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Biochimica et Biophysica Acta 1777 (2008) 74–83
Biochimica et Biophysica Acta
BBA
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Differential regulation of *psbA* and *psbD* gene expression, and the role of the different D1 protein copies in the cyanobacterium *Thermosynechococcus elongatus* BP-1

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Received 15 August 2007; received in revised form 28 October 2007; accepted 30 October 2007
Available online 12 November 2007

Abstract

In *Thermosynechococcus elongatus* BP-1, which is the preferred organism in recent structural studies of PSII, three *psbA* and two *psbD* genes code for three D1 and one D2 protein isoforms, respectively. The regulation and function of these genes and protein products is largely unknown. Therefore, we used quantitative RT-PCR to follow changes in the mRNA level of the respective genes, in combination with biophysical measurements to detect changes in the electron transport activity of Photosystem II under exposure to different visible and UV light, and temperature conditions. In cells which are acclimated to 40 μmol $m^{-2}s^{-1}$ growth light conditions at 40 °C the main populations of the *psbA* and *psbD* transcripts arise from the *psbA1* and *psbD1* genes, respectively. When the temperature is raised to 60 °C *psbA1* becomes the single dominating *psbA* mRNA species. Upon exposure of the cells to 500 μmol $m^{-2}s^{-1}$ intensity visible light *psbA3* replaces *psbA1* as the dominating *psbA* mRNA species, and *psbD2* increases at the expense of *psbD1*. UV-B radiation also increases the abundance of *psbA3*, and *psbD2* at the expense of *psbA1* and *psbD1*, respectively. From the different extent of total D1 protein loss in the absence and presence of lincomycin it was estimated that the PsbA3 protein isoform replaces PsbA1 in about 65% of PSII centers after 2 h of high light acclimation. Under the conditions of different *psbA* transcript distributions chlorophyll fluorescence and thermoluminescence measurements were applied to monitor charge recombination characteristics of the $S_2Q_A^-$ and $S_2Q_B^-$ states. We obtained faster decay of flash-induced chlorophyll fluorescence in the presence of DCMU, as well as lower peak temperature of the Q and B thermoluminescence bands when PsbA3 replaced PsbA1 as the main D1 protein isoform. The relevance of dynamic changes in the abundance of *psbA* and *psbD* transcript levels, as well as D1 protein isoforms in the acclimation of *T. elongatus* to changing environmental conditions is discussed.

Keywords: *psbA* gene; *psbD* gene; D1 protein; Photosystem II; Photo-oxidative stress

1. Introduction

In cyanobacteria the D1 and D2 proteins are encoded by small multigene families of the *psbA* and *psbD* genes, respectively [1]. The *psbA* family contains 1–5 gene copies, which encode 1–3 different D1 protein sequences in the cyanobacteria characterized so far. Whereas, the *psbD* family usually has two copies which encode one D2 protein form. The main physiological role of the different *psbA* and *psbD* genes is most likely related to acclimation to various stress conditions. The best characterized example is *Synechococcus* PCC7942 with three *psbA* gene copies that encode two different D1 protein isoforms (called D1:1 and D1:2) [2–5]. Under environmental stress conditions such as high light [3,6], blue light [7], low temperature [5,8], UV-B [9], or oxygen depletion [10] *psbA* expression is altered to selectively exchange the D1:1 isoform encoded by *psbA1* with the D1:2 isoform, encoded by *psbA2* and *psbA3*. When PSII has the high light isoform of D1 (D1:2) it has 25% higher quantum yield and shows increased tolerance to photoinhibition as compared to PSII centers with the low light isoform of D1 (D1:1) [5]. By expressing the two D1 isoforms of *Synechococcus* PCC7942 in *Synechocystis* PCC6803 background it has been

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PAR, photosynthetically active radiation; PSII, photosystem II

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doi:10.1016/j.bbabio.2007.10.015

shown that increased light tolerance is related partly to decreased extent of photodamage of PSII centers containing the D1:2 protein isoform, and partly to its enhanced ability to incorporate into the PSII complex during the repair cycle of D1 [11]. Further examples for light and UV dependent differential *psbA* regulation have been observed in *Anabaena* sp. PCC7120 [12] and *Gloeobacter violaceus* PCC7421 [13] both having five *psbA* genes, which encode 3 different D1 isoforms.

Although cyanobacteria usually contain only two different *psbD* genes, which encode identical D2 polypeptides their expression is also differentially regulated by changing light conditions. This has been demonstrated for *Synechococcus* PCC7942 as well as *Synechocystis* PCC6803. In both organisms the relative contribution of *psbD1* represents the dominating transcript under low light conditions, which is decreased at the expense of *psbD2* when the cells are exposed to high light [14] or UV-B radiation [15].

Thermosynechococcus elongatus BP-1 is a thermophilic cyanobacterium, which has gained importance recently as the source of PSII complexes suitable for crystallization [16–18]. This species has three *psbA* genes, which if all transcribed would encode three different D1 protein isoforms, and two *psbD* genes, which encode identical D2 polypeptides. Under normal growth conditions the dominating *psbA* transcript should arise from the *psbA1* gene, since the crystallized PSII complexes contain the corresponding PsbA1 protein isoform. Based on the analogy with other cyanobacteria having different D1 isoforms it is expected that the *psbA* gene copies and the corresponding D1 protein isoforms are also expressed differentially under different environmental conditions in *T. elongatus* as shown by preliminary results [19,20].

In the present work we studied the expression pattern of the *psbA* and *psbD* genes of *T. elongatus* by quantitative RT-PCR in parallel with measurements of PSII electron transport characteristics. Our results show that the mRNA levels of the *psbA1* and *psbA3*, as well as of the *psbD1* and *psbD2* genes are changing dynamically in response to changes in temperature, light intensity and ultraviolet radiation. These effects are accompanied by changes in PSII charge recombination characteristics, which indicate the role of PsbA1 and PsbA3 protein in acclimation to photo-oxidative stress conditions.

2. Materials and methods

2.1. Culture conditions

T. elongatus BP-1 cells were routinely grown in BG-11 medium in a rotary shaker at 40 °C under CO_2-enriched atmosphere. The intensity of PAR during growth was 40 μmol $m^{-2}s^{-1}$. Cells in the exponential growth phase (OD_{750} of 0.8–1.2) were harvested by centrifugation for 15 min at 4000 ×*g* at room temperature and resuspended at a concentration of 10 μg Chl mL^{-1} in a fresh culture medium.

2.2. Sample treatments

High light illumination experiment was performed in open, square glass containers in which the cell suspension formed a 14 mm high layer, with continuous stirring at 40 °C. An array of 50 W halogen lamps with adjustable light intensities provided the homogenous white light illumination. During light treatment sample aliquots were collected at certain time points, and stored at −80 °C until they were used for various measurements.

UV-B light was provided by a Vilbert–Lourmat lamp, with maximum emission at 312 nm, in combination with 0.1 mm cellulose acetate filter (Clarfoil, Courtalouds Chemicals, UK) yielding an intensity of 12 μmol $m^{-2}s^{-1}$ at the sample surface.

2.3. Gene expression analysis

10 mL of samples were harvested by centrifugation and total RNA was isolated by hot phenol method [21] with minor modifications. The crude RNA was further purified and freed from DNA contamination using NucleoSpinRNA kit (Macherei Nagel, Düren, Germany). 2 μg of the RNA was reverse transcribed using H-MuLV (Fermentas). Aliquots of the resulted cDNA were used in the Q-PCR reaction as template.

Quantitative RT-PCR (RT-PCR) was carried out on an ABI 7000 Sequence Detection System (Applied Biosystems Inc, Foster City, CA, U.S.A.) using SYBR green PCR Master mix of the same manufacturer. Primer pairs for the individual sequences were designed using Primer Express 2.0 program (ABI) as follows. psbaA1-sense: TTTGGACTTATCACGACTATGACCA, psbA1-antisense: CGGTGCTCGTCACCCAGT, psbA2-sense: GTCCCGTTGTTATTTATGGATTCAT, psbA2-antisense: ACGCTCCCACAGATTCGC, psbA3-sense: GGTTTGTAACATTCATTCAT ATTGTTCAA, psbA3-antisense: CAGCCCACATAGAGACGGTTG, psbD1-sense: TCTATATCTGCAAGAGGATTTAATTCCA, psbD1-antisense: ATGTCAAACCAT CCCCGTTC, psbD2-sense: TGCCCTTCGGAGTTGAATTTTA, psbD2-antisense: TCCCCGTTCCGCTGG.

Alignments of the homologous sequences of the *psbA* and *psbD* families, respectively, were carried out for aiding the selection of primers mapping to unique sequence regions of the respective mRNA sequences.

2.4. D1 protein degradation

Changes in the amount of the D1 protein were followed by immunoblotting, as described earlier [22].

2.5. Oxygen evolution measurements

PSII activity was assessed by measuring the light-saturated rate of oxygen evolution from whole cells, in the presence of 0.5 mM 2,5-dimethyl-*p*-benzoquinone as electron acceptor, using a Hansatech DW2 oxygen electrode. Usually, 1 mL of cells at 10 μg Chl mL^{-1} was used in each measurement.

2.6. Fluorescence relaxation kinetics

Flash-induced increase and subsequent decay of chlorophyll fluorescence yield was measured by a double-modulation fluorometer (PSI, Brno), in the 150 μs–100 s time range, in samples which were dark acclimated for 3 min prior to measurements. Analysis of the fluorescence relaxation traces was performed as described earlier [23]. The sample concentration was 10 μg Chl mL^{-1}.

2.7. Thermoluminescence measurements

Thermoluminescence curves were measured with a home built apparatus as described earlier [24]. Cells were harvested by gentle filtration through Whatmann GF/C glass microfibre filters to achieve 50 μg Chl on a filter disc. After 3 min of dark acclimation at 20 °C the samples were excited by a single saturating flash at 5 °C in the absence and at 0 °C in the presence of 10 μM DCMU. This was followed by a fast cooling to −20 °C, from where the slow heating with a rate of 20 °C min^{-1} was initiated, and thermoluminescence was detected.

3. Results

3.1. Effects of external conditions on psbA and psbD transcript levels

In control cells grown at 40 °C and low intensity (40 μmol $m^{-2}s^{-1}$) of photosynthetically active radiation (PAR) 94–95%

of the *psbA* transcript comes from *psbA1*, with 4–5% contribution from *psbA3* and 1–2% of *psbA2* (Fig. 1A). Acclimation of cells at 60 °C under the same low PAR increases even further the contribution of *psbA1* (to 99%). Exposure of cells to high PAR (1000 μmol $m^{-2}s^{-1}$) at 40 °C induces an almost complete exchange of the distribution of *psbA1* and *psbA3*, with the latter representing 98% of transcripts. However, the amount of *psbA2* transcript remained below 1%. Low intensity UV radiation (12 μmol $m^{-2}s^{-1}$) induced a change with the same tendency as high intensity PAR, i.e. decrease of *psbA1* and increase of *psbA3* transcript levels. However, in that case the contribution of the *psbA3* transcript in the total mRNA pool increased only to about 20%.

The distribution of *psbD* transcripts also responded to changing culture conditions (Fig. 1B). In control cells, grown at 40 °C and 40 μmol $m^{-2}s^{-1}$ PAR the dominating transcript was *psbD1* (88%), which increased slightly (90%) when the temperature is raised to 60 °C. Increase of PAR intensity to 1000 μmol $m^{-2}s^{-1}$ at 40 °C induced a decrease in the relative abundance of *psbD1*

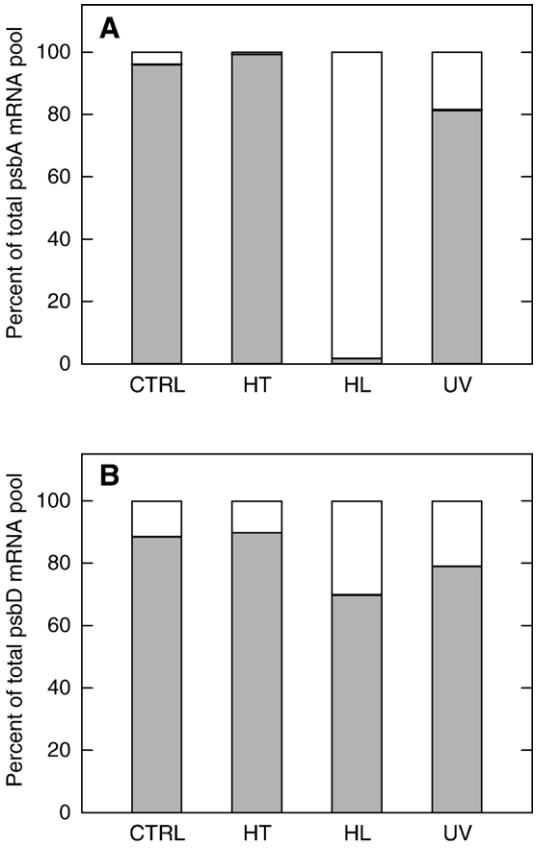

Fig. 1. mRNA distribution of the *psbA* and *psbD* genes under various light and temperature conditions. *T. elongatus* cells grown at 40 °C and 40 μmol m^{-2} s^{-1} light intensity (CTRL) were exposed to different conditions: High temperature of 60 °C, for 2 days (HT); High intensity visible light of 1000 μmol m^{-2} s^{-1} PAR, for 4 h (HL); UV-B light of 12 μmol m^{-2} s^{-1} intensity for 3 h (UV). Relative abundance of transcript levels was determined by RT-PCR. A, *psbA* transcript distribution with *psbA1* (gray), *psbA2* (black) and *psbA3* (white). B, *psbD* transcript distribution with *psbD1* (gray) and *psbD2* (white). The data are shown after normalizing the sum of the different mRNA homologues of the same gene family to 100%.

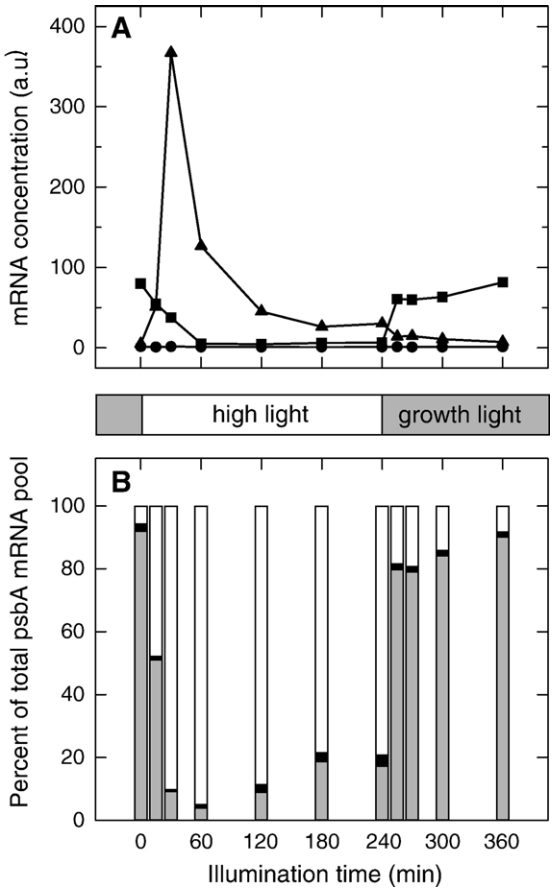

Fig. 2. Light induced changes in the mRNA level of the *psbA* homologues. *T. elongatus* cells grown at 40 μmol m^{-2} s^{-1} and 40 °C were subjected to 500 μmol m^{-2} s^{-1} light for 4 h, followed by 2 h recovery period under growth light. A, concentration of the mRNA species after normalization to the level in the control culture. *psbA1* (squares), *psbA2* (circles) and *psbA3* (triangles). B, Distribution of the *psbA* mRNA homologues shown after normalizing the sum of the three *psbA* mRNA concentrations to 100%: *psbA1* (grey), *psbA2* (black) and *psbA3* (white).

to 70% with the corresponding increase of *psbD2* to 30%. Similar effect was induced by exposing the cells to 12 μmol $m^{-2}s^{-1}$ UV-B radiation, although to a smaller extent, which resulted in the decrease of the relative abundance of *psbD1* mRNA from 88 to 81% (Fig. 1B).

We have also studied the kinetics of transcript changes. After shifting cells from growth light to high light (500 μmol $m^{-2}s^{-1}$) the *psbA3* mRNA responded by an about 20-fold increase in 30 min, followed by gradual decline reaching steady state after 3–4 h of high light exposure at about two-fold higher level than observed in the growth light acclimated cells (Fig. 2A). These changes were accompanied by decrease of the *psbA1* mRNA amount from the beginning of the high light exposure, which showed about 10-fold decrease during the first 60 min of high light exposure and kept this level practically unchanged during the following 3 h (Fig. 2A). When cells were shifted back to growth light following the high light exposure both the *psbA3* and *psbA1* mRNA amount was restored to the control level in 30 min. In contrast to the dynamic changes observed for *psbA3* and *psbA1*, the amount of *psbA2* mRNA remained at the

same very low level seen in the growth light acclimated control cells.

The above described dynamic changes of *psbA* amounts resulted in significant rearrangement of the relative distribution of the different *psbA* mRNA species during the course of the experiment. The *psbA3* mRNA replaced *psbA1* as dominating *psbA* species in the first 30 min of high light exposure (Fig. 2B). The increase of *psbA3* contribution continued until 60 min, and reached about 95% of the total *psbA* mRNA pool. After that the relative contribution of *psbA1* started to increase again at the expense of *psbA3* and reached steady state level at about 20:80% distribution, respectively. After shifting the cells back from high to growth light conditions the rapid increase of *psbA1* and concomitant decrease of *psbA3* amount resulted in the restoration of dominating contribution of *psbA1* in 30 min. The abundance of the *psbA2* transcripts was practically unchanged. However, due to the change of the total transcript pool size some fluctuation can also been seen in the relative amount of *psbA2* mRNA.

3.2. Correlation of psbA transcript distribution and D1 protein synthesis

In order to assess whether the light induced rearrangement of the *psbA* transcript distribution is followed by the exchange of the PsbA1 and PsbA3 protein isoforms we measured the total D1 protein amount during high light exposure. In these, and the following, experiments 3 h illumination with 500 μmol $m^{-2}s^{-1}$ intensity light was used, which was sufficient to induce steady state level of *psbA* transcript exchange (Fig. 2.), but had only a small extent of photoinhibitory effect that could interfere with the protein isoform exchange. In intact cells capable of *de novo* protein synthesis the total D1 protein pool decreased by 20% during 3 h exposure to high light, which corresponded to the same extent of decrease in the oxygen evolution rate in the presence of PSII acceptor (Fig. 3.). This shows that the cells were well acclimated to the high light conditions and retained the majority of their functional PSII centers. However, when the high light illumination was performed in the presence of the protein synthesis inhibitor lincomycin the loss of both the total D1 pool and of the oxygen evolution rate was accelerated and only 20% of their initial value was retained by the end of the three hours illumination period. The difference between the extent of D1 protein loss in the absence and presence of lincomycin must be compensated by *de novo* synthesis of D1. Since the *psbA* transcript pool was dominated by *psbA3* after the first 30 min of high light exposure, it is highly likely that the newly synthesized D1 copies were transcribed mainly from *psbA3* leading to a significant exchange of the PsbA1 and PsbA3 protein isoforms.

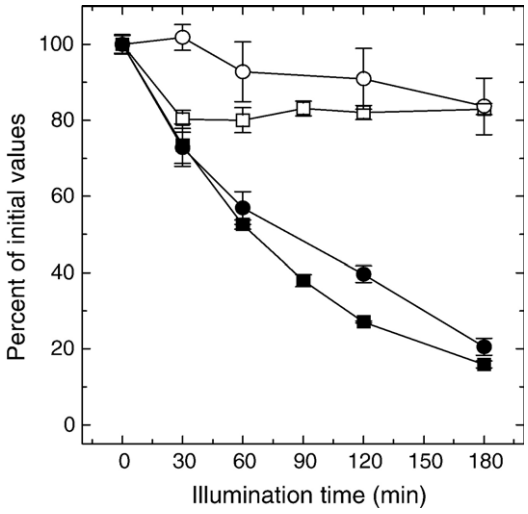

Fig. 3. Effect of high light on oxygen evolution rate and D1 protein amount. *T. elongatus* cells grown at 40 μmol $m^{-2}s^{-1}$ were exposed to high (500 μmol $m^{-2}s^{-1}$) PAR and the rate of oxygen evolution (squares) as well as the amount of the D1 protein (circles) was measured in the absence (open symbols) and presence (closed symbols) of 300 μg/mL lincomycin.

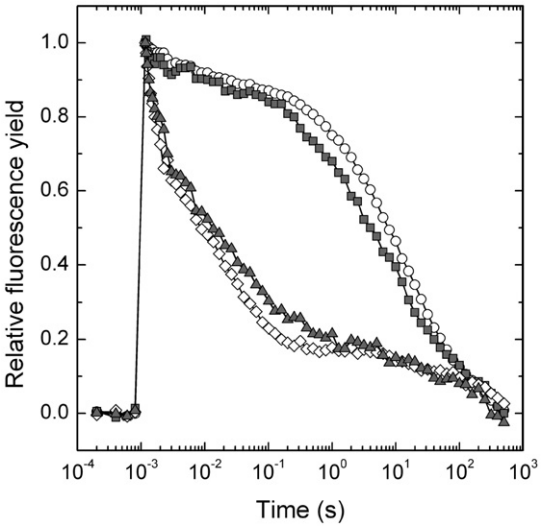

Fig. 4. Effect of high light on flash-induced fluorescence decay in *T. elongatus*. The kinetic traces were measured in low light grown cells before (empty symbols) and after 3 h illumination by 500 μmol $m^{-2}s^{-1}$ visible light (closed symbols). The measurements were performed in the absence (circles, diamonds) and in the presence (squares, up triangles) of 10 μM DCMU. The curves are normalized to the same initial amplitudes.

3.3. Correlation of psbA transcript distribution and PSII electron transport characteristics

Exchange of different D1 protein isoforms has been shown to affect charge separation efficiency, charge recombination characteristics, and phototolerance in *Synechococcus* PCC7942 [5,25]. Differential light tolerance, and recombination rate of the $S_2Q_A^-$ charge pair has also been observed in transformed *Synechocystis* PCC6803 cells, which contained either the D1:1, or D1:2 protein isoform of *Synechococcus* PCC7942 [11]. Therefore, we also checked the characteristic features of PSII electron transport in *T. elongatus* cells under different illumination conditions.

Illumination of cells with a single turnover saturating flash reduces Q_A by an electron extracted from the water oxidizing complex, which leads to increased fluorescence yield. Reoxidation of Q_A^- in the dark results in the relaxation of fluorescence

yield exhibiting three main decay phases (see [23]). The fast phase, $T_1 \sim 1$ ms arises from the reoxidation of Q_A^- by PQ molecules bound to the Q_B site before the flash. The middle phase, $T_2 \sim 30$ ms, originates from Q_A^- reoxidation by PQ molecules in centers where the Q_B site was empty at the time of the flash. Finally the slow phase, $T_3 \sim 50$ s, arises from back reaction of the S_2 state of the water oxidizing complex with Q_A^-, which is populated via the equilibrium between $Q_A^-Q_B$ and $Q_AQ_B^-$. Exposure of cells to high light for 3 h, which was sufficient to induce the exchange of the *psbA1* mRNA with *psbA3*, and about 65% of PsbA1 with PsbA3 (see below) resulted in a slow down of fluorescence relaxation, which affected mainly the middle phase (Fig. 4.). This observation indicates that the Q_B binding site might be modified as a result of the replacement of the PsbA1 protein isoform with PsbA3, although a high light induced modification of the Q_B site can not be completely excluded.

When the Q_A to Q_B electron transfer step is blocked by DCMU, the reoxidation of Q_A^- proceeds via charge recombination with donor side components, mainly with the S_2 state of the water oxidizing complex. The overall $T_{1/2}$ of this process is about 15 s in low light acclimated cells, which contain PsbA1 in PSII. High light induced exchange of the *psbA1* and *psbA3* transcripts and partial replacement of PsbA1 with PsbA3 was accompanied with a slight acceleration of fluorescence decay ($T_{1/2} \sim 9$ s) (Fig. 4.) indicating that recombination of the $S_2Q_A^-$ charge pair is faster in the presence of the PsbA3 than in the PsbA1 protein isoform.

Charge recombination characteristics were also checked by thermoluminescence (TL). After a single turnover flash the so-called B band is observed, which arises from the $S_2Q_B^-$ recombination [26,27] and appears at 53 °C in the low light acclimated cells (Fig. 5.). In high light acclimated cells the peak position of the B band shifts slightly lower, to about 50 °C. In the presence of DCMU the main TL component arises from the $S_2Q_A^-$ recombination and designated as the Q band [26,28], which appears at around 27 °C in the low light acclimated cells and shifted to 22 °C in the high light acclimated cells.

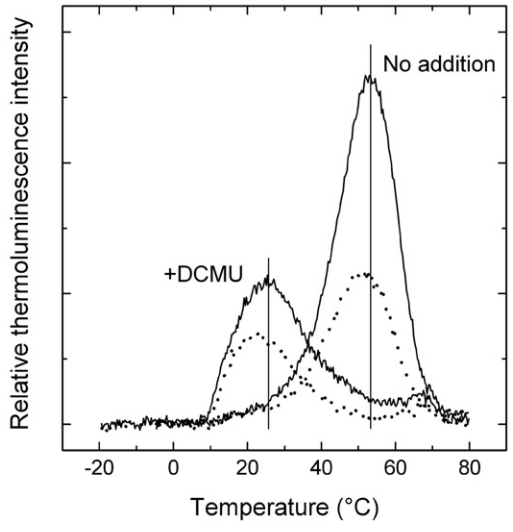

Fig. 5. Effect of high light on thermoluminescence of *T. elongatus*. The TL curves were measured in low light grown cells before (solid lines) and after 3 h illumination by 500 μmol $m^{-2}s^{-1}$ visible light (dotted lines). The measurements were performed in the absence and in the presence of 10 μM DCMU as indicated.

It is also interesting to note that the amplitudes of both the B and Q bands are significantly lower in cells acclimated to high light as compared to those acclimated to low light. Such an effect could be caused by photoinhibition due to high light exposure. However, the rate of oxygen evolution decreased only by 20% after the same high light treatment when measured in the presence of PSII acceptor, which is not sufficient to explain the total loss of TL intensity. Thus, the TL intensity difference between the low and high light acclimated cells, which remains after correction for the decrease of oxygen evolution, is probably related to the different charge recombination characteristics of PSII in the presence of the PsbA1 and PsbA3 protein isoforms as will be discussed below.

4. Discussion

4.1. Differential regulation of psbA and psbD genes under photo-oxidative stress conditions

Similar to most cyanobacterial species *T. elongatus* utilizes small *psbA* and *psbD* gene families to code for the D1 and D2 reaction centre subunits, respectively. The *psbA* transcript pool is consisted almost exclusively of *psbA1* (99%) with negligible contribution from *psbA2* and *psbA3* when cells are grown at low light intensities (40 μmol $m^{-2}s^{-1}$) and optimal temperatures (55–60 °C). Different environmental conditions, which lead to photo-oxidative stress, such as an increase of light intensity, a decrease of temperature, or the presence of ultraviolet radiation, all tend to increase the contribution of the *psbA*3 transcripts at the expense of *psbA1*. The most pronounced effect is induced by light intensity changes as shown by the rapid (<30 min) and almost complete replacement of the *psbA1* with *psbA3* mRNA under exposure to 500–1000 μmol $m^{-2}s^{-1}$ illumination. The transcript level of *psbA2*, which codes for a highly divergent D1 isoform, remains at trace levels at all conditions tested. This provides a further example of a lowly expressed *psbA* genes, encoding divergent D1 sequences with unclear function as detected earlier in *Synechocystis* PCC6803, *Anabaena* PCC7120 [12] and *G. violaceus* [13].

The *psbD* transcript pool is dominated by *psbD1* (>90%), at optimal growth conditions, but its contribution decreases under photo-oxidative stress conditions. Similar to the case of the *psbA* transcripts the most pronounced effect is induced by high intensity visible light. However, even in that case *psbD1* remains the dominating form with about 70% contribution to the total *psbD* transcript pool.

Although our methods did not permit direct detection of the actual change in the amount of the PsbA1 and PsbA3 protein isoforms our results show that *de novo* protein synthesis occurs from the stress induced transcript form in a similar way as demonstrated for *Synechococcus* PCC7942 [29], *Synechocystis* PCC6714 [30] and *Synechocystis* PCC6803 [31] leading to the

partial replacement of PsbA1 with PsbA3. This conclusion is supported by the observation that about 75% of D1 was synthesized from a transcript pool containing 80–90% *psbA3* in PSII centres present after 3 h of high light illumination. Assuming proportionality between transcript abundance and protein synthesis we can estimate that in about 65% of PSII the PsbA1 protein isoform was replaced with the PsbA3 isoform in the high light acclimated cells. In addition, the modification of charge recombination characteristics in the high light cells shows that in a large fraction of PSII centres the charge stabilization and recombination energetics is changed as a consequence of PsbA1 to PsbA3 exchange as discussed below.

4.2. Effect of the PsbA1 to PsbA3 protein exchange on charge recombination characteristics of PSII

The PsbA1 and PsbA3 protein isoforms of *T. elongatus* differ at 21 amino acid positions (Fig. 6.). The most affected regions are the N-terminus (6 changes), the 121–130 range (4 changes), the 151–155 range (3 changes), the 281–283 range (2 changes) and the 307–310 range (2 changes). Four additional single amino acid changes are found at the 36th, 184th, 212th and 270th positions.

The N-terminus is not involved in any cofactor binding. However, very recent data indicate that the first 20–25 amino acid

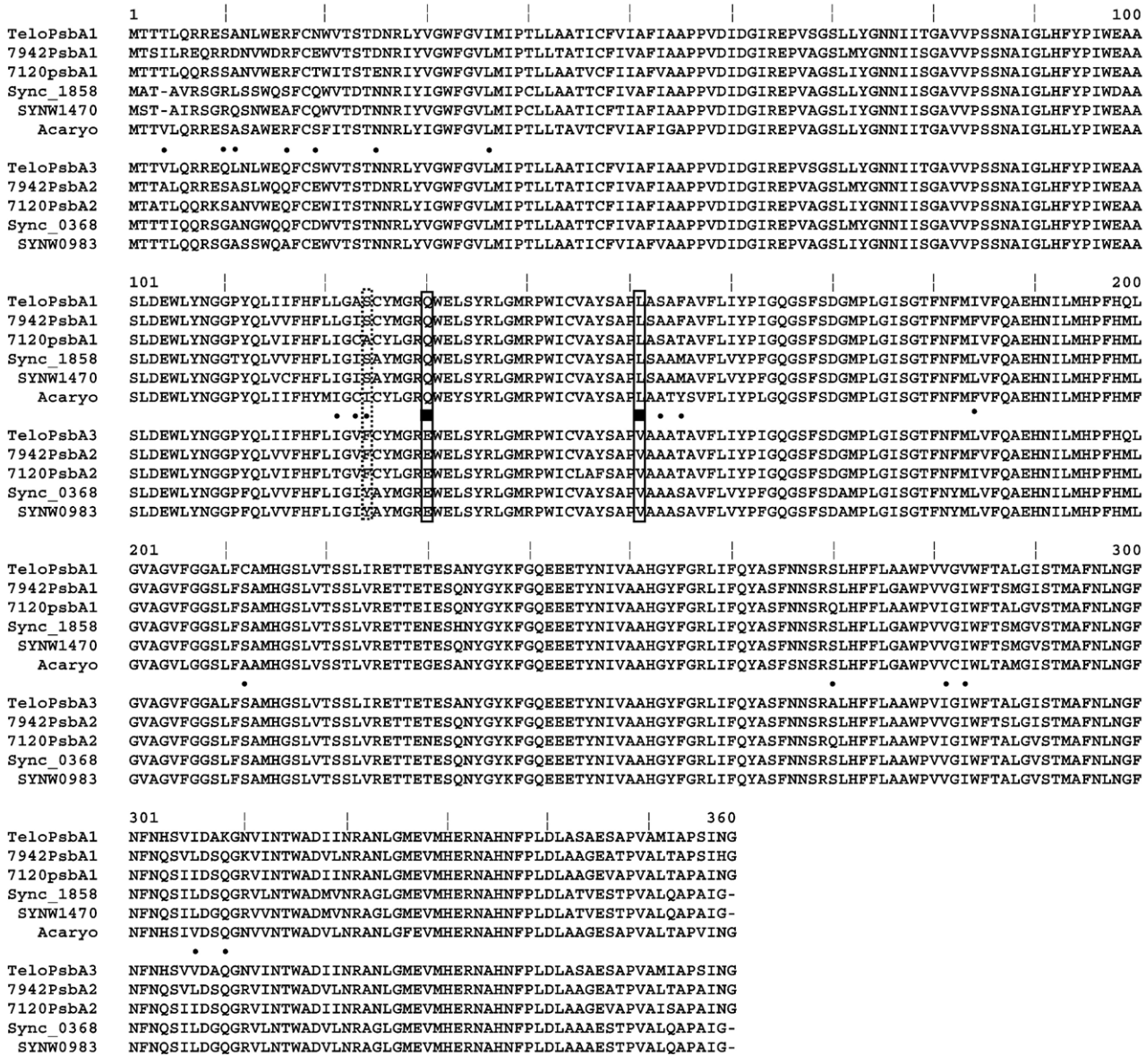

Fig. 6. Sequence comparison of D1 protein isoforms in different organisms. The sequences shown in the upper 6 rows correspond to the low light D1 isoforms, whereas the sequences in the lower 5 rows correspond to the high light (stress-responsive) D1 isoforms of different species. The position of amino acids, which are different in the PsbA1 and PsbA3 sequences are indicated by dots. Amino acids that are conserved in all stress-responsive D1 isoforms and changed to other conserved residue in the low light D1 isoforms are boxed with continuous line. The dotted box shows an important semi-conserved position. All amino acid sequences have been deduced from the nucleotide sequences of corresponding *psbA* genes, whose names are shown in front of the rows. The sequences of *T. elongatus* BP-1(Telo), *Nostoc* (formerly classified as *Anabaena*) sp PCC7120 (7120), *Synechococcus* WH8102 (SYNW) and *Synechococcus* CC9311 (Sync) originate from CyanoBase [42]. *Synechococcus* PCC7942 (formerly classified as *Anacystis nidulans* R2) (7942) and *A. marina* MBIC11017 (Acaryo) *psbA* sequences were obtained from GenBank [40].

residues at the N-terminus are involved in the degradation of photodamaged D1 protein via interaction with the FtsH protease [32]. Previous data obtained with *Synechocystis* PCC6803 mutants, which express either the D1:1 (low light) or D1:2 (high light) isoform of *Synechococcus* PCC7942, showed that increased phototolerance of D1:2 containing PSII arises partly from enhanced repair capacity and partly from decreased extent of photodamage [11]. Thus, modification of the N-terminal region of PsbA3 relative to PsbA1 in *T. elongatus* might play a role in more efficient D1 degradation, which is a prerequisite of PSII repair.

The 121–130 sequence range affects the binding pocket of $Pheo_{D1}$ with the Glu or Gln at 130th residue position acting as H-bond donor that modulates the redox potential of Pheo [33] (Fig. 7.). Previous site directed mutagenesis studies in *Synechocystis* PCC6803 have demonstrated that the replacement of D1-Gln130 with Glu shifts the redox potential of Pheo to more positive values by about 35 mV [33]. This effect in turn induces a 4-fold faster recombination of the $S_2Q_A^-$ charge pair [34,35], and a 4-fold decrease in thermoluminescence intensity [35]. Similar effects were observed in *Chlamydomonas reinhardtii* containing D1-Glu130 or D1-Gln130 [36,37]. The D1-Gln130Glu change occurs also between the low and high light (stress inducible) isoforms of D1 in other cyanobacteria (Fig. 6.) and correlated with accelerated $S_2Q_A^-$ recombination in *Synechococcus* PCC7942 [11], and *Anabaena* PCC7120 [12].

Our chlorophyll fluorescence decay and thermoluminescene data show that partial exchange of the PsbA1 and PsbA3 protein isoforms in high light acclimated *T. elongatus* cells also leads to accelerated recombination of the $S_2Q_A^-$ (and $S_2Q_B^-$) charge pair and accompanied with the decrease of TL intensity. According to a recent model TL intensity is modulated by $\Delta G(P^*_{680} \leftrightarrow P^+_{680}Pheo^-)$, which regulates the yield of non-radiative charge recombination via the primary radical pair[35]. This phenomenon can be used to estimate the effect induced by the modification of the $Pheo_{D1}$ pocket on the redox potential of Pheo. The intensities of the Q and B bands in high light acclimated cells are 60–62 and 48–50% of those in low light acclimated cells, respectively. The rate of oxygen evolution in the presence of PSII acceptors is 20% smaller in the high- than in the low light acclimated cells indicating the same extent of decrease in the amount of functional PSII centers. Considering further the estimation that about 65% of high light acclimated cells has PsbA3, the intensity of the Q band in the presence of PsbA3 is 45–49% (or 36–40% for the B band) of that in the presence of PsbA1. According to the recent model of TL [35] these values correspond to 18–20 (or 24–26) meV increase of $\Delta G(P^*_{680}-$

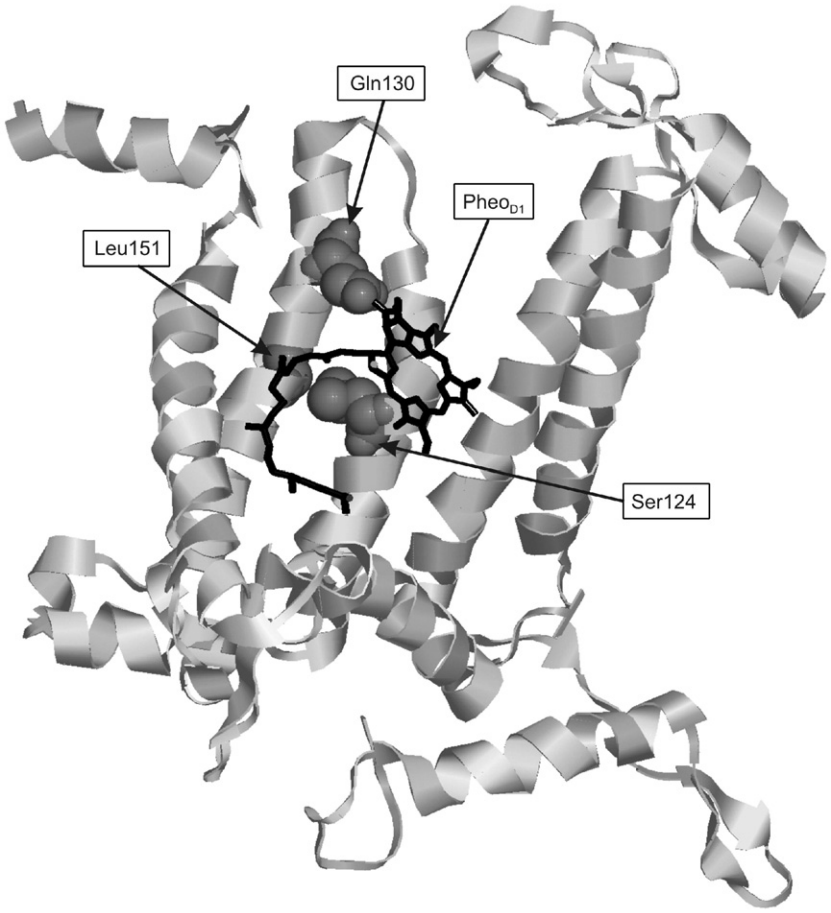

Fig. 7. Amino acid changes in the D1 sequence which may influence the redox potential of $Pheo_{D1}$. The figure shows the structure of the D1 protein within the PSII reaction center of *T. elongatus* based on the three-dimensional structure of the PSII reaction center [18], and shows the positioning of amino acids whose replacement in the vicinity of $Pheo_{D1}$ during the PsbA1 to PsbA3 exchange is highly conserved in all species investigated (Fig. 6).

$P^{+}_{680}Pheo^{-}$). This results in the same extent of shift of the free energy level of the $P^{+}_{680}Pheo^{-}$ radical pair to more positive values in the presence of PsbA3 since the free energy of the P^{*}_{680} excited state will not change.

The energetic shift of $P^{+}_{680}Pheo^{-}$ affects also the overall recombination rate of $S_2Q^{-}_{A}$, which is determined by $\Delta G(P^{+}_{680}Pheo^{-} - S_2Q^{-}_{A})$ as reflected by the kinetics of fluorescence decay in the presence of DCMU. Considering that $T_{1/2} \sim 15$ s in low light acclimated cells (100% PsbA1) and ~10 s in high light acclimated cells (~35% PsbA1 + 65% PsbA3) $T_{1/2} \sim 7$ s can be estimated for PSII containing PsbA3. From the $T_{1/2,\ PsbA3}/T_{1/2,\ PsbA1}$ ratio ~20 meV is obtained for the decrease of the total free energy span between the $P^{+}_{680}Pheo^{-}$ and $S_2Q^{-}_{A}$states. This can be assigned to an effect on $P^{+}_{680}Pheo^{-}$ since the amino acid changes between PsbA3 and PsbA1 do not indicate a significant modification in the vicinity of the Mn cluster or Q_A, which could affect the energetics of $S_2Q^{-}_{A}$ charge stabilization.

The above values of $\Delta G(P^{*}_{680} \leftrightarrow P^{+}_{680}Pheo^{-})$ in PsbA1 and PsbA3 containing *T. elongatus* cells are smaller than the 35 [33], or 30–38 meV [35] obtained in the D1-Gln130Glu mutant of *Synechocystis* PCC6803 . Therefore, we have to assume that amino acid differences between PsbA3 and PsbA1 other then the Gln130Glu exchange can partly compensate the effect on $E_m(Pheo/Pheo^{-})$. Such changes could be the Leu151Val, and Ser124Phe, which are also located in the vicinity of $Pheo_{D1}$ (Fig. 7.), and occur in parallel with the Gln130Glu exchange in *T. elongatus* and other species (Fig. 6.).

4.3. Functional role of psbA and psbD transcript regulation

Regulation of different *psbA* and *psbD* transcript levels and PsbA protein isoforms appears to be widespread among cyanobacteria. As discussed above this phenomenon occurs as a response not only to exposure to high light, but also to UV-B radiation and suboptimal temperatures, which all represent photo-oxidative stress for cyanobacteria under dynamically changing environmental conditions.

Although members of *psbA* gene families often code for different D1 isoforms, differential stress induced *psbA* transcription takes place even if all active *psbA* genes code for the same D1 polypeptide as occurs in *Synechocystis* PCC6714 [30] and *Synechocystis* PCC6803 [38]. Therefore, the physiological role of the stress inducible *psbA* gene copies without making another D1 isoform is the rapid increase of the *psbA* transcript pool under the conditions which require rapid D1 repair. Similar role can be assigned to the differential transcription of the *psbD* genes, which code for identical D2 polypeptides. The dominant *psbD1* gene is co-transcribed with *psbC*, which codes for the CP43 subunit of PSII. CP43 is damaged much less by light than D2 (or D1), therefore, more D2 than CP43 has to be replaced during PSII repair. Thus, it is an energy saving strategy of cyanobacterial cells to increase the *psbD* transcript pool by upregulation of the monocistronic *psbD2* message instead of increasing the transcription of *psbD1* together with *psbC*.

The importance of using different D1 sequences to cope with photo-oxidative stress has been shown by the increased photo-tolerance in *Synechococcus* PCC7942 [5] and *Synechocystis* PCC6803 mutants [11], which contained either the low- or high light isoform of *Synechococcus* PCC7942. Provided that this is a general strategy among cyanobacteria, it is expected that amino acid differences between low- and high light D1 isoforms share crucial similarities among different species. In Fig. 6 we present a sequence comparison, which includes the low- versus high light D1 isoforms of species, which are known to respond with D1 protein isoform exchange to light and other photo-oxidative stress conditions. It is remarkable to note that all low light sequences have D1-Gln130, whereas all high light sequences have D1-130Glu, which points to a critical role of this residue in photoprotection. In addition to the well characterized cyanobacteria we also included further species in the sequence comparison whose genome has been sequenced, but their *psbA* regulation has not been studied yet. Their grouping with the low- or high light D1 isoforms was based on their ecological characteristics: *Acaryochloris marina* lives in a steady, low light environment [39]. It has two *psbA* genes with identical amino acid sequence [40], which was compared to the low light D1 isoforms. Coastal and near surface species, like *Synechococcus* CC9311 and *Synechococcus* WH8102, live in a dynamic environment and have 4 *psbA* genes. One of their *psbA* genes codes for a D1 sequence with Gln130, which were grouped with the low light sequences. The other 3 *psbA* genes in both species code for one mature D1 sequence with Glu130 (in *Synechococcus* WH8102 there is one amino acid difference in the processed C-terminal region), which were grouped with the high light sequences. Comparison of these D1 sequences revealed only two sites, which show conserved changes between the low- and high light D1 isoforms. These are Gln130Glu as well as Leu151Val. The sequence comparison also reveals a semi-conserved change at position 124 from Ser (Ala/Ile) (low light forms) to Phe (Tyr) with bulky aromatic side chains (high light forms). Gln130 is in the $Pheo_{D1}$ pocket and the other two residues are also close to $Pheo_{D1}$ (Fig. 7.).

The presence of D1-Glu130 instead of D1-Gln130 in the high light D1 isoforms is most likely related to its effect on the redox potential of Pheo as has been shown in *Synechocystis* PCC6803 [33]. Our recent data indicated that this point mutation confers increased phototolerance in *Synechocystis* PCC6803 [41] probably due to enhanced non-radiative charge recombination of the singlet form of the $P^{+}_{680}Pheo^{-}$ primary radical pair that leads to decreased singlet oxygen formation [35]. The Leu151Val and Ser(Ala/Ile)124Phe(Tyr) changes in the vicinity of the $Pheo_{D1}$ pocket might also contribute to the regulation of the Pheo redox potential as discussed above, and could be involved in the phototolerance mechanism.

It is also of note that besides increased phototolerance the quantum yield of PSII was also increased by about 25% in *Synechococcus* PCC7942 in the presence of the high light D1 isoform as compared to the low light isoform [5]. In addition, a significant increase in the quantum yield of primary charge separation in the *Synechocystis* PCC6803 strain carrying the Gln130Glu point mutation was also observed relative to the wild type *Synechocystis* PCC6803 [33]. These findings indicate that regulation of $Pheo_{D1}$ redox potential also serves for better utilization of captured light energy under high light conditions.

The observations which show that D1 protein isoform exchange occurs not only under high light, but also under other environmental conditions (UV light, low temperature) leading to photo-oxidative stress as reported here and previously in the literature [9,12,13] indicate that modulation of PSII quantum yield is an important, but not an exclusive function of D1 protein isoform exchange.

4.4. Concluding remarks

Our results demonstrate differential expression of *psbA* and *psbD* genes in *T. elongatus* under changing visible and UV light, and temperature conditions. The accompanied changes in the distribution of the PsbA1 and PsbA3 protein isoforms result in accelerated charge recombination from the $S_2Q_A^-$ (and $S_2Q_B^-$) states. This effect probably arises from the modification of the protein environment around $Pheo_{D1}$, especially due the Gln130Glu, and Leu151Ser as well as Ser124Phe replacements, resulting in an increased free energy gap between P_{680}^* and the $P_{680}^+Pheo^-$ primary radical pair. Such modification can enhance the quantum yield of primary radical pair formation [33] together with harmless dissipation of captured light energy via non-radiative charge recombination of the singlet radical pair [33,35] leading to a photoprotective effect. Based on the highly conserved nature of these amino acid changes between low- and high light D1 isoforms of different species, it is likely that regulation of D1 protein isoform distribution represent an important strategy of acclimation to conditions of high light and photo-oxidative stress in cyanobacteria. The high occurrence of amino acid changes in the N-terminal part of the D1 protein, which has been implicated in D1 degradation [32], points to the possibility that the photoprotective effect of D1 isoform exchange involves also regulation of PSII repair rate.

Acknowledgements

This work was supported by grants from European Union (MRTN-CT-2003-505069 and STREP-SOLAR-H-516510).

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