NUCLEAR DNA CODES FOR THE PHOTOSYSTEM II CHLOROPHYLL–PROTEIN OF CHLOROPLAST MEMBRANES

S.D. KUNG, J.P. THORNBER and S.G. WILDMAN
Department of Botanical Sciences and Molecular Biology Institute
University of California, Los Angeles, Calif. 90024, USA

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1. Introduction

The use of various reciprocal, interspecific, Nicotiana hybrid combinations has made it possible to study the mode of inheritance of chloroplast macromolecular constituents in order to reveal whether the coding information for such proteins is in the chloroplast or nucleus. In the case of constituents located within the mobile phase [1] of chloroplasts, Kawashima and Wildman [2] demonstrated that nuclear DNA contains the genetic information for the sequence of amino acids of the small subunit of Fraction I protein. Bourque and Wildman [3] showed that three proteins in the 50 S subunit of chloroplast ribosomes were also coded by nuclear DNA. The availability of a homogeneous Photosystem II chlorophyll–protein complex of higher plants [4], which is a known major protein component of chloroplast lamellae [5], now permits an insight into the location of DNA coding for a structural protein of the thylakoid membrane. The present paper demonstrates a Mendelian mode of inheritance of fingerprints of tryptic peptides produced upon digestion of this protein moiety obtained from *N. tabacum, N. glauca* and their reciprocal hybrids.

2. Materials and methods

Photosystem II chlorophyll–protein complex was isolated as described previously [4]. Pigments were removed from the complex by two extractions with 80% acetone, and the protein moiety collected by centrifugation. The proteins were washed with distilled water (twice) and dried by lyophilization. Three mg of lyophilized protein was suspended in 0.1 ml of 0.1 N NH_4HCO_3 (pH 8.0) and digestion with chymotrypsin-free trypsin (Calbiochem) was carried out at 30° for 30 hr. The final enzyme to substrate ratio was 1:50 (w/w). After incubation, the reaction mixture was lyophilized. In some experiments, the G-25 Sephadex–paper chromatographic method of fingerprinting [6] was used. In others, half of the digested material (1.5 mg) was spotted on Whatman No. 3MM paper, and then chromatographed with n-butanol–acetic acid–water (4:1:5) for 40 hr. The thoroughly dried paper was subsequently electrophoresed in pyridine–acetic acid–water (1:10:289), pH 3.6 at 3000 V for 30 min [7]. The chromatographs were dipped through buffered ninhydrin solutions, air-dried, and then placed in a cool oven which was then heated to 70–80° to develop the spots [8].

3. Results

The Photosystem II chlorophyll–proteins prepared from *N. tabacum, N. glauca* and their reciprocal hybrids migrated as homogeneous material during polyacrylamide gel electrophoresis. No additional components were observed even when the gel was overloaded (fig. 1a). The purity was confirmed by their sedimentation as a single symmetrical component in the analytical centrifuge, the Schlieren pattern in fig. 1b being typical of the preparations. The pigment in the sample sedimented simultaneously with the protein demonstrating that the preparation is a

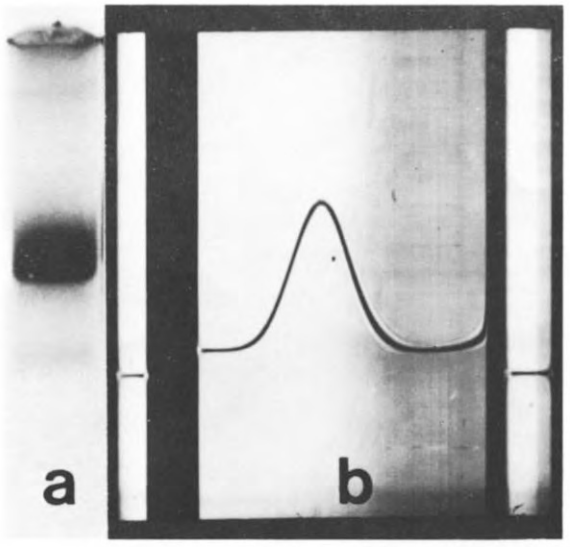

Fig. 1. a) Electrophoretic pattern of a heavy loading of the Photosystem II chlorophyll–protein preparation from *N. tabacum*. Electrophoresis and stain were carried out as described in [5]. b) Schlieren photograph of Photosystem II chlorophyll–protein preparation from *N. Tabacum*. Photograph was taken 8 min after attaining a speed of 44,770 rpm using synthetic boundary cell.

chlorophyll–protein complex, and is therefore a structural element of the thylakoid membrane system.

The amino acid composition of the *N. tabacum* complex has been determined previously [9]; the protein contains about 35 moles of arginine plus lysine per subunit weight of 35,000 g, so that about 35 peptides were to be expected on tryptic digestion. Initial experiments using the technique of Kawashima and Wildman [6] showed that the fingerprints of Photosystem II chlorophyll–protein from *N. tabacum* differed by at least two peptides from the fingerprint from *N. glauca*. Analysis of Photosystem II chlorophyll–protein from the reciprocal hybrids, *N. tabacum* × *N. glauca*, indicated that inheritance of the Photosystem II chlorophyll–protein was independent of the maternal parent. Confirmation of this observation was obtained by resolving the tryptic peptides by the conventional fingerprinting technique [7]. A fingerprint representing a typical tryptic peptide pattern obtained from the Photosystem II chlorophyll–protein of *N. tabacum* and the two reciprocal hybrids, *N. tabacum* × *N. glauca*, is shown in fig. 2. About 35 tryptic peptides were observed in the digest of the above protein moieties. The peptide fingerprints of protein from *N. tabacum* and the two reciprocal hybrids are identical. On the other hand, the digest of *N. glauca* protein exhibits a fingerprint showing a difference in at least two spots. These differences are quite clear and reproducible. Fig. 3 shows the tracings of the spots in the marked region of fig. 2; it is in this area that the differences occur between the tryptic peptides of the *N. glauca* component and those of *N. tabacum* and the reciprocal hybrids. Peptide "G" is present in *N. glauca* only, whereas peptide "T" occurs only in *N. tabacum* and the two hybrids. In order to confirm this difference, a mixture of the tryptic digests of Photosystem II chlorophyll–protein from *N. tabacum* and *N. glauca* was prepared. The peptide pattern of this mixture is also illustrated in fig. 3; both peptides "T" and "G" are present. Since both hybrids carry the peptide "T" of *N. tabacum*, but not peptide "G" of *N. glauca*, transmission of information of the *N. tabacum* peptide "T" was independent of the maternal parent. From this we conclude that the Photosystem II chlorophyll–protein is coded by nuclear DNA. As was found for the small subunit of Fraction I protein [2], information conveyed by *N. tabacum* pollen appears to dominate the expression of information already in *N. glauca* egg cells for *N. glauca* Photosystem II chlorophyll–protein.

4. Conclusion and discussion

A certain amount of evidence has accrued in the literature that some proteins of the chloroplast membranes are coded by chloroplast DNA and some by nuclear DNA. Herrmann [10,11] has concluded that the Photosystem I chlorophyll–protein and some proteins associated with Photosystem II activity are regulated by chloroplast DNA. This conclusion was based upon studies on plastome mutants of *Antirrhinum majus*. However, it has not been rigorously established whether or not such mutation actually resides in chloroplast DNA; in the case of a plastome mutant of Oenothera, which also had an impaired Photosystem II activity, the nature of the plastome lesion could not be identified [12]. Furthermore,

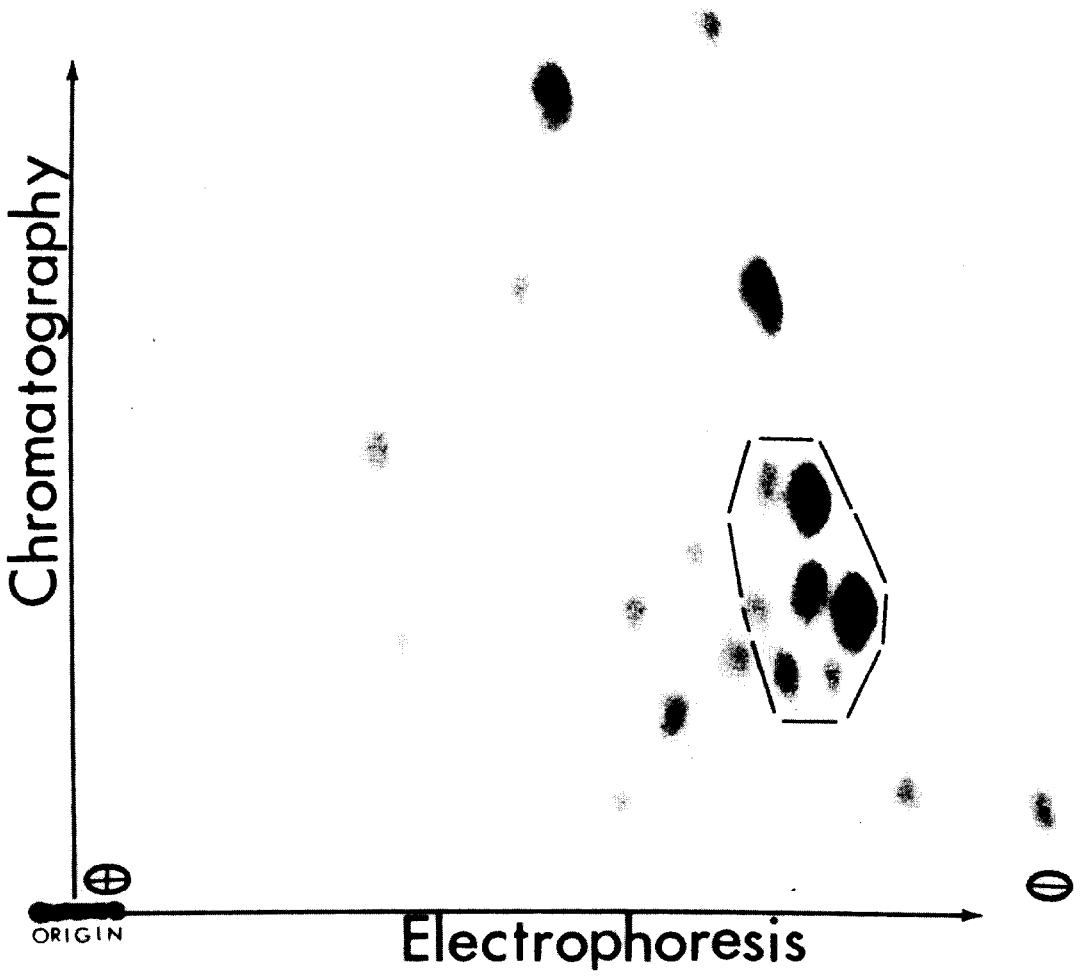

Fig. 2. Fingerprint of tryptic digest of Photosystem II chlorophyll–protein preparation from Nicotiana chloroplasts. The marked area is shown in detail in fig. 3.

Walles [13] has pointed out that present knowledge about the function of the plastome is restricted to what can be inferred from studies of chloroplast ultrastructure and physiological behavior of plastome mutants.

Kirk [14] has summarized the earlier evidence that some thylakoid proteins of unspecified function are made on cycloheximide-sensitive, cytoplasmic ribosomes; this implies that they are coded by nuclear DNA. More recent work by Apel and Schweiger [15] has substantiated the observation that nuclear DNA codes for some thylakoid proteins in Acetabularia; however, no function has been ascribed to these thylakoid proteins.

Our work provides the first conclusive proof that nuclear DNA codes for a major thylakoid protein of known function. How the information for the protein is transcribed as mRNA and whether it is translated into protein by chloroplast ribosomes remains still to be discovered. Our own prejudices are in favor of an

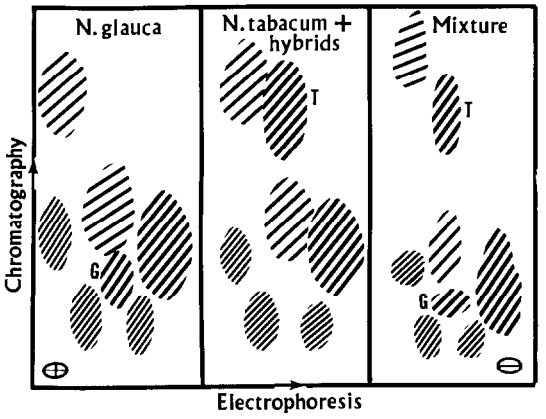

Fig. 3. The tryptic peptide pattern of the Photosystem II protein of *N. glauca, N. tabacum* and of a mixture of these two proteins. Only that area of the fingerprint marked in fig. 2 is shown. "G" marks the peptide which occurs in *N. glauca* only. "T" marks the peptide which occurs in *N. tabacum* and the two reciprocal hybrids.

extensive degree of cooperation and interaction between syntheses performed by chloroplast and cytoplasmic ribosomes during biogenesis of chloroplasts, and nuclear and chloroplast DNA both provide the information necessary for regulating chloroplast protein synthesis. It is quite possible that the pathway of biosynthesis of Photosystem II chlorophyll–protein may have as many unresolved possibilities as that proposed by Kawashima and Wildman [2] for the small subunit of Fraction 1 protein of chloroplasts.

Acknowledgements

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References

[1] S.G. Wildman, in: Autonomy and Biogenesis of Mitochondria and Chloroplasts, eds. N.K. Boardman, A.W. Linnane and R.M. Smillie (North-Holland, Amsterdam-London, 1971) p. 402.
[2] N. Kawashima and S.G. Wildman, Biochim. Biophys. Acta 262 (1972) 42.
[3] D.P. Bourque and S.G. Wildman, Science, submitted for publication, 1972.
[4] S.D. Kung and J.P. Thornber, Biochim. Biophys. Acta 253 (1971) 285.
[5] J.P. Thornber, R.P.F. Gregory, C.A. Smith and J.L. Bailey, Biochemistry 6 (1967) 391.
[6] N. Kawashima and S.G. Wildman, Biochim. Biophys. Acta 229 (1971) 749.
[7] A.M. Katz, W.J. Dreyer and C.B. Anfinsen, J. Biol. Chem. 234 (1959) 2897.
[8] C.W. Easley, Biochim. Biophys. Acta 107 (1965) 386.
[9] J.P. Thornber, J.C. Stewart, M.W.C. Hatton and J.L. Bailey, Biochemistry 6 (1967) 2006.
[10] F. Herrmann, FEBS Letters 19 (1971) 269.
[11] F. Herrmann, Exp. Cell Res. 70 (1972) 452.
[12] D.C. Fork and U.W. Heber, Plant Physiol. 43 (1968) 606.
[13] B. Walles, in: Structure and Function of Chloroplasts, ed. M. Gibbs (Springer-Verlag, New York, 1971) p. 54.
[14] J.T.O. Kirk, Ann. Rev. Biochem. 40 (1971) 161.
[15] K. Apel and H.G. Schweiger, European J. Biochem. 25 (1972) 229.