhcb1  | LHCII type 1 | Lhcb1.1 | Atlg29920 |                           |
| Lhcb2  | LHCII type 2 | Lhcb2.1 | Atg05100  |                           |
| Lhcb3  | LHCII type 3 | Lhcb3  | At5g54270 |                           |

^aPlastid gene names start with a lower-case letter; nuclear genes are referred to by gene names starting with a capital letter. C/T: inactivation of the corresponding gene was performed in *Chlamydomonas/tobacco.*
3. Intersystemic Electron Transport and the ATPase

The cytochrome $b_6/f$ complex and the chloroplast ATPase are very similar to their prokaryotic pendants. Plastocyanin, the soluble protein that transports electrons from the cytochrome $b_6/f$ complex to PSI, is also found in both prokaryotes and plants. However, although it was once thought that cytochrome $c_6$—which can replace plastocyanin in cyanobacteria and some algal species—is absent in higher plants, a cytochrome $c_6$-like protein has recently been identified in *Arabidopsis* chloroplasts (Gupta et al., 2002).

III. Mutational Dissection of Photosynthesis

The sequencing of the complete DNA complement of *Arabidopsis thaliana* and the availability of large collections of mutant lines have recently stimulated efforts to systematically identify all the genes that encode photosynthetic proteins and characterize their products. This dissection of photosynthesis can be carried out efficiently by isolating and characterizing mutants with altered photosynthetic performance. In higher plants, a number of strategies have been developed for the identification of photosynthetic mutants (Somerville, 1986; Pesaresi et al., 2001a).

Reverse genetics, in addition, offers new opportunities for the study of photosynthesis. The most popular methods for the identification of lines defective in the function of genes of interest involve the use of antisense, cosuppression, or RNAi approaches to inhibit their expression, or screening of large collections of *Arabidopsis* lines bearing random transposon or T-DNA insertions (Parinov and Sundaresan, 2000). The screens are based on polymerase chain reactions (PCRs) primed by oligonucleotides that are complementary to both the target genes and the insertional DNA. In some collections, sequences flanking the insertions have been isolated and arranged in arrays, allowing the large-scale identification of mutant lines by hybridization procedures (Tissier et al., 1999; Steiner-Lange et al., 2001). More recently, the systematic sequencing of DNA sequences that flank insertions and their organization into searchable databases have allowed for even more efficient mutant identification (Parinov et al., 1999; Tissier et al., 1999; J. Ecker and co-workers: http://signal.salk.edu/; and others). As an alternative to insertional mutagenesis, loss-of-function alleles induced by EMS mutagenesis can be identified by the TILLING approach, a gel-based method for the identification of mismatched heteroduplexes (McCallum et al., 2000; Colbert et al., 2001).

For plastome-encoded genes, mutant alleles can be generated by homologous recombination, allowing wild-type genes to be replaced by knockout alleles and their mutant phenotypes to be studied in homotransplastomic
plants. Targeted inactivation of plastome genes has been successfully performed in tobacco and in the green alga *Chlamydomonas reinhardtii*.

A. The Photosynthetic Apparatus

1. PSII

   a. Plastome-Encoded Proteins  Fifteen of the 26 known subunits of the *A. thaliana* PSII complex (excluding Lhcb1-3) are encoded by plastid genes (Table 1). Because knockout of plastome genes is not yet practical in *Arabidopsis*, the phenotypes of corresponding mutants in tobacco and/or *Chlamydomonas* are briefly described here.

   The function of the D1 protein, encoded by the *psbA* gene, has been addressed by replacing the wild-type form of *psbA* in *Chlamydomonas* with alleles that carried specific point mutations (Erickson *et al.*, 1989; Przibilla *et al.*, 1991; RoVeY *et al.*, 1991). The effects of these point mutations on herbicide action, as well as on PSII activity, were then studied in detail, leading to the identification and characterization of a region in the D1 polypeptide involved in herbicide binding, which defines a binding niche for the second quinone acceptor. In the nuclear *nac2* mutant of *C. reinhardtii*, no *psbD* transcript for the D2 protein accumulates, whereas all other PSII proteins are synthesized normally (Kuchka *et al.*, 1989). However, the entire PSII complex is unstable in *nac2* cells, and all major PSII polypeptides, including the three proteins of the oxygen-evolving complex, are absent or greatly reduced as a result of posttranslational degradation.

   The role of cytochrome *b*$_{559}$ has been investigated in a *psbE* null mutant in *C. reinhardtii* (Morais *et al.*, 1998). No PSII activity could be detected, either in oxygen evolution assays or by the analysis of chlorophyll fluorescence. Immunoblotting experiments showed that the absence of PSII activity in the mutant was due to loss of the PSII complex in both light-grown and dark-grown cultures.

   Lack of editing of the *psbF* mRNA following the introduction of a point mutation leads to slower growth, a lower chlorophyll content, and high chlorophyll fluorescence in tobacco plants (Bock *et al.*, 1994). In *Chlamydomonas* mutants lacking *psbH*, translation and thylakoid insertion of PSII core proteins are unaffected. However, high-molecular-weight forms of PSII do not accumulate to any significant extent, indicating a primary role for PSII-H in the assembly/stability of PSII through dimerization (Summer *et al.*, 1997; O’Connor *et al.*, 1998).

   The chloroplast *psbI* gene, the product of which is associated with the PSII reaction center, has been disrupted in *Chlamydomonas* (Kunstner *et al.*, 1995). The loss-of-function lines are still able to grow photoautotrophically
in dim, but not in bright, light, and remain photosensitive when grown heterotrophically. The levels of both the PSII complex and oxygen-evolving activity are substantially reduced in the PSII-I-deficient mutants, indicating that this polypeptide plays a role in ensuring the stability of PSII, and possibly also in modulating electron transport or energy transfer in the complex.

Characterization of $\Delta psbK$ lines in *C. reinhardtii* shows that the *psbK* product is required for the stable assembly and/or stability of the PSII complex and for photoautotrophic growth (Takahashi *et al.*, 1994). These results differ from those previously reported for similar cyanobacterial *psbK* mutants, which were capable of photoautotrophic growth (Ikeuchi *et al.*, 1991). PSII-T is a small, chloroplast-encoded, hydrophobic polypeptide associated with the PSII core complex. A *psbT*-deficient mutant of *C. reinhardtii* can grow photoautotrophically; but growth is significantly impaired in strong light (Ohnishi and Takahashi, 2001).

The *ycf9* gene has been knocked out repeatedly (Higgs *et al.*, 1998; Mänpää *et al.*, 2000; Ruf *et al.*, 2000; Baena-Gonzalez *et al.*, 2001; Swiatek *et al.*, 2001), but Wollman and co-workers were the first to provide strong evidence for the localization of its gene product to PSII. The protein is now designated PSII-Z (Swiatek *et al.*, 2001) and controls the interaction of the PSII core with its light-harvesting antenna. In the absence of PSII-Z, tobacco plants show severe growth defects and increased sensitivity to light (Swiatek *et al.*, 2001). More recently, the plastid genes coding for the PSII subunits J and L were inactivated (Regel *et al.*, 2001; Hager *et al.*, 2002). Tobacco $\Delta psbL$ lines without a functional PSII can be propagated only under heterotrophic conditions, whereas absence of PSII-J leads to the deregulation of photosynthetic electron flow and perturbs the assembly of the oxygen-evolving complex.

**b. Nucleus-Encoded Proteins** The proteins CP24, CP26, and CP29, which are encoded by members of the nuclear LHC gene family, are located between the PSII core and the major LHCII complex (Bassi *et al.*, 1997). Another protein encoded by the LHC family, PSII-S, is present in many oxygen-evolving PSII preparations depleted of other LHC proteins, indicating that PSII-S may be closely associated with the PSII reaction center (Kim *et al.*, 1994). Mutagenesis or silencing of several of the nuclear genes coding for LHC proteins of PSII has markedly improved our understanding of their functions. The specific roles of CP29 and CP26 in light harvesting and energy dissipation were investigated in *A. thaliana* by using antisense lines (Andersson *et al.*, 2001). The decrease in the level of the CP24 protein observed in CP29 antisense lines indicates a physical interaction between the two proteins. The CP29 and CP26 antisense lines have distinct chlorophyll fluorescence characteristics, indicating a change in the organization of the
light-harvesting antenna. However, the overall rate of photosynthesis in both lines is similar to that in the wild type, with a normal qE type of nonphotochemical fluorescence quenching (feedback deexcitation), indicating that CP29 and CP26 are unlikely to be sites of nonphotochemical quenching.

In the course of a screen for *A. thaliana* strains that are unable to dissipate excess light energy by nonphotochemical quenching (NPQ mutants; see Section III.B.2.b), the mutant line npq4 was isolated. This mutant does not accumulate the PSII-S protein (Li *et al.*, 2000), and characterization of this line showed that PSII-S is necessary for nonphotochemical quenching, but not for efficient light harvesting or photosynthesis. Subsequent studies showed that plants with a two-fold increase in qE capacity can be produced by overexpressing PSII-S, demonstrating that the level of PSII-S limits the qE capacity in wild-type *Arabidopsis* (Li *et al.*, 2002). These results link PsbS to natural or induced variation in qE capacity. In addition, because increased qE is associated with decreased PSII excitation pressure and with changes in chlorophyll a fluorescence lifetimes, it was suggested that qE protects from photoinhibition by preventing excessive reduction of PSII electron acceptors.

In *Chlamydomonas*, a nuclear mutant that is deficient in oxygen-evolving activity cannot synthesize the PSII-P protein, but is not impaired in the accumulation of other PSII polypeptides or their mRNAs (Mayfield *et al.*, 1987).

### 2. PSI

The PSI complex of higher plants is a mosaic of five plastid- and at least nine nucleus-encoded protein subunits (Fig. 2). Furthermore, in *A. thaliana*, there are two expressed genes for each of the subunits D, E, and H (Pesaresi *et al.*, 2001a).

#### a. Plastome-Encoded Proteins

In *Chlamydomonas*, four of the five plastid genes coding for PSI polypeptides have been inactivated (the exception being *psaI*): *psaA* and *psaB* (Redding *et al.*, 1998, 1999), *psaC* (Rochaix *et al.*, 1989; Takahashi *et al.*, 1991; Redding *et al.*, 1999), and *psaJ* (Fischer *et al.*, 1999). Cells that lack PSI-A, -B, or -C are incapable of photoautotrophic growth (Redding *et al.*, 1999), whereas Δ*psaJ* lines can grow photoautotrophically, but show altered kinetics of oxidation of plastocyanin or cytochrome c6 (Fischer *et al.*, 1999). In cyanobacteria, knockout of *psaI* leads to a substantial decrease in the amount of PSI-L and to a destabilization of PSI trimers (*Xu et al.*, 1995). Similar experiments have not yet been carried out in plants or algae.

By targeted inactivation of the “hypothetical” plastome ORFs *ycf3* and *ycf4* in *Chlamydomonas*, the role of their products in the assembly of PSI has
been clarified (Boudreau et al., 1997). YCF3 is a chaperone that interacts directly and specifically with at least two of the PSI subunits during assembly of the PSI complex (Naver et al., 2001). Both YCF proteins are located on the thylakoid membrane of the *Chlamydomonas* chloroplast, but are not stably associated with a multiprotein complex.

**b. Nucleus-Encoded Proteins** Scheller and co-workers have generated a collection of *A. thaliana* lines in which individual nucleus-encoded subunits of PSI can be down-regulated at the protein level by antisense and co-suppression strategies (Naver et al., 1999; Haldrup et al., 1999, 2000; Lunde et al., 2000; Jensen et al., 2000, 2002). This strategy has proven to be effective, even in cases in which the same subunit is encoded by two functional genes. Such analyses have revealed that PSI-K plays a role in organizing LHCI (Jensen et al., 2000), PSI-N is necessary for the interaction of plastocyanin with PSI (Haldrup et al., 1999), and PSI-H appears to provide an attachment site for LHCII (Lunde et al., 2000) during state transitions (changes in the distribution of light energy between the two photosystems; see below). Loss of PSI-F has far more dramatic consequences on photoautotrophic growth in *Arabidopsis* than do the corresponding mutations in cyanobacteria or algae: in *Arabidopsis*, deficiency of PSI-F results in significantly decreased levels of most PSI polypeptides (Haldrup et al., 2000), making it difficult to distinguish between primary and secondary effects of the loss of these subunits in *A. thaliana*.

The analysis of stable knockout mutants generated by T-DNA or transposon insertions is required to dissect the impact of each of the two functional gene copies for the subunits D, E, and H in *Arabidopsis*. Such mutants make it possible to generate multiple-knockout lines that allow us to understand the degree of functional redundancy between the subunits forming PSI, as well as to carry out complementation experiments using modified versions of cyanobacterial or higher plant genes.

The *psae1-1* mutant of *Arabidopsis* was identified on the basis of a decrease in the effective quantum yield of PSII ($\Phi_{II}$) (see Section III.B.2.c), and the mutation responsible was localized to *PsaE1*, one of two *Arabidopsis* genes that encode subunit E of the reaction center of PSI. The entire stromal side of PSI is affected by disruption of the *PsaE1* gene (Varotto et al., 2000a), and, furthermore, the interaction between PSI and LHCII (see Sections III.A.3 and IV.A) is perturbed in an unusual way (Pesaresi et al., 2002). In the *psae1* mutant, a fraction of LHCII becomes stably associated with PSI when plants are exposed to low-light conditions, giving rise to a high-molecular-mass protein–pigment complex detectable in native protein gels. The formation of this abnormal LHCII–PSI complex is associated with the almost complete suppression of state transitions, a drastic increase in the levels of phosphorylated LHCII under all light regimens tested, and a permanent reduction in
PSII antenna size. All these observations indicate that in the absence of PSI-E, a docking site is exposed on PSI that mediates stable binding of phosphorylated LHCII to the complex.

Plants that have a PSI complex that lacks PSI-G have been generated in two laboratories (Jensen et al., 2002; Varotto et al., 2002a). Their analysis shows that PSI-G plays a role in stabilizing the binding of the peripheral antenna (Jensen et al., 2002; Varotto et al., 2002a). The increased activity of PSI in the absence of PSI-G suggests that this subunit could play an important role in the regulation of PSI (Jensen et al., 2002).

The application of hierarchical clustering to the data on the abundance of individual polypeptides of PSI in the various PSI mutants permitted a further test of previously proposed protein–protein relationships (Pesaresi et al., 2003) (Fig. 4). This analysis confirmed that levels of PSI-H and -L, the stromal subunits -C, -D, and -E, and the light-harvesting proteins Lhca2 and 3 are interdependent, which is consistent with chemical cross-linking data (Jansson et al., 1996). Also the levels of the luminal subunits PSI-F and -N behave coordinately in the genotypes tested (Pesaresi et al., 2003). This can be explained either by physical contacts between the two proteins that protect both from rapid turnover, or by a regulatory mechanism that adjusts the relative abundances of the two subunits, which are both involved in plastocyanin binding (Haldrup et al., 1999, 2000). Cluster analysis incorporating both polypeptide levels and parameters reflecting photosynthetic performance strongly support the suggestion, based on the study of two genotypes (Lunde et al., 2000), that PSI-H is involved in state transitions (Pesaresi et al., 2003). In the genotypes tested, levels of Fr (which is a measure for the level of state transitions; see below) and of PSI-H covary, supporting an earlier suggestion that the two are related.

3. The Light-Harvesting Complexes

The proteins that make up LHCII and II belong to the large LHC multi-protein family, which comprises about 20 members (Jansson, 1999). For some LHC proteins, more than one gene copy exists, e.g., there are five Lhcb1 genes and three Lhcb2 genes (Table I). The expression of LHC genes responds to changes in illumination (Anderson et al., 1988), and for this reason, constitutive or decreased Lhc gene expression has been used as a tool to screen for several types of mutants that are defective in plastid-to-nucleus signaling (Susek et al., 1993; Li et al., 1995) (see Section IV.E).

a. LHCII

Six different proteins contribute to the light harvesting antenna of PSII. The three minor proteins CP29, CP26, and CP24 (see Section III.A.I) are encoded by the genes Lhcb4, 5 and 6, whereas the major
LHCII complex consists of three proteins encoded by the genes \textit{Lhcb}1, 2, and 3, of which \textit{Lhcb}1 and 2 are by far the most abundant. LHCII binds chlorophyll \textit{a}, chlorophyll \textit{b}, and xanthophylls.

A fraction of LHCII, the so-called mobile pool of LHCII, plays a distinct role in balancing the allocation of energy between the two photosystems via the so-called state transitions (see Section IV.A). This involves the reversible association of the mobile pool of LHCII with either PSII (state 1) or PSI (state 2). During the transition from state 1 to 2—induced by either
preferential excitation of PSII or by low-light conditions—the mobile LHCII pool becomes phosphorylated, migrates from the PSII-containing grana to the PSI-rich stromal lamellae, and attaches to PSI. When PSI is preferentially excited or plants are exposed to high light, LHCII is dephosphorylated and reattaches to PSII (state 2 → 1 transition).

Transgenic lines in which Lhcb1 and Lhcb2 genes have been silenced almost completely lack LHCII, but appear unchanged in their growth behavior (St. Jansson, personal communication). However, lack of the Lhcb1 and 2 polypeptides suppresses state transitions and reduces the capacity for nonphotochemical quenching.

b. LHCI At least four different LHC proteins, Lhca1 to 4, make up the light-harvesting complex of PSI. Eight such Lhca polypeptides are organized as dimers in the PSI–LHCI complex (Croce et al., 2002). LHCI contains the Lhcal–4 heterodimer, but also dimers containing Lhca2 and/or Lhca3 complexes. According to the current working model for the structure and topology of PSI (Scheller et al., 2001) (Fig. 5), PSI-G is located peripherally, and contacts an Lhca2 homodimer (Jansson et al., 1996). PSI-K is at the opposite pole and interacts with an Lhca3 homodimer, whereas the two Lhca1/Lhca4 heterodimers are associated with the PSI-F/J region (Haldrup et al., 2000; Boekema et al., 2001; Scheller et al., 2001).

The analysis of lines down-regulated for specific LHCI proteins, or with mutated versions of the PSI core, has provided valuable information on the interaction of Lhca proteins with each other and with the PSI core. The function of Lhca4 was investigated based on antisense technology (Zhang et al., 1997): Lhca4 amounts were reduced in several transgenic lines and the protein was eliminated in one. No depletion of Lhca1 was observed, but low-temperature fluorescence emission was altered: the emission maximum was blue-shifted by 6 nm, indicating that Lhca4-bound chlorophyll molecules are responsible for most of the long-wavelength fluorescence emission. In addition, some Lhca4 antisense lines show a delay in flowering and an increase in seed weight.

Antisense inhibition of either Lhca2 or 3 results in a concomitant decrease in the levels of both proteins (Ganeteg et al., 2001). The slight decrease in Lhca2 noted in a PSI-G knockout mutant does not support the idea that Lhca2 is exclusively bound to PSI-G (Varotto et al., 2002a). However, the marked drop in levels of Lhca3 in plants lacking PSI-K (Jensen et al., 2000; Varotto et al., 2002a) implies that in the wild type, homodimers of Lhca3 are bound to PSI-K. Cross-linking experiments provide no evidence that Lhca2 and 3 form heterodimers (Jansson et al., 1996), which would otherwise explain the interdependence of the proteins observed in several genotypes (Ganeteg et al., 2001; Varotto et al., 2002b). However, wild-type levels of Lhca3 are present in PSI-G knockout plants, whereas the
concentration of Lhca2 is decreased. Taken together, these data suggest that Lhca2 = Lhca3 heterodimers may exist in vivo but do not accumulate in significant amounts.

Overall, the changes in LHCI observed in PSI mutants indicate a complex quantitative interdependence between the various Lhca proteins, and suggest that some degree of flexibility in its composition is compatible with photosynthetic function. One example is provided by the PSI-G knockout mutant, in which—in addition to the decrease in Lhca2—Lhca4 levels are slightly increased (Varotto et al., 2002a). Furthermore, antisense PsaF plants (Haldrup et al., 2000) exhibit multiple changes in LHCI composition (Lhca1 drops to 70%, Lhca2 to 40%, and Lhca3 to 30% of normal, whereas Lhca4 reaches 120% of wild-type levels).

FIG. 5 The current working model for PSI topology in higher plants, adapted from Scheller et al. (2001). A top view from the stroma is shown. The luminal subunits PSI-N and -F are not shown. Plastome-encoded subunits (PSI-A, -B, -C, -I, and -J) are highlighted in gray, and nucleus-encoded subunits in white. Hatching indicates the availability of Arabidopsis lines deficient for the corresponding subunits: "\"" symbolizes insertional mutants and "/" gene-silenced lines. Interactions of individual subunits detected by cross-linking analysis (Jansson et al., 1996) are symbolized by zig-zag lines, whereas subunit level interdependencies described in Section III.A.2 are indicated by arrows.
According to the current working model (Fig. 5), the psag psak double mutant should lack the attachment sites for both Lhca2 and 3 homodimers; but about half the normal level of Lhca2 and Lhca3 is still detectable (Varotto et al., 2002a). This indicates that Lhca2 and Lhca3 can bind to other PSI proteins, in addition to PSI-G and -K, and supports the idea that alternative dimeric forms and stoichiometries of Lhca proteins may occur, depending on environmental conditions (Bailey et al., 2001; Ganeteg et al., 2001). However, barley mutants that accumulate LHCI, but not the PSI core (Nielsen et al., 1996), to wild-type levels demonstrate that LHCI can exist independently of the core of PSI, so that additional experiments will be necessary to characterize unambiguously the extent of LHCI reorganization.

3. Intersystemic Electron Transport and the ATPase Complex

The cytochrome \( b_6/f \) complex catalyzes electron transport from plastoquinol to plastocyanin or cytochrome \( c_6 \). Cytochrome \( b_6/f \) is very similar to the cytochrome \( b/c_1 \) complex of mitochondria; however, in contrast to cytochrome \( b/c_1 \), cytochrome \( b_6/f \) is thought to be able to switch between two different types of coupling between electron transfer and proton transport. Targeted inactivation of ORFs of the plastid genome with unknown functions in tobacco and in Chlamydomonas has led to the discovery of two novel subunits of the cytochrome \( b_6/f \) complex: YCF7/PetL (Takahashi et al., 1996) and YCF6/PetN (Hager et al., 1999) (Fig. 2). Orthologs of PetL and PetN are also present in the plastome of A. thaliana (Table I). Recently, a 15.2-kDa polypeptide, encoded by the nuclear gene PetO, was identified as a novel cytochrome \( b_6/f \) subunit in C. reinhardtii. This subunit is reversibly phosphorylated upon state transition, pointing to a possible role in signal transduction during redox-controlled adaptation of the photosynthetic apparatus (Hamel et al., 2000). Because sequences homologous to the PetO gene have so far been found only in the green alga Volvox, it remains to be seen whether this subunit exists in higher plants.

To study the function of the three major proteins of the cytochrome \( b_6/f \) complex—cytochrome \( f \) (cyt \( f \)), cytochrome \( b_6 \) (cyt \( b_6 \)), and subunit IV (suIV)—corresponding deletion strains were constructed in Chlamydomonas, each lacking one of the major chloroplast pet genes (Kuras and Wollman, 1994). The \( \Delta petA, \Delta petB, \) and \( \Delta petD \) strains are unable to synthesize cyt\( f \), cyt\( b_6 \), and suIV, respectively. The rates of synthesis of cyt\( b_6 \) and suIV are independent of the presence of the other subunits of the complex, but their stabilization in the thylakoid membranes is a concerted process, with suIV stability being markedly dependent on the presence of cyt\( b_6 \) (Kuras and Wollman, 1994). In contrast, mature cyt\( f \) is stable in the absence of either suIV or cyt\( b_6 \), but its rate of synthesis is severely decreased under these conditions.
conditions. It was concluded that the stoichiometric accumulation of the chloroplast-encoded subunits of the cyt\textsubscript{b6/f} complex results from two regulatory processes: (1) a posttranslational mechanism leading to the proteolytic removal of unassembled cyt\textsubscript{b6} and suIV, and (2) a cotranslational, or early posttranslational, regulation that ensures the production of cyt\textsubscript{f} next to its site of assembly.

Three \textit{C. reinhardtii} mutants that are affected in the nuclear PetC gene encoding the Rieske iron-sulfur protein have been characterized (de Vitry \textit{et al.}, 1999). One carries a stable deletion that eliminates the protein; the other two encode the substitutions Y87D and W163R, which result in low accumulation of the protein. Interestingly, attenuated expression of the stromal protease ClpP increases accumulation and assembly of the Y87D and W163R mutant Rieske proteins into cytochrome \textsubscript{b6/f} complexes. Rates of electron transfer by these complexes are 10- to 20-fold slower than those recorded for the wild type (de Vitry \textit{et al.}, 1999).

Plastocyanin is a soluble copper-containing protein that serves to transfer electrons from cytochrome \textsubscript{b6/f} to PSI. In cyanobacteria and some algae, the heme protein cytochrome \textsubscript{c6} can replace plastocyanin. \textit{Arabidopsis} has a cytochrome \textsubscript{c6}-like protein, which was recently suggested to function as an alternative electron carrier to plastocyanin (Gupta \textit{et al.}, 2002). However, analysis of \textit{Arabidopsis} mutants defective for both of the plastocyanin genes in the nuclear genome indicate that under normal physiological conditions plastocyanin cannot be replaced by cytochrome \textsubscript{c6} (Weigel \textit{et al.}, 2003).

The chloroplast ATP synthase utilizes the energy stored in a transmembrane electrochemical proton gradient to drive the synthesis of ATP from ADP and phosphate. This multisubunit, thylakoid membrane-bound enzyme consists of a proton channel (CF\textsubscript{0}) and an extrinsic catalytic complex (CF\textsubscript{1}). The structure of the chloroplast ATPase is very similar to that of its bacterial and mitochondrial counterparts. It consists of nine different subunits organized in the two separate sectors. The extrinsic catalytic CF\textsubscript{1} complex contains five different polypeptides, and the intrinsic F\textsubscript{0} complex contains four different proteins (Groth and Strotmann, 1999) (Table I).

With respect to the regulation of its catalytic activity, the chloroplast ATP\textsubscript{ase} differs from its prokaryotic and mitochondrial counterparts. The activity of the chloroplast ATP synthase is controlled by the proton gradient between stroma and the thylakoid lumen, by thiol modulation, and by nucleotide binding. Thiol modulation refers to activation of the ATP synthase by the reduction of a disulfide bond in the \(e\)-subunit. Cyanobacteria lack the regulatory sequence containing the disulfide group characteristic of higher plants and green algae, and thus do not display thiol modulation.

Only three subunits of the chloroplast ATP\textsubscript{ase} of \textit{A. thaliana} are encoded by nuclear genes: the b\textsuperscript{1}, the \(\delta\)-, and the \(\gamma\)-subunit. A knockout allele of the
Arabidopsis AtpD gene has been identified: plants carrying this allele in the homozygous state are seedling-lethal when grown on soil (Budziszewski et al., 2001) (see Section III.B.1), indicating that the δ-subunit is indispensable for ATP synthesis. In Chlamydomonas, the nonautotrophic AtpC mutant T1-54, generated by insertional mutagenesis (Smart and Selman, 1991, 1993), does not accumulate the γ-subunit, exhibits polymorphisms at the AtpC locus compared with the parental strain, and lacks AtpC mRNA.

Mutations in the plastome-encoded polypeptides of the chloroplast ATPase of Chlamydomonas have also been described (Lemaire and Wollman, 1989; Robertson et al., 1989). They allow us to conclude that in Chlamydomonas, in the absence of ATP synthase assembly, the rate of synthesis of the β-subunit is controlled by the α-subunit (Lemaire and Wollman, 1989; Robertson et al., 1989) and, furthermore, that α and β interact in the stroma of the chloroplast, with the α-subunit being protected from proteolytic degradation (Lemaire and Wollman, 1989). In addition, in the absence of ATP synthase assembly, CF₀ cannot accumulate in the thylakoid membranes of Chlamydomonas, whereas α- and β-subunits accumulate in the stroma, presumably as part of a soluble form of CF₁ (Lemaire and Wollman, 1989).

B. Photosynthetic Mutants

1. Seedling-Lethal and Pigmentation Mutants

The largest class of chloroplast mutations comprises those that affect leaf coloration (albino, bleached, pale green, etc.) and/or seedling viability. These phenotypes are easily identified by visual inspection of plants in the greenhouse. Mutants with altered leaf coloration frequently show pleiotropic effects. Most of the genes affected in such mutants code for chloroplast proteins that are related to, among other functions, protein translocation, proteolysis, enzyme activity, protein complex assembly, and metal ion homeostasis. Examples include proteins that are involved in the translocation of polypeptides across the chloroplast envelope or the thylakoid membrane, such as Toc33 (Jarvis et al., 1998), Toc159 (Bauer et al., 2000), Tic20 (Chen et al., 2002), and CAO (Klimyuk et al., 1999); the chloroplast FtsH-type protease VAR2 (Chen et al., 2000), which plays a role in thylakoid membrane biogenesis; and the IMMUTANTS protein (Carol et al., 1999; Wu et al., 1999), which is thought to function early in chloroplast biogenesis as a component of a redox chain responsible for phytoene desaturation. In addition, absence of the chloroplast ALB3 protein (Sundberg et al., 1997), which is homologous to the yeast OXA1 protein that is required in mitochondria for proper assembly of the cytochrome oxidase complex, results in
an albino phenotype, whereas perturbations in metal ion homeostasis, such as those found in the absence of the iron transporter IRT1 in the plasma membrane (Henrique et al., 2002; Varotto et al., 2002b; Vert et al., 2002), lead to chlorosis.

Scoring of seedling lethality is a relatively unspecific method of screening for mutants in chloroplast function. Mutants with a seedling-lethal phenotype are most likely to be affected in proteins specifically required during early seedling development. In the course of several mutagenesis programs various collections of seedling-lethal mutants have been isolated, but a large-scale mutant screen combined with the molecular cloning of affected genes has been attempted only recently (Budziszewski et al., 2001). Budziszewski and colleagues screened collections of both T-DNA and transposon mutagenized Arabidopsis plants by examining their development and growth on a synthetic medium lacking sugars. In total, 505 lines segregating seedling-lethal mutants were isolated, of which 80% exhibit a change in pigmentation and 9% a change in morphology, with 11% affected in both pigmentation and morphology. Genetic analyses of 246 seedling-lethal lines showed that 162 have a single-locus insertion, 40 lines have two insertion loci, and 44 have more than two. Based on cosegregation analysis of 210 lines, 64 tagged lines were identified, and for 39 mutants the disrupted gene is now known. Sequence analysis of the tagged loci revealed that a diverse set of genes, most of which code for plastid-targeted proteins, is essential for seedling viability. This set includes three genes that were previously shown to have seedling-lethal mutant phenotypes: CLA1 (Mandel et al., 1996), PALE CRESS (Reiter et al., 1994), and TatC (Motohashi et al., 2001). In addition, mutant alleles of the AtpD gene for the δ-subunit of the chloroplast ATPase, PetC encoding the Rieske protein of the cytochrome b6/f subunit, three genes involved in the plastid non-mevalonate isoprenoid pathway, and a gene similar to the pea Tic40 (Stahl et al., 1999) have been identified. Stahl et al. (1999) also addressed the question of how many genes in the entire A. thaliana genome code for essential genes with a seedling-lethal mutant phenotype. It is estimated by extrapolation that between 320 and 480 such genes should exist in Arabidopsis.

2. Chlorophyll Fluorescence Mutants

Screens based on the measurement of parameters of chlorophyll fluorescence as an indicator of overall photosynthetic performance more specifically identify mutations in chloroplast functions. Alterations in chlorophyll fluorescence reflect defects in photosynthetic electron flow, due to perturbations in the thylakoid protein complexes. Depending on the specific fluorescence parameter monitored, different types of mutations can be accessed, ranging from subtle defects that have no significant consequences for plant
morphology or growth to more dramatic structural changes that lead to the disappearance of entire thylakoid protein complexes.

**a. High-Chlorophyll Fluorescence (hcf) Mutants** The first systematic studies based on altered chlorophyll fluorescence characteristics considered the “high chlorophyll fluorescence” phenotype (hcf), which is characterized by the emission of red fluorescence in response to illumination of plants with UV light. hcf mutants have been identified in *C. reinhardtii*, maize, and *Arabidopsis* (Bennoun and Delepelaire, 1982; Miles, 1994; Meurer *et al*., 1996) (Table II). In most such lines, photosynthesis is severely affected, often resulting in seedling lethality.

Since the isolation of the first *Arabidopsis* HCF gene, *HCF136* (Meurer *et al*., 1998), a number of additional *Arabidopsis* HCF genes involved in essential chloroplast functions, such as thylakoid biogenesis (Kroll *et al*., 2001; Lennartz *et al*., 2001) and mRNA processing (Felder *et al*., 2001), have been cloned.

Although synthesis of plastid PSII proteins is normal in the T-DNA-tagged *hcf136* line, the mutant fails to accumulate subunits of PSII due to a decrease in their stability (Meurer *et al*., 1998). Homologs of the *HCF136* gene are present in cyanobacteria and in the cyanelle genome of *Cyanophora paradoxa*, but not in the plastomes of chlorophytes, metaphytes, rhodophytes, or chromophytes. The HCF136 protein is a luminal protein, found only in stromal thylakoid lamellae. AtHCF136 is produced in dark-grown seedlings and its levels do not increase dramatically during light-induced greening. This, together with the finding that in both *Synechocystis* and *Cyanophora*, the HCF136 homologs are located directly upstream of the *psbEFLJ* operon, indicates that HCF136 encodes a stability and/or assembly factor for PSII that dates back to the cyanobacterial endosymbiont.

Another type of hcf mutant affected in the assembly of the PSII complex is represented by *hcf107* of *Arabidopsis* (Felder *et al*., 2001). In this mutant, the *psbB* gene product (CP47) and an 8-kDa phosphoprotein, the *psbH* gene product (PSII-H), are absent. PSII-B/CP47 and PSII-H are essential for the assembly of PSII in photosynthetic eukaryotes, and their absence in *hcf107* is consistent with the PSII-minus mutant phenotype. The *hcf107* mutation specifically impairs the accumulation of some, but not all, oligocistronic *psbH* transcripts that are processed from the pentacistronic *psbB–psbT–psbH–petB–petD* precursor RNA by intergenic endonucleolytic cleavage. In contrast, *psbB*-containing RNAs are not affected. A more detailed analysis of the pattern of oligocistronic *psbH* transcripts indicates that only those *psbH*-containing transcripts that have been cleaved at position −45 (relative to the initiation codon of *psbH*) can be translated. Secondary structure analysis of the 5′ *psbH* leader supports the idea that stable
stem–loop structures form in the nonprocessed transcripts, which are unfolded by processing at the –45 site. It appears plausible that unfolding of the psbH leader segment by RNA processing should be essential for the translation of the psbH reading frame, suggesting that HCF107 has a

<table>
<thead>
<tr>
<th>Mutant phenotype</th>
<th>Number of mutants</th>
<th>Genes cloned</th>
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<tbody>
<tr>
<td>Viability/pigmentation</td>
<td>Several dozens</td>
<td>CAO (Klimyuk et al., 1999), VAR2 (Chen et al., 2000), IM (Carol et al., 1999; Wu et al., 1999), ALB3 (Sundberg et al., 1997), HOI (Davis et al., 1999; Muramoto et al., 1999)</td>
</tr>
<tr>
<td>leaf coloration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>seedling lethality</td>
<td>505</td>
<td>CLAI (Mandel et al., 1996), PAC (Reiter et al., 1994), TatC (Motohashi et al., 2001); AtpD, TIC40-LIKE, PetC, and others (Budziszewski et al., 2001)</td>
</tr>
<tr>
<td>Chlorophyll fluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hcf (high chlorophyll fluorescence)</td>
<td>67</td>
<td>HCF136 (Meurer et al., 1998), HCF164 (Lennartz et al., 2001), VIPP1 (Kroll et al., 2001), HCF107 (Felder et al., 2001)</td>
</tr>
<tr>
<td>npq (nonphotochemical quenching)</td>
<td>119</td>
<td>ZEAXANTHIN EPOXIDASE (Niyogi et al., 1998), VIOLAXANTHIN DEEPOXIDASE (Niyogi et al., 1998), PsbS (Li et al., 2000), PetC (Munekage et al., 2001)</td>
</tr>
<tr>
<td>ΦH (effective quantum yield of PSII)</td>
<td>63</td>
<td>PsaE1 (Varotto et al., 2000b), PrpI1 (Pesaresi et al., 2001a), IRT1 (Varotto et al., 2002a)</td>
</tr>
<tr>
<td>Plastid signaling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gun (genome uncoupled)</td>
<td>9</td>
<td>GUN2/HY1 (Davis et al., 1999; Muramoto et al., 1999; Mochizuki et al., 2001), GUN3/HY2 (Kohchi et al., 2001; Mochizuki et al., 2001), GUN5 (Mochizuki et al., 2001)</td>
</tr>
<tr>
<td>cue (chlorophyll a/b-binding protein underexpression)</td>
<td>38</td>
<td>CUE1/PPT (Li et al., 1995; Streatfield et al., 1999)</td>
</tr>
<tr>
<td>flu (fluorescence after etiolation)</td>
<td>4</td>
<td>FLU (Meskauskiene et al., 2001)</td>
</tr>
<tr>
<td>laf (long after far-red)</td>
<td>20</td>
<td>LAF6 (Møller et al., 2001)</td>
</tr>
</tbody>
</table>
dual function: (1) it is involved in intercistronic processing of the psbH 5' untranslated region and/or in the stabilization of 5' processed psbH RNAs, and (2) it is required for the synthesis of PSII-B/CP47. Furthermore, the hcf107 mutant is similar to the mbb1 mutant of *C. reinhardtii*: mbb1 also does not synthesize PSII-B/CP47 (the synthesis of PSII-H has not yet been analyzed) and is defective in the assembly of PSII complexes (Monod *et al.*, 1992). With respect to their effects on RNA accumulation, however, the two mutations are clearly different: the mbb1 mutant accumulates neither dicistronic psbB–psbT transcripts nor monocistronic psbH RNAs (Vaistij *et al.*, 2000a), whereas the hcf107 mutation affects only the accumulation of psbH RNAs with the −45 leader and leaves all other psbH RNAs, as well as all psbB transcripts, unaffected. The Mbb1 (Vaistij *et al.*, 2000b) and HCF107 (Felder *et al.*, 2001) genes are orthologous. The tetrameropptide repeat motif present in both proteins is known to mediate protein–protein interactions, and the Mbb1 protein has been shown to be part of a large complex (Vaistij *et al.*, 2000b).

The hcf164 mutant is deficient in the accumulation of the plastid cytochrome b$_6$/f complex (Lennartz *et al.*, 2001). In vivo protein labeling experiments indicate that the mutation acts posttranslationally, interfering with the assembly of the complex. The HCF164 gene encodes a thioredoxin-like protein that possesses disulfide reductase activity, which is anchored to the thylakoid membrane on its luminal side. HCF164 is closely related to the Tx1A protein of *Synechocystis*, and also shows limited similarity to the eubacterial proteins CcsX and CcmG, which are required for the maturation of periplasmic c-type cytochromes.

A novel transport pathway involving vesicle budding from the inner membrane was discovered during the characterization of the hcf155 mutant (Kroll *et al.*, 2001). In this mutant, the lack of vesicle formation is paralleled by the inhibition of thylakoid formation. The mutant responsible for the hcf155 phenotype, named VIPPI1 (vesicle-inducing protein in plastids 1), encodes a protein of 330 amino acids whose N-terminal domain exhibits features typical of plastid transit sequences. Of the several VIPPI1 homologs in cyanobacteria and plants, the closest similarity is observed with the IM30 protein of pea, which has been described as a plastid protein of unknown function associated with both the inner envelope and thylakoids (Li *et al.*, 1994).

**b. Nonphotochemical Quenching (NPQ) Mutants** The quantification of additional parameters of chlorophyll fluorescence using more complex measuring systems allows the identification of mutations that cause relatively minor alterations in photosynthetic performance, which do not necessarily result in seedling lethality. For this class of mutants the parameter “nonphotochemical quenching” (NPQ), which reflects the energy dissipated
as heat following energization of the thylakoid membrane due to the accumulation of protons in the lumen, is monitored. NPQ mutants were first identified in *Chlamydomonas* by using a video imaging system (Niyogi et al., 1997), and more recently two laboratories have described *Arabidopsis* mutants that show changes in NPQ (Niyogi et al., 1998; Shikanai et al., 1999) (Table II). The *npq1* mutants are unable to convert violaxanthin to zeaxanthin when exposed to excessive light, whereas the *npq2* mutants accumulate zeaxanthin constitutively (Niyogi et al., 1998). The *npq2* mutations are new alleles of *aba1*, the zeaxanthin epoxidase gene. The high levels of zeaxanthin in *npq2* affect the kinetics of induction and decay, but not the extent, of nonphotochemical quenching. Genetic mapping, DNA sequencing, and complementation of *npq1* have demonstrated that this mutation affects the structural gene encoding violaxanthin deepoxidase (Niyogi et al., 1998). Lack of violaxanthin results in greatly reduced nonphotochemical quenching, which implies that deepoxidation of violaxanthin is required in *Arabidopsis* for the bulk of rapidly reversible nonphotochemical quenching. Altered regulation of photosynthetic energy conversion in *npq1* is associated with increased sensitivity to photoinhibition. These results, in conjunction with the analysis of *npq* mutants of *Chlamydomonas*, suggest that the role of the xanthophyll cycle in nonphotochemical quenching has been conserved, although different photosynthetic eukaryotes rely to different extents on the xanthophyll cycle for the dissipation of excess absorbed light energy.

The isolation and characterization of the *NPQ4/PsbS* mutant allele (Li et al., 2000) have been described above. An additional genetic locus necessary for normal nonphotochemical quenching of chlorophyll fluorescence was isolated by Munekage et al. (2001). The corresponding mutant, *pgr1* (*proton gradient regulation 1*), is virtually incapable of nonphotochemical quenching. Map-based cloning showed that *pgr1* has a point mutation in the *PetC* gene for the Rieske subunit of the cytochrome *b6/f* complex. Although the rate of electron transport is not affected at low light intensity, it is significantly restricted at high light intensity in *pgr1*, indicating that the degree of luminal acidification is not sufficient to induce thermal dissipation. This view is supported by the slow rate of deepoxidation of violaxanthin, which is closely related to luminal acidification, and the reduction in 9-aminoacridine fluorescence quenching observed in the mutant. Although luminal acidification is insufficient to induce thermal dissipation, growth rate is not affected under low light conditions in *pgr1*. These results suggest that thermal dissipation is precisely regulated by luminal pH to maintain maximum photosynthetic activity. The authors concluded that maximum activity of the cytochrome *b6/f* complex is indispensable for short-term acclimation (Munekage et al., 2001).
c. Effective PSII Quantum Yield ($\Phi_{II}$) Mutants  
An approach that exploited a second chlorophyll fluorescence parameter employed an automated screening system to pinpoint individuals with alterations in $\Phi_{II}$ (Varotto et al., 2000b), the effective quantum yield of PSII ($\Phi_{II}$), a measure of the overall efficiency of PSII reaction centers in the light. In this screen, a pulse amplitude modulation (PAM) fluorimeter was combined with a computerized numerical control (CNC) router, allowing the screening of several thousand plants per week.

More than 15,000 lines derived from insertion-mutagenized *A. thaliana* collections at the MPI für Züchtungsforschung (Wisman et al., 1998; B. Reiss and B. Weisshaar, unpublished), as well as about 36,000 individual plants obtained from the *Arabidopsis* stock centers, have been analyzed. About 70 mutants with altered $\Phi_{II}$ were identified, revealing defects in plastid and nonplastid proteins. Of the 12 independent mutant alleles associated with changes in $\Phi_{II}$ for which the tagged gene locus has been identified so far, six code for plastid proteins and six for proteins that are not targeted to the chloroplast.

In the first $\Phi_{II}$ mutant characterized in detail, the *PsaEl* gene coding for the E subunit of PSI (see Section III.A.2.b) is disrupted by an *En* insertion (Varotto et al., 2000a). The steady-state redox level and the rate of reoxidation of P700 are significantly altered in the *psae1*-1 mutant. An additional mutant allele of this locus, *psae1*-2, was identified by reverse genetics. In wild-type plants, the *PsaEl* transcript is expressed at a higher level than *PsaE2* mRNA. In the mutants, however, the E1 transcript is barely detectable, and is expressed only in small groups of wild-type cells resulting from somatic reversions. Consequently, the amount of PSI-E protein present in the mutant is significantly reduced. Concomitantly, the levels of other stromal PSI subunits (PSI-C and -D) are also affected. Mutant plants show a marked increase in light sensitivity and photoinhibition. Additional effects of the *psae1* mutation include light green pigmentation, an increase in chlorophyll fluorescence, and a decrease of approximately 50% in growth rate under greenhouse conditions. More recently, further characterization of the *psae1* mutant has revealed that state transitions are perturbed in this genotype, leading to the formation of an abnormal LHCII–PSI complex (see Section III.A.2.b) (Pesaresi et al., 2002).

The analysis of the $\Phi_{II}$ mutant *prpl11*-1 demonstrated that defects in the machinery responsible for the expression of the plastid genome can have a deleterious effect on photosynthetic performance (Pesaresi et al., 2001b). The *prpl11*-1 mutant was identified among a collection of T-DNA tagged lines, and the insertion was localized to *Prpl11*, a single-copy nuclear gene that encodes PRPL11, a component of the large subunit of the plastid ribosome. The amino acid sequence of *Arabidopsis* PRPL11 is very similar to those of L11 proteins from spinach and prokaryotes. In the *prpl11*-1 mutant,
photosensitivity and chlorophyll fluorescence parameters are significantly altered, owing to changes in the levels of thylakoid protein complexes and stromal proteins. The abundance of most plastome transcripts examined, such as those of genes coding for the PSII core complex and RbcL, is not decreased. Plastid ribosomal RNA accumulates in wild-type amounts, and the assembly of plastid polysomes on the transcripts of the rbcL, psbA, and psbE genes remains largely unchanged in mutant plants, indicating that lack of PRPL11 affects neither the abundance of plastid ribosomes nor their assembly into polysomes. However, in vivo translation assays demonstrate that the rate of translation of the large subunit of Rubisco (RbcL) is significantly reduced in prpl11-1 plastids. Taken together, these data suggest a major role for PRPL11 in plastid ribosome activity per se, which is consistent with its location near the GTPase-binding center of the chloroplast 50 S ribosomal subunit. Additional effects of the mutation, including the pale green color of the leaves and a drastic reduction in growth rate under greenhouse conditions, are compatible with reduced levels of protein synthesis in plastids.

Extraplastid functions can also be limiting for photosynthetic electron flow. An example of this is provided by the two ΦII mutants irtI-1 and irtI-2 (Varotto et al., 2002b). These were identified among a collection of T-DNA tagged lines, and the mutations responsible interfere with expression of IRT1, a nuclear gene that encodes the metal ion transporter IRT1. In the irt1 mutants, photosensitivity and chlorophyll fluorescence parameters, as well as the abundance and composition of the photosynthetic apparatus, are significantly altered. Additional effects of the mutation under greenhouse conditions include chlorosis and a drastic reduction in growth rate and fertility, which are compatible with a deficiency in iron transport. Indeed, propagation of irt1 plants on medium supplemented with iron salts rescues the phenotype almost completely. The irt2-1 mutant, which carries an En insertion in the highly homologous IRT2 gene of A. thaliana, was identified by reverse genetics. Interestingly, this mutant line shows no symptoms of iron deficiency. This, together with the finding that irt1-1 can be complemented by 35S::IRT1, but not by 35S::IRT2, demonstrates that although the products of the two genes are closely related, only AtIRT1 is required for iron homeostasis under physiological conditions.

In addition to the three mutants described above, two other ΦII mutant lines are currently being characterized at the molecular and physiological levels. The psad1-1 mutant has reduced levels of the PSI-D protein, whereas the atmak3-1 mutant is defective for a specific type of cotranslational modification of cytoplasmic proteins (Pesaresi et al., 2003). This supports the view that among the screens for photosynthetic mutants outlined here, the ΦII screen is the least specific, as it can identify more general chloroplast and cell functions that can become limiting for photosynthesis.
3. Specialized Mutant Screens

More specialized screens designed for large-scale identification of gene functions have the potential to uncover further and very specific chloroplast functions related to photosynthesis. Thus, the dissection of plastid-to-nucleus signaling (see Section IV.E) was approached by screening for mutants that express nuclear photosynthetic genes such as \(Lhb\) (coding for LHCII) and \(RbcS\) (coding for the small subunit of Rubisco) in the absence of chloroplast development (genome-uncoupled or \(gun\) mutants; Susek et al., 1993). Other types of plastid-to-nucleus signaling mutants either underexpress light-regulated nuclear genes encoding chloroplast proteins (chlorophyll \(a/b\)-binding protein \(under\)expression or \(cue\) mutants; Li et al., 1995), or show a change in both light-dependent morphological responses and gene expression (long after \(far\)-red or \(laf\) mutants; reviewed in Rodermel, 2001).

Some of the corresponding genes were recently cloned and found to have roles in porphyrin metabolism [\(GUN2/HY1\) (Davis et al., 1999; Muramoto et al., 1999), \(GUN3/HY2\) (Kohchi et al., 2001), \(GUN5\) (Mochizuki et al., 2001)], and in the transport of porphyrins [\(LAF6\) (Møller et al., 2001)] or other metabolites [\(CUE1\) (Li et al., 1995; Streatfield et al., 1999)].

\(Arabidopsis\) mutants affected in plastid division have been identified by a microscopy-based screen. Twelve accumulation and replication of chloroplasts (\(arc\)) mutants have been isolated so far (summarized in Pyke, 1999). \(arc\) mutants show a variety of chloroplast division phenotypes, and include lines that have a larger number of smaller chloroplasts per cell than does the wild-type (\(arc1\) and \(arc7\)), whereas \(arc6\) has only two very large chloroplasts per mesophyll cell, instead of over 100 as in the wild type.

Dark-grown (etiolated) \(Arabidopsis\) mutant seedlings that accumulate protochlorophyllide, an intermediate in chlorophyll biosynthesis, can be identified by their red fluorescence when exposed to blue light (\(flu\) mutants). Cloning of \(FLU\) revealed that it encodes a negative regulator of chlorophyll biosynthesis (Meskauskiene et al., 2001). State transition mutants, which are deficient in the shuttling of LHCII between the two photosystems, have been identified using chlorophyll fluorescence imaging systems in \(C.\ reinhardtii\) (Fleischmann et al., 1999; Kruse et al., 1999), and more recently, in \(Arabidopsis\) (O. Kruse, personal communication).

IV. Regulation of Photosynthesis and Cross-Talk between Chloroplast and Nucleus

Fluctuations in light conditions affect the photochemical reactions of photosynthesis in chloroplasts, as well as posttranslational protein modifications and plastid gene expression. Light conditions also modulate nuclear
transcription, cytosolic protein synthesis, and the import of precursor proteins into chloroplasts.

A. State Transitions

To maximize the efficiency of photosynthesis and minimize damage to the delicate infrastructure that absorbs and converts light energy within the chloroplast thylakoid membrane, photosynthetic organisms are able to direct photons preferentially to either PSI or PSII. The posttranslational mechanisms that mediate adaptations to different wavelengths of light lead to so-called “state transitions,” as described above, and this represents one of the most rapid adaptive mechanisms known. The thylakoids of chloroplasts of all plants contain LHCII, an intrinsic membrane protein that binds chlorophyll $a$ and $b$ and functions as the major light harvesting antenna of photosynthesis (Kühlbrandt et al., 1994). This protein can be phosphorylated (Bennett, 1977) to modulate its light-harvesting function. Phosphorylated LHCII supplies absorbed light energy to PSI, and unphosphorylated LHCII supplies light energy to PSII. When electrons leave PSII faster than they can be used by PSI, plastoquinone and other electron carriers connecting the two photosystems become reduced. This reduction activates one or more LHCII kinases (Allen and Race, 2002), and phosphorylation of LHCII ensues, effectively diverting light energy away from PSII to PSI, and thus tending to balance the rates of electron transport through the two photosystems. If the light available then favors PSI, plastoquinol is oxidized, the LHCII kinase is switched off, and an LHCII phosphatase reaction predominates, reattaching LHCII to PSII. The redox control of the LHCII kinase thus maintains maximal efficiency of photosynthesis by distributing chlorophyll molecules between the two photosystems in proportion to their capacity to use light energy to drive electron transport (Fuyita, 1997; Allen and Forsberg, 2001).

In green algae, in addition to balancing excitation energy, state transitions have other functions such as increasing ATP synthesis by favoring cyclic photophosphorylation (Wollman, 2001). Although the mobile pool of LHCII is significantly smaller in higher plants than in green algae (15–20% versus ca. 80%; Allen, 1992; Delosme et al., 1994), a similar mechanism is assumed to operate in higher plants (Vallon et al., 1991).

B. Control of Plastid Gene Expression

Transcription of plastid chromosomes in vascular plants is accomplished by at least two RNA polymerases of different phylogenetic origin: the ancestral (endosymbiotic) cyanobacterial-type RNA polymerase (PEP), the core of
which is encoded on the organelle chromosome, and an additional phage-type RNA polymerase (NEP) of nuclear origin. Disruption of PEP genes in tobacco leads to off-white phenotypes. A macroarray-based study of the rates and patterns of transcription of the entire plastid chromosome in leaves of wild-type tobacco and transplastomic tobacco plants lacking PEP showed that the plastid chromosome is transcribed fully in both wild-type and PEP-deficient plastids, although the profiles are polymerase specific (Legen et al., 2002). Legen and co-workers showed that frequently no correlation exists between transcription rate, transcript level, transcript patterns, and amounts of the corresponding polypeptides. Run-on transcription, as well as steady-state RNA concentrations, can increase, decrease, or remain similar in wild-type relative to PEP-deficient plants, independent of the nature of the encoded gene product or of the multisubunit assembly considered (thylakoid membrane or ribosome). Legen et al. (2002) concluded that the absence of photosynthesis-related, plastome-encoded polypeptides in PEP-deficient plants is not directly caused by the lack of transcription by PEP, and that the functional integration of PEP and NEP into the genetic system of the plant cell during evolution has been substantially more complex than previously supposed.

A counterpart to the rapid posttranslational adjustments known as state transitions is the adjustment of photosystem stoichiometry at the transcriptional level. As shown previously, the redox state of plastoquinone controls the rate of transcription of genes that code for reaction-center apoproteins of PSI and PSII (Pfannschmidt et al., 1999). This control is specific, only modulating the transcription of the psaAB and psbA genes of the plastome, whereas the expression of other chloroplast genes remains unaffected (Pfannschmidt et al., 1999). In its reduced state (i.e., when PSI is rate limiting) the plastoquinone pool activates the expression of psaAB. Conversely, in its oxidized state (when PSII is rate limiting) the plastoquinone pool activates the expression of psbA and represses that of psaAB. As a result of this control, the stoichiometric relationship between the two photosystems changes so as to counteract the inefficiency that results when either photosystem limits the rate of action of the other. Whether an organellar two-component system is part of this redox signaling is currently a matter of debate (Forsberg et al., 2001; Allen and Race, 2002).

The photosynthetic stoichiometry can also be modified by variation in the number of PSI particles. In the cyanobacterium Synechocystis, it has been shown that this type of adjustment is controlled by the redox state of the cytochrome b6/f complex, which controls the translation of the psaAB RNA (Fuyita, 1997).

Translation in chloroplasts is also modulated by the redox state of thioredoxin, which in turn is controlled by the light-driven reduction of ferredoxin by PSI (Danon and Mayfield, 1994). In fact, protein synthesis in chloroplasts
increases by 50- to 100-fold upon exposure to light. However, there is no concomitant increase in the abundance of chloroplast mRNAs, suggesting that light controls the rate of translation rather than transcription. In the chloroplast, nucleus-encoded activator proteins bind to the 5' untranslated regions of transcripts, promoting their translation. The binding of translation-activating proteins to the 5'-untranslated region of psbA is controlled by the redox state of thioredoxin. Experiments with a *Chlamydomonas* mutant (cc703) that lacks the PSI reaction center showed that light could not induce the translation of wild-type levels of the psbA gene product. Taken together, these data permit us to conclude that the redox state of PSI, and consequently of thioredoxin, controls the rate of translation of certain proteins in the chloroplast (*Surpin et al.*, 2002).

C. Control of Nuclear Gene Expression

Besides redox control within the chloroplast, a number of experiments have revealed that redox signals from the chloroplast influence the expression of specific nuclear genes involved in photosynthesis. Redox control of nuclear gene expression is an adaptational, not a developmental, process. The latter is modulated by the photoreceptors phytochrome and cryptochrome. Dark–light shift experiments with transgenic tobacco plants show that photosynthetic electron transport controls the transcription of the ferredoxin gene, as well as the loading of ferredoxin mRNA onto ribosomes (*Petracek et al.*, 1998). Escoubas and co-workers (1995) demonstrated that the redox status of the plastoquinone pool influences nuclear photosynthetic gene expression. Thus the green alga *Dunaliella tertiolecta* adjusts the abundance of LHCs to changing light intensities, a process known as photoacclimation. These alterations in the abundance of LHC are reversible and occur in fully differentiated cells, suggesting that they are not coupled with developmental changes in the chloroplast. Escoubas *et al.* (1995) also measured alterations in cellular chlorophyll content; a three-fold increase in the amount of cellular chlorophyll occurs in *D. tertiolecta* cells within 24 hours of transfer from high to low light intensity (*Escoubas et al.*, 1995). Changes in pigment content are a measure of LHC apoprotein abundance, and a three- to four-fold increase in *Lhcb* mRNA can be observed within 9 hours of a shift to low light.

The addition of DCMU, which blocks electron flow from PSII to plastoquinone, to *Dunaliella* cultures mimics the effects of acclimation to low light. Neither uncoupling of photophosphorylation nor suppression of water oxidation mimics these effects, suggesting that neither ATP synthesis nor water splitting is involved directly in modulating *Lhcb* expression (*Escoubas et al.*, 1995).
More recently, the Oelmüller group (2002) showed that polysome loading of spinach mRNAs for PSI subunits is controlled by photosynthetic electron transport. The 5'-untranslated region of spinach PsaD is sufficient to make loading of the uidA transcript onto polysomes dependent upon light and photosynthesis, whereas the 5'-UTR of tobacco PsaD RNA directs constitutive loading of ribosomes (Sherameti et al., 2002), prompting the authors to conclude that light- and plastid-derived signals can operate via the same cis-acting elements in a responsive RNA segment.

Pfannschmidt and colleagues (2001) demonstrated that the redox state of the plastoquinone pool also affects nuclear photosynthetic gene expression in higher plants. This study measured the transcriptional response of selected nuclear photosynthetic genes to changes in photosystem stoichiometry (Pfannschmidt et al., 2001). The promoters of four genes, PetE and H, PsaD and F, were fused to the β-glucuronidase reporter gene and used to construct transgenic tobacco lines. These lines were then examined for their response to excitation pressure applied to the two photosystems and also to inhibitors of photosynthetic electron transport. It emerged that the PetH promoter does not respond to redox signals, whereas the PsaD and F promoters appear to respond to redox signals originating from between the plastoquinone pool and PSI or react to the electron transport capacity in general. The PetE promoter seems to be regulated specifically by the redox state of the plastoquinone pool.

The Arabidopsis cue1 mutant also offers substantial evidence for the involvement of the redox state of the plastoquinone pool in the regulation of nuclear photosynthetic genes (Streatfield et al., 1999). CUE1 codes for the phosphoenol/pyruvate translocator in the inner envelope of the plastid. The biosynthesis of aromatic compounds is reduced in cue1 plants, resulting in a reduced flux through the shikimate pathway—the source of phenolic UV protectants, such as flavonoids, hydroxycinnamic acids, and phenolics. Plastoquinone is also derived from the shikimate pathway, and the relative size of the plastoquinone pool in the cue1-1 and cue1-6 mutants is 10–50% lower than that of wild-type plants. Furthermore, measurements of photosynthetic electron transport and of photochemical and nonphotochemical quenching show that plastoquinone is more susceptible to reduction by brief illumination in the cue1 mutant, and that altered redox poise may, in fact, affect Lhcb expression (Streatfield et al., 1999). In addition, the reduced levels of phenolics in the mutant may make cue1 more susceptible to high-light-induced repression of Lhcb gene transcription.

A large-scale approach to characterize mRNA expression of Arabidopsis nuclear genes coding for chloroplast proteins (the nuclear chloroplast transcriptome) has been established recently (Kurth et al., 2002). Profiling of the nuclear chloroplast transcriptome under different genetic or environmental conditions may allow us to assign functions to genes that have
not been characterized so far, as well as to correlate the transcriptional effects of different mutations or stimuli with the physiological perturbations observed under these conditions. In *A. thaliana*, a set of 1827 nuclear genes coding for chloroplast proteins has been amplified from genomic DNA by PCR and spotted on nylon membranes to generate arrays of gene sequence tags (GSTs) (Kurth *et al.*, 2002; Varotto *et al.*, 2002). This 1827-GST array was employed to compare mRNA levels in dark- versus light-grown seedlings, as well as in wild-type versus *prpl11* mutant plants. In the *prpl11* mutant, the nuclear gene coding for the L11 subunit of the plastid ribosome is disrupted, severely affecting translation in plastids, which in turn causes a drop in the levels of components of the photosynthetic apparatus and Rubisco, as monitored by PAGE and Western analyses (Pesaresi *et al.*, 2001b). *prpl11* mRNA profiling showed that transcript levels of nuclear genes coding for proteins of the plastid ribosome, of the photosynthetic apparatus, and of the small subunit of Rubisco were up-regulated (Kurth *et al.*, 2002), indicating that the mutant plant is able to monitor the altered physiological state of the chloroplast and to react by up-regulating appropriate nuclear genes. This supports the idea that regulatory networks operate in plant cells that can sense the levels of key proteins in the chloroplast and transmit a signal to the nucleus, which then acts to compensate for the relevant deficit. In the case of the photosystems and of Rubisco, which contain nuclear- and plastome-encoded subunits in a fixed stoichiometry, however, up-regulation of respective nuclear genes cannot repair the structural defect of *prpl11*, since the noted decrease in the level of plastome-encoded proteins seems also to limit the amount of nucleus-encoded protein subunits.

In our laboratory, the 1827-GST array was recently replaced by a 3300-GST array, which covers almost all nuclear *Arabidopsis* genes encoding chloroplast-targeted proteins, and which is currently being used to analyze mRNA expression under a variety of conditions, as well as to characterize mutants. As an example of the characterization of transcriptional regulation in a mutant genotype, the classification of differential mRNA expression of 2700 nuclear chloroplast genes and 600 nonchloroplast genes in the *prpl11* mutant (see above) is shown in Fig. 6.

When gene expression profiles observed under 35 different genetic/environmental variables have been compared in our laboratory, covarations in the expression of a large set of genes have been interpreted as evidence for the existence of a major switch that regulates the composition of the nuclear chloroplast transcriptome following changes in the metabolic state of plants. The 35 conditions tested elicit only three major classes of response in the nuclear chloroplast transcriptome, of which two responses reflect diametrically opposed reactions to a signaling mechanism that targets essentially the same large set of genes (Richly *et al.*, 2003). Such coordinate expression of nuclear genes in response to various treatments has often been described for
FIG. 6  DNA array analysis of nucleus-encoded chloroplast genes in prpl1-1. Complex cDNA derived from total RNA isolated from 4-week-old wild-type and prpl1-1 mutant plants was used to probe a DNA array carrying 3292 gene-specific tags. (A) All 622 genes showing differential expression in prpl1-1 were placed into 10 different categories. The proportion of genes within each category showing up-regulation (gray bars) and down-regulation (white bars) in prpl1-1 is shown. (B) Signal intensity in prpl1-1 was plotted against signal intensity in the wild type.
prokaryotes and eukaryotes. Examples include the SOS response in *Escherichia coli*, in which at least 30 (but probably many more) genes exhibit a coordinate increase in expression level upon treatments leading to DNA damage (Sutton *et al.*, 2000; Khil and Camerini-Otero, 2002), and the so-called environmental stress response (ESR) in yeast—in which a set of about 900 genes appears to be activated on exposure to multiple stressful stimuli (Gasch *et al.*, 2000).

D. Sugar Signaling

The regulatory effect of sugars on photosynthetic activity and on plant metabolism is well known. In general, a low sugar status enhances photosynthesis and induces reserve mobilization and export, whereas an abundant supply of sugars promotes growth and carbohydrate storage (Koch, 1996). Photosynthetic adaptation to increased CO₂ levels is likely to be the result of sugar repression, especially under conditions of nitrogen deficiency. In contrast, repression of photosynthetic gene expression, chlorophyll accumulation, and seedling development by sugars can be antagonized by nitrate signals (Moore *et al.*, 1999; Stitt and Krapp, 1999).

More recently, Oswald *et al.* (2001) investigated the relationship between photosynthetic electron transport and sugar signaling. Using *Arabidopsis* cell cultures, as well as transgenic *Arabidopsis* lines carrying luciferase reporter genes driven by the CAB2 or plastocyanin promoter, it was found that the transcriptional activation of *Lhcb* and *RbcS* depends on photosynthetic electron transport, and not on the sugar status of the cells (Oswald *et al.*, 2001). Oswald *et al.* (2001) concluded that a redox signal from the plastid overrides the sugar-related control of the expression of nuclear photosynthetic genes. For a more comprehensive description of sugar sensing and signaling we refer to the recent review of Sheen and co-workers (Rolland *et al.*, 2002).

E. Tetrapyrrole Signaling

Besides redox signaling, a tetrapyrrole-dependent pathway acts to control the expression of nucleus-encoded chloroplast proteins with functions in photosynthesis. Tetrapyrroles, which are synthesized in the plastids, are

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wild type, on a logarithmic scale, for all 622 genes showing differential expression. Each gene is represented by a small gray circle. Additionally, genes of four of the classes listed in (A) are highlighted using the indicated symbols. Symbols lying above the solid diagonal line represent genes that are up-regulated in *prpl11-1*, and symbols lying below the line correspond to genes that are down-regulated in *prpl11-1*. 
the intermediates and end-products of heme, chlorophyll, and phytochrome biosynthesis. In *Chlamydomonas*, studies of protoporphyrin accumulation in mutants, and the results of feeding of inhibitors of the chlorophyll biosynthetic pathway to wild-type cells, suggest that intermediates in the chlorophyll biosynthetic pathway inhibit the expression of chloroplast proteins (Johanningmeier and Howell, 1984; Johanningmeier, 1988). In this context, conditions or compounds that are presumed to cause Mg-protoporphyrin IX monomethyl ester (Mg-ProtoMe) to accumulate appear to be the most effective inhibitors of Lhcb mRNA expression, whereas protoporphyrin IX (ProtoIX) and protochlorophyllide (Pchlide) are less effective (Johanningmeier and Howell, 1984; Johanningmeier, 1988).

The levels of chlorophyll biosynthetic intermediates correlate with the rate of transcription of nuclear genes that encode chloroplast proteins in higher plants. For example, Mg-Proto and Mg-ProtoMe levels, as well as Mg-chelatase activity and Mg-Proto methyltransferase activity, all peak at dark-to-light transitions, and decline throughout a subsequent 12-h-light period, whereas the expression of *Lhc* genes is strongly induced by light (Gibson *et al.*, 1996; Jensen *et al.*, 1996; Papenbrock *et al.*, 1999; Pöpperl *et al.*, 1998). Procedures that cause accumulation of chlorophyll biosynthetic intermediates by increasing the level of 5-aminolevulinic acid can inhibit nuclear gene expression: e.g., feeding of 5-aminolevulinic acid represses *Lhc* mRNA levels in *Arabidopsis* (Vinti *et al.*, 2000) and in cress seedlings (Kittsteiner *et al.*, 1991). Another example concerns the herbicide amitrole, which causes Mg-Proto accumulation and inhibits the light-dependent enhancement of *Lhc* and *RbcS* mRNA levels, and decreases *RbcS* transcript levels also in dark-grown seedlings (La Rocca *et al.*, 2001).

The Mg-chelatase inserts Mg$^{2+}$ into the porphyrin ring of Proto IX and is composed of three subunits. These subunits are referred to as ChlD, ChlH, and ChlI (Walker and Willows, 1997). Tobacco plants that express antisense transcripts for ChlI show no change in the expression of ChlD and ChlH, but exhibit reduced levels of Mg-chelatase activity and chlorophyll, a reduction in ALA-synthesizing capacity and ALA dehydratase activity, and a fall in the concentrations of ProtoIX and heme, and of steady-state amounts of mRNAs for glutamyl-tRNA-reductase, ALA dehydratase, and Lhcb (Papenbrock *et al.*, 2000).

A deeper insight into tetrapyrrole signaling has come from a mutational analysis involving a screen for *Arabidopsis* mutants that do not repress *Lhc* transcription upon photooxidative damage (Susek *et al.*, 1993). The isolated mutants, “*genomes uncoupled 1 to 5*,” are referred to as gun1–5 (Susek *et al.*, 1993; Mochizuki *et al.*, 2001). Because none of the gun mutations affects the tissue- and cell-specific, light-dependent, or circadian regulation of *Lhc* genes, these genotypes appear to be specifically impaired in the plastid-mediated regulation of nuclear transcription (Susek *et al.*, 1993). In contrast
to GUN1, GUN2–5 are essential for normal tetapyrrole metabolism (Vinti et al., 2000; Mochizuki et al., 2001). The products of GUN2 and GUN3 form part of the “iron branch” of tetapyrrole biosynthesis, whereas GUN5 encodes the ChlH subunit of the Mg-chelatase (Mochizuki et al., 2001). A role for the ChlH subunit of Mg-chelatase as a tetapyrrole sensor in an inter-organellar signaling pathway that coordinates the expression of nuclear genes for chloroplast proteins with the functional state of the plastid has been discussed recently (Mochizuki et al., 2001; Surpin et al., 2002). In addition, the most recent studies by the same group suggest that the tetapyrrole intermediate Mg-ProtoIX acts as a signaling molecule in one of the signaling pathways between chloroplast and nucleus (Strand et al., 2003). Thus, accumulation of Mg-ProtoIX is both necessary and sufficient to repress the expression of a large number of nuclear genes that code for chloroplast proteins associated with photosynthesis. Strand et al. (2003) detected an increase of several fold in Mg-protoIX content when wild-type seedlings were subjected to photooxidative damage. In gun2 and gun5 mutants, considerably less of the intermediate accumulates due to reduced flux through the tetapyrrole pathway; the gun phenotype can be rescued by treating mutant seedlings growing under conditions of photooxidative stress with dipyridyl, which is an Fe-chelator that is known to increase the levels of Mg-proto IX and of its methyl esters (Strand et al., 2003). This treatment forces gun2 and gun5 seedlings to accumulate higher amounts of Mg-ProtoIX. Array-based expression profiling revealed that in gun2 and gun5 mutants, in addition to Lhcb genes, the expression of a set of about 70 genes directly associated with photosynthetic reactions is repressed (Strand et al., 2003).

V. Concluding Remarks

For photosynthesis, novel mutant screens and reverse genetics are currently identifying new protein–function relationships in Arabidopsis thaliana, as well as in the two other model species for chloroplast research, maize and Chlamydomonas. These analyses serve to identify functions housed in the chloroplast that can be limiting for photosynthesis. It was estimated that as many as 1000 genes are essential for chloroplast functions in Arabidopsis and can be identified in mutant screens (Leister, 2003); and many if not most of these gene functions will also directly or indirectly affect photosynthesis.

Physiological and transcriptomics-based analyses of photosynthetic mutants show that photosynthetic lesions can result in changes in many other, and apparently unrelated, chloroplast functions. Conversely, mutations in nonplastid proteins can cause defects in photosynthesis. Photosynthesis also plays a role in communication between the chloroplast and other
cellular compartments, and it is expected that chloroplast-wide mRNA profiling will open a new chapter in plastid signaling research.

The ability to predict the chloroplast localization of proteins based on their amino acid sequence is playing a crucial role in chloroplast functional genomics. A further important step in photosynthesis research will be the systematic identification of proteins that are targeted to the photosynthetic subcompartments of the chloroplast: stroma, thylakoid membrane, and lumen. The first step toward this goal involves proteomics based on two-dimensional electrophoresis and mass spectrometry, combined with genome-wide searches for lumenal targeting motifs. Thus, 30–35 lumenal proteins, identified by experimental proteome analysis, and 30–56 additional proteins encoded by the Arabidopsis nuclear genome and containing a tentative TAT motif are, respectively, listed in two independent studies (Peltier et al., 2002; Schubert et al., 2002). This allowed the authors to predict that the thylakoid lumen should contain at least 80 different proteins, and based on the annotation of the novel lumenal proteins, new functions in addition to the accumulation of protons necessary for ATP synthesis and the equilibration of ion currents through the thylakoid membrane are expected to emerge.

Furthermore, systematic screens for protein interactions, such as those now available for the entire proteome of yeast, should in the future further extend the range of tools available for automated experimental proteomics. Last but not least, the sequencing of the genome of Chlamydomonas (a draft sequence was scheduled for release by the end of 2002), which has long been a classic model for the reverse genetics of chloroplast-related genes, will further stimulate forward and reverse genetic analyses of nuclear genes for the dissection of photosynthetic functions.

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References


FUNCTIONAL GENOMICS OF PHOTOSYNTHESIS


