SPIRODELA OLIGORHIZA CHLOROPLAST DNA CODES FOR ATPase SUBUNITS α AND β

Immunological evidence from a coupled transcription–translation system

J. T. DE HEIJ and G. S. P. GROOT

Biochemical Laboratory, Free University, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

Received 15 May 1981; revision received 21 September 1981

1. Introduction

The chloroplast genetic system contributes a small, but essential part to the biosynthesis of the organelle [1]. Chloroplast DNA, a covalently closed circular molecule with a circumference of 40–54 μm, codes for the RNA constituents of the chloroplast ribosomes [2]. These ribosomes synthesize a number of polypeptides amongst which the large subunit of ribulose-1,5-bisphosphate carboxylase [3–5] and a 32 000 M_r shielding protein [6]. The genes for these polypeptides have been precisely localized on cpDNA. The coding site of other translation products, like subunits of CF_1 [7] and photosystem I [8], is unknown.

Here, we report on the coupled transcription–translation of cpDNA of *Spirodela oligorhiza*, a small aquatic plant, very suitable for biosynthetic studies with a well-characterized cpDNA [9–11]. An extract of *Escherichia coli* was used to express the genetic information of *Spirodela* cpDNA. The products were analyzed by immuneprecipitation with heterologous antisera raised against spinach α- and β-subunits of CF_1. It appears that *Spirodela* cpDNA codes for the α- and β-subunit of CF_1. Our results suggest that the method used is a powerful tool in elucidating the coding capacity of cpDNA.

2. Materials and methods

Spirodela oligorhiza was cultured as in [11]. Plants were labeled by growing on a low sulphate medium in the presence of $Na_2{}^{35}SO_4$ (Amersham). After 5 generations (~10 days) the plants were harvested and washed with sterile water. Plants were homogenized in a Waring Blendor with 3 4 vol. 0.33 M sorbitol, 10 mM EDTA, 50 mM Tricine–KOH (pH 8.0), 2 mM $MgCl_2$ and 4 mM β-mercaptoethanol. The homogenate was filtered through nylon cloth and chloroplasts were pelleted by centrifugation at 2000 × *g* for 5 min at 4°C. Chloroplasts were lysed by resuspending in sterile water in the presence of a freshly prepared solution containing: 1 mM *p*-aminobenzamidine, phenylmethyl sulfonyl fluoride, *N*-tosyl-L-phenylalanyl chloromethane and *N*-tosyl-L-lysyl chloromethane and fractionated on a discontinuous sucrose gradient as in [12]. Thylakoid membranes were pelleted by centrifugation at 200 000 × *g*. These membranes appeared to be free of ribulose-1,5-bisphosphate carboxylase contamination. Solubilization of thylakoid membranes was done by resuspending in 2.5% SDS (Serva), 6.25 mM Tris–HCl (pH 8.5), 50 mM DTT and 15% glycerol, to 1 mg chl/ml [13].

Polyacrylamide gel electrophoresis was performed according to [14]; the gradients were stabilized by 0.2% polyacrylamide. Gels were stained with CBB-R 250 and destained in 25% methanol, 7.5% acetic acid. Fluorography with Kodak Royal X-O-Mat XR_1 was used to detect labeled proteins [15]. Phosphorylase *b* (M_r 94 000), bovine serum albumin (M_r 67 000), ovalbumin (M_r 43 000), carbonic anhydrase (M_r 30 000), soybean trypsin inhibitor (M_r 20 000) and lactalbumin (M_r 14 000) were used as M_r markers.

Immuneprecipitation was done as in [16] with the modification in [17]. Chloroplast coupling factor (CF_1) was isolated and its enzymatic activity determined as in [27]. Protein blotting on nitrocellulose (Sartorius, 0.1 μm) and immunodetection of protein

Abbreviations: cp, chloroplast; chl, chlorophyll; CF_1, chloroplast coupling factor 1; SDS, sodium dodecylsulphate; app. M_r, apparent relative molecular mass; CP_I, chlorophyll protein complex I; CBB, Coomassie brilliant blue

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.75

was done according to [28] as modified [18].

cpDNA was isolated as in [11] and transcribed–translated in the *E. coli* PR_7 extract [19]. In vitro protein synthesis with isolated chloroplasts was performed as in [20]. Protein fractions were isolated as in [12].

3. Results

The subunit composition of CF_1, isolated from *Spirodela* chloroplasts was analyzed by SDS–polyacrylamide gel electrophoresis. Fig.1 shows that it contains 5 subunits, like CF_1 of other organisms, with M_r-values similar to those reported for other coupling factor preparations [21]. On the basis of this analysis the *Spirodela* CF_1 preparation is rather pure and, in accordance with this, the specific ATPase activity of this preparation is 20–40 μmol ATP hydrolyzed . min^{-1} . mg $protein^{-1}$.

To investigate the feasibility of the use of antisera raised against purified CF_1 subunits from spinach, to detect the corresponding *Spirodela* CF_1 subunits, we have analyzed the immunoprecipitates of a thylakoid extract of *Spirodela* plants grown in the presence of [^{35}S]sulphate. Fig.2 shows the autoradiogram of the SDS–polyacrylamide gel analysis of the immunoprecipitate. Besides a non-specifically precipitated band with M_r 29 500 (which is also precipitated by a non-immune serum) we do find that the heterologous spinach antisera precipitate the corresponding *Spirodela* CF_1 subunits. The extra bands in lanes 3 and 4 are caused by precipitation of subunit β by the anti α-serum and by precipitation of a breakdown

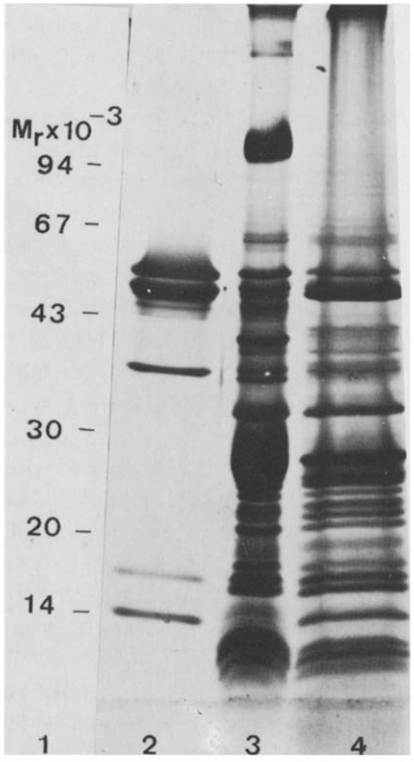

Fig.1. *Spirodela oligorhiza* chloroplast proteins separated on a 7.5–15% polyacrylamide gradient SDS gel after staining with CBB-R 250: (1) marker proteins; (2) purified CF_1; (3) purified thylakoid proteins; (4) total chloroplast proteins.

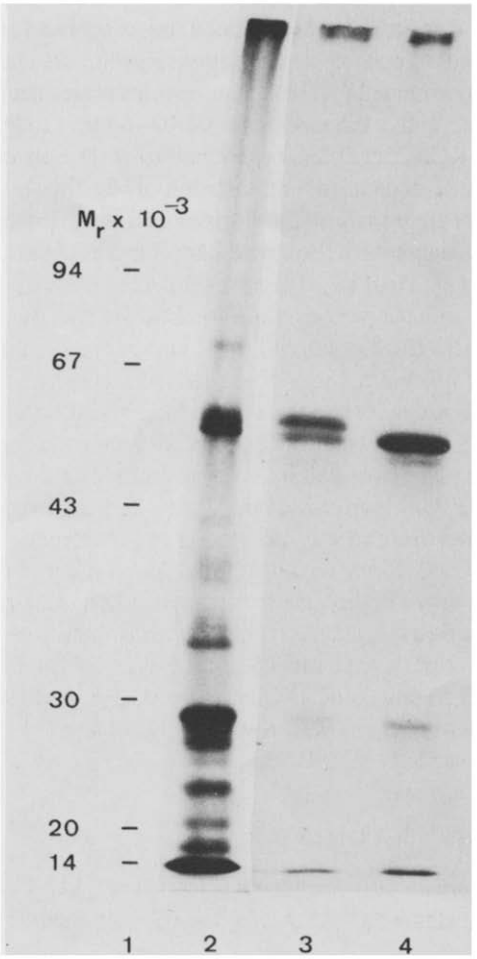

Fig.2. Fluorogram of a 7.5–15% polyacrylamide gradient SDS gel of immunoprecipitated ^{35}S-labeled *Spirodela* thylakoid proteins: (1) marker proteins; (2) total thylakoid proteins; (3,4) immuneprecipitate with anti-subunit α and β sera, respectively.

product of subunit β [22] by the anti β-serum. The M_r-values of the precipitated bands are in very good agreement with the values from fig.1. Similar results were obtained with the anti-subunits γ, δ and ϵ sera (not shown). The cross-reactivity of the spinach antisera with CF_1 subunits of other organisms like pea (*Pisum sativum*), or garden cress (*Lepidium sativum*), could also be detected using a protein blot method (not shown). Apparently there is a close antigenic similarity between all coupling factor subunits of these non-related plants. Only minor differences between the M_r-values of the CF_1 subunits could be detected in these different organisms.

The finding that spinach antisera are capable of detecting specifically *Spirodela* CF_1 subunits enabled us to confirm that also in this organism subunits α, β and ϵ are translated on chloroplast ribosomes. Therefore we incubated isolated chloroplasts under conditions optimal for light-driven protein synthesis [20] in the presence of [^{35}S]methionine and subjected a thylakoid extract, free of chlorophyll [23], to immunoprecipitation. Subunits α, β and ϵ incorporated radioactivity (fig.3), and are therefore translated on chloroplast ribosomes. Subunits γ and δ do not have incorporated radioactivity and most likely are cytoplasmic translation products. In all lanes a minor band is seen, caused by coprecipitation of the heavily labeled large subunit of ribulose-1,5-bisphosphate carboxylase. These observations confirm and extend those in [7,8,24,25].

To establish the coding site for subunits α and β we have expressed the genetic information present on *Spirodela* cpDNA in a coupled transcription–trans

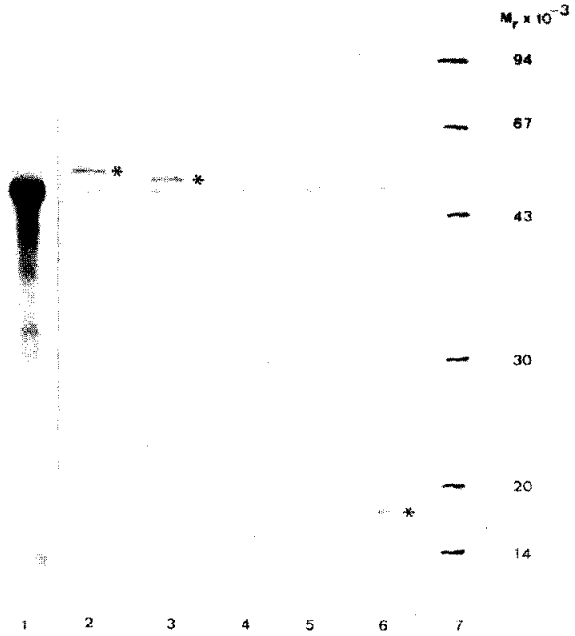

Fig.3. Fluorogram of a 8–20% polyacrylamide gradient SDS gel of immunoprecipitated ^{35}S-labeled products of light-driven protein synthesis by isolated chloroplasts: (1) total chloroplast translation products; (2–6) immunoprecipitate with anti-subunit α, β, γ, δ and ϵ sera, respectively; (7) marker proteins.

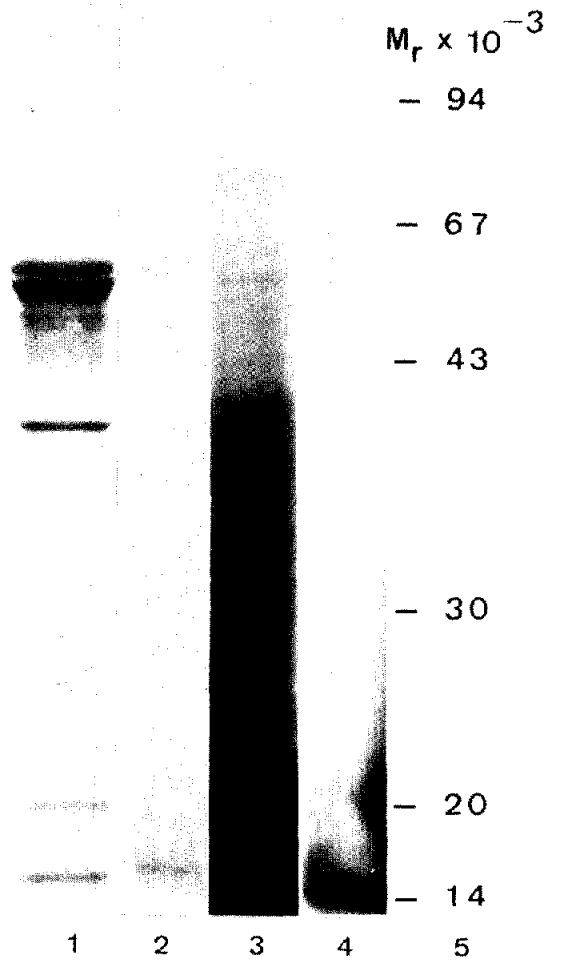

Fig.4. Fluorogram of a 8–20% polyacrylamide gradient SDS gel of immunoprecipitated ^{35}S-labeled products of coupled transcription–translation of *Spirodela* cpDNA: (2) total translation products immunoprecipitated with anti-subunit α serum; (3) total translation products; (4) total translation products with anti-subunit β-serum. For comparison (1) shows the protein stain of a CF_1 preparation run on the same gel; (5) marker proteins.

lation system, isolated from *E. coli* [4,19]. The [^{35}S]methionine labeled products were subjected to immuneprecipitation and the precipitate analyzed by SDS–polyacrylamide gel electrophoresis. Fig.4 shows the results. When the *E. coli* system is programmed with isolated cpDNA, a number of discrete polypeptides is synthesized in addition to a fair amount of low-M_r polypeptides. The latter are most likely caused by the fact that our cpDNA is in part randomly broken down so that artificial starts and stops during transcription may have occurred. Analysis of the immunoprecipitates shows that polypeptides with M_r-values corresponding to subunits α and β of CF_1 are recognized by the respective antisera. The reliability of the approach is further demonstrated by the fact that digestion of the cpDNA with *Xho*I specifically leads to the disappearance of subunit α. Digestion of cpDNA with *Sac*I, prior to programming the extract, leads to the disappearance of subunit β (not shown). We interpret these results in such a way that the genes for subunits α and β lie on cpDNA with a restriction site for *Xho*I and *Sac*I in the genes for α and β, respectively.

4. Discussion

Our results suggest that there is an antigenic conservation of all 5 subunits of CF_1. This is in apparent contrast with the results obtained in [22] who found cross-reactivity only with subunits α and β of CF_1 of different organisms and not with the other subunits. This discrepancy, however, can be explained by the different sensitivities of the techniques used. The immunoprecipitation procedure we have used is independent of secondary network formation and therefore inherently more sensitive than techniques such as crossed immunoelectrophoresis used in [22]. The cross-reactivity of antisera raised against certain polypeptides with the corresponding antigen from a different organism is not limited to subunits of the chloroplast coupling factor. Other examples, e.g., with CP_I and ferredoxin, have been found ([26], our unpublished results). These results suggest that heterologous antisera can be used more universally in developmental studies, e.g., dark–light transition and in studies such as this.

The prokaryotic nature of chloroplast DNA makes expression of the genetic information present on it, possible in a bacterial coupled transcription–translation system [4]. The accuracy of this system is shown by the fact that polypeptides are synthesized with an M_r and antigenic determinants identical to the original antigen. This enabled us to establish the coding site of subunits α and β of CF_1.

Soon it should be possible to establish the exact location of the genes for these 2 subunits on cpDNA by expressing the information of suitable cloned chloroplast DNA restriction fragments and to study the expression of these genes in more detail.

Acknowledgements

The authors are very grateful to Dr G. Schatz for his generous gift of the antisera. We wish to thank P. R. Abraham for preparing the garden cress chloroplast preparation and H. Lustig for expert technical assistance.

References

[1] Gilham, N. W. (1978) in: Organelle Heredity, pp. 539–560, Raven, New York.
[2] Kirk, J. T. O. (1978) in: The Plastids (Kirk, J. T. O. and Tilney-Basset, R. A. E. eds) pp. 525–554, Elsevier/North-Holland, Amsterdam, New York.
[3] Coen, D. M., Bedbrook, J. R., Bogorad, L. and Rich, A. (1977) Proc. Natl. Acad. Sci. USA 74, 5487–5491.
[4] Bottomley, W. and Whitfeld, P. R. (1979) Eur. J. Biochem. 93, 31–39.
[5] Barraclough, R. and Ellis, R. J. (1980) Biochim. Biophys. Acta 608, 19–31.
[6] Driesel, A. J., Speirs, J. and Bohnert, H. J. (1980) Biochim. Biophys. Acta 610, 297–310.
[7] Mendolia-Morgenthaler, L. R., Morgenthaler, J. J. and Price, C. A. (1976) FEBS Lett. 62, 96–100.
[8] Nechushtai, R., Nelson, N., Mattoo, A. K. and Edelman, M. (1981) FEBS Lett. 125, 115–118.
[9] Edelman, M. and Reisfeld, A. (1980) in: Genome Organization and Expression in Plants (Leaver, C. J. ed) pp. 353–361, Plenum, New York.
[10] Van Ee, J. H., Vos, Y. J. and Planta, R. J. (1980) Gene 12, 191–200.
[11] Van Ee, J. H., Man in 't Veld, W. A. and Planta, R. J. (1980) Plant Physiol. 66, 572–575.
[12] Douce, R., Holtz, R. B. and Benson, A. A. (1973) J. Biol. Chem. 248, 7215–7222.
[13] Whatley, F. R. and Arnon, P. J. (1963) Methods Enzymol. 6, 1308–1313.
[14] Laemmli, U. K. (1970) Nature 227, 680–685.
[15] Bonner, W. M. and Laskey, R. A. (1974) Eur. J. Biochem. 46, 83–88.
[16] Kessler, S. W. (1975) J. Immunol. 115, 1617–1624.

[17] Bollen, G. H. P. M., Cohen, L. H., Mager, W. H., Klaassen, A. W. and Planta, R. J. (1981) Gene 14, 279–287.
[18] Vaessen, R. T. M., Kreike, J. and Groot, G. S. P. (1981) FEBS Lett. 124, 193–196.
[19] Whitfeld, P. and Bottomley, W. (1980) Biochem. Intl. 1, 172–178.
[20] Jagendorf, A. T. and Bouthyette, P. Y. (1980) 5th Int. Congr. Photosynthesis, abst. p. 274.
[21] Baird, B. A. and Hammes, G. C. (1979) Biochim. Biophys. Acta 549, 31–53.
[22] Høyer-Hansen, G., Møller, B. L. and Pan, L. C. (1979) Calrsberg Res. Commun. 44, 337–351.
[23] Nelson, N., Nelson, H. and Schatz, G. (1980) Proc. Natl. Acad. Sci. USA 77, 1361–1364.
[24] Ellis, R. J. (1977) Biochim. Biophys. Acta 463, 185–215.
[25] Bouthyette, P. Y. and Jagendorf, A. T. (1978) Plant Cell Physiol. 19, 1169–1174.
[26] Simpson, D., Høyer-Hansen, G., Chua, N. H. and Von Wettstein, D. (1978) in: Proc. 4th Int. Congr. Photosynthesis (Hall, D. O. et al. eds) pp. 537–548, The Biochemical Society, London.
[27] Verheijen, J. H. (1980) PhD Thesis, Rodopi, Amsterdam.
[28] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.