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Functional analysis of the two homologous *psbA* gene copies in *Synechocystis* PCC 6714 and PCC 6803

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Abstract

The cyanobacteria *Synechocystis* 6803 and 6714 contain three genes (*psbA*) coding for the D_1 protein. This protein is an essential subunit of photosystem II (PSII) and is the target for herbicides. We have used herbicide-resistant mutants to study the role of the two homologous copies of the *psbA* genes in both strains (the third copy is not expressed). Several herbicide resistance mutations map within the *psbAI* gene in *Synechocystis* 6714 (G. Ajlani *et al.*, Plant Mol. Biol. 13 (1989): 469–479). We have looked for mutations in copy II. Results show that in *Synechocystis* 6714, only *psbAI* contains herbicide resistance mutations. Relative expression of *psbAI* and *psbAII* has been measured by analysing the proportions of resistant and sensitive D_1 in the thylakoid membranes of the mutants. In normal growth conditions, 95% resistant D_1 and 5% sensitive D_1 were found. In high light conditions, expression of *psbAII* was enhanced, producing 15% sensitive D_1. This enhancement is specifically due to high light and not to the decrease of D_1 concentration caused by photoinhibition. Copy I of *Synechocystis* 6714 corresponds to copy 2 of *Synechocystis* 6803 since it was always *psbA2* which was recombined in *Synechocystis* 6803 transformants. PSII of the transformant strains was found to be 95% resistant to herbicides as in resistant mutants of *Synechocystis* 6714.

Introduction

Cyanobacteria, like higher plants, carry out oxygenic photosynthesis, which requires photochemical reactions at two multiprotein complexes termed photosystems I and II. The reaction centre core of photosystem II contains a heterodimer of two proteins, D_1 and D_2. D_1, encoded by the *psbA* gene, is the target for herbicides like DCMU, atrazine and ioxynil. These herbicides interrupt photosystem II electron transfer by displacing Q_B, the secondary quinonic electron acceptor, from its site on the D_1 protein (for a review, see [24]).

In cyanobacteria, *psbA* belongs to a multigene family with three or more copies. For example, in *Anabaena* PCC 7120, there are four copies of *psbA*. Three copies (*psbAII–IV*) are very similar and encode identical D_1 polypeptides which differ from the *psbAI* product by 21 amino acids. *psbAI* and one or more of the other copies are expressed, so that two forms of D_1 can be present in the thylakoids [26].

In *Synechococcus* PCC 7942, there are three

copies of *psbA*. Two of these (*psbAII* and *III*) encode identical D_1 polypeptides, which differ from the *psbAI* product by 25 amino acids [7]. These three copies are expressed with different efficiencies, so that two different forms of D_1 are present in the thylakoids. Each of the copies is capable of producing sufficient functional D_1 protein to support normal photoautotrophic growth [7]. Mutations conferring herbicide resistance were introduced in each of the three copies [4]. Strains with varying combinations of R (resistant) and S (sensitive) alleles of *psbA* were analysed and expression of the genes was measured by RNA determination and by quantification of R and S PSII using variable fluorescence [4, 21]. These experiments allowed for analysis of dominance in cyanobacteria containing R and S alleles of *psbA*. Strains containing R *psbA* are resistant to herbicide proportionally to the expression of the R gene [4]. The relative expression of the copies can be modified by light intensity [22, 5].

In *Synechocystis* PCC 6803, there are three copies of *psbA* [10]. Two of these (*psbA2* and *psbA3*) are highly homologous (99.4% identity at the nucleotide level) [20, 16], produce stable transcripts of 1.3 kb [17] and encode identical gene products. The third copy (*psbA1*) is divergent [18] and no transcripts are detected [17]. Therefore only one form of D_1 can be found in thylakoids. Strains containing only one of the two homologous gene copies are still capable of photosynthetic growth [17].

In *Synechocystis* PCC 6714, the situation is very similar to that of *Synechocystis* 6803. Two homologous copies, *psbAI* and *psbAII*, and a divergent copy, *psbAIII*, have been detected [1]. *psbAI* has been cloned and sequenced [1]. This gene is highly homologous to *psbA2* and *psbA3* of *Synechocystis* 6803 (97% identity at the nucleotide level) and encodes a D_1 protein with only two different amino acids.

In *Synechocystis* 6803, the presence of only one of the two homologous copies is sufficient for photosynthesis [17]. This result shows that each of the two homologous copies can be functional in the absence of the other copy. However, when the two homologous copies are present, their relative expression is unknown, their messengers being identical. We are interested in knowing the expression of the two homologous copies of *psbA* in *Synechocystis* 6714 and *Synechocystis* 6803. For this purpose, we have used our collection of herbicide-resistant mutants of *Synechocystis* 6714. Molecular analysis of these mutants has revealed point mutations in the *psbAI* copy [1, 11, 2, 6, 3, 19]. In this paper we present the genetic and molecular analysis of the *psbAII* copies of several of these mutants and the characterization of their PSII centres. Transformants of *Synechocystis* 6803 into which herbicide resistance has been transferred were analysed to correlate the two homologous copies of the two strains and to evaluate their relative expression.

Materials and methods

Cyanobacterial strains

Synechocystis 6714 and 6803 (wild type, mutants and transformants) were grown photoautotrophically. The mineral medium was that described [9] with twice the concentration of nitrate. For the solid medium, 1.5% agar autoclaved separately was added. Standard growth was achieved by incubation in a Gallenkamp rotary shaker at 34 °C in a CO_2-enriched atmosphere, under 70 μE m^{-2} s^{-1}.

Measurements of herbicide resistance

The inhibition by herbicides of photosystem II electron transport between Q_A and Q_B was evaluated by measuring the changes in chlorophyll fluorescence as described [3]. The chlorophyll fluorescence yield is known to be controlled by the redox state of Q_A. In conditions where photosystem I is preferentially excited, there are very few PSII centres in the Q_A^- state and the fluorescence yield is low (F_0 level). Addition of herbicide blocking electron transfer between Q_A and Q_B produced an increase of the fluorescence quasi proportional to the number of PSII centres

blocked in the Q_A^- state by the herbicide. Complete inhibition raises fluorescence to its maximum level F_m. The apparatus was previously described [25]. Cell suspensions contained 1 μg chlorophyll per ml.

High-light experiments

Cell suspensions (20 ml at 30 μg chlorophyll per ml) were illuminated with an Atralux spot of 150 W, in a stirred thermostated cuvette in the absence or the presence of streptomycin (250 μg/ml). At various times samples were withdrawn and diluted at 1 μg/ml in growth medium buffered at pH 7.1 (50 mM Hepes) for fluorescence measurements.

DNA preparation

Genomic DNA was isolated from exponentially growing cultures as described [1]. DNA fragments containing copies 1, 2 and 3 of the *psbA* gene were separated by electrophoresis on agarose and electroelution.

Transformation of Synechocystis *6803 wild type*

0.5 ml containing 2×10^8 cells per ml of wild-type *Synechocystis* 6803 growing exponentially was mixed in 2.5 ml top agarose and plated onto minimal medium. DNA (4 to 25 μg in 10 μl) was dotted onto this lawn of recipient cells. After 16 h incubation in light at 34 °C, selective herbicide was added under the agar. Surviving colonies were individually streaked onto herbicide-containing plates and further allowed to grow on minimal medium and their phenotypes analysed.

Southern analysis

2 kb *Eco* RI-*Hin*d III *psbAI* fragment and 0.7 kb *Kpn* I-*Kpn* I *psbAI* fragment were nick-translated to produce radioactive probes.

Synthetic oligonucleotides were obtained with a Milligen-Biosearch 7500 DNA synthesizer. ^{32}P-end-labelled probe was prepared with T4 polynucleotide kinase.

After electrophoresis, DNA restriction fragments were transferred to nitrocellulose filters. For *psbA* probes, hybridizations were performed under high-stringency conditions (42 °C, 3 × SSC, 0.1% SDS and 50% formamide, overnight) and filters were washed for 2 × 30 min in 3 × SSC, 0.5% SDS at 60 °C. For oligomeric probes, hybridizations were performed under low-stringency conditions (42 °C, 2 × SSC, 0.1% SDS, overnight) and filters were washed for 5 min in 2 × SSC at room temperature.

Results

Mapping of the psbA *mutations to the gene copies of* Synechocystis *6714 resistant mutants.*

We found mutations in the *psbAI* gene of all *Synechocystis* 6714 mutants we have isolated [1, 11, 2, 6, 3, 19]. Mutations and phenotypes of the mutants analysed in this paper are described in Table 1. As interchromosomal recombination has been shown to occur in *Synechocystis* 6803 [8], a strain very closely related to *Synechocystis* 6714, and as nothing is known about the relative expression of the two homologous copies, we searched for mutations in *psbAII* by various methods.

Transformation experiments

DNA fractions of 2, 4.5 and 3 kb containing copies 1, 2 and 3, respectively, were isolated from mutant DCMU-IIA, which is resistant to DCMU [1] and was used to transform wild-type *Synechocystis* 6803 cells. Figure 1 shows that only genomic DNA and the fraction containing *psbAI* were able to transfer resistance to wild-type cells. No transformants resistant to DCMU were isolated with DNAs from fractions containing *psbAII* and *psbAIII*.

Molecular analysis

The mutant IOX-IIA, which is resistant to ioxynil [6], presents a mutation in the *psbAI* gene giving

Table 1. Mutations and phenotypes of various herbicide-resistant mutants of *Synechocystis* 6714. I_{50} is the herbicide concentration needed to block half of the variable fluorescence (see Materials and methods), and R/S is the ratio between the I_{50} of the mutant and the I_{50} of the wild type.

Strains	Mutation(s)	R/S			
		DCMU	Atrazine	Metribuzin	Ioxynil
DCMU IIA	264Ser/Ala	≈1000	100	≈1000	0.8
DCMU IIB	264Ser/Ala-255Phe/Leu	≈1000	2	≈1000	3
Iox IA	266Asn/Thr	0.8	1	7	10
Iox IIA	266Asn/Asp	0.7	1		4

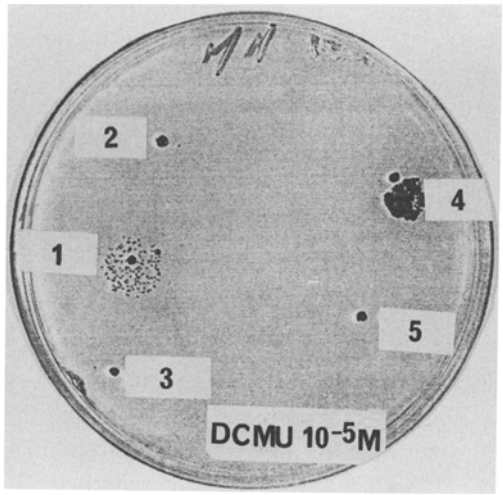

Fig. 1. Dot transformation of *Synechocystis* 6803 wild type by DNA fractions containing one of the copies of the *psbA* gene of the DCMU-resistant mutant of *Synechocystis* 6714 (DCMU-IIA). 4 μg of DNA fraction containing *psbAI*, *psbAII* and *psbAIII* were deposited in 1, 2 and 3, respectively. 25 μg of genomic DNA of DCMUII-A and buffer alone as controls were deposited in 4 and 5, respectively. Plates contained 10^{-5} M DCMU.

an Asp instead of an Asn at amino acid 266 of D_1. This mutation (A→G) introduces a unique *Taq* I restriction site in the *Kpn* I-*Kpn* I fragment (0.7 kb) of the *psbAI* gene (see Fig. 2). Digestion of genomic DNAs by *Taq* I and hybridization, in high-stringency conditions, with the *Kpn* I-*Kpn* I 0.7 kb fragment of the wild-type *psbAI* gene as a probe, gave 2 bands (1.3 and 1.1 kb) for mutants DCMU-IIA and IOX-IA and 3 bands (1.1, 0.7 and 0.6 kb) for mutant IOX-IIA (Fig. 3). As only one of the two copies in the IOX-IIA mutant was cut by *Taq* I, we can conclude that only *psbAI* possesses the mutation.

To eliminate any doubt, we further characterized the two copies [4] by specific hybridization with a synthetic oligomer corresponding to nucleotides 784–802 (amino acids 262–281) of wild-type *psbAI* (Fig. 2). Genomic DNAs from the wild type and IOX-IIA were digested by *Hin*d III and *Eco* RI and hybridizations with the 0.7 kb (*Knp* I-*Knp* I) fragment and the 784–802 oligomer as probes were performed. Figure 4 shows that for the wild type both copies showed good hybridization with the 0.7 kb probe as well as with the oligomer probe. In contrast, in the IOX-IIA mutant, both copies hybridized with the 0.7 kb probe, but only *psbAII* showed hybridization with the oligomer probe, thus confirming the presence of a mutation only in *psbAI*.

Analysis of the proportion of PSII centres resistant in mutant strains

Mutations conferring herbicide resistance have been found only in *psbAI*. If both copies *psbAI* and *psbAII* were expressed, two types of D_1 would be present in the mutant thylakoids in proportions depending upon their relative expression. It is known that under selective conditions, *psbAI* is dominant because mutant cells are able to grow in the presence of herbicide. In the absence of selective pressure, we checked the proportion of resistant and sensitive PSII in the mutant cells by measuring the PSII inhibition produced by various herbicide concentrations. Figure 5 shows in-

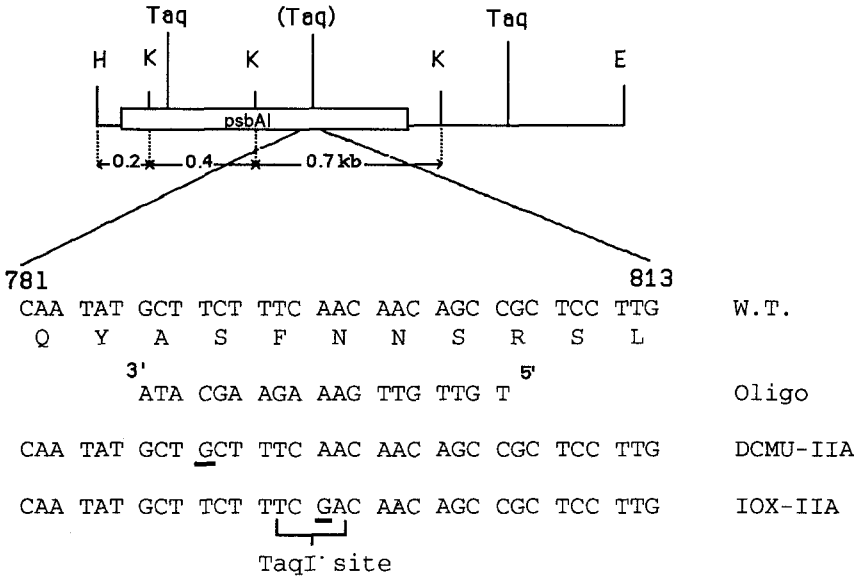

Fig. 2. Restriction map of *Synechocystis* 6714 *psbAI* fragment and sequences of part of the *psbA* gene of the wild type and two mutants. The sequence of the synthesized oligonucleotide, complementary to the wild-type sequence and used as a probe for the presence of the mutations (see text), is also shown. Underlined nucleotides indicate point mutations.

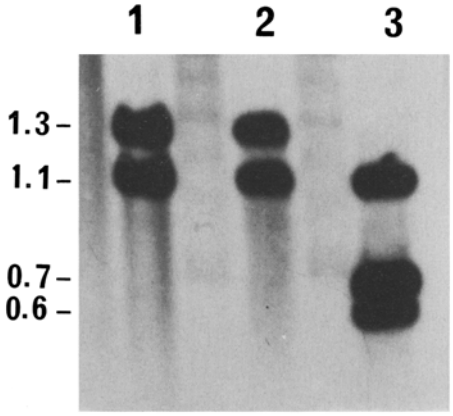

Fig. 3. Southern blot analysis of genomic DNA from three herbicide-resistant mutants of *Synechocystis* 6714 digested by *Taq* I. 1, DNA of DCMUII-A; 2, DNA of IoxI-A; 3, DNA of IoxII-A. DNAs (10 μg) were digested by *Taq* I, separated by agarose gel electrophoresis and transferred to nitrocellulose membranes. The probe used was the 0.7 kb fragment of *psbAI* of *Synechocystis* 6714 wild-type. Hybridization was carried out under high-stringency conditions.

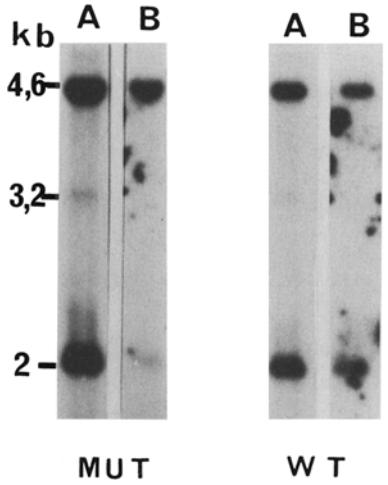

Fig. 4. Southern blot analysis of genomic DNA from *Synechocystis* 6714 wild type and IoxI-A mutant digested by *Eco* RI + *Hin*d III. A. Hybridization with the 0.7 kb fragment of *psbAI* of *Synechocystis* 6714 wild type at high-stringency conditions. B. Hybridization with the synthetic oligonucleotide (described in Fig. 2) at low-stringency conditions.

hibition curves obtained by addition of DCMU to wild-type and DCMU-IIA mutant cells (closed symbols). Wild-type cells present a high sensitivity to DCMU ($I_{50} = 0.13\ \mu M$) and DCMU-IIA cells a low sensitivity to DCMU ($I_{50} = 0.1$ mM), I_{50} being the DCMU concentration which blocks half of the variable fluorescence. In the mutant, little inhibition ($5 \pm 2\%$) was observed in the 1–10 μM DCMU region where sensitive D_1 is

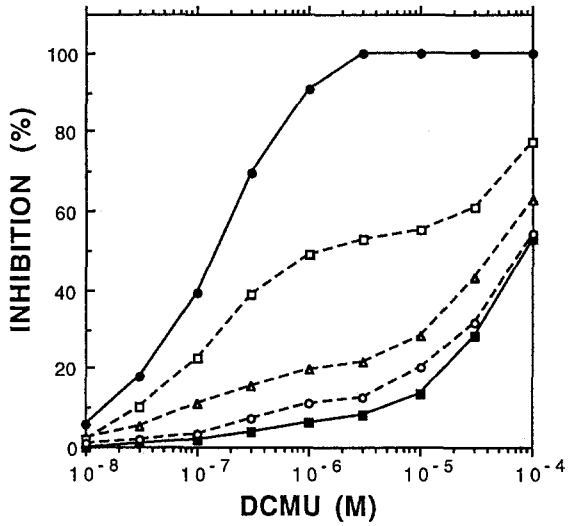

Fig. 5. Photosystem II inhibition by DCMU of *Synechocystis* 6714 wild-type cells (●) of DCMUII-A cells (■) and of mixtures of mutant and wild-type cells in the following proportions: 95%/5% (○), 90%/10% (△) and 50%/50% (□). For experiments with mutant cells, 100% inhibition was obtained by addition of 50 mM ioxynil.

completely inhibited. To test the precision of our method for detection of sensitive PSII centres in the mutant, we measured inhibition curves of mixtures of mutant and wild-type cells in various proportions: 95%/5%, 90%/10% and 50%/50% (Fig. 5, open symbols). The presence of sensitive cells clearly increased the inhibition in the $-10\,\mu$M DCMU region proportionally to the percentage of wild-type cells present. These results indicate that *psbAI* is expressed much more strongly than *psbAII*, as the maximum quantity of sensitive D_1 which can be estimated in the mutant is $5 \pm 2\%$ of the total D_1 concentration. Similar experiments were performed with the DCMU-IIB mutant [1] (inhibition by DCMU and metribuzin) and with the ID mutant [19] (inhibition by metribuzin) and similar results were obtained.

Relative expression of the two copies in Synechocystis *6714 mutant strains under different light intensities*

DCMU-IIB cells were incubated in high light ($1000\,\mu$E m^{-2} s^{-1}) for various times, at 34 °C,

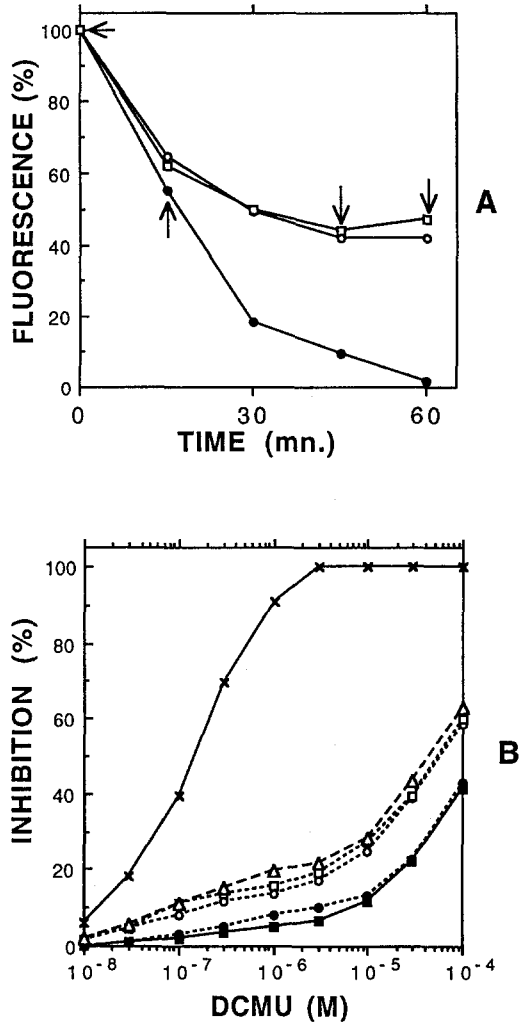

Fig. 6. Effect of high light on the relative expression of the two copies *psbAI* and *psbAII* in the *Synechocystis* 6714 DCMU-II-B mutant. A. Effect of high light at 34 °C on PSII variable fluorescence versus time of illumination in the presence (●) and the absence (○, □) of streptomycin. ○, □ represent two different experiments. Arrows indicate the samples for which inhibition curves by DCMU were performed. B. Inhibition curves by DCMU of DCMUII-B cells unilluminated (■), illuminated for 15 min in the presence of streptomycin (●), illuminated for 45 min (○) and 60 min (□) in the absence of streptomycin. For comparison, the inhibition curve of wild-type cells (x) and of a mixture of 90% mutant and 10% wild-type cells (△) are shown. Other details as in Fig. 5.

in the presence or the absence of the translation inhibitor streptomycin. Under such illumination, photoinhibition occurred, producing a decrease in PSII fluorescence. The maximum level (F_m)

decreased whereas the F_0 level did not change. We have previously shown that the decrease of variable fluorescence F_m-F_0 is closely related to the decrease of the PSII electron flow [13]. Figure 6A shows that the decrease of variable fluorescence was greater in the presence (closed symbols) than in the absence of streptomycin (open symbols). The difference between the two curves represents fluorescence of PSII containing high-light-synthesized D_1. We compared the DCMU inhibition curves (Fig. 6B) of samples exposed or not exposed to high light in the presence and in the absence of streptomycin (the samples analysed are indicated by arrows in Fig. 6A). Comparison of unilluminated samples with samples illuminated in the presence of streptomycin shows that DCMU inhibition curves are similar (presence of about 5% of sensitive D_1). Therefore both forms of D_1 were similarly damaged by high light treatment. DCMU inhibition curves of samples illuminated for 45 and 60 min show the presence of about 15% sensitive D_1. For comparison, a DCMU inhibition curve of a mixture of 90% mutant and 10% wild-type cells is presented in Fig. 6B.

To determine whether the enhancement of *psbAII* expression (giving sensitive D_1) is induced by high light *per se* or by the decrease of D_1 concentration after this high illumination, the following experiment was performed. D_1 concentration was decreased by high light at 20 °C, a temperature too low to permit efficient synthesis [12] (Fig. 7A). The samples were then incubated at 34 °C either in low light (50 μE m^{-2} s^{-1}) or in high light (1000 μE m^{-2} s^{-1}). Figure 7B shows the DCMU inhibition curves of cells that have resynthesized D_1 in both conditions. Clearly, only after recovery in high light do cells have an increased proportion of sensitive D_1.

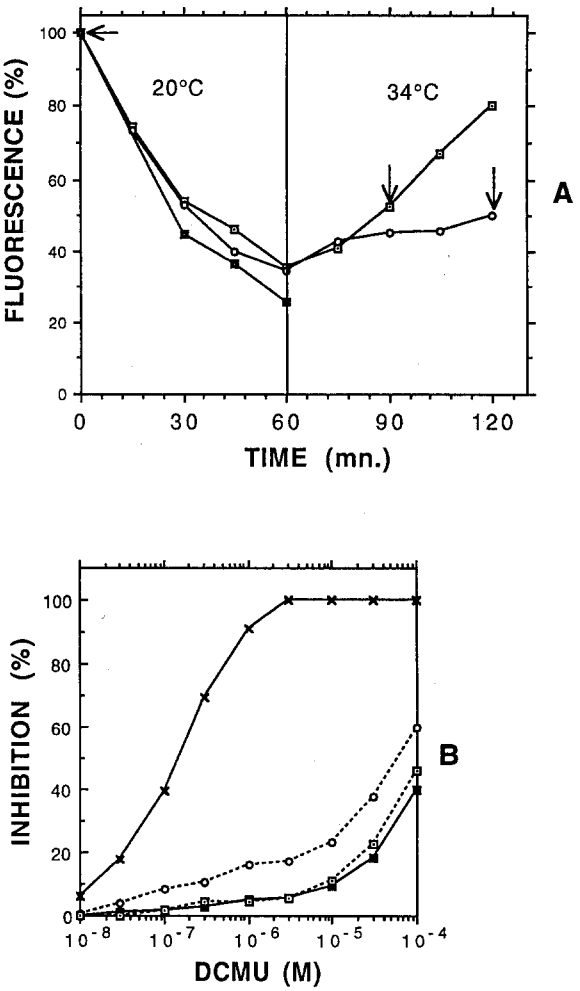

Fig. 7. Effect of light intensity on the relative expression of the two *psbA* copies of *Synechocystis* 6714 DCMUII-B mutant after an illumination which has destroyed 70% of fluorescent PSII. A. Effect of high light at 20 °C on PSII variable fluorescence in the presence (■) and in the absence (○, □) of streptomycin. After 60 min samples (without streptomycin) were warmed up to 34 °C and recovery was followed either in medium light (□) or in high light (○). Arrows indicate samples for which inhibition curves by DCMU were performed. B. Inhibition curves by DCMU of DCMUII-B cells not illuminated (■), and which have recovered about 50% of fluorescent PSII at 34 °C in medium light (□) or high light (○). Other details as in Fig. 6.-

Analysis of Synechocystis *6803 DCMU-resistant transformants*

The two homologous copies have been called *psbAI* and *psbAII* in *Synechocystis* 6714 and *psbA2* and *psbA3* in *Synechocystis* 6803. In resistant *Synechocystis* 6803 transformants, obtained by transformation with *Synechocystis* 6714 DNA, we determined which one of the two copies bears the mutations as well as the level of resistance.

Mapping of the psbA *mutations in the* Synechocystis *6803 transformants*

Transformants were obtained with DNA from the *Synechocystis* 6714 mutant DCMU-IIB. Genomic DNAs from the wild type and seven transformants were digested by *Hin*d III. According to Jansson *et al.* [10], *psbA3* and *psbA2* were found in 9.4 and 2.1 kb fragments respectively. As shown in Fig. 8, in the wild type the two bands at 9.4 and 2.1 kb both hybridize with the oligonucleotide probe (see Fig. 2), but in the transformants only the 9.4 kb fragment hybridizes with the probe. Therefore mutations are present exclusively in the *psbA2* copy.

DCMU-resistant PSII centres in the transformant strains

As for *Synechocystis* 6714 mutants, we performed inhibition curves (not shown) for *Synechocystis* 6803 wild type and the transformants described above. These experiments pointed out that 95% of the PSII centres are resistant to herbicide in the transformant cells and therefore that *psbA3* is much less expressed than *psbA2*.

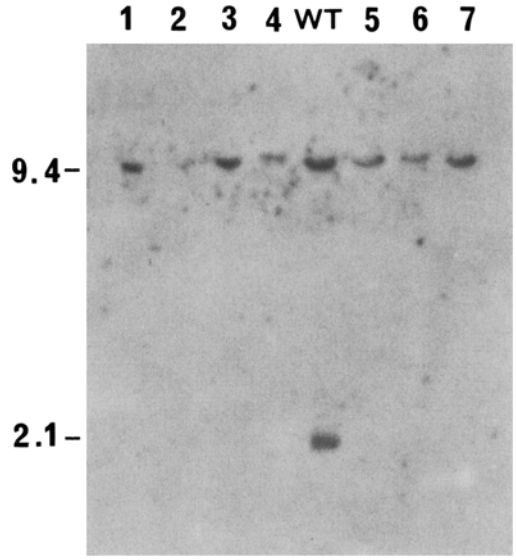

Fig. 8. Southern blot analysis of genomic DNAs from *Synechocystis* 6803 wild type and seven transformants (by DNA from *Synechocystis* 6714 DCMUII-B) digested by *Hin*d III. Hybridization was done with the synthetic oligonucleotide described in Fig. 2 at low-stringency conditions.

Discussion

In *Synechocystis* 6714 we have established that the *psbAII* copy in different herbicide-resistant mutants was not mutated. Indeed, transformations of the wild-type *Synechocystis* 6803 with *Synechocystis* 6714 DNA fractions showed that only *psbAI* transferred resistance. In a *Synechocystis* 6714 ioxynil-resistant mutant, a point mutation introduced a new restriction site in *psbAI*. This restriction site is absent in *psbAII*. Mapping the mutations in various mutants through oligonucleotide hybridization showed their presence exclusively in copy I.

Therefore, in the mutants we were able to distinguish the products of *psbAI* and *psbAII*. Two forms of D_1 can be synthesized and are present in the thylakoids, a herbicide-resistant D_1 encoded by a mutated *psbAI* and a herbicide-sensitive D_1 encoded by *psbAII* devoid of mutation. These two different forms of D_1 can be detected by carefully performing herbicide-induced inhibition curves in the mutant strains as described [21]. Results of such experiments (Fig. 5) point out that only very few sensitive D_1 were present in the mutant thylakoids, even in the total absence of selective pressure. The proportion is about 5%, so that in normal growth conditions *psbAII* is much less expressed than *psbAI*.

We checked if the relative expression of the two copies can be modulated by the light intensity, as was shown to occur for the three copies of the *psbA* gene in *Synechococcus* 7492 [22, 5]. Experiments described in Fig. 6 showed that in high-light conditions (1000 $\mu E\ m^{-2}\ s^{-1}$) the proportion of sensitive D_1 is significantly enhanced, increasing from 5 to 15% in 45–60 min. Since the light intensity needed to observe this effect produces photoinhibition [13, 12], we checked if the increase of *psbAII* expression was due to the decrease of D_1 concentration or to the light intensity *per se*. Experiments described in Fig. 7, in which in a first step the D_1 concentration decreased due to illumination at 20 °C and in a second step D_1 was synthesized *de novo* in high and medium light, allow us to conclude that the enhancement of *psbAII* expression is modulated

by light intensity and not by the D_1 concentration remaining in the thylakoids.

All *Synechocystis* 6803 transformants obtained with *Synechocystis* 6714-mutated DNA contained the mutation of the donor DNA in *psbA2*. Only recombinations with *psbA2* seem to be efficient, possibly due to a preferential recombination of *psbAI* of *Synechocystis* 6714 with *psbA2* of *Synechocystis* 6803 because of a greater similarity or to the fact that only recombination in copy 2 has been selected because of a higher transcription level of this copy than of copy 3. Preliminary experiments done with *Synechocystis* 6803 strains containing only *psbA2* or *psbA3* (kindly provided by C. Jansson) have shown that similar transformation efficiencies of the two strains were obtained either with genomic DNA or with the 0.7 kb fragment of the *psbAI* gene from *Synechocystis* 6714 mutants. This indicates that copy 3, like copy 2, can be transformed by *Synechocystis* 6714 DNA. In spite of this equal probably, in all selected transformants of wild-type *Synechocystis* 6803, only *psbA2* has been mutated. Therefore, under selective pressure *psbA3* could not be expressed enough to permit growth in the presence of high concentrations of herbicide. Brusslan and Haselkorn [4] have shown that the *Synechococcus* TAQ3 strain, in which the mutation was on a slightly expressed copy, was poorly resistant to herbicide, in contrast to the strain TAQ1 in which the mutation was on a highly expressed copy. We are probably observing the same phenomenon in *Synechocystis*.

What could be the advantages of the presence of multiple copies of the *psbA* gene? In *Synechococcus* 7942, the *psbA* family encodes two different forms of D_1, form I encoded by *psbAI* and form II encoded by *psbAII* and *psbAIII*. In the wild type, light intensity has been shown [23] to influence the ratio of the forms of D_1 as high light promotes increased expression of *psbAII* and *psbAIII*. In mutants deleted for *psbAII* and *psbAII* (R2S2C3) or *psbAI* (R2K1) it has been shown that PSII of R2K1 is more resistant to photoinhibition than that of R2S2C3 due to an increased turnover of D_1 and also to an increased intrinsic resistance of PSII [14, 15].

In contrast, in *Synechocystis* 6714 or 6803 wild types, only one form of D_1 is encoded by the *psbA* genes but, as in *Synechococcus* 7942, high light increases the expression of the copy which is only slightly expressed in normal conditions (medium light). Therefore it seems that the advantage of a family of *psbA* genes is probably conferred by differential expression of the different genes during stress rather than by the ability to encode different products.

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