Transcription control of the 32 kDa-Q_B protein of photosystem II in differentiated bundle sheath and mesophyll chloroplasts of maize

Gadi Schuster, Iris Pecker*, Joseph Hirschberg*, Klaus Kloppstech° and Itzhak Ohad+

*Department of Biological Chemistry and *Department of Genetics, Institute of Life Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel* and °*Institute of Botany, University of Hannover, Herrenhauser Strasse 2, D-3000 Hannover 21, FRG*

Received 23 December 1985

Bundle sheath chloroplasts of maize, a C_4 plant, lack a functional herbicide-binding site and the 32 kDa-Q_B thylakoid protein of photosystem II. Measurements of the amounts of Q_B-protein mRNA in bundle sheath and mesophyll chloroplasts by hybridization to a cloned psbA probe, and analysis of in vitro translation products of bundle sheath and mesophyll RNA show that during differentiation of maize leaf cells to bundle sheath and mesophyll, the expression of the chloroplast gene, psbA, which encodes the 32 kDa-Q_B protein, is controlled at the transcriptional level.

psbA gene *Q_B-protein* *C_4 plant* *(Mesophyll, Bundle sheath)* *Chloroplast* *Gene expression*

1. INTRODUCTION

The differentiation of the C_4 plants' chloroplasts into two types, those of mesophyll (M) and those of bundle sheath (B) cells, offers an attractive experimental system for studying gene expression in the chloroplast and regulation of photosynthetic activity [1–5].

It has previously been demonstrated that in maize, both the nuclear-encoded RuBP carboxylase small subunit (SSu) and the chloroplast-encoded large subunit (LSu) polypeptides are absent in M cells, while B-type cells lack the nuclear-encoded LHC II polypeptides and the phospho*enol*pyruvate carboxylase [1–3,5]. In both cell types, the expression of these genes appears to be regulated at the transcriptional level, the corresponding genes being 'shut off' or 'turned on', as differentiation progresses during cell division and leaf growth [1–5]. In fully differentiated B cells, the PS II reaction center polypeptides are present [3,5] but PS II activity is drastically reduced in vivo, probably due to the absence of the polypeptides of the water-oxidizing complex on the donor side, and the absence of the Q_B-protein (secondary acceptor side) [5], resulting in the disconnection of PS II from the plastoquinone pool.

The Q_B-protein also forms the binding site of the triazine- and urea-type herbicides such as atrazine and DCMU, respectively [6,7]. The chloroplast gene coding for this protein, psbA, has been cloned and sequenced, and a remarkable conservation of the predicted amino acid sequence has been demonstrated in a variety of photosynthetic organisms [6,8–10]. The expression of this gene appears to be light-induced in etiolated maize plants after growth in the dark for 7 days [11].

+ To whom correspondence should be addressed

Abbreviations: B, bundle sheath; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; LHC II, light-harvesting chlorophyll *a,b*-protein complex of photosystem II; M, mesophyll; PS, photosystem; RuBP carboxylase, ribulose-1,5-bisphosphate carboxylase

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

However, light does not seem to affect its transcription in fully differentiated chloroplasts in which the amount of the psbA mRNA remains high and constant, even after prolonged incubation in dark- or light-grown plants [12].

The rapid turnover of the Q_B-protein in the light and its stability in the dark, together with the fact that the rate of its degradation and synthesis is proportional to light intensity [13,15,16], suggest that the expression of this protein is also under translational control. It has been shown in the green alga *Chlamydomonas*, that when the rate of degradation exceeds that of its synthesis, the thylakoids become depleted of Q_B-protein and PS II activity is drastically reduced (photoinhibition) [15,16]. The translation and/or integration of this protein into the thylakoids is subject to pleiotropic effects. In various nuclear gene mutants of both higher plants [17] and algae [18,19], the amount of Q_B-protein, as well as other PS II complex polypeptides, is significantly reduced or completely lost. Thus, the possibility should be considered that the absence of the Q_B-protein in the B-type chloroplasts of maize is not due to regulation of psbA transcription, as was shown to be the case for the other genes mentioned above, but merely a secondary translation or integration effect.

Here, we provide evidence for transcriptional control of the psbA gene expression following differentiation of maize B and M chloroplasts.

2. MATERIALS AND METHODS

Zea mays (Neve Ya'ar 170) plants (2–3 weeks old) were grown on vermiculite in a 4-fold diluted Hoagland solution [5], and M and B thylakoids isolated as reported [5]. B strands were checked by microscopy to be free of M cells, and the chlorophyll *a* to *b* ratio of isolated B thylakoids was >5.5. Measurements of [^{3}H]DCMU (3.54 Ci/mmol) binding were performed according to Shochat et al. [20]. Total cellular RNA, purified according to Fromm et al. [12], was isolated from M or B cells as in [5].

Thylakoid proteins were separated by SDS-polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue R [5]; alternatively, the resolved proteins were electrotransferred to nitrocellulose paper, and the Q_B-protein detected using specific antibodies, as described [16]. A purified 780 bp *Pst/Xba* DNA fragment of the plasmid PAH484, containing the psbA gene from *Amaranthus hybridus* [9], was used as a specific probe for the Q_B-protein mRNA. The high conservation of the nucleotide sequence between *Amaranthus* and maize (L. McIntosh and L. Bogorad, unpublished) allows hybridization of mRNA/DNA from the two species even under high stringency conditions. The LSu RuBP carboxylase DNA probe was a 2.16 kb *Bam*HI/*Eco*RI fragment of the plasmid pZmc 37 [2]. The pZmc 100 plasmid, containing the genes for the chloroplast 16 S and 23 S ribosomal RNA [17], was used as an rRNA-specific probe. Total RNA was measured by absorption at 260 nm.

The DNA probes were labeled with ^{32}P by nick-translation [21], and mRNA levels were assessed by dot-blot hybridization to total RNA isolated from M or B cells [22]. The hybridization was carried out at 42°C for 15–20 h. Following hybridization, the nitrocellulose paper was washed 4 times (10 min each) with 2 × SSC buffer [22] at 42°C and twice with 0.1 × SSC at 60°C.

In vitro translation of total leaf M and B RNA in a reticulocyte lysate system, kindly provided by Dr M. Edelman, Weizmann Institute of Science, Rehovot, Israel, was performed according to Pelham and Jackson [23]. [^{35}S]Methionine was used for labeling in vitro translation products which were resolved by SDS-polyacrylamide gel electrophoresis, followed by fluorography with sodium salicylate [24].

Immunoprecipitation of the precursor polypeptide of the Q_B-protein from the in vitro translation products by antibodies specific to the Q_B-protein [16] and goat anti-rabbit IgG (Miles-Yeda), as a second antibody, was performed according to Cullen and Schwartz [25].

3. RESULTS AND DISCUSSION

3.1. *Isolation of B and M cells and thylakoids*

To check the B- and M-type preparations used here, the electrophoretic pattern of thylakoid polypeptides, the presence of active herbicide-binding sites and the presence of the Q_B-protein in thylakoids were assessed. As reported in [5], the B thylakoids did not show specific DCMU binding, whereas a normal reciprocal plot of 1/free vs 1/bound DCMU as a function of DCMU concen-

tration was obtained for the M thylakoids (fig.1). Electrophoretic separation of thylakoid polypeptides, followed by transfer to nitrocellulose paper and probing with Q_B-protein-specific antibodies, showed a strong positive reaction in the M thylakoids, while no detectable reaction was obtained in the B thylakoids (fig.2). These results demonstrate the absence of a detectable Q_B-protein as well as a functional herbicide-binding site in the thylakoid of B cell preparations. The differences which were observed in thylakoid polypeptides of the B and M preparations are in agreement with previous data [3,5].

3.2. *Measurements of the Q_B-protein mRNA in B and M cells*

The Q_B-protein mRNA is found in relatively large amounts in chloroplasts of both higher plants and algae [6]. Measurements of the relative amounts of Q_B-protein mRNA in B and M cells were performed by hybridizing ^{32}P-labeled cloned psbA gene to total RNA bound to nitrocellulose (dot blot). As shown in fig.3, the level of the Q_B-protein mRNA in B cells is lower than that of M cells. Counting the radioactivity of the spots revealed an 8-fold reduction of the Q_B-protein mRNA in B cells, using the rRNA probe as an internal standard. The low level of hybridization of the psbA probe still found in B cells might reflect a low level of transcription or, alternatively, might be an artifact of nonspecific binding due to the use of total cellular RNA. The latter possibility is more

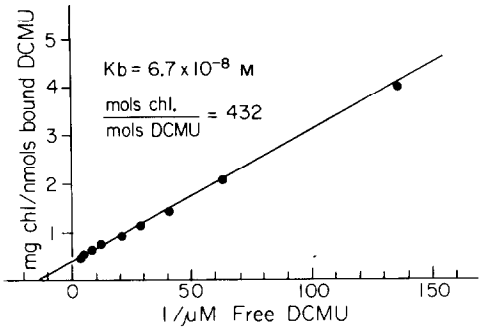

Fig.1. Reciprocal plot of [^{3}H]DCMU binding to maize M thylakoids. The K_b and the ratio DCMU bound/chlorophyll were calculated from the intercepts with the ordinate and abscissa, respectively. No specific binding of DCMU could be detected when B thylakoids were used.

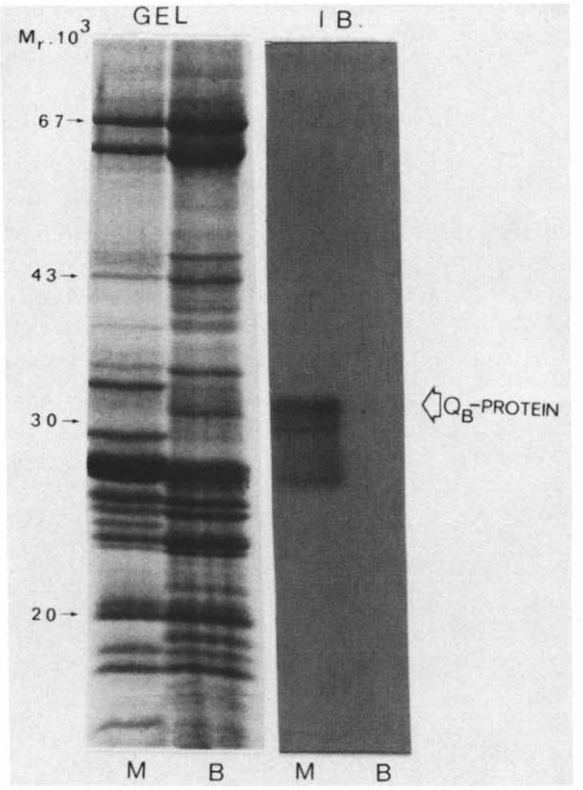

Fig.2. Polypeptide pattern of B and M thylakoids and immunoblotting (IB) using Q_B-protein antibodies after transfer of the polypeptide to nitrocellulose. A positive reaction with the Q_B-protein antibodies is observed only in the M thylakoids.

likely, since it has been demonstrated before that the mRNA of LSu RuBP carboxylase is practically undetectable in pure M cells' RNA [1–3,5], while the data shown in fig.3 indicate that apparently a low but detectable level of hybridization of LSu RuBP carboxylase specific probe is found in our RNA preparation of M cells. Thus, it is possible that the amount of mRNA for the Q_B-protein in B cells is even lower than that shown in fig.3. Further proof of the reduction or absence of the Q_B-protein mRNA in B cells is provided by fig.4, in which the in vitro translation products of B and M cells' RNA were analyzed. Synthesis of Q_B-protein precursor was detected by [^{35}S]methionine labeling and immunoprecipitation only when RNA from M cells was used.

The present results indicate that the regulation of the psbA gene expression, following differentia-

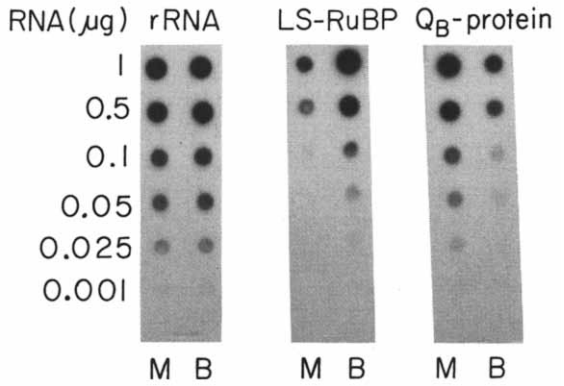

Fig.3. Differential distribution of mRNA of the LSu RuBP carboxylase and Q_B-protein in M and B thylakoids of maize. Total B or M cells' RNA was hybridized with nick-translated DNA probes for the rRNA and LSu RuBP carboxylase of maize and the Q_B-protein of *Amaranthus*.

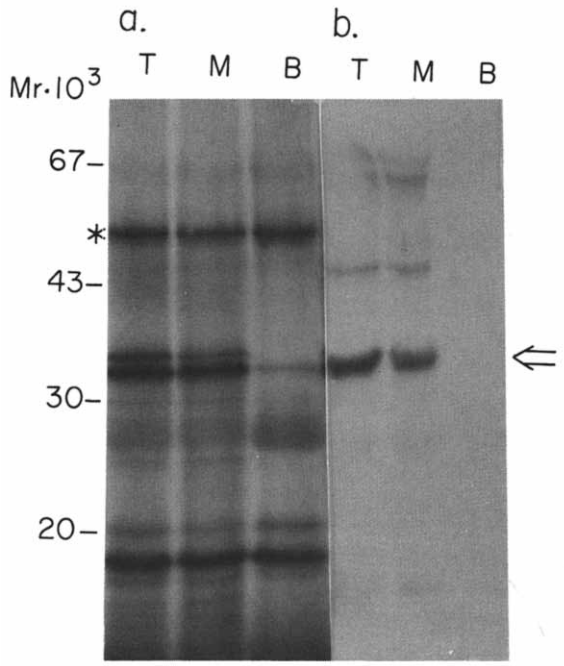

Fig.4. Absence of Q_B-protein in the in vitro translation products of total RNA obtained from whole maize leaf. RNA of B and M cells of maize (2 μg) was added to the reticulocyte lysate translation system. A radioactive band, corresponding to the Q_B-protein (a), as identified by immunoprecipitation with Q_B-protein antibodies (b, arrow), is present only in the translation products of whole leaf (T) and M total RNA; * [^{35}S]methionine incorporation into a band specific to the reticulocyte lysate translation system, independent of exogenous RNA addition.

tion of maize chloroplasts into B and M type, occurs at the transcriptional level, as shown to be the case for LHC II, SSu and LSu RuBP carboxylase and phospho*enol*pyruvate carboxylase [1–3,5].

It has been previously reported that the specific loss of the 43 or 47 kDa PS II polypeptides of *Synecocystis* by specific gene deletion does not cause loss of other PS II thylakoid polypeptides [26], while deletion of the psbA gene in *Chlamydomonas* resulted in the instability and loss of the 43 and 47 kDa polypeptides [19]. Our results show that in maize B chloroplasts, the absence of the Q_B-protein does not induce loss of 43 and 47 kDa polypeptides of the PS II complex which is still able to reduce silicomolybdate, using 1,5-diphenylcarbazide as a donor, a photochemical activity considered to occur at the reaction center of PS II prior to the Q_B site [5].

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