## The *ctpA* gene encodes the C-terminal processing protease for the D1 protein of the photosystem II reaction center complex

(photosynthesis/oxygen evolution/Synechocystis 6803/posttranslational modification/interphotoreceptor retinoid-binding protein)

P. R. Anbudurai<sup>\*†</sup>, Tsafrir S. Mor<sup>\*‡</sup>, Itzhak Ohad<sup>§</sup>, Sergey V. Shestakov<sup>¶</sup>, and Himadri B. Pakrasi<sup>\*||</sup>

\*Department of Biology, Box 1137, Washington University, St. Louis, MO 63130; <sup>§</sup>Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel; and <sup>§</sup>Department of Genetics, Moscow State University, Moscow 119899, Russia

Communicated by Joseph E. Varner, March 25, 1994

ABSTRACT The D1 protein of the photosystem II (PSII) complex in the thylakoid membrane of oxygenic photosynthetic organisms is synthesized as a precursor polypeptide (pD1) with a C-terminal extension. Posttranslational processing of the pD1 protein is essential to establish water oxidation activity of the PSII complex. We have recently identified a gene, ctpA, a mutation in which resulted in a loss of PSII activity in the cyanobacterium Synechocystis sp. PCC 6803. To study the function of the CtpA protein, we inactivated the ctpA gene by inserting a kanamycin-resistance gene into its coding sequence. The resultant mutant strain, T564, had no PSII-mediated water oxidation activity, but it had normal cytochrome  $b_6 f$  and photosystem I activities. Measurements of thermoluminescence profiles and rates of reduction of 2,6-dichlorophenolindophenol indicated that PSII complexes in the mutant cells had functional reaction centers that were unable to accept electrons from water. Immunoblot analysis showed that D1, D2, CP47, CP43, and the  $\alpha$  subunit of cytochrome  $b_{559}$ , five integral membrane proteins of PSII, were present in T564 cells. Interestingly, the D1 protein in the mutant cells was 2 kDa larger than that in wild-type cells, due to the presence of a C-terminal extension. We conclude that the CtpA protein is a processing enzyme that cleaves off the C-terminal extension of the D1 protein. Interestingly, the CtpA protein shows significant sequence similarity to the interphotoreceptor retinoid-binding proteins in the bovine, human, and insect eye systems.

Many proteins in prokaryotic as well as eukaryotic cells are synthesized in their precursor forms. They are converted to the mature forms after proteolytic processing and removal of short peptides at the N- or C termini of the precursor proteins. Many proteins have cleavable N-terminal signal sequences that are necessary for their translocation across specific membranes. Signal peptidases responsible for the endoproteolytic cleavage of such precursor proteins have been identified and studied extensively (1). A number of proteins are synthesized with cleavable C-terminal extensions (2, 3), the functions of most of them being largely unknown. Moreover, the processing proteases for such C-terminal extensions have not been identified in most cases.

Linear electron transport during oxygenic photosynthesis is mediated by three protein complexes—photosystem II (PSII), cytochrome  $b_6 f$ , and photosystem I (PSI)—in the thylakoid membranes of green plants, algae, and cyanobacteria. PSII mediates light-induced electron transfer from water to plastoquinone, with concomitant evolution of molecular oxygen (see ref. 4 for a recent review). Upon excitation with light, P680, the reaction center chlorophyll(s) (Chl), releases electrons that move successively through a

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

pheophytin and two plastoquinone ( $Q_A$  and  $Q_B$ ) molecules on the acceptor side of PSII. The oxidized P680 molecule is subsequently reduced by electrons arriving from water via a cluster of four Mn atoms and a redox-active tyrosine residue ( $Y_Z$ ) on the donor side of PSII (4). The catalytic core of PSII comprises two proteins, D1 and D2. Closely associated with these polypeptides are other integral membrane proteins namely, CP47, CP43, cytochrome  $b_{559}$ , PsbL, and a number of other small polypeptides (5).

In most oxygenic photosynthetic organisms, the D1 protein is synthesized with a C-terminal extension (6) anywhere between 8 and 16 amino acid residues long (7). The D1 protein is present in its precursor (pD1) form in LF1, a photosynthesis-deficient mutant strain of the eukaryotic alga *Scenedesmus obliquus* (7, 8). In the presence of the C-terminal extension on D1, a functional Mn cluster is not formed, as a result of which the PSII complex in the LF1 mutant is unable to oxidize water. The C-terminal processing enzyme for the D1 protein has been partially purified from pea and spinach (9, 10). However, biochemical purification of this processing enzyme to homogeneity has not yet been reported.

We have used a genetic approach to identify the *ctpA* gene that complements SK18, a spontaneous PSII-deficient mutant strain of the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (11). Interestingly, the sequence of the CtpA protein exhibited a significant homology to Prc (or Tsp), a recently identified C-terminal processing protease in the bacterium *Escherichia coli* (2, 12), suggesting that the CtpA protein may also be a C-terminal processing enzyme in *Synechocystis* 6803. The data presented in this report demonstrate that CtpA is the processing protease for the D1 protein of PSII.

## **MATERIALS AND METHODS**

Cell Culture. Synechocystis sp. PCC 6803 cultures were grown at 30°C as described (13). The T564 strain was grown in medium supplemented with 5 mM glucose and 25  $\mu$ g of kanamycin (Km) per ml. Cultures of wild-type and LF1 mutant strains of the green eukaryotic alga Scenedesmus obliquus were grown in the dark in a minimal medium supplemented with glucose as described (14).

Protein Electrophoresis and Immunodetection. To obtain total protein extracts, Synechocystis 6803 cells were ruptured

Abbreviations: PSI, photosystem I; PSII, photosystem II; Chl, chlorophyll; DCIP, 2,6-dichloroindophenol; DPC, diphenyl carbazide; IRBP, interphotoreceptor retinoid-binding protein; Km<sup>r</sup>, kanamycin resistance; Y<sub>2</sub>, redox-active tyrosine in PSII.

<sup>&</sup>lt;sup>†</sup>Present address: Department of Botany, The American College, Madurai, 625002, India.

<sup>&</sup>lt;sup>‡</sup>Present address: Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

To whom reprint requests should be addressed.

by sonication as described (15). Thylakoid membranes from cyanobacterial cells were isolated essentially as described (16). Concentrations of Chl were measured in methanol (17). Fractionation of proteins on SDS/polyacrylamide gels and immunodetection of specific proteins on Western blots were performed as described (18). Rabbit polyclonal antibodies raised against D1, D2, CP47, and CP43 proteins were kind gifts from M. Ikeuchi and Y. Inoue (The Institute of Physical and Chemical Research, Tokyo). Antibodies against the  $\alpha$ subunit of cytochrome  $b_{559}$  (F. Sutterwala and H.B.P., unpublished data) were raised in our laboratory. For production of antibodies against the CtpA protein, we used the pET plasmid system (19) to overexpress the *ctpA* gene in *E. coli* cells essentially as described (20).

Steady State Electron Transport Rates. A Clark-type oxygen electrode was used to measure the rates of light-mediated oxygen evolution or consumption from intact cyanobacterial cells at 30°C as described (21). Rates of PSII-mediated electron transfer from diphenyl carbazide (DPC) to 2,6dichloroindophenol (DCIP) were measured at 25°C on a modified Hitachi model 160 spectrophotometer. The concentration of P700, the reaction center Chl of PSI, was estimated from ascorbate-reduced minus ferricyanide-oxidized chemical difference spectra of thylakoid samples as described (22).

**Thermoluminescence.** The intensity of thermoluminescence from intact cyanobacterial and algal cells as a function of temperature was measured as described (23).

**Comparisons of Sequences.** The BLAST (24) service at the National Center for Biotechnology Information was used to search for proteins with sequence similarities to the CtpA protein. For a detailed comparison of the sequences of the CtpA protein and the interphotoreceptor retinoid-binding protein (IRBP), the BESTFIT program in the Genetics Computer Group software package (25) was used.

## RESULTS

**Insertional Inactivation of the** ctpA Gene. The chromosome of *Synechocystis* 6803 contains a single copy of the ctpA gene. Molecular cloning of this gene and determination of its nucleotide sequence (GenBank accession no. L25250) have been described elsewhere (11).

The scheme for inactivation of the ctpA gene is shown in Fig. 1A. In the plasmid pSL523, a 1407-bp EcoRI fragment of Synechocystis 6803 genomic DNA contains the coding region of the ctpA gene. A 1.1-kbp BamHI fragment from the plasmid pRL442, containing a Km-resistance (Km<sup>r</sup>) gene (26), was inserted at a unique Bsm I site in the coding region of ctpA. The resultant plasmid, pSL564, was used to transform wild-type Synechocystis 6803 cells. T564, one of the resultant Km<sup>r</sup> transformants, was used for further analysis.

Southern hybridization analysis of the chromosomal DNA demonstrated a complete segregation of the insertion-bearing chromosome in the T564 strain. As shown in Fig. 1*B*, the *ctpA* probe hybridized to a 1.4-kbp *Eco*RI fragment of the chromosomal DNA from wild-type cells (lane 1) as well as to an *Eco*RI fragment of similar size in the parent plasmid pSL523 (lane 3). In contrast, the same probe hybridized to a 2.5-kbp *Eco*RI fragment of the genomic DNA from the mutant T564 cells (lane 3), as expected for an interrupted *ctpA* gene (Fig. 1*A* and Fig. 1*B*, lane 4).

Absence of the CtpA Protein in T564 Cells. Antibodies raised against a CtpA fusion protein recognized a relatively diffuse band on a Western blot of a cellular extract of the wild-type strain (Fig. 2, lane 1). The mobility of this band on this SDS/urea gel system was 39 kDa. No such protein band was observed when the corresponding preimmune sera were used (data not shown). This immunostained band was absent in a cellular extract from the T564 mutant strain (lane 2), demonstrating that the insertion of the Km<sup>r</sup> cartridge in the



FIG. 1. (A) Construction of the pSL564 recombinant plasmid for insertional inactivation of the ctpA gene. (B) Southern hybridization analysis of chromosomal DNA from wild-type (WT) and T564 mutant strains of *Synechocystis* 6803 as well as the plasmids pSL523 and pSL564. Each DNA sample was digested with *Eco*RI, fractionated on an agarose gel, blotted onto nitrocellulose paper, and hybridized to a <sup>32</sup>P-labeled 1.4-kbp *Eco*RI fragment of pSL523.

ctpA gene has resulted in loss of the CtpA protein from these cyanobacterial cells.

Absence of Water-Oxidation Activity in the T564 Mutant Cells. Mutant strains of Synechocystis 6803 with no functional PSII complex cannot survive if their growth media are not supplemented with glucose. As shown in Table 1, the T564 strain was unable to grow in the BG11 medium. However, it grew well in glucose-supplemented medium. Moreover, there was no detectable PSII-mediated oxygen evolution activity in the T564 mutant cells. In contrast, the rates of light-induced electron transfer from duroquinol to methyl viologen, a reaction mediated by both cytochrome  $b_6 f$  and PSI complexes as well as the soluble carrier protein(s) between these two complexes (21), were similar in the wild-type and the T564 cells. The P700 contents in the thylakoid membranes of the wild-type and mutant cells were



FIG. 2. Immunoblot analysis of proteins in total cellular extracts from wild-type (WT) and T564 mutant strains of *Synechocystis* 6803. Protein samples were fractionated on denaturing SDS/polyacryl-amide gel, transferred to nitrocellulose filter, and immunostained with rabbit antibodies raised against a T7 gene 10-CtpA fusion protein. Samples were loaded at 100  $\mu$ g of protein per lane.

Table 1.Comparison of growth rates, Chl/P700 ratios, andlight-mediated electron transport activities of Synechocystis6803 wild-type (WT) and T564 mutant strains

	WT	T564
Doubling time in BG11, hr	9.5	NG
Chl/P700, mol/mol	122	119
Rates of electron transport		
H <sub>2</sub> O to DCBQ*	410	<10
$DQH_2$ to $MV^*$	-305	-322
DPC to DCIP <sup>†</sup>	260	150

NG, no growth; DCBQ, 2,6-dichloro-*p*-benzoquinone; DQH<sub>2</sub>, duroquinol; MV, methyl viologen.

\*Expressed as  $\mu$ mol of O<sub>2</sub> per mg of Chl per hr. Negative numbers indicate O<sub>2</sub> uptake.

<sup>†</sup>Expressed as microequivalent electrons per mg of Chl per hr.

also similar. These data clearly showed that the insertion mutation in the ctpA gene in the T564 strain affected the function of the PSII complex only.

To further determine whether the lesion in the mutant cells was on the acceptor or the donor side of the PSII reaction center, we measured the rates of light-induced electron transfer from DPC (an electron donor to  $Y_Z$ ) to DCIP in isolated thylakoid membranes. As shown in Table 1, the T564 thylakoids had  $\approx 60\%$  activity as compared to that of the wild-type thylakoids, indicating that the mutation has specifically affected electron transfer from water to  $Y_Z$ . These data also implied that the PSII protein complex was present at a significant level in the thylakoid membranes of these mutant cells.

Immunoblot Analysis of PSII Proteins. Western blot analysis showed that D2, CP43, CP47, and the  $\alpha$  subunit of cytochrome  $b_{559}$ —four integral membrane protein components of the PSII complex—were present in the T564 mutant cells. Moreover, the steady state levels of these polypeptides in the T564 cells were between 50% and 65% of their wild-type levels.

As shown in Fig. 3B, the D1 protein of PSII was also present at a reduced but significant level in the thylakoid membranes of T564 cells (lane 2). Interestingly, the mobility of D1 in the mutant cells was significantly slower (34 kDa) than that (32 kDa) in the wild-type cells (lane 1). A similar increase in the molecular mass of D1 has previously been observed in the LF1 mutant strain of the eukaryotic alga Scenedesmus (8) in which the pD1 protein accumulates (7). To examine whether the D1 protein in T564 cells was in its precursor form, we digested membranes isolated from wildtype and T564 cells with Lys-C, a lysine-specific endoprotease, and examined the D1 protein in these digested samples (lanes 3 and 4). The D1 protein in Synechocystis 6803 has a single lysine residue (at position 238) exposed to the stroma (27, 28). Digestion at this site is expected to produce two fragments of the D1 protein-a longer N-terminal fragment and a shorter C-terminal fragment. As shown in lane 3, digestion of the mature D1 protein in the wild-type thylakoids resulted in a 20.5- and a 13.5-kDa fragment, both of which were recognized by the D1-specific antibodies. (Interestingly, these antibodies recognized the C-terminal shorter fragment better than the N-terminal longer fragment of D1.) As shown in lane 4, the longer fragment of D1 in the T564 cells had a mobility identical to that of the wild-type D1 protein, whereas the shorter fragment had an apparent molecular mass of 15.5 kDa, 2 kDa larger than that of the shorter fragment of the wild-type D1 protein. Based on these data, we concluded that the D1 protein in the T564 mutant cells had a C-terminal extension. We also inferred that the CtpA protein is the processing protease that cleaves this C-terminal extension in the wild-type cells.

Thermoluminescence Analysis. Fig. 4 shows the profiles of



FIG. 3. (A) Immunoblot analysis of PSII polypeptides in membrane preparations. Thylakoid proteins from Synechocystis 6803 wild-type (W) and T564 mutant (T) cells were fractionated on denaturing SDS/polyacrylamide gel, transferred to nitrocellulose filter, and immunostained with rabbit antibodies raised against D2, CP43, CP47, and the  $\alpha$  subunit of cytochrome  $b_{559}$  (cyt b559- $\alpha$ ), respectively. (B) Immunoblot analysis of membrane proteins from wild-type and T564 cells of Synechocystis 6803. Blot was probed with antibodies raised against the D1 protein. Otherwise, the experimental conditions were as described in A. Samples in lanes 1 and 2 were untreated thylakoid proteins, whereas those in lanes 3 and 4 were similar thylakoid samples digested with a Lys-C lysine-specific endoprotease. Lanes: 1 and 3, wild-type samples; 2 and 4, T564 samples. Positions of molecular mass markers (kDa) are shown on the left.

temperature-dependent emission of luminescence from intact cells of *Synechocystis* 6803 and *Scenedesmus*. The glow peaks at 23.5°C in the wild-type *Synechocystis* cells and at 16°C in the wild-type *Scenedesmus* cells originated from recombination between  $S_{2,3}/Q_B^-$  whereas the shoulder at 7°C in the former sample originated from  $S_{2,3}/Q_A^-$  recombination (23). These peaks were missing from the T564 as well as the LF1 mutant cells. In addition, both of these mutant strains gave rise to peaks centered around  $-35^{\circ}$ C. A similar peak (A<sub>T</sub>



FIG. 4. Thermoluminescence intensity as a function of temperature in intact cells of wild-type (solid line) and T564 mutant (triangles) strains of *Synechocystis* 6803 as well as in cells of wild-type (squares) and LF1 mutant (circles) strains of *Scenedesmus*. cps, Counts per second.

215 738	OGYDRO 000DOR • PGOSVGYIRLSQFSA-NAYKEVAHALHQLEEQGADGYILDLRNNPGG     :  :  ::            : PGQ-L GYIRFDAMAFI FTVKAVGPQI VQI VWGKI VDTAAI VVDI RYNPGS
,	
261	LLOAGIDIARLWLPESTIVYTV-NROGTOESFTANGEAATDR
787	YSTAVPLLCSYFFEAEPRRHLYSVFDRATSRVTEVWTLPHVTGQRYGSHK
	• • 00GE • •
302	OOGE • • PLVYLVNQGTASASE ILAGALQDNQRATLVGEKTFGKGLIQSLFELSDGA
302 837	• 00GE • • PLVVLVNQGTASASE ILAGALODNORATLVGEKTFGKGLIGSLFELSDGA      :  .  : .   +   :  .   :  ::::::  DLVVLVSHTSGSAAEAFAHTMODLQRATIIGEPTAGGALSVGIYQVGSSA
302 837 352	OOGE •• PLVVLVNQGTASASEILAGALQDNQRATLVGEKTFGKGLIQSLFELSDGA .                       DLVVLVSHTSGSAAEAFAHTMQDLQRATIIGEPTAGGALSVGIYQVGSSA GIAVTVAKYETPQHHDIHKLGIMPDEVYEQPLISFAEITSPADYQYQA

400 ALD--LLTGGVAIAHKSSSIPAMAT 422 :. I I: I : I.. I I.::.

934 KVPTVLQTAGKLVADNYAS-PELGV 957

FIG. 5. Sequence similarities between the CtpA protein (top line) in Synechocystis 6803 and one of the four repeated domains ( $\approx$ 300 residues) of bovine IRBP (bottom line; ref. 34). Regions with invariant sequences OGYORO, OOODOR, and OOGE (where O represents a hydrophobic residue) are indicated. Solid circles above the sequence indicate additional residues that are completely conserved in bovine and human IRBPs (12) as well as in the CtpA protein in Synechocystis 6803. Hyphens between amino acids on the same line indicate gaps introduced to optimize alignments, vertical bars indicate amino acid identity, and colons and periods indicate conservative amino acid replacements.

band) has been observed in the LF1 thylakoids (29) and has been attributed to recombination between P680 and a redoxactive histidine residue on the donor side of PSII (30). Based on the remarkable similarity of the thermoluminescence glow curve of T564 cells to that of LF1 cells, we conclude that PSII centers are indeed assembled in the mutant cells and these centers are capable of charge separation and recombination between the reduced quinone acceptors and the  $Y_Z$ /histidine donors.

## DISCUSSION

Processing of the C-terminal extension of the precursor form of the D1 protein is an important event in the biogenesis of water-oxidizing PSII centers in thylakoid membranes. In this study, we have demonstrated that ctpA, a recently discovered gene in Synechocystis 6803 (11), encodes the processing protease that cleaves off this C-terminal extension. The predicted molecular mass of the ctpA gene product is 47 kDa (11). If the putative signal peptide is removed, the molecular mass of the mature protein is expected to be 43 kDa. Interestingly, our immunoblot analysis showed that the apparent molecular mass of this protease is 39 kDa (Fig. 2). This discrepancy between the predicted and the experimentally determined molecular masses may simply be a result of an aberrant migration of this protein in the SDS/urea gel system used in our experiment. Another possibility is that the 43-kDa form of this protein is further processed in vivo to a 39-kDa form. Such processing events are known to occur with many proteases (31).

Our data showed that in the absence of the CtpA protein, the D1 protein was present in the pD1 form (Fig. 3B). Interestingly, pD1 was incorporated in the PSII complexes present in the thylakoid membranes of the T564 mutant strain. Similar observations have been made with a mutant strain of *Synechocystis* 6803 in which the Ser-345 residue in the D1 protein has been converted to a proline residue (32). As mentioned earlier, the LF1 mutant of *Scenedesmus* assembles a PSII complex with the pD1 protein (7, 8). Similar to LF1, the T564 mutant strain of *Synechocystis* 6803 cannot catalyze water oxidation, but it can mediate electron transfer from the artificial electron donor DPC to the acceptor DCIP. Our data also indicated that the level of assembled but non-oxygen-evolving PSII centers is between 50% and 60% of the wild-type level. The molecular basis for this decrease in the number of PSII centers in the T564 mutant strain is currently unknown and may be due to an accelerated degradation of D1 as well as other PSII proteins due to the inactivation of donor side activities (33). It should also be noted that the Ser-345 to Pro mutant strain of *Synechocystis* 6803 (see above) assembles PSII centers at 20–25% of the wild-type level (32).

Analysis of the GenBank sequence data base revealed an unexpected but highly intriguing sequence similarity between the CtpA protein and the IRBPs in human, bovine, and insect eye systems. As shown in Fig. 5, 50% of the residues in these two proteins are similar, with 30% being identical. Silber and coworkers (12) have also noted a similar sequence similarity between IRBP and Prc (or TSP), a C-terminal processing protease in E. coli. In particular, the sequence of the CtpA protein (Fig. 5) showed the presence of three motifs-OGYORO, OOODOR, and OOGE (O being a hydrophobic residue)—that are present in all of the IRBP sequences analyzed to date (35). The IRBP binds retinols, retinoic acids, cholesterol, and fatty acids, all of which are hydrophobic molecules (36). The observed sequence similarities between these two proteins may simply indicate that the CtpA protein binds to hydrophobic stretches of the pD1 protein. However, this may also indicate that molecules structurally similar to retinoids (e.g., carotenoids) may bind to the CtpA protein. Based on these data, it is also reasonable to postulate that the IRBP may have a processing protease function. It is noteworthy that three Arg residues in the CtpA protein (residues 223, 255, and 327) are completely conserved in all of the IRBPs. One of these residues in CtpA may form a salt bridge with the Asp-342 residue (of the D1 protein), the negative charge on which has recently been implicated in playing a critical role in binding of the C-terminal region of pD1 to the processing protease (37).

We thank Dr. L. K. Lind and J. Han for collegial assistance. This work was supported by a grant from the National Institutes of Health (GM 45797) to H.B.P. The visits of T.S.M. and I.O. to Washington University were supported by an Israel-U.S.A. Bionational Agricultural Research and Development grant to I.O. and H.B.P.

- 1. Dalbey, R. E. & von Heijne, G. (1992) Trends Biochem. Sci. 17, 474-478.
- Hara, H., Yamamoto, Y., Higashitani, A., Suzuki, H. & Nishimura, Y. (1991) J. Bacteriol. 173, 4799-4813.
- Islam, M. R., Grubb, J. H. & Sly, W. S. (1993) J. Biol. Chem. 268, 22627–22633.
- 4. Debus, R. J. (1992) Biochim. Biophys. Acta 1102, 269-352.
- Pakrasi, H. B. & Vermaas, W. F. J. (1992) in Current Topics in Photosynthesis. The Photosystems: Structure, Function and Molecular Biology, ed. Barber, J. (Elsevier, The Netherlands), Vol. 11, pp. 231-256.
- Marder, J. B., Goloubinoff, P. & Edelman, M. (1984) J. Biol. Chem. 259, 3900-3908.
- Diner, B. A., Ries, D. F., Cohen, B. N. & Metz, J. G. (1988) J. Biol. Chem. 263, 8972–8980.
- Metz, J. G., Pakrasi, H. B., Seibert, M. & Arntzen, C. J. (1986) FEBS Lett. 205, 269-274.
- Bowyer, J. R., Packer, J. C. L., McCormack, B. A., Whitelegge, J. P., Robinson, C. & Taylor, M. A. (1992) J. Biol. Chem. 267, 5424-5433.
- Inagaki, N., Fujita, S. & Satoh, K. (1989) FEBS Lett. 246, 218-222.
- 11. Shestakov, S. V., Anbudurai, P. R., Stanbekova, G. E., Gadzhiev, A. & Pakrasi, H. B. (1994) J. Biol. Chem., in press.
- 12. Silber, K. R., Keiler, K. C. & Sauer, R. T. (1992) Proc. Natl. Acad. Sci. USA 89, 295-299.
- Pakrasi, H. B., Williams, J. G. K. & Arntzen, C. J. (1988) EMBO J. 7, 325-332.
- 14. Gong, H. & Ohad, I. (1991) J. Biol. Chem. 266, 21293-21299.
- Zhang, L., McSpadden, B., Pakrasi, H. B. & Whitmarsh, J. (1992) J. Biol. Chem. 267, 12489-12495.

- Shukla, V. K., Stanbekova, G. E., Shestakov, S. V. & Pakrasi, H. B. (1992) Mol. Microbiol. 6, 947-956.
- 17. Lichtenthaler, H. K. (1987) Methods Enzymol. 148, 350-382.
- 18. Anbudurai, P. R. & Pakrasi, H. B. (1993) Z. Naturforsch. Teil C 48, 267-274.
- Studier, F. W., Rosenberg, A. H., Dunn, J. H. & Dubendorff, J. W. (1990) Methods Enzymol. 185, 60-89.
- Lind, L. K., Shukla, V. S., Nyhus, K. J. & Pakrasi, H. B. (1993) J. Biol. Chem. 268, 1575-1579.
- 21. Zhang, L., Pakrasi, H. B. & Whitmarsh, J. (1994) J. Biol. Chem. 269, 5036-5042.
- Mannan, R. M., Whitmarsh, J., Nyman, P. & Pakrasi, H. B. (1991) Proc. Natl. Acad. Sci. USA 88, 10168-10172.
- Carpenter, S. D., Ohad, I. & Vermaas, W. F. J. (1993) Biochim. Biophys. Acta 1144, 204–212.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) J. Mol. Biol. 215, 403-410.
- Devereux, J., Haeberli, P. & Smithies, O. (1984) Nucleic Acids Res. 12, 387–395.
- 26. Elhai, J. & Wolk, C. P. (1988) Gene 68, 119-138.
- 27. Ravnikar, P. D., Debus, R., Sevrinck, J., Saetaert, P. & McIntosh, L. (1989) Nucleic Acids Res. 17, 3991.
- Metz, J., Nixon, P. & Diner, B. (1990) Nucleic Acids Res. 18, 6715.
- 29. Seibert, M., Ono, T.-A., Koike, H., Ikeuchi, M., Inoue, Y. &

Bishop, N. I. (1990) in *Current Research in Photosynthesis*, ed. Baltscheffsky, M. (Kluwer, The Netherlands), Vol. 1, pp. 507–510.

- Ono, T.-A. & Inoue, Y. (1992) Biochim. Biophys. Acta 1099, 185-192.
- Stroud, R. M., Kossiakoff, A. A. & Chambers, J. L. (1977) Annu. Rev. Biophys. Bioeng. 6, 177–193.
- Nixon, P. J., Trost, J. T. & Diner, B. A. (1992) Biochemistry 31, 10859-10871.
- Prasil, O., Adir, N. & Ohad, I. (1992) in Current Topics in Photosynthesis. The Photosystems: Structure, Function and Molecular Biology, ed. Barber, J. (Elsevier, The Netherlands), Vol. 11, pp. 295-348.
- Borst, D. E., Redmond, T. M., Elser, J. E., Gonda, M. A., Wiggert, B., Chader, G. J. & Nickerson, J. M. (1989) J. Biol. Chem. 264, 1115-1123.
- Fernandez-Gonzalez, F., Kittredge, K. L., Rayborn, M. E., Hollyfield, J. G., Landers, R. A., Saha, M. & Grainger, R. M. (1993) J. Cell Sci. 105, 7-21.
- Stavenga, D. G., Schwemer, J. & Hellingwerf, K. J. (1991) in *Photoreceptor Evolution and Function*, ed. Holmes, M. G. (Academic, London), pp. 261-349.
- Taguchi, F., Yamamoto, Y., Inagaki, N. & Satoh, K. (1993) FEBS Lett. 326, 227-231.