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Light-regulated expression of the *psbA* transcript in the cyanobacterium *Anacystis nidulans*

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The level of photoinhibition as well as the rate of recovery of photosynthesis increases with increasing light intensities in *Anacystis nidulans*. In addition, the recovery from photoinhibition is insensitive to rifampicin. We found that the amount of *psbA*-specific mRNAs, coding for the D1 protein, was higher in cells grown in high rather than in low photon flux densities of white light. The results support the hypothesis that the rate of synthesis of the D1 protein plays a role both in the susceptibility of photosynthesis to photoinhibition and during recovery from photoinhibition. In addition, we found that a majority of the mRNAs specific for the *psbA* gene could be detected intact 90 min after the addition of rifampicin.

Rifampicin; Photosynthetic recovery; Photoinhibition; Transcript; *psbA* Gene; Protein D1; (*Anacystis nidulans*)

1. INTRODUCTION

When light is absorbed by the photosynthetic antenna in excess to that which can be deexcited by photosynthesis, a reversible damage of the photosynthetic apparatus, photoinhibition, may occur [1]. The overexcitation can be triggered by light intensities higher than the intensity to which plants are acclimated [2,3] or by a combination of light and other stress-factors. Such stress factors can be low temperatures [4–7] or drought [8,9]. In combination with secondary stress factors, photoinhibition may become evident even at relatively low light intensities. If a photoinhibited organism is placed under favourable growth conditions it will recover its photosynthetic capacity after some time [5,10]. The recovery process requires light [11] and is also temperature dependent [5].

Much interest has centered around the question of the location of the primary site of photoinhibition in PS II [1]. The D1 protein (also called the Q_B- or herbicide-binding protein) of PS II has been suggested as a candidate for the primary site of photoinhibition or being closely linked to the primary site [12–15].

We have previously shown that the cyanobacterium *Anacystis nidulans* responds to photoinhibition in a similar way to higher plants [10]. In *A. nidulans*, both the rate of photoinhibition and the following rate of recovery of photosynthesis were dependent on the growth light regime: the level of photoinhibition was decreased and the rate of recovery was increased with increasing growth light intensities [10,11]. The recovery process in *A. nidulans* was also shown to be inhibited by the translation inhibitors streptomycin and kanamycin, but not by rifampicin, an inhibitor of transcription [10,11]. It was concluded that the degree of net photoinhibition was determined by a balance between the photoinhibitory process and the operation of a repair mechanism. The capacity of the repair mechanism significantly determined the difference in the susceptibility of photosynthesis to photoinhibition of high and low light grown *A. nidulans*.

Here we present data on how rifampicin affects the amount of total RNA, and how rifampicin and different growth light intensities influence the amount of transcripts for phycocyanin and the D1

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protein. The results give support for the suggestion that the susceptibility to photoinhibition of photosynthesis is correlated with the net synthesis of the D1 protein.

2. MATERIALS AND METHODS

2.1. *Culture conditions*

Axenic cultures of *A. nidulans* 625 (*Synechococcus* 6301) [16] were grown as has been described before [10,11,17]. The photon flux densities were 10 and 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the low-intensity white light (LW) and high-intensity white light (HW) growth conditions, respectively. Reactivation of photosynthesis after photoinhibition was carried out at 38°C in dim light of 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Rifampicin was added to cultures to a final concentration of 250 μg/ml. When needed, additional rifampicin (100 μg/ml) was added at 30 min intervals.

2.2. *DNA probes*

The *psbA* clone, pPSII32/1, from spinach was kindly provided by Professor R. Herrmann. Plasmid pPSII32/1 contains an internal *psbA* fragment. The β-phycocyanin probe, pPCYSP45, from *A. nidulans* is a pUC9 clone carrying a 1 kb long β-phycocyanin specific insert, constructed in our laboratory. Only purified, gene-specific fragments were used in the hybridization experiments.

2.3. *Total RNA measurements*

Total RNA was measured with the Orcinol method [18].

2.4. *^{32}P-pulse treatment*

From an exponentially growing culture of *A. nidulans*, four aliquots of 50 ml were taken and to each aliquot, 1 mCi of ^{32}P-orthophosphate was added. In the control culture, ^{32}P was added 20 min before the harvest of the culture. To the other three aliquots, rifampicin (250 μg/ml) was added at time zero. ^{32}P was added 5, 30 and 60 min after the addition of rifampicin to the three cultures, respectively. The cultures were harvested 20 min after the addition of ^{32}P. When needed, additional rifampicin (100 μg/ml) was added at 30 min intervals.

2.5. *RNA preparation*

RNA was prepared from steady-state grown *A. nidulans* by the hot phenol extraction method [19].

2.6. *Northern blot analyses*

Extracted RNA was subjected to Northern blot analysis using denaturing formaldehyde gels [20]. RNA samples were denatured at 60°C for 15 min before loading to the formaldehyde gel [21].

2.7. *Slot blot*

RNA samples were applied in a slot blot apparatus (Schleicher & Schuell, GFR) on nitrocellulose filters. The filters were then treated as described above for the Northern blot analyses. RNA samples were denatured before the addition to the filters as described above [21].

2.8. *Hybridization*

The RNA-containing filters were prehybridized at 45°C and hybridized at 42°C as described by Schleicher & Schuell. After hybridization, the filters were washed 4 × 5 min in 2 × SSPE (0.36 M NaCl, 20 mM Na-phosphate, pH 7.7, 2 mM Na_2EDTA) plus 0.1% SDS and 2 × 15 min in 0.1 × SSPE plus 0.1% SDS at 45°C, before they were autoradiographed.

3. RESULTS AND DISCUSSION

We have previously reported that the recovery of photosynthesis after photoinhibition of *A. nidulans* is insensitive to the transcription inhibitor rifampicin [10,11]. Rifampicin-insensitive processes are rare in bacterial cells and it was therefore of interest to investigate this process in further detail. It has been shown that purified RNA-polymerase from *A. nidulans* is sensitive to rifampicin [22]. In order to study the effect on growing cells, rifampicin (250 μg/ml) was added to steady-state grown cultures in HW and samples for A_{750} and total RNA determinations were taken before and after addition of the antibiotic (fig.1). It is clearly shown that the increase in both cell mass and RNA immediately stopped when rifampicin was added to the *A. nidulans* culture, which has also been shown to be the case for other cyanobacteria [23]. No decrease in RNA content could be detected during the time course of the experiment.

To study more precisely the effect of rifampicin, ^{32}P-orthophosphate was added to growing cultures of *A. nidulans* for different times after the addition of rifampicin. Without rifampicin treatment, 3 distinct RNA bands, 3.2, 1.6 and 1.1 kb in size, and a non-distinct area around the length of 0.1 kb appeared when the RNA was analyzed on a fractionating gel (not shown). The three bands correspond to 23 S rRNA, 16 S rRNA and to a degradation product of 23 S rRNA [24]. The non-distinct area is tRNA and/or 5 S rRNA [24]. Transcription was completely inhibited when the cultures were treated with rifampicin (250 μg/ml) for 5 or 30 min. No bands could be seen on the autoradiogram. Treatment for 60 min with the inhibitor generated transcription at a low level but with the same general pattern as in the untreated samples, except that the degradation of 23 S rRNA had become more prominent. These results show that rifampicin inhibits most if not all of the

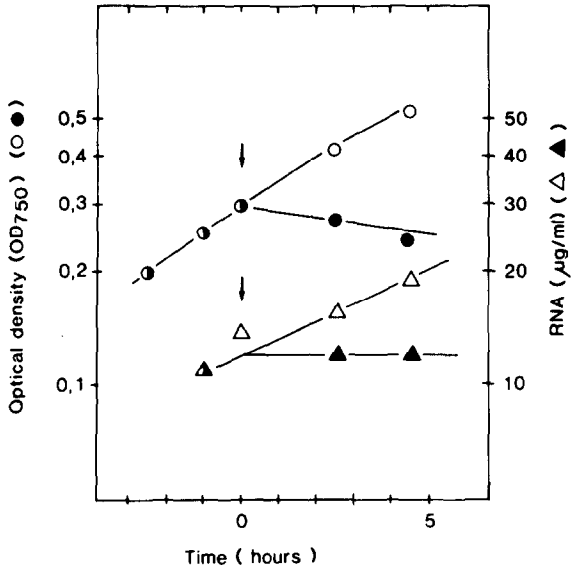

Fig.1. A_{750} and RNA content in an exponentially growing culture before and after the addition of rifampicin. Rifampicin was added at time zero. A_{750} (circles) and RNA content (triangles) were measured as described in section 2. Filled and open symbols describe results obtained with and without the addition of rifampicin, respectively.

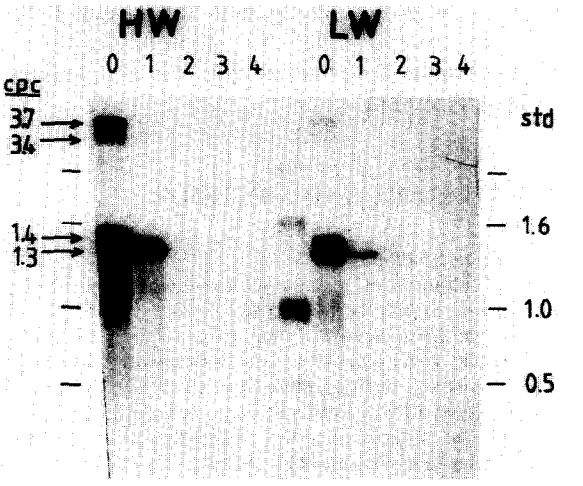

Fig.2. Hybridization of a phycocyanin specific probe to size fractionated RNA prepared from HW- and LW-grown cells with or without the addition of rifampicin. To each lane 10 μg of RNA was added. Panel HW: High, white light grown cells. Panel LW: Low, white light grown cells. Rifampicin was added at time 0 min. Samples were taken at different time intervals after the rifampicin addition. Lanes: 0, control; 1, 20 min; 2, 60 min; 3, 90 min; 4, 240 min. Single-stranded DNA was used as a molecular size standard (std). Molecular sizes are given as kilonucleotides. Sizes of phycocyanin (cpc) specific mRNAs are given.

transcription. However, after 60 min rifampicin starts to become inefficient. Further additions every 30 min were made to overcome this problem in the experiments described below.

To elucidate in more detail whether or not rifampicin had any effect on specific transcripts, we analyzed levels of phycocyanin mRNA with the Northern blot technique. We used an internal *SmaI-PstI* fragment from plasmid pPCYSP45 as a probe which is specific for the gene coding for the β-subunit of phycocyanin. The probe was hybridized to RNA samples from HW and LW grown cultures with or without rifampicin treatment for different time periods (fig.2). The RNA was prepared from cultures grown as described in section 2 and exposed to recovery light for 15 min. In both samples not treated with rifampicin, weak bands, 3.4–3.7 kb in size, and two strong bands of 1.3 kb and 1.4 kb could be seen. However, in the samples taken from the cultures treated with rifampicin all of the bands disappeared and no degradation products could be detected after 60 min (fig.2). The bands on the autoradiogram were scanned in a densitometer and from the values obtained, the half-life of the phycocyanin mRNA was calculated and was found to be 7–10 min. The half-life was the same for the culture grown in high white as that grown in low white light. The results show that transcription of β-phycocyanin was completely inhibited by rifampicin and the turnover rate of the mRNAs was so rapid that all β-phycocyanin specific mRNAs were completely degraded within 60 min.

RNA was prepared from HW- and LW-grown cultures of *A. nidulans*, and probed with a *psbA* gene from spinach in a Northern blot experiment. The internal fragment of *psbA* from spinach hybridized to one fragment with a size of 1.2 kb in a Northern blot experiment (fig.3). Hybridization with this fragment could in this experiment only be detected in the samples taken from the HW-grown cells. The result shows that more *psbA* mRNA is present in HW-grown cultures than in cultures grown in LW light. Considerable amounts of mRNA are also present after 90 min treatment with rifampicin. The result indicates that the *psbA*

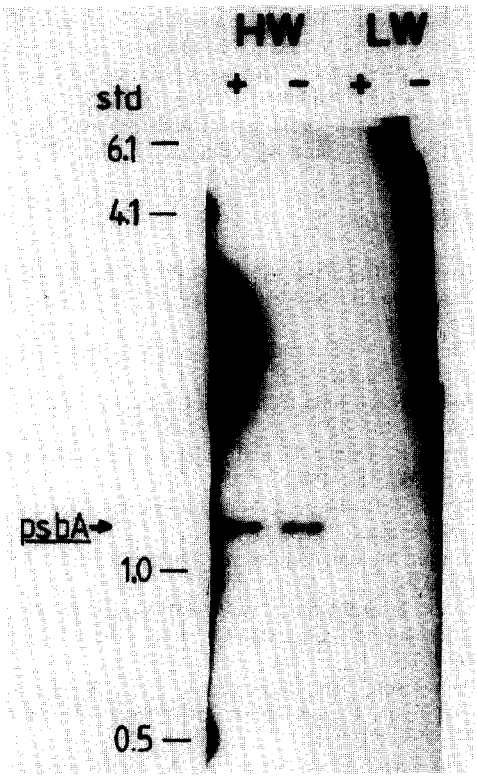

Fig.3. Hybridization of a *psbA* gene-specific probe to size fractionated RNA prepared from HW- and LW-grown cells with or without the addition of rifampicin. Rifampicin was added 90 min before RNA preparation. To each lane 10 μg of RNA was added. Lanes: HW +, HW grown cells + rif; HW −, HW grown cells − rif; LW +, LW grown cells + rif; LW −, LW grown cells − rif. Single-stranded DNA was used as a molecular size standard (std). Molecular sizes are given as kilonucleotides. The location of the *psbA* specific mRNA is indicated.

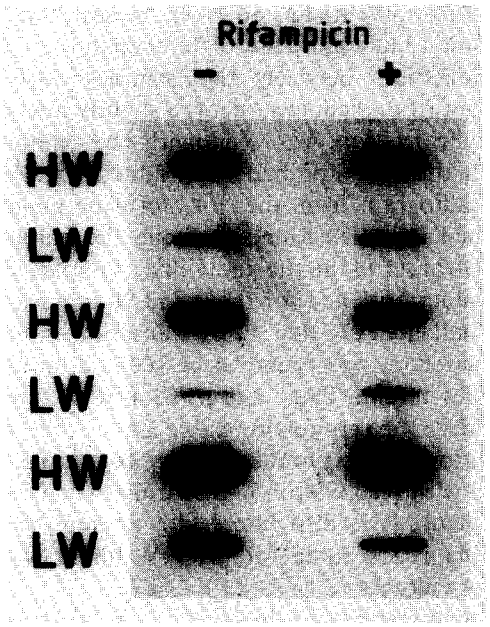

Fig.4. Hybridization of a *psbA* gene-specific probe to RNA in a slot blot experiment. RNA was prepared from HW- and LW-grown cells with (+) or without (−) the addition of rifampicin. Rifampicin was added 90 min before RNA preparation, 2 μg of RNA was added to each slot. Six different RNA preparations, treated identically, from three different cultures are shown.

mRNA is stable under the growth conditions used. As no unspecific hybridization could be detected, the hybridization and washes were considered stringent enough to permit quantitative measurements of the *psbA* mRNA in slot blot experiments. The results from such experiments are shown in fig.4. Cultures were grown and RNA was prepared from six different cultures (three grown in HW and three in LW) with or without the addition of rifampicin. From each treatment it can be clearly seen that the D1-specific RNA existed in all samples. As was evident in fig.3, the slot blot experiment also shows that the amount of *psbA*-specific RNA was higher in the samples from HW-grown cells than in those from LW-grown cells.

In earlier studies performed with *A. nidulans* and *C. reinhardii*, we have concluded that the net photoinhibitory damage results from the balance between the photoinhibitory process and the operation of a recovery process [10,11,25]. Furthermore, the lower susceptibility of HW- than of LW-grown *A. nidulans* was related to a relatively high rate of rifampicin-insensitive recovery in HW-grown cells as compared with LW-grown cells. These earlier results fit nicely to the findings that the transcript of the D1 protein was expressed at a higher level in HW- than in LW-grown cells (figs 3 and 4). These results agree with the suggestion of Ohad et al. [26] that the D1 protein is damaged in photoinhibited *C. reinhardii* and that photoinhibition occurs when the inactivation and removal of the D1 protein exceeds the rate of its resynthesis.

The amount of *psbA* probe that hybridized to the RNA species in HW-grown cells in the Northern blot experiment (fig.3) appeared to be about the same irrespective of whether the culture was treated with rifampicin or not. In the slot blot

hybridizations, it can, however, be seen that there is a small but significant difference in the level of hybridization between the samples with and without rifampicin treatment; a slightly lower level of hybridization was seen with the samples to which the inhibitor had been added (fig.4). The autoradiogram from the slot blot experiment was scanned in a densitometer giving the amount of *psbA*-transcript in the individual samples. From the values obtained for the 0 and 90 min samples, the half-life of the *psbA*-transcript could be calculated. It was found to be 100–120 min in all samples tested. Thus, the *psbA* mRNA must be considered exceptionally stable under the growth conditions used: photoinhibitory conditions combined with reactivation light during the rifampicin treatment. It is also interesting to note that the stability is the same for the HW and LW light-grown cultures.

A high stability of the level of the D1 transcript has been shown before in experiments with the plant *Spirodela oligorhiza* [14]. In mature chloroplasts there was no substantial effect of light on the level of D1 transcripts while the level of the protein itself was dramatically affected by light. As the D1 protein is chloroplast encoded in plants, and chloroplasts are believed to be of a prokaryotic origin, it is not unexpected if the synthesis of the D1 protein is regulated in a similar way in the chloroplasts of *S. oligorhiza* as in the cyanobacterium *A. nidulans*.

A stable transcript coding for the D1 protein indicates that this protein is mainly regulated at the translational level. However, the higher level of D1 transcript in HW compared to LW cells suggests that there is in addition a long term acclimation at the transcriptional level.

In summary, our results suggest that the D1 protein of *A. nidulans* is encoded by a stable mRNA species and that the D1 protein can play a significant role in photoinhibition of photosynthesis and its recovery. The results support the hypothesis that the rate of synthesis of the D1 protein can be a factor determining the susceptibility of photosynthesis to photoinhibition and the rate of recovery from photoinhibition.

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