

Exposure of *Synechocystis* 6803 cells to series of single turnover flashes increases the *psbA* transcript level by activating transcription and down-regulating *psbA* mRNA degradation

Taina Tyystjärvi^a, Esa Tyystjärvi^a, Itzhak Ohad^b, Eva-Mari Aro^{a,*}

^aDepartment of Biology, University of Turku, BioCity A 6th floor, Tykistökatu 6, FIN-20520 Turku, Finland

^bMinerva Avron Evenari Center for Photosynthesis Research, Department of Biological Chemistry, Silberman Institute of Life Sciences, The Hebrew university of Jerusalem, Jerusalem 91904, Israel

Received 13 August 1998

Abstract Exposure of *Synechocystis* sp. PCC 6803 cells to series of single turnover flashes increases specifically the level of *psbA* and *psbD2* messages, encoding the D1 and D2 proteins of photosystem II, as compared to light exposed cells. This increase is due to maintenance the transcription rate as high as in growth light and to the down-regulation of transcript degradation as in darkness. Inhibition of the plastoquinone pool reduction by DCMU or its oxidation by DBMIB does not diminish the transcription of the *psbA* gene under growth conditions. However, the degradation rate of *psbA* transcript, as well as of other transcripts encoding proteins of thylakoid complexes, is down-regulated in all conditions leading to the oxidation of the plastoquinone pool. We conclude that single turnover flashes are sensed as 'light' by transcription machinery of the cells irrespective of the plastoquinone pool reduction state and as 'dark' by the transcript degradation system.

© 1998 Federation of European Biochemical Societies.

Key words: *psbA* gene; *psbD* gene; Regulation of photosynthetic genes expression; mRNA stability; $Q_B^-/S_{2,3}$ charge recombination; *Synechocystis* sp. PCC 6803

1. Introduction

The D1 protein of PSII in the cyanobacterium *Synechocystis* 6803, is encoded by a small multigene family [1]. The *psbA1* gene is not functional [1] while *psbA2* and *psbA3* genes produce identical D1 proteins [2,3], among which the *psbA2* gene product predominates [4,5]. Transcription initiation has been proposed to be the main regulatory level of the *psbA* gene expression in *Synechocystis* 6803 [6], and a direct correlation between the transcription activity and light intensity has been proposed [6,7]. However, later studies have shown that the *psbA* gene expression in *Synechocystis* is not only regulated at the level of transcription, but also at the level of message stability [8,9] and translation [10]. Furthermore, studies with mutant strains have shown that the transcription rate of the *psbA* gene is not directly dependent on light [10,11].

The regulation of *psbA* message level is of particular interest since *psbA* gene transcripts and their translation products are needed for the PSII repair cycle that maintains the PSII ac-

tivity under varying light conditions [12,13]. In addition to continuous light treatments, single turnover light flashes (STF) that induce charge separation but produce only small amount of reduced plastoquinone, have been shown to induce degradation of the D1 protein in *Chlamydomonas* cells [14,15] and PSII photoinactivation and degradation of the D1 protein in isolated thylakoids [16]. Thus, it was of interest to test whether STF treatment may also regulate the level of the *psbA* message in vivo.

In this study we have used *Synechocystis* 6803 cells to investigate the effect of STF that induces charge separation followed by recombination during the dark interval between flashes, on the level of the *psbA* message. As a control we have also tested the effect of such treatments on the level of the *psbD* gene family, encoding the PSII reaction center protein D2 that is also degraded in high light exposed cells in vivo [17]. The *psbD* gene family consists of two functional members [18]: *psbD2*, transcribed into a monocistronic message and *psbD1* that is co-transcribed with the *psbC* gene encoding the chlorophyll *a* binding protein CP43 of PSII. Furthermore, we have also measured the changes induced in the transcript levels of the *psaAB* operon encoding the reaction center proteins of PSI [19] and the *petAC* operon encoding cytochrome *f* and Rieske Fe-S center subunits of the cytochrome *bf* complex [20]. The results show that exposure of the cells to single turnover flashes results in a specific increase in the level of the *psbA* and *psbD2* messages.

2. Materials and methods

2.1. Cell growth and exposure to short light flashes

Synechocystis sp. PCC 6803 cells were grown in BG-11 medium under continuous illumination of 70 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ as reported before [21]. For single turnover flash treatments, cell suspensions (10 $\mu\text{g chlorophyll ml}^{-1}$, 20 ml) in 6 cm diameter glass dishes were placed in a temperature controlled (32°C) drum maintained in the dark. The cell suspension was stirred by magnetic bars and dark adapted for 5 min before treatments. The drum could be rotated to bring the cell suspension under a Xenon arc discharge lamp (Micro-pulser, Xenon Corporation, MA). The rotation of the drum and light pulses were computer controlled to deliver to the desired dish, series of single or two consecutive (1 Hz) light pulses at time as desired or maintain the cells in darkness. The lamp discharged a 2 μF capacitor charged at 9 kV and delivering about 6 μs light pulses. The light was filtered through a Perspex filter to remove UV radiation. Since such light pulses are compatible with the generation of single turnover cycles of the photochemical reaction centers, they will be referred to in an abbreviated form as single turnover flashes (STF).

2.2. Determination of *psbA* message levels and half-life times

RNA extraction was performed as described by Tyystjärvi et al. [10]. For DNA probes, the whole coding regions of the *psbA2*,

*Corresponding author. Fax: +358 (2) 3338075.
E-mail: evaaro@utu.fi

Abbreviations: PSII, photosystem II; PSI, photosystem I; cyt *bf*, cytochrome *bf* complex; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; STF, single turnover light flashes

psbD2, *petA* genes and the N-terminal half of *psaA* gene were amplified by PCR from genomic DNA of *Synechocystis* 6803. The PCR products were purified with QIAquick purification kit for PCR fragments. *Pst*I fragment of the pAN4 [22] was used as a *rrn* probe. Probes were radiolabeled with [α - 32 P]dCTP using a multiprime DNA labeling kit (Amersham). Prehybridization, hybridization and washing after hybridization were performed as described earlier [10]. To measure the amount of transcripts, the X-ray films were scanned with a laser densitometer (Ultrosan XL, LKB) and the area of the peak was measured. The equal loading of the samples was verified with a *rrn* probe. For determination of message stability, rifampicin, an inhibitor of transcription initiation, was added to a final concentration of 500 μ g ml $^{-1}$.

2.3. Inhibition of electron flow

Reduction of the plastoquinone pool in cells exposed to continuous light was inhibited by addition of 15 μ M DCMU and the activity of cytochrome b/f complex was inhibited by adding 100 μ M DBMIB. Measurements of oxygen evolution using a Clark type oxygen electrode were used to confirm that both inhibitors totally prevent whole chain dependent oxygen evolution in vivo.

3. Results and discussion

3.1. Single turnover flashes induce a rise in the level of *psbA* and *psbD* transcripts

Light-grown cells were exposed to STF delivered at 8, 32 or 120 s intervals for 5 h. Alternatively the cells were exposed to trains of two consecutive flashes (1 Hz) at 32 s intervals. The level of the *psbA* message was measured in growth light conditions, and in cells incubated in darkness for 5 h, or after STF treatments. The *psbA* message level increased 3- to 4-fold during the 5 h of STF treatment if the dark interval between the flashes was 8 s, 32 s or 32 s for two consecutive STF. However the messages remained at growth light level if the dark interval between the flashes was 120 s or if the cells were incubated in darkness (Fig. 1A, B).

Activation of the *psbA* gene transcription upon shift of *Synechocystis* cells from low to high light conditions is a well documented phenomenon [6,10]. This phenomenon was suggested to be related to the light-induced degradation of the D1 protein [10]. However the degradation rate of the D1 protein induced by the STF treatments was very low as compared with that observed under growth light conditions (data not shown). The induction of *psbAIIIIII* gene expression by high light in another cyanobacterium, *Synechococcus* sp. PCC 7942, has been proposed to result from binding of high light dependent regulatory proteins to 5' upstream region of the *psbAIIIIII* genes [23]. To test whether the elevated *psbA* message levels in flash treated *Synechocystis* cells were due to a similar activation mechanism, the cells were illuminated with a low continuous background light (20 μ mol m $^{-2}$ s $^{-1}$) during the STF treatments. Similar *psbA* message levels were found in cells exposed to the background light in the presence and

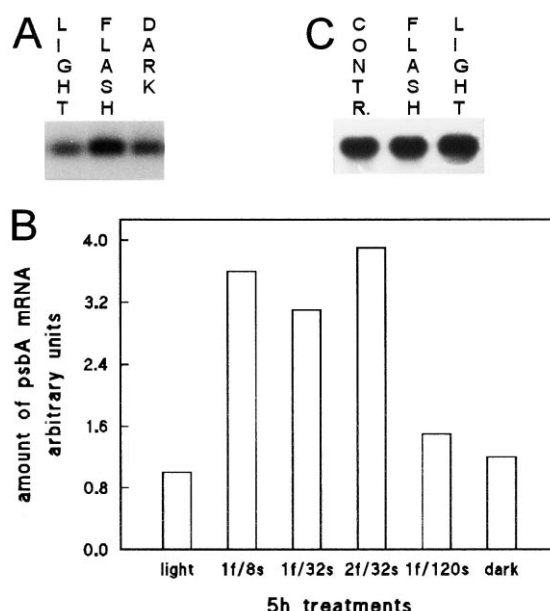


Fig. 1. Single turnover flashes induce an increase in the *psbA* transcript level. A: Northern blot of *psbA* messages in cells exposed to the growth light (LIGHT), after 5 h exposure to STF delivered at 32 s interval (FLASH) or in the dark (DARK). B: Level of *psbA* mRNA of cells STF treated for 5 h with single (1f) or two consecutive (2f) light flashes delivered at 8, 32 or 120 s interval between the flash series. The amount of *psbA* messages in cells maintained in growth light and after 5 h incubation in darkness are shown as controls. C: Level of *psbA* messages before (CONTR.), after 5 h exposure to STF delivered at 32 s interval under continuous background illumination (FLASH) and after 5 h exposure only to background light (LIGHT).

absence of STF (Fig. 1C). These results rule out the role of intense flashes as a signal for high light induction factors.

3.2. Exposure of *Synechocystis* cells to single turnover flashes increases the stability of the *psbA* transcript

Since the STF treatments did not enhance the rate of *psbA* gene transcription above the growth light level we considered the possibility that the increased *psbA* message levels were due to stabilization of the messages by the STF treatments. To test this possibility the life time of the *psbA* transcript was measured in cells exposed to series of one or two consecutive STF (1 Hz) delivered at 32 s intervals as well as in cells maintained in growth light or in darkness. Rifampicin was added to all cell suspensions to prevent transcription initiation. The samples were taken at various times (0, 20, 60 and 120 min) after addition of the inhibitor and RNAs were isolated and analyzed by Northern blotting. The result of such experiments (Fig. 2) shows a considerably slower decay of the *psbA* mes-

Table 1
Relation between the state of Q_A and PQ reduction and the activation of *psbA* message transcription and degradation

Exp. conditions	Light+DCMU	Light+DBMIB	Light	Dark	Flashes
Redox conditions					
Q_A	Q_A^-	Q_A^-	Q_A^-	Q_A	$(Q_A^-)^a$
PQ	PQH $_2$	PQ	PQH $_2$	PQ	PQ
<i>psbA</i>					
Transcription	Fast	Fast	Fast	Slow	Fast
Degradation	Fast	Slow	Fast	Slow	Slow

^a(Q_A^-) indicates that Q_A is reduced transiently during the process of charge recombination following every flash excitation (see text).

sage in cells exposed to single or consecutive flashes or maintained in darkness than in cells exposed to growth light. The calculated half-life of the *psbA* messages was 40 min in darkness and in the flash treated cells, and about 10 min under continuous light.

A simulation of the *psbA* message level (Fig. 2C), by assuming the same transcription rate as in the growth light and the half-life of 40 min for the messages, predicts an increase in the *psbA* mRNA level by a factor of 3.8 times during the 5 h STF treatment, in agreement with the experimental data from STF treated cells (Fig. 1). Thus we can conclude that both, the maintenance of transcription rate at growth light level and the stabilization of *psbA* messages contribute to the observed

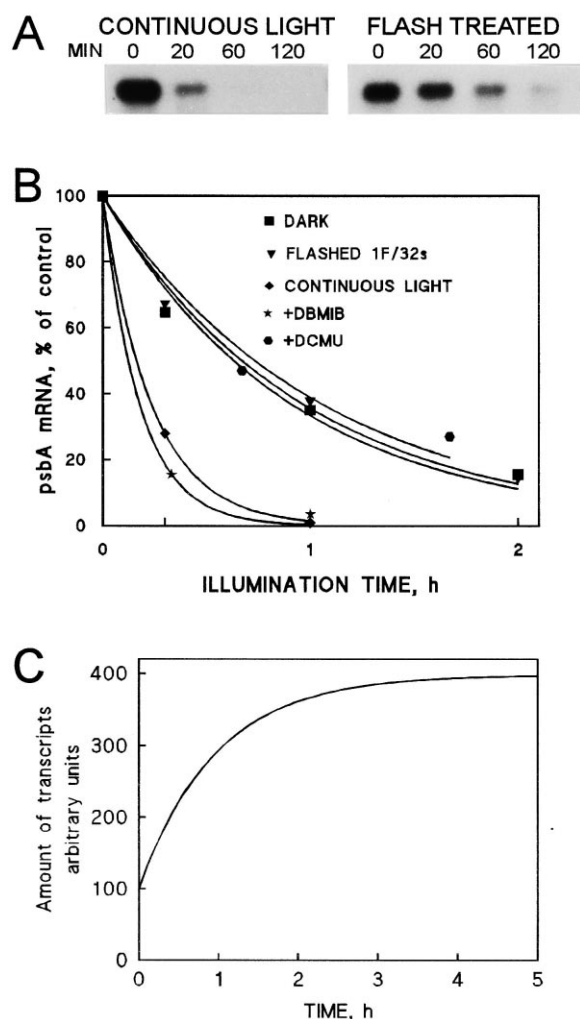


Fig. 2. The *psbA* message stability increases in cells exposed to single turnover flashes. **A**: Northern blot demonstrating the degradation of *psbA* messages under continuous light and in STF treated cells. **B**: Degradation of *psbA* messages under different conditions as indicated. The amount of *psbA* mRNA was quantified from Northern blots and the lines were drawn according to the best first order fits. **C**: Simulation of the changes in the level of *psbA* messages in cells exposed to STF, based on the following assumptions: the rate of transcription is constant and the same under STF and growth light conditions; the $t_{1/2}$ of the transcripts calculated from panel B is 40 min. The equation used is: $dT/dt = s - k_{deg}T$ where T represents amount of transcripts, s the rate of synthesis and k_{deg} the degradation rate constant.

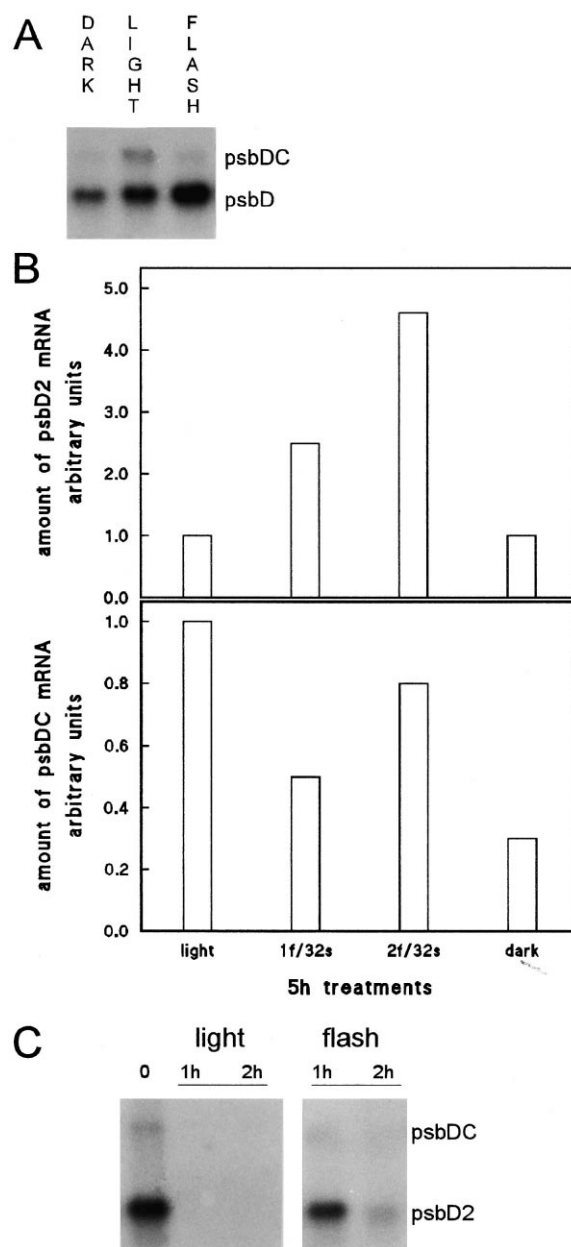


Fig. 3. Effect of single turnover flashes on the level and stability of *psbD* and *psbDC* messages. **A**: A Northern blot showing the level of *psbD* and *psbDC* transcripts in cells maintained in growth light conditions (LIGHT), after 5 h incubation in darkness (DARK) or exposed STF (FLASH). **B**: Changes in the level of *psbD* and *psbDC* messages in cells exposed for 5 h to series of STF (1f) or two consecutive STF (1 Hz) (2f) delivered at 32 s intervals. As a control the amount of the transcripts was also measured in cells maintained in growth light (light) and in darkness (dark). **C**: Degradation of *psbD* and *psbDC* messages in cells maintained in continuous growth light (light) or exposed to STF (flash). The samples were taken 0, 1 and 2 h after addition of rifampicin.

increase in the message level in the cells exposed to the flash treatments.

In cells exposed to STF only one plastoquinol molecule is generated in 50% of the PSII centers at every flash event, and PQH₂ produced by the flashes at long intervals is rapidly oxidized by ambient oxygen. Under these conditions, one can consider the PQ pool being in the oxidized state as it is in the dark incubated cells (Table 1). To test whether the

oxidation of the plastoquinone pool may serve as a signal for the stabilization of *psbA* messages, the effect of DCMU and DBMIB treatments were measured in light exposed cells. Inhibition of plastoquinone pool reduction by DCMU stabilized *psbA* messages like dark treatments (Fig. 2B), while fast degradation of messages was measured in the cells exposed to light in the presence of DBMIB that prevents oxidation of the plastoquinone pool. Thus we conclude that a good correlation exists between the plastoquinone pool oxidation and the increase in the *psbA* transcript stability (Table 1).

3.3. Linear electron flow is not required for active *psbA* gene transcription

Similar transcription activity of the *psbA* genes occurs under growth light when photosynthetic electron transfer is fully active, and in cells exposed to STF treatments (dark intervals 32 s or 8 s) or treated with DCMU and DBMIB. These results are in agreement with the earlier result reported by Mohamed and Jansson [8] indicating that maintenance of continuous photosynthetic electron flow of the whole electron transfer path is not required for the transcription of the *psbA* genes in *Synechocystis* 6803. As shown in Table 1 the primary electron acceptor of PSII, Q_A , is reduced in the light exposed cells in the absence or presence of DCMU or DBMIB. In the cells exposed to series of one or two flashes, Q_B^- is generated in 50% of the PSII population at every flash and decays via back electron flow in the dark period between the flashes by recombination with the $S_{2,3}$ states. In this process Q_A^- is transiently generated via the equilibrium between $Q_B^-:Q_B/Q_A:Q_A^-/Pheo:Pheo^-$ that is responsible for the charge recombination process [15,16]. As shown in Table 1, growth light level of *psbA* transcription occurs in all situations in which Q_A undergoes transient reduction/oxidation cycles. Thus one can not discard the possibility that in photosynthetically competent cells, transient formation of Q_A^- may represent one of the signals in the transcription regulation (D. Kirilovsky, personal communication). However, Q_A reduction can not be the only or main signal for increase of *psbA* transcription when cells are transferred from dark to light. The light effect occurs in mutant strains that do not contain PSII centers [11]. Thus additional signal transduction pathways possibly involving photoreceptors may operate in cyanobacteria. One can also consider the existence of photoreceptors that can be activated by short light pulses and can operate if the activated state is maintained by excitations at the frequencies as used in this work. Furthermore one should also note that *psbA* transcription continues during 5 h dark treatment, albeit at a slower rate relative to the growth light conditions, as the level of *psbA* messages remains constant during the dark treatment and the half-life of the messages is 40 min.

3.4. STF treatment effect is specific for *psbA* and *psbD2* transcripts

To test the specificity of the flash effect on the expression of *psbA* gene, the expression of the *psbD* gene family was studied under the same conditions. The transcript level of the *psbD2* gene and its life time in cells exposed to STF treatment closely resembled that of the *psbA* genes (Fig. 3). Contrary to the *psbD2* messages, the level of the *psbDC* transcript was lower in the cells exposed to flashes as compared to that of cells maintained in continuous light and resemble that in darkness

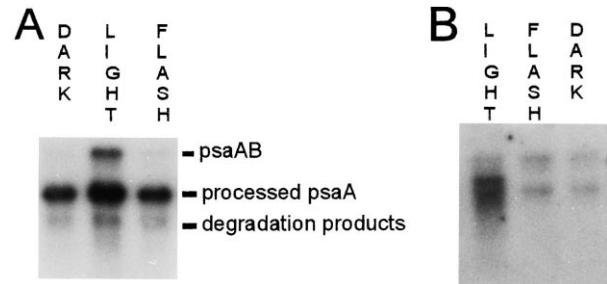


Fig. 4. Effect of STF on the level of transcripts encoding PSI and cytochrome bf subunits. Pattern of transcripts recognized by A: *psaA* probe and B: *petA* probe in cells maintained in growth light conditions (LIGHT), in darkness (DARK) and in cells exposed to single turnover flashes delivered at 32 s interval (FLASH).

(Fig. 3B). However, the stabilization of *psbDC* messages occurred in darkness and in STF treated cells (Fig. 3C).

As a further control we have also determined the effect of STF on the transcription of the *psaAB* operon encoding the PSI reaction center proteins and that of the *petAC* operon encoding the cytochrome f and Rieske Fe-S subunits of the cytochrome bf complex. The results (Fig. 4A) demonstrate that the *psaA* probe recognized two main transcripts. Comparison of the hybridization pattern with those published earlier [19] suggests that the largest transcript (5 kb) contains a dicistronic message *psaAB*, and the other main transcript is the *psaA* transcript. The transcripts that are seen as faint bands in Fig. 4A are too short to contain the whole coding region of the *psaA* and are most probably degradation products. The *psaAB* transcript almost disappeared in both STF and dark treated cells, while the amount of the *psaA* transcripts decreased by about 20%. The *petA* probe recognized multiple transcripts. The transcript patterns were similar in cells maintained in the dark or illuminated with single turnover flashes. Cells maintained under growth light conditions had at least one additional transcript species suggesting differential posttranscriptional processing of *petAC* message in light and in darkness or under the STF conditions (Fig. 4).

We conclude that the oxidation of the plastoquinone pool acts as a signal for the stabilization of messages encoding subunits of the photosynthetic protein complexes in *Synechocystis* 6803. Although transfer of *Synechocystis* cells from darkness to light enhances the transcription of all genes and operons studied in this work, it seems that the mechanism of *psbA* and *psbD2* genes transcription is subjected to additional controlling factors as evidenced by the STF effect reported in this study.

Acknowledgements: This work was supported by EMBO short term fellowship (T.T.), Academy of Finland (T.T., E.T. and E.-M.A.) and by HFSP and the Israel Science Foundation, administrated by the Israel Academy of Sciences and Humanities (I.O.).

References

- [1] Jansson, C., Debus, R.J., Osiewacz, H.D., Gurevitz, M. and McIntosh, L. (1987) *Plant Physiol.* 85, 1021–1025.
- [2] Ravnikar, P.D., Debus, R., Sevrinck, J., Saetaert, P. and McIntosh, L. (1989) *Nucleic Acids Res.* 17, 3991.
- [3] Metz, J., Nixon, P. and Diner, B. (1990) *Nucleic Acids Res.* 18, 6715.
- [4] Bouyoub, A., Vernotte, C. and Astier, C. (1993) *Plant Mol. Biol.* 21, 249–258.

- [5] Mohamed, A., Eriksson, J., Osiewacz, H.D. and Jansson, C. (1993) *Mol. Gen. Genet.* 238, 161–168.
- [6] Mohamed, A. and Jansson, C. (1989) *Plant Mol. Biol.* 13, 693–700.
- [7] Kanervo, E., Mäenpää, P. and Aro, E.-M. (1993) *J. Plant Physiol.* 142, 669–675.
- [8] Mohamed, A. and Jansson, C. (1991) *Plant Mol. Biol.* 16, 891–897.
- [9] Mulo, P., Laakso, S., Mäenpää, P. and Aro, E.-M. (1998) *Bot. Acta* 111, 71–76.
- [10] Tyystjärvi, T., Mulo, P., Mäenpää, P. and Aro, E.-M. (1996) *Photosynth. Res.* 47, 111–120.
- [11] Mulo, P., Tyystjärvi, T., Mäenpää, P. and Aro, E.-M. (1997) *Plant Mol. Biol.* 33, 1059–1071.
- [12] Prasil, O., Adir, N. and Ohad, I. (1992) in: J. Barber (Ed.), *Topics in Photosynthesis*, Vol. 11, Elsevier, Amsterdam, pp. 293–348.
- [13] Aro, E.-M., Virgin, I. and Andersson, B. (1993) *Biochim. Biophys. Acta* 1143, 113–134.
- [14] Ohad, I., Keren, N., Zer, H., Gong, H., Mor, T.S., Gal, A., Tal, S. and Domovich, Y. (1994) in: N.R. Baker and J.R. Bowyer (Eds.), *Photoinhibition of Photosynthesis from Molecular Mechanism to the Field*, BIOS Scientific Publishers Ltd, Oxford, pp. 161–177.
- [15] Keren, N., Gong, H. and Ohad, I. (1995) *J. Biol. Chem.* 270, 806–814.
- [16] Keren, N., Berg, A., van Kan, P.J.M., Levanon, H. and Ohad, I. (1997) *Proc. Natl. Acad. Sci. USA* 94, 1579–1584.
- [17] Zer, H. and Ohad, I. (1995) *Eur. J. Biochem.* 231, 448–453.
- [18] Vermaas, W.F.J., Charite, J. and Shen, G. (1990) *Biochemistry* 29, 5325–5332.
- [19] Smart, L.B. and McIntosh, L. (1991) *Plant Mol. Biol.* 17, 959–971.
- [20] Mayers, S.R., Dubbs, J.M., Vass, I., Hideg, E., Nagy, L. and Barber, J. (1993) *Biochemistry* 32, 1454–1465.
- [21] Tyystjärvi, T., Aro, E.-M., Jansson, C. and Mäenpää, P. (1994) *Plant Mol. Biol.* 25, 517–526.
- [22] Tomioka, N., Shinozaki, K. and Sugiura, M. (1981) *Mol. Gen. Genet.* 184, 359–363.
- [23] Li, R., Dickerson, N.S., Mueller, U.W. and Golden, S.S. (1995) *J. Bacteriol.* 177, 508–516.