

## Cyanobacterial *psbA* gene family: optimization of oxygenic photosynthesis

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**Abstract** The D1 protein of Photosystem II (PSII), encoded by the *psbA* genes, is an indispensable component of oxygenic photosynthesis. Due to strongly oxidative chemistry of PSII water splitting, the D1 protein is prone to constant photodamage requiring its replacement, whereas most of the other PSII subunits remain ordinarily undamaged. In cyanobacteria, the D1 protein is encoded by a *psbA* gene family, whose members are differentially expressed according to environmental cues. Here, the regulation of the *psbA* gene expression is first discussed with emphasis on the model organisms *Synechococcus* sp. and *Synechocystis* sp. Then, a general classification of cyanobacterial D1 isoforms in various cyanobacterial species into D1<sub>m</sub>, D1:1, D1:2, and D1' forms depending on their expression pattern under acclimated growth conditions and upon stress is discussed, taking into consideration the phototolerance of different D1 forms and the expression conditions of respective members of the *psbA* gene family.

**Keywords** Adaptation · Cyanobacteria · Cyanophage · D1 protein · *psbA* gene · Regulation of gene expression · Stress responses

### Introduction

Cyanobacteria, algae, and higher plants have a unique capacity to use water as a source of electrons in reducing CO<sub>2</sub> to various organic compounds. In organisms performing oxygenic photosynthesis, the linear electron transfer (light reactions) takes place in the thylakoid membrane-embedded protein complexes Photosystem II (PSII), Cytochrome b<sub>6</sub>f (Cytb<sub>6</sub>f), and Photosystem I (PSI). These multiprotein complexes harness solar energy and, together with ATP synthase, produce reducing power (NADPH) and chemical energy (ATP) for production of carbohydrates. These carbohydrates together with oxygen, the side product of photosynthetic electron transfer, enable all heterotrophic life on Earth.

The core of PSII multisubunit pigment protein complex is composed of the D1 and D2 proteins, which are involved in ligating most of the redox active components of PSII including the Mn<sub>4</sub>Ca cluster, the site of water oxidation. The primary charge separation in PSII results in highly oxidating chlorophyll (Chl) cation P680<sup>+</sup>, which is the only biological compound strong enough to drive water oxidation. The recombination of Chl cation P680<sup>+</sup> with downstream electron transport cofactors pheophytin (Phe)<sup>-</sup> or the primary stable electron acceptor plastoquinone Q<sub>A</sub><sup>-</sup> can lead to the formation of triplet Chl states and ultimately to the formation of singlet oxygen, which in turn may damage the photosynthetic machinery. In addition to various protective mechanisms [1, 2], the PSII repair cycle functions to replace the damaged reaction centre protein

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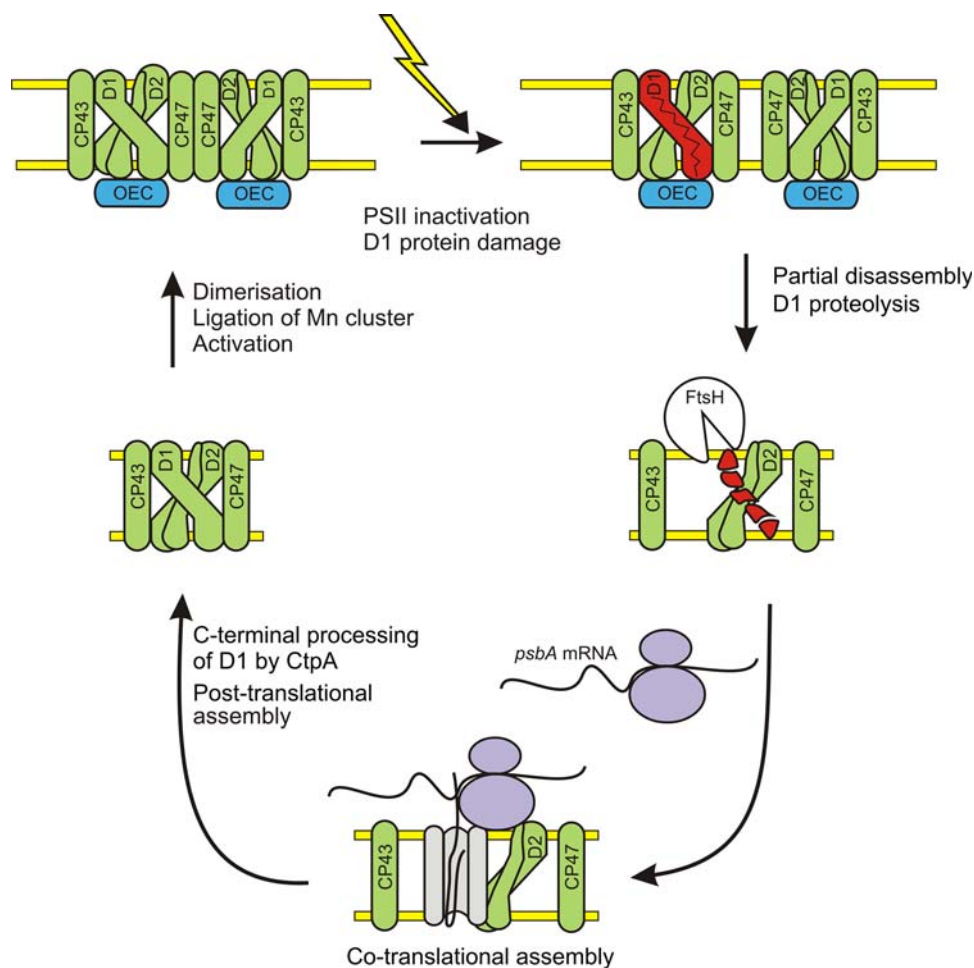
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D1 with a de novo synthesized copy [3, 4] (Fig. 1). The D1 protein is degraded and replaced by a new copy every 5 h under low light growth conditions, and every 20 min under intense illumination [5], to guarantee the maintenance of a steady-state level of the D1 protein in PSII complexes. Due to the capacity of photosynthetic organisms to increase the turn-over rate of the D1 protein upon increasing light intensity, a decrease in the total amount of D1 protein occurs only upon prolonged and severe light stress, which results in impairment of the photosynthetic capacity, i.e., photoinhibition [3, 4]. Hence, the expression of the *psbA* gene(s) encoding the D1 protein must be under strict control to guarantee the function of the photosynthetic machinery under ever-changing environmental conditions.

In higher plants, the *psbA* gene encoding the PSII reaction centre protein D1 is present only in one copy, while all cyanobacteria have a small *psbA* gene family ranging from one to six members (Table 1; <http://www.kazusa.or.jp/cyano/>, <http://genome.jgi-psf.org/>). In the chloroplast genome of some conifers, however, the *psbA* gene has been duplicated [6]. Despite the difference in gene number, the similarity of the plant and cyanobacterial

strategies in *psbA* gene expression is amazing, and is exemplified by the studies showing the suitability of higher plant *psbA* gene promoter to control the expression of the *psbA* gene in cyanobacteria [7, 8]. Still, the presence of multiple *psbA* genes encoding different D1 isoforms in cyanobacteria is an indication of their importance in regulatory mechanisms responsible for maintaining a functional PSII upon changing environmental conditions in natural habitats of cyanobacteria. Regulation of the *psbA* gene family members in cyanobacteria follows at least two distinct mechanistic principles. One strategy is to replace the D1 protein present in PSII centres under unstressed conditions with a different form when the stress is detected (Fig. 2a). The other strategy is, upon stress conditions, to increase the turn-over of the same D1 protein produced under basic growth conditions (Fig. 2b). Both of these strategies have been demonstrated in more than one cyanobacterial species. Yet a new regulation mechanism was recently documented in several cyanobacterial species concerning the divergent and “silent” *psbA* genes, which were proven to be induced by microaerobic/low oxygen conditions [9, 10].

**Fig. 1** Simplified scheme of the PSII repair cycle. Functional PSII dimers are inactivated by light, and the D1 protein is damaged. After partial disassembly of PSII, the damaged D1 protein is accessed by the FtsH protease, and degraded. Subsequently, the ribosome-nascent D1 chain complex is targeted to the thylakoid membrane, and the D1 protein is co-translationally inserted into the membrane and the PSII complex. The C-terminus of the D1 protein is post-translationally processed, PSII is re-assembled, activated, and the PSII dimers are formed



**Table 1** The *psbA* genes and the D1 isoforms present in various cyanobacterial species, classified according to their functional features

D1 forms	D1 <sub>m</sub>	D1:1	D1:2	D1'	Selected references
<i>Synechocystis</i> sp. PCC 6803	1 protein <i>psbA2</i> <i>psbA3</i>			1 protein <i>psbA1</i>	[9, 10, 52, 54–58]
<i>Anabaena</i> sp. PCC 7120		1 protein <i>psbA1</i>	1 protein <i>psbA2 psbA3 psbA4</i>	1 protein <i>psbA0</i>	[9, 10, 95]
<i>Thermosynechococcus elongatus</i> BP-1		1 protein <i>psbA1</i>	1 protein <i>psbA3</i>	1 protein <i>psbA2</i>	[9, 91, 96]
<i>Synechococcus</i> sp. PCC 7942		1 protein <i>psbA1</i>	1 protein <i>psbA2</i> <i>psbA3</i>		[22–24, 27, 28, 35]
<i>Gloeobacter violaceus</i> PCC 7421	1 protein <i>psbA1</i> <i>psbA2</i> <i>psbA3</i>			1, maybe 2 proteins <i>psbA4 psbA5</i>	[88]

A vast number of studies focusing on regulation of cyanobacterial *psbA* gene expression have been published during the past two decades. Although more and more details are currently being disclosed, the ultimate mechanisms concerning especially the trans-acting factors regulating the *psbA* gene expression still remain to be revealed. It has also become clear that, although the increase in *psbA* transcripts is a general response of cyanobacteria upon shift of the cells to high light intensity, each strain seems to have its own characteristic regulation mechanisms which cannot be directly generalized to other strains. Here, we have summarized the current knowledge about regulation of *psbA* gene expression with major focus on studies performed with model organisms *Synechococcus elongatus* sp. PCC 7942 (hereafter, *Synechococcus* 7942) and *Synechocystis* sp. PCC 6803 and 6714 (hereafter, *Synechocystis* 6803 and 6714, respectively). Although at least in some species also valid to the *psbA* genes, we have here excluded the circadian regulation of gene expression, which was recently reviewed in [11]. We apologize for not being able to present results from all published experimental systems due to space limitation and for the sake of clarity.

### Regulation of *psbA* gene expression in cyanobacteria

As typical to eubacteria, the *psbA* gene expression can be expected to be regulated at the levels of transcription initiation, elongation, and termination, mRNA stability as well as translation. In cyanobacteria, the initiation of transcription is considered to be the most crucial determinant of gene expression. RNA polymerase holoenzyme, composed of the catalytically active core and one of the

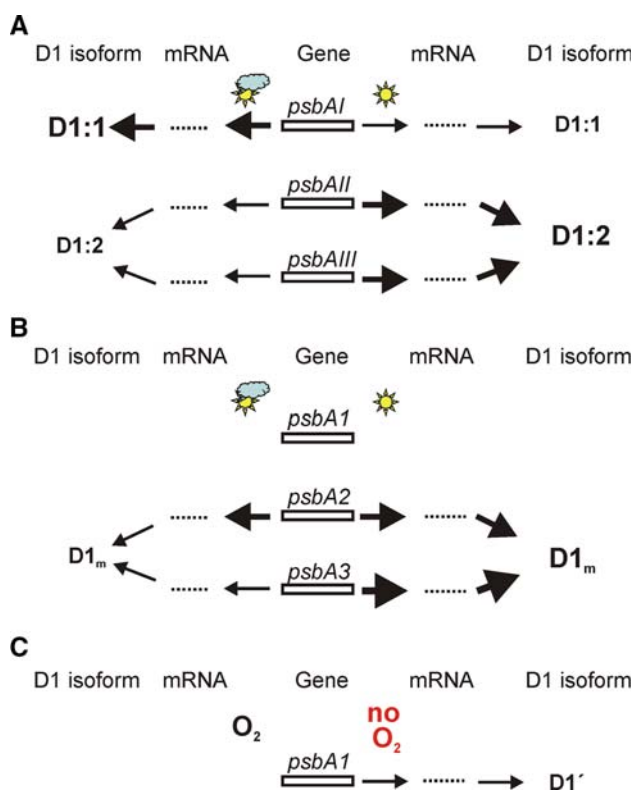
several sigma factors, initiates transcription. The sigma factors are responsible for promoter recognition, and the chromatin structure along with various cis-acting elements up- and downstream from the transcription start site regulates the level of gene expression. It has been shown that the principal sigma factor (Group 1) specifically recognizes the hexameric –35 and –10 regions located in the promoter region of many cyanobacterial *psbA* genes [12–14]. The light-responsive expression of the *psbA* gene, however, seems to require the function of SigB, SigD, and SigE [15–18].

The tertiary structure of DNA is known to have a marked effect on gene expression [19]. AT repeats on one face of the DNA, often found in the region –240 to –40 from the transcriptional start, influence the formation of DNA double helix, and these bends may modulate transcriptional activity. An intrinsic curvature composed of several AT tracts has been found in the upstream region of *psbA* genes in many organisms including *Synechocystis* 6803 and *Microcystis aeruginosa* K-81 [20, 21]. Modification of these tracts was observed to severely downregulate the transcription of the *psbA* gene, which, however, still remained light-responsive in nature.

Specific features of *psbA* gene regulation have mainly been addressed in studies with *Synechococcus elongatus* PCC 7942 and *Synechocystis* 6803 and 6714. Below, we focus on regulation of *psbA* gene expression in these species.

#### *psbA* gene expression in *Synechococcus elongatus* PCC 7942

In *Synechococcus* 7942, the three *psbA* genes encode two distinct D1 protein isoforms, D1:1 being encoded by *psbA1*



**Fig. 2** Mode of expression of the *psbA* genes. **a** Regulation of the *psbA* genes in *Synechococcus* 7942, which contains the D1:1 and D1:2 forms. Upon standard conditions, the *psbA1* gene is actively transcribed while the *psbAII* and *psbAIII* genes are repressed, and accordingly the D1:1 isoform is synthesized and accumulates in PSII complexes. High light (or other stresses resulting in thiol-reducing conditions) activates the transcription of the *psbAII* and *psbAIII* genes with concomitant inactivation of *psbA1*. Consequently, the D1:2 isoform accumulates in PSII complexes. Adaptation to the new ambient conditions reverses the situation. **b** Regulation of the *psbA2* and *psbA3* genes in *Synechocystis* 6803, which contains the D1<sub>m</sub> and D1' forms. Under standard conditions, the *psbA1* gene is silent and most of the *psbA* transcripts and the D1<sub>m</sub> protein are produced by the *psbA2* gene. Intense illumination results in enhanced transcription rate of the *psbA2* and especially that of *psbA3*, providing transcripts for rapid D1 turnover. Transcription of trace amounts of *psbA1* gene also occurs at high light intensity, but no D1' protein has been found to accumulate in PSII complexes. **c** Regulation of the “silent” *psbA1* gene, which is induced under low O<sub>2</sub> pressure and encodes the D1' form. Transcript or protein products of the *psbA1* gene in *Synechocystis* 6803, previously thought to be a silent gene, cannot be detected under standard growth conditions. Microaerobic or anaerobic conditions result in activation of transcription

and D1:2 by *psbAII* and *psbAIII* [22–27]. Under low light conditions (125 μmol photons m<sup>-2</sup> s<sup>-1</sup>), more than 80% of total *psbA* transcripts originate from *psbA1*, but a shift of *Synechococcus* 7942 cells to high light (750 μmol photons m<sup>-2</sup> s<sup>-1</sup>) decreases the transcription of *psbA1* while the transcription of *psbAII* and *psbAIII* increases [28–31]. Due to enhanced turnover and differences in transcription activity between the *psbA1* and the *psbAII* and *psbAIII* genes, a rapid interchange of the D1:1 form by D1:2 occurs

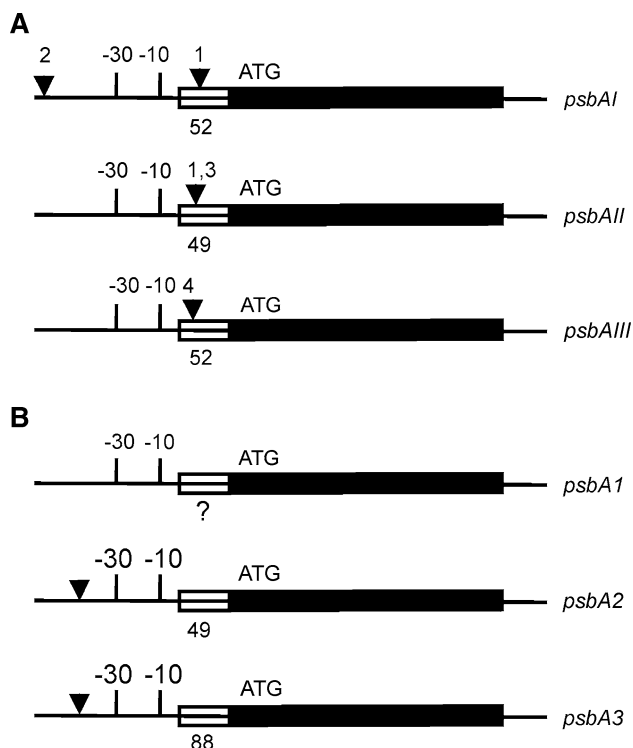
upon shift of cells to high light, which in turn is important for adaptation of cells to changing environmental cues [23, 24, 27, 32–34]. Moreover, mutant strains of *Synechococcus* 7942 [22] in which the exchange of D1:1 to D1:2 is blocked suffer enhanced inhibition of PSII under UVB and high light illumination [35], showing that the two isoforms are functionally distinct.

All three *psbA* genes in *Synechococcus* 7942 give rise to transcripts of 1.2 kb with 5' ends comprising 49–52 bases upstream from the coding region (Fig. 3a; [22]). The constitutive expression of the *psbAII* and *psbAIII* genes is driven by basal  $\sigma^{70}$  type promoter elements residing between –39 and +12 for *psbAII* and positions –38 and –1 for *psbAIII* [30], whereas in *psbA1* the  $\sigma^{70}$  promoter TATAAT is replaced by an atypical –10 element, TCTCCT [22]. In addition to the promoter of the *psbA* gene, there are regulatory elements within the transcribed region, which enhance gene expression and confer light-responsiveness.

*psbA1* promoter region encompasses nucleotides –54 to +1, and one or more proteins bind specifically to the *psbA1* upstream region (+1 to +43; [36, 37]). At least one of the regulatory factors is shared with *psbA1* and *psbAII* [37], whereas the gel mobility shift experiments have shown binding of a de novo synthesized protein factor specifically to the 5' end (66 bp) of the *psbA1* coding region [38]. This so far uncharacterized protein factor is essential for transcriptional activation of the *psbA1* gene [38]. Moreover, PsfR protein has been identified as a regulator of *psbA1* gene expression [39]. Overexpression of *psfR* results in enhanced expression of *psbA1* without an effect on *psbAII* and *III*. However, knock-out of *psfR* did not prevent *psbA1* expression. Thomas and coauthors [39] have suggested that PsfR may rather regulate gene expression via protein–protein interactions than via direct binding to the *psbA1* promoter. Moreover, a recent study suggested that the D1 protein might regulate its own synthesis: gel mobility shift experiments provided evidence that the degradation products of the D1:1 protein bind to the upstream region (–106 to –10) of the *psbA1* gene, thereby possibly regulating the efficiency of transcription [40]. Additional upstream sequences enhance expression but are not needed for light-responsive regulation.

Both *psbAII* and *psbAIII* also bind regulatory proteins in the regions +1 to +41 in *psbAII* and –2 to +38 in *psbAIII*. One such protein is CmpR, which is required for expression of the best-characterized low-CO<sub>2</sub> inducible operon *cmpABCD* [41]. The exact binding site of the CmpR protein has been shown to be the palindromic TTA-N<sub>7</sub>-TAA and TTA-N<sub>8</sub>-TAA sequences in the enhancer elements of *psbAII* and *psbAIII*, respectively [41]. Knock-out of CmpR, however, did not completely stall the expression of the *psbA* genes. Moreover, the region between the –10 basal





**Fig. 3** Regulatory elements of the *psbA* genes. **a** Regulatory elements of the *psbAI*, *psbAII* and *psbAIII* genes in *Synechococcus* 7942 (not in scale). The region composing the 1.2 kb transcript of each *psbA* gene is shown as a thick line. The coding region (starting with ATG) is marked as a solid black line and the 5'UTR as a striped line with a number below indicating the length in base pairs (bp). For each gene, the  $-10$  and  $-30$  regulatory elements (atypical TCTCCT in *psbAI*) are shown. The black triangles show the approximate binding sites for various (putative) trans-acting regulatory factors. (1) One *psbAI*-specific and at least one regulatory factor shared with *psbAI* and *psbAII* bind to the 5' end of the *psbAI* coding region. (2) The degradation products of the D1:1 protein bind to the upstream region of the *psbAI* gene. (3–4) CmpR increases the expression of *psbAII* and *psbAIII* by interacting with the TTA-N<sub>7/8</sub>-TAA sequence. Other uncharacterized regulatory proteins may be involved as well, and the AT-rich region downstream from the basal elements of the *psbAII* gene may additionally affect the gene expression. Additional negative and positive elements upstream of the basal promoter have been identified (not shown), but the interacting trans-factors remain to be elucidated. **b** Regulatory elements of the *psbA1*, *psbA2* and *psbA3* genes in *Synechocystis* 6803 (not in scale). The region composing the 1.2 kb transcript of each *psbA* gene is shown as a thick line. The coding region (starting with ATG) is marked as a solid black line, and the 5'UTR as a striped line with a number below indicating the length in base pairs (bp). The transcription start site of the *psbA1* gene is not known. For each gene, the  $-10$  and  $-30$  regulatory elements are shown. The  $-30$  site in *psbA1* differs significantly from those of *psbA2* and *psbA3*. The black triangles show the binding sites (TTCAA-N<sub>4</sub>-TTACAA) of at least one putative transcriptional repressor, which stalls transcription of the *psbA2* and *psbA3* genes in the dark

promoter and the Shine–Dalgarno sequence of the *psbAII* gene in *Synechococcus* 7942 shares similarity with that in *Microcystis aeruginosa* K-81 [42]. This AT-rich region

upstream from the Shine–Dalgarno sequence seems to function as a negative cis-element, which might bind regulatory factors and/or ribosomes affecting accumulation of the *psbAII* transcripts [42]. Additionally, upstream of the basal promoters are negative elements that depress the expression [30].

Besides the 1.2-kb *psbAII* transcripts, the *psbAII* gene also produces 1.6-kb transcripts which originate 419 bp upstream from the start site for the 1.2-kb *psbAII* mRNA. This dicistronic message contains a 342-bp ORF immediately upstream of the *psbAII* coding region [28]. Expression of the 1.6-kb transcript, however, is not light dependent [43]. Whether the gene product of this ORF is somehow involved in the regulation of *psbA* gene expression, for example as a regulatory factor, remains to be elucidated.

Induction of *psbAII/III* messages occurs not only at high light intensity, but also upon a shift of the cells to low light intensity, to UVB irradiation as well as to anoxia and to light favoring excitation of PSI [35, 38, 44–46]. However, neither heat shock nor oxidative stress produced similar responses as generated by an increase in light intensity [32]. It is conceivable that the reducing power, produced by the two photosystems, cannot be used efficiently in carbon assimilation under stress conditions, thus leading to elevated levels of thiol reductants. Reducing conditions, in turn, are likely to be sensed as a signal to induce the expression of *psbAII/III*. Indeed, addition of reduced DTT to the cell suspension was shown to induce the *psbAII/III* gene expression and down-regulation of the *psbAI* gene expression. Inhibitors of photosynthetic electron transfer chain (DCMU and DBMIB) did not cause any changes in *psbA* gene expression when added under low light conditions, but both chemicals dramatically reduced the induction of *psbAII/III* when added upon a high light shift. These results indicate that the thiol redox state rather than the redox state of the plastoquinone pool regulates *psbA* gene expression in *Synechococcus* 7942 [46].

Besides redox regulation, the expression of the *psbA* genes has been suggested to be controlled via a blue light photoreceptor. Shift of cells to low-fluence blue light was shown to induce transcription of the *psbAII/III* genes, and this induction could be reversed by a subsequent pulse of red light [47]. Since blue light has been shown to regulate chloroplast gene expression in higher plants, it is possible that components of an ancient blue light photosensory pathway are evolutionarily conserved during the divergence of plant chloroplasts from cyanobacteria [48, 49]. One possible candidate for a blue light receptor is NbLS, a putative histidine kinase, which is involved—among other things—in controlling *psbA* gene expression [50].

Concomitantly with upregulation of *psbAII* and *psbAIII* the amount of *psbAI* transcripts decreases as an immediate

reaction when *Synechococcus* cells are shifted to bright light [29, 51]. This is mostly due to destabilization of the *psbAI* transcript ( $T_{1/2} = 10\text{--}12$  min), which is dependent on the 52-nt untranslated leader sequence [29, 51]. Destabilization of the *psbAI* transcript is accompanied by a decrease in transcription activity of the *psbAI* gene [37]. Similarly, the degradation of the *psbAIII* mRNA has been shown to be accelerated at high light intensity, but the *psbAII* transcripts are long-lived and apparently not subject to post-transcriptional regulation [29]. Determinants of *psbA* mRNA turnover have been shown to reside within the untranslated leader regions of the *psbA* genes as well as within the coding region [51]. The region encoding the first membrane span of the D1 protein is essential for the stability of both the *psbAI* and *psbAII* transcripts, probably via pausing of ribosomes, which protects the mRNAs [51].

In contrast to an immediate response of cells to high light, prolonged exposure of *Synechococcus* 7942 cells to high light leads to an increased accumulation of all *psbA* transcripts, including *psbAI*. This was shown to result from restabilization of the *psbAI* transcript after several hours at high light intensity [32]. Nevertheless, despite vigorous reaccumulation of *psbAI*, no corresponding increase in the amount of D1:1 could be detected in the thylakoid membrane of high light-adapted *Synechococcus* 7942 cells [32] probably due to a high sensitivity of D1:1-containing PSII centres to photoinhibition.

#### *psbA* gene expression in *Synechocystis* sp. PCC 6803 and 6714

In *Synechocystis* sp., only one type of D1 ( $D1_m$ , see below) protein, encoded by both the *psbA2* and *psbA3* genes, has been detected under normal growth conditions as well as under most stress conditions [52]. However, recent studies have revealed anaerobiosis-induced expression of the *psbAI* gene [9, 10], which was previously thought to be a silent pseudogene. The transcript from *psbA2* accounts for 90% and from *psbA3* for 3–10% of the total *psbA* transcript pool under normal growth conditions (ca.  $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), whereas under these conditions the *psbAI* gene remains silent [52–55]. Intense illumination increases the transcription of the *psbA2* and *psbA3* genes, and this induction is not affected by addition of electron transfer inhibitors [56–61]. Studies using S1 nuclease protection assay, micro-arrays [62] and northern blotting [63] have shown that UVB exposure of the cells also induces an increase in the total *psbA* transcript pool primarily through increased accumulation of *psbA3* transcripts [57, 64]. However, inactivation of either *psbA2* or *psbA3* up-regulates the expression of the intact gene to the normal wild type level without marked effects on cell metabolism, indicating that either gene alone is sufficient to support

autotrophic growth of the cells [54, 63]. Thus, *Synechocystis* pattern of supplemental expression of an identical protein isoform under excitation stress is distinct from the D1 isoform exchange found in *Synechococcus* 7942.

In *Synechocystis* 6803, both the  $-35$  and  $-10$  elements are present in the upstream regions of the *psbA2* and *psbA3* genes (Fig. 3b). The transcription start points for *psbA2* and *psbA3* have been mapped to positions  $-49$  and  $-88$ , respectively, relative to the ATG site [52]. The promoter region of *psbA1* differs significantly from those of *psbA2* and *psbA3*, especially in the  $-35$  element, which is identical in *psbA2* and *psbA3*. *psbA1* also lacks a Shine–Dalgarno sequence, which, however, is not absolutely required for successful translation [65]. The transcription start site of *psbA1* has not yet been defined and hence the location of promoter is uncertain. In addition to the possible promoter next to the coding region of the *psbA1* gene, there is a distal promoter-like region.

*Synechocystis* 6803 mutants with modified degradation rates of the D1 protein have provided evidence that not only light intensity but also the rate of D1 synthesis regulates transcription of the *psbA2* and *psbA3* genes [5, 66–69], which additionally seems to require de novo synthesized protein factors [70, 71]. Transcription of the *psbA* genes during the recovery process after photoinhibitory treatment of *Synechocystis* 6714, however, was not prevented by inhibition of translation, and the photoinhibitory treatment longer than 40 min finally resulted in increased stability of the *psbA* messages [58]. Transcription of the *psbA* genes ceases rapidly upon shift of the cells to darkness, even in the presence of an external energy source, indicating that the energy status of the cells does not directly affect the transcription activity of the *psbA* genes in *Synechocystis* [60].

Numerous studies during the past 10 years have suggested an involvement of the intersystem redox status in the regulation of *psbA* gene expression in *Synechocystis* 6803. These experiments mainly followed the accumulation of *psbA* transcripts in cells exposed to different nutrient regimes, light quantities and qualities as well as to electron transfer inhibitors, DCMU and DBMIB. The presence of DCMU or DBMIB upon illumination of cells results in accumulation of PSII reaction centres with a reduced quinone at a  $Q_A$  site, which was suggested to act as a signal to transiently increase the amount of *psbA* transcripts in *Synechocystis* 6803 and 6714 [59]. A more recent study by the same authors suggested that the occupancy of the plastoquinone binding  $Q_O$  site in the Cyt  $b_6/f$  complex might be involved in regulation of the *psbA* gene expression. This conclusion was deduced from the fact that the reduction of the intersystem carriers activated the transcription of the *psbA* gene and destabilized the message, whereas oxidation of the electron transfer chain decreased transcription and stabilized the *psbA* message [72]. Li and

Sherman have shown that long-term (6 h) treatment of *Synechocystis* 6803 cells with either DCMU or DBMIB has strong effects on accumulation of *psbA* transcripts and indicated that reduction of the plastoquinone pool (presence of DBMIB) decreases, and oxidation (presence of DCMU) increases the expression of the *psbA* genes [69]. RppA, a response regulator of a two-component system, was suggested to sense the changes in the redox poise and accordingly to adjust the stoichiometry between PSII and PSI via regulating the expression of photosynthetic genes, e.g., *psbA* [69]. The effects of blue, orange, and far-red light on the expression of *psbA* gene in *Synechocystis* 6803 made El Bissati and coworkers conclude that light quality regulates the expression of photosynthetic genes via a redox control occurring in the Cyt  $b_6/f$  complex [73]. However, data not supporting this interpretation also exist. Comparison of the action spectra of *psbA* transcription to that of PSII activity, photosynthesis and photoinhibition [63], as well as subjecting *Synechocystis* 6803 cells to oversaturating single turn-over flashes inducing photoinhibition but without affecting the oxidation state of the intersystem redox carriers [61], made the authors conclude that the redox state of the electron transfer chain is an unlikely candidate to carry information for regulation of *psbA* expression.

The half-life of *psbA2* and *psbA3* transcripts in *Synechocystis* 6803 under illumination is around 10–20 min, and independent of light intensity or the rate of PSII electron transfer [56, 58, 59, 66, 72, 74], whereas the stability of *psbA* transcripts increases remarkably in darkness [56, 60, 71, 75]. Stabilization of transcripts is dependent on cessation of photosynthetic electron transfer rather than on light per se [56, 60, 61, 72]. Other factors, such as polyamines, have also been suggested to affect the stability of *psbA* transcripts [71]. Transfer of dark-treated cells back to light induces rapid protein-synthesis-independent accumulation of *psbA* transcripts [60]. Indeed, the 170-bp upstream region of the *psbA2* gene was shown to bind protein factors in the dark, suggesting that the transcription of the *psbA2* gene is down-regulated in darkness via transcriptional repressor proteins [60]. Specifically, a hexanucleotide direct repeat, TTACAA-N<sub>4</sub>-TTACAA, found in the promoter region of *Synechocystis* 6803 *psbA2* and *psbA3* genes as well as in *Anabaena* 7120, has been shown to act as a binding site for a putative repressor in the dark (Fig. 3b; [76]).

### Translational regulation of D1 synthesis in cyanobacteria

It is well known that translation is a key regulatory step in the chloroplasts of higher plants, and many trans-acting

factors involved in translation of the D1 protein in chloroplasts have been characterized since the 1980s [77]. Evidence is accumulating indicating that the D1 protein synthesis in cyanobacteria is also not solely controlled at the transcriptional level, e.g., in *Synechococcus* 7942, practically no D1:1 protein accumulated upon stress in the thylakoid membrane, even though high amounts of *psbAI* transcripts were present [32, 35, 46, 78]. Moreover, upon anoxia and under thiol-reducing conditions in the presence of electron transfer inhibitors, a substantial amount of *psbAII/III* messages accumulates without synthesis of the D1:2 protein [45, 79]. It has been shown that, if there are *psbA* transcripts present in *Synechococcus* 7942, the messages are always efficiently associated with ribosomes, suggesting that initiation of translation does not play a significant role in regulation of *psbA* gene expression, whereas membrane targeting of nascent D1 protein ribosome complex might, at least under some conditions, be a rate-limiting step for D1 protein synthesis [5]. Elongation of translation is also under strict regulation in *Synechocystis* 6803: upon shift of the cells from light to darkness, the abundant *psbA* transcripts keep attached to ribosomes and D1 translation continues up to a distinct pausing site. However, the newly formed ribosome-nascent D1 chain complexes are not targeted to the thylakoid membrane, and therefore no complete D1 synthesis takes place in the dark [80]. Indeed, the ribosome complexes are targeted to the thylakoid membranes and D1 synthesis can be completed only in light to replace the damaged D1 protein, indicating that it is not the initiation of translation but rather the translational elongation that is an important regulatory step in expression of the *psbA* genes [80]. Translational elongation of proteins in general and in particular that of the D1 protein is also sensitive to singlet oxygen, which is generated especially during photosynthesis [81, 82]. An additional regulatory factor is the availability of chlorophyll: although the accumulation of *psbA* transcripts is independent of chlorophyll availability, the lack of chlorophyll seems to affect the initiation of *psbA* translation [83]. Further complexity to the regulation of *psbA* gene expression is provided by the membrane insertion and C-terminal processing of the D1 protein as well as the assembly of PSII (see [84] and references therein). The different steps of PSII assembly and function are assisted by a number of auxiliary proteins, including factors involved in degradation of the damaged D1 protein, in translation and membrane insertion of the new D1 copy, and in PSII (super) complex formation and activation [85, 86]. Putative antisense mRNAs against various *psbA* genes in *Synechocystis* 6803 might represent a novel regulatory network in *psbA* gene expression and are presently under intense research (Wolfgang Hess, unpublished results).

## Functional classification of cyanobacterial D1 proteins

Nearly 40 fully sequenced cyanobacterial genomes together with functional characterization of several *psbA* gene families make it now possible to attempt to classify this important gene family and its product, the D1 protein. This, however, faces a serious difficulty since the *psbA* genes within a species tend to be more closely related to their family relatives than to functionally similar members of other species. Therefore, the classification of D1 isoforms based solely on the genomic data is unavoidably prone to errors. In order to be accurate, one has to take into consideration the specific response and behavior of the individual members of the gene family as a response to a variety of factors. From evidence accumulated so far, we attempt here a classification of the D1 isoforms based on the manner that their expression is regulated under typical growth conditions as well as under various environmental stresses (Fig. 2; Table 1).

From a functional point of view cyanobacterial D1 proteins can be divided into the following four categories:

- i) D1<sub>m</sub> is a D1 form expressed and present in the PSII centres under normal growth conditions. D1<sub>m</sub> is also induced under most stress conditions (m denotes for “major”);
- ii) D1:1 is a D1 form expressed and assembled into PSII under normal growth conditions, but repressed under stress;
- iii) D1:2 is a D1 form repressed under normal growth conditions, but induced and accumulated into PSII by stress;
- iv) D1' is a D1 form virtually silent under standard growth conditions, but induced under microaerobic/low oxygen conditions

In the following section, we will characterize the above-mentioned D1 isoforms one by one. The individual functional distinctiveness of each D1 form, and the justification of placing them in these distinct categories, is also discussed.

### The D1<sub>m</sub> isoform

Characteristic to the D1<sub>m</sub> isoform is its presence in PSII centres both under normal growth conditions and when the cells are exposed to stress. This isoform thus contributes to the maintenance of functional PSII, but the defining trait is its increased expression under environmental stress conditions that speed up D1 degradation. A typical example of D1<sub>m</sub> is the D1 isoform encoded by the *psbA2* and *psbA3* genes in *Synechocystis* 6803 (see “[psbA gene expression in \*Synechocystis\* sp. PCC 6803 and 6714](#)”). In this cyanobacterium, the *psbA2* transcript is responsible for almost all

the D1 protein produced under regular growth. When the rate of D1 degradation increases, e.g., upon exposure of the cells to high light or UVB stress, the transcription of the *psbA3* gene is considerably enhanced thereby enabling an increased turnover rate of the D1 protein (Fig. 2b). When the stress condition is removed, the pattern of the *psbA2* and *psbA3* gene expression reverses. Both genes are alone sufficient to support normal autotrophic growth mode of the cells [54, 87].

Interestingly, *Gloeobaacter violaceus* PCC 7421, a cyanobacterium that shows deep molecular and ultrastructural divergence from other cyanobacteria and is considered very primitive, regulates its five *psbA* genes in a similar manner as *Synechocystis* 6803 [88]. Under standard growth conditions (10 μmol photons m<sup>-2</sup> s<sup>-1</sup>, 25°C), the D1 protein is produced by *psbAI* (*glr2322*) and *psbAII* (*glr0779*) both showing considerably high levels of expression, whereas under stress, *psbAIII* (*gll3144*) is induced supplementing the available transcripts. All three genes are encoding the same D1 isoform. The other two *psbA* genes in *Gloeobaacter* encode distinctly different D1 proteins of unclear function. One of them, *psbAV* (*glr2656*), encodes the most divergent *psbA* sequence known to date [88].

### The D1:1 isoform

D1:1, also known as D1 form 1, is best described in *Synechococcus* 7942 and is, together with D1:2, part of a specific regulation mechanism in response to stress conditions. Recently, a similar stress-response mechanism was revealed in *Anabaena* PCC 7120, *Thermosynechococcus elongatus* BP-1, and the marine *Synechococcus* WH 7803 [89]. These are diverse cyanobacterial species with no easily traceable phylogenetic relationship. The defining characteristic of D1:1 is its high level of expression in well-acclimated cells, and a distinct down-regulation upon abrupt changes in the standard growth conditions (Fig. 2a). Once the cell acclimates to the new status quo or the stress is removed the expression of the genes encoding D1:1 increases and it becomes once again the dominant D1 form in PSII centres. For some time, D1:1 was also associated with the presence of a specific amino acid at position 130 proven to influence the potential of the redox active D1-Phe residue (see discussion in the next section). This is situated in a conserved portion of the D1 protein and it has been proven true, so far, that the D1:1 protein always contains a glutamine residue at this position. While this is true, the reverse is not: not every D1 with a glutamine at 130 is a D1:1. A clear example is the low-expressed, divergent D1' encoding *psbA* genes from *Anabaena* 7120 (*psbA0*) and *Synechocystis* 6803 (*psbA1*) that have a glutamine at position 130 but functionally are clearly not D1:1 forms (see the following section on D1').



## The D1:2 isoform

The D1:2 isoform reflects the mirror image of its “sister” form D1:1 in its expression. D1:2 is expressed at low level in well-acclimated cells, but upon stress conditions the expression is markedly induced and D1:2 replaces D1:1 in PSII centres (Fig. 2a). Upon acclimation or removal of stress, the D1:2 will be repressed and replaced by D1:1. This D1 form exchange is one of the clearest mechanisms documented so far on gene regulation as adaptation to changing environmental conditions, and it was well documented in *Synechococcus* 7942 by numerous studies (see “*psbA* gene expression in *Synechococcus elongatus* PCC 7942”). The exchange is facilitated by the fast turnover rate of the D1 protein, establishing a correlation between the expression levels of distinct *psbA* genes, the form of D1 protein present in PSII centres, and the functional characteristics of PSII. The evolutionary development and maintenance of this regulation mechanism speaks for a direct requirement of the functional characteristics of both D1 forms, thus offering unique advantages under their specific expression conditions. A good question raised by the presence of D1:2 under stress conditions is why the cells do not always possess D1:2 in the PSII centres? The answer, while not clear yet, has to do with a discreet advantage of D1:1 over D1:2 under standard growth conditions. Maintenance of D1:1 in the PSII centres seems to give an evolutionary advantage over mutants containing only the D1:2 form, generated by random mutagenesis.

D1:2 is a functionally well-defined form and different from its counterpart D1:1, as shown by artificially made mutant strains of *Synechococcus* 7942 [22]. In these strains, the exchange of D1:1 to D1:2 is blocked, which results in enhanced PSII photoinhibition under UVB and high light illumination [35]. Furthermore, it has been shown that a transgenic *Synechocystis* 6803 strain expressing the *Synechococcus* 7942 D1:2 isoform possesses a faster decay of variable fluorescence in the presence of DCMU, reflecting faster recombination of reduced  $Q_A$  with positive charges on the donor side of PSII, compared to a *Synechocystis* 6803 mutant expressing only the D1:1 isoform from *Synechococcus* 7942 [25]. Thermoluminescence [27] and fluorescence life time data [26] from *Synechococcus* 7942 cells containing only the D1:1 or the D1:2 isoform also support enhanced charge recombination in PSII centers containing D1:2. It is important to note that this functional difference between the D1:1 and D1:2 isoforms is correlated with the presence of a glutamate residue in D1:2 instead of a glutamine residue at position 130 in the D1 protein sequence, which interacts with a key Phe co-factor [7, 90]. It was recently shown that Glu occupies D1-130 position in all high-light D1 isoforms identified so far, whereas Gln is found in the low light D1 isoforms, like

D1:1 [91]. The correlation of the D1 Gln130Glu amino acid replacement with phototolerance is apparently related to accelerated charge recombination, i.e., to the existence of an  $E_m$  (Phe/Phe<sup>-</sup>)-dependent photoprotection [92]. It is worth noting here that the single D1 protein in higher plants has strictly conserved Glu130 residue.

## The D1' isoform

The term D1' was for a long time used only for the product of *psbA1* gene in *Synechocystis* 6803. This gene was considered enigmatic as its open reading frame was intact but its transcript could not be detected by classic hybridization methods [54, 56, 75]. However, artificial activation of *psbA1* in *Synechocystis* 6803 [53], via replacement of the 320-bp upstream fragment of the *psbA1* gene with a 160-bp upstream fragment of *psbA2*, leads to production of a functional and light-responsive, albeit aberrant, D1 protein [93, 94]. Whole genome sequences and functional studies recently published from several cyanobacterial species (<http://www.kazusa.or.jp/cyano/>, <http://genome.jgi-psf.org/>) have revealed that, apart from *Synechocystis* 6803, a *psbA1*-type divergent, low-expressed and non-responsive *psbA* gene also exist in *Anabaena* sp. PCC 7120 (*psbA0*-alr3742) [95], *Thermosynechococcus elongatus* BP-1 (*psbA2*-tlr1844) [91, 96], *Cyanothece* sp. ATCC 51142 (*psbA2*) [10], and possibly *Gloeobacter violaceus* PCC 7421 [88]. So far, in cyanobacterial species where the expression of the *psbA* gene family has been characterized, *Synechococcus* 7942 is the only species that does not contain such a gene [32].

During the past year, two independent studies demonstrated induction of the *psbA* gene encoding the D1' isoform under microaerobic [9] or low oxygen [10] conditions in *Synechocystis* 6803, *Anabaena* 7120, *Thermosynechococcus elongatus* BP-1, and *Cyanothece* sp. ATCC 51142 (Fig. 2c). Already earlier, the *Anabaena psbA0* (alr3742) gene was found to be expressed at a very low constitutive level and was not responsive to photo-oxidative UVB stress, light stress, or nitrogen stress [95]. This behavior of *psbA0* of *Anabaena* 7120 is very similar to that of *psbA1* of *Synechocystis* 6803. Indeed, both these genes are in fact not silent even under normal growth conditions, but transcribed to a very low level insufficient to play a significant role as part of the cellular *psbA* transcript pool [95].

While still we do not have an answer regarding the direct functional role of the D1' isoform, we can now with a high degree of confidence establish D1' as a widely distributed D1 isoform important for adaptation of cyanobacterial cells to specific environmental conditions, particularly to a microaerobic pressure. The conditions requiring the presence of D1' thus have a sufficient evolutionary importance to ensure that the gene has been selectively maintained in the genome. There are no major

sequence characteristics that would individualize the D1' from the other D1 forms. In the D1 forms functionally characterized [9], there are only three amino acid residues that are both conserved in all traditional D1 forms and mutated but conserved across all the D1' proteins. These modifications are Gly80Ala, Phe158Leu, and Thr286Ala [9]. The low-oxygen-pressure-induced D1' form encoded by *psbA2* in *Cyanothece* [10] also has the same consistent modifications. Some initial molecular modelling studies did not show any major D1 conformational changes [9], and it is not yet clear what, if any, are the implications of these modifications on the general PSII function. It is worth mentioning, however, that at least two of the conserved modifications in D1' (Phe158Leu and Thr286Ala) are located in the binding pockets of important Chl residues: ChlD1, the place of initial charge separation, and Chl PD1, the most probable location of the stable cation P680<sup>+</sup>. The fact that the genes encoding D1' are present in such a diverse array of cyanobacterial species and that all these genes apparently respond to the same environmental cue makes it very likely that the encoded D1 protein belongs to a distinct category: D1'. The clear selective advantage of D1' over the regular D1 is not obvious and remains to be identified. Also, while under standard laboratory growth conditions the cells do not usually experience periods of microaerobic growth, in the natural environment they do occur as a result from imbalanced cellular metabolism, specific environmental conditions or due to niche a given cyanobacteria inhabits, e.g., in microbial mats [97].

### *psbA* genes in cyanophages

Cyanobacteria are responsible for a majority of primary production in oligotrophic regions of oceans. Cyanophages, viruses that infect cyanobacteria, are equally abundant in marine ecosystems, and probably exert major ecological effects on the marine environment [98, 99]. The first cyanophages were isolated in 1963 [100], but only recently it has been found that many of the cyanophages infecting *Synechococcus* and *Prochlorococcus* hosts possess *psbA* and *psbD* genes encoding the D1 and D2 proteins, respectively [101–107]. The photosynthesis genes in cyanophages originate from their host cyanobacteria, and thus show marked (up to 95%) identity to their host homologs at amino acid level [103, 105, 108, 109]. Accordingly, *Prochlorococcus* myoviruses and podoviruses encode the only D1 form found in *Prochlorococcus*, whereas in *Synechococcus* myoviruses, the stress-responsive D1:2 form of *Synechococcus* D1 protein has been selected over D1:1 [107].

It has been shown that the cyanophage *psbA* gene is indeed both transcribed and translated in cyanobacteria

during infection [104, 110], and at the same time the expression of the host photosynthesis genes declines [104]. Since all bacteriophages rely on their hosts to provide energy and carbon sources for replication and assembly, it is conceivable that the expression of the cyanophage *psbA* gene allows continuous operation of the PSII repair cycle (Fig. 1), even if infection down-regulates the expression of the host *psbA* gene. Thus, the D1 protein encoded by the cyanophage is likely to replace the light-sensitive D1 protein of the host (despite possessing some unique features in structure [111]), thus allowing photosynthesis to continue efficiently even under bright light [104, 110, 112, 113], and thereby provide the energy needed by the virus for its replication. Obviously, the prevalence of photosynthesis genes in cyanophages serves as a valuable genetic reservoir for the host, and has probably played a role in driving host niche differentiation [105].

### Summary

The D1 protein of PSII is a specialized protein component targeted to photodamage and rapid turnover in all organisms performing oxygenic photosynthesis. In cyanobacteria, which can inhabit various extreme habitats, the D1 protein is encoded by a small gene family. Obviously, the range of environmental factors cyanobacteria may face during their life cycle requires the possibility to modify the expression of the *psbA* genes, and more importantly the presence of a proper D1 isoform in PSII centres, in order to guarantee the best adaptation and fitness under given conditions. Optimal adaptation to varying environmental conditions seems to be obtained via complex regulation of not only one, but several, *psbA* genes. During the past two decades, it has become clear that there is not a single, universal pattern of regulation of *psbA* gene expression in cyanobacteria, but rather the ecological niche occupied by the individual strain has guided the evolution towards proper adaptation and satisfactory fitness. Ongoing release of genomic data from a vast number of cyanobacterial species will certainly provide additional tools to further study the regulatory factors of *psbA* gene expression in different cyanobacterial species. Understanding the adaptation processes of the photosynthetic machinery to changing environmental cues will be of utmost importance when biotechnological applications, for example the production of biofuels and improvement of crop yields, are to be developed.

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