Biochemical Genetics, Vol. 15, Nos. 11/12, 1977

Cytoplasmic Inheritance of Chloroplast Coupling Factor 1 Subunits

R. D. Durbin[1] and T. F. Uchytil[1]

Received 13 Apr. 1977—Final 1 May 1977

An analysis of interspecific hybrids of Nicotiana *spp. in which one of the parents was sensitive to tentoxin showed that this sensitivity was transmitted only through the female parent. Since tentoxin acts by selectively binding to the α,β subunit complex of chloroplast coupling factor 1, the gene(s) specifying either one or both of these subunits is located in the cytoplasm.*

KEY WORDS: coupling factor 1; chloroplasts; maternal inheritance; *Nicotiana.*

INTRODUCTION

Chloroplast coupling factor 1 (CF_1) is required in the terminal stages of photophosphorylation for light-dependent ATP synthesis. It is situated on the chloroplast thylakoids and is composed of five different polypeptide subunits designated as α, β, γ, δ, and ε in order of decreasing molecular weight (Nelson *et al.*, 1973). The mode of inheritance of these subunits is unknown, although, based on differential inhibitor studies, Horak and Hill (1972) concluded that both cytoplasmic and chloroplastic protein-synthesizing systems are required. Subsequent results indicated that the chloroplast was not the site of synthesis (Eaglesham and Ellis, 1974). More recently, though,

Research cooperative with the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and the Agricultural Research Service, U.S. Department of Agriculture. Financial support was provided in part by a specific cooperative agreement between ARS and the University of Wisconsin.

[1] Plant Disease Resistance Research Unit, ARS, USDA, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

Mendiola-Morgenthaler *et al.* (1976) presented evidence based on differential extraction of CF_1 and its SDS-PAGE mobility patterns that the α, β, and possibly ε subunits can be synthesized by isolated chloroplasts. However, because of the complexity of the systems and the ambiguity associated with such studies, a definitive interpretation of inheritance cannot be made from these data.

An opportunity to directly localize the gene(s) responsible for the synthesis of a portion of CF_1's subunits was recently afforded by the findings that (1) tentoxin [cyclo(L-leucyl-*N*-methyl(*Z*)-dehydrophenylalanyl-glycyl-*N*-methyl-L-alanyl)] acts by specifically binding to CF_1 and inhibiting its Ca^{2+}-dependent ATPase (Steele *et al.*, 1976); (2) this binding involves only the α and β polypeptides (Steele *et al.*, 1977); and (3) interfertile *Nicotiana* spp. have been identified that are either sensitive or insensitive to the action of tentoxin (Durbin and Uchytil, 1977). Thus reciprocal hybrids involving combinations of sensitive and insensitive species should provide evidence for determining whether the gene(s) coding for these subunits resides in the nucleus or cytoplasm.

MATERIALS AND METHODS

The reciprocal interspecific hybrids were made using as parents two sensitive *Nicotiana* species (*N. raimondii* Macrb. and *N. solanifolia* Walp.) and two insensitive species (*N. knightiana* Goodsp. and *N. paniculata* L.). In addition, a number of interspecific crosses utilizing other species were tested. Those used as the sensitive parent were *N. benthamiana* Domin, *N. bigelovii* (Torr.) Wats., *N. clevelandii* Gray, *N. cordifolia* Phil., *N. glutinosa* L., *N. plumbaginifolia* Viv., *N. repanda* Willd. ex Lehm., and *N. suaveolens* Lehm.; those used as the insensitive parent were *N. fragrans* Hook., *N. sylvestris* Speg. & Comes, and *N. tabacum* L.

The hybrid seedlings were classified as either sensitive (chlorotic) or insensitive (normal) to tentoxin after 7–12 days' growth at 26 C using the seedlings germination test previously described (Durbin and Uchytil, 1977). Seedlings from crosses classed as insensitive were grown in the greenhouse, the CF_1 was isolated from the chloroplasts, and the reaction of its Ca^{2+}-dependent ATPase to tentoxin was determined (Steele *et al.*, 1976). This was done in order to be sure that a phenotypic expression of insensitivity was indeed due to CF_1 insensitivity. Two concentrations of tentoxin were used: 0.1 μg/ml, which is sufficient to cause complete inhibition of the ATPase activity of CF_1 from sensitive plants, and 10 μg/ml, which has no effect on the activity of CF_1 ATPase isolated from insensitive plants.

Table I. Reaction of Interspecific *Nicotiana* Hybrid Seedlings to Tentoxin

Hybrid	Reaction[a]
solanifolia × *knightiana*	−
solanifolia × *paniculata*	−
benthamiana × *gossei*	−
bigelovii × *tabacum*	−
clevelandii × *glutinosa*	−
cordifolia × *tabacum*	−
glutinosa × *sylvestris*	−
glutinosa × *tabacum*	−
plumbaginifolia × *tabacum*	−
raimondii × *knightiana*	−
raimondii × *paniculata*	−
repanda × *sylvestris*	−
suaveolens × *megalosiphon*	−
suaveolens × *tabacum*	−
fragrans × *tabacum*	+
knightiana × *solanifolia*	+
knightiana × *raimondii*	+
paniculata × *solanifolia*	+
paniculata × *raimondii*	+
sylvestris × *tomentosiformis*	+
tabacum × *alata*	+
tabacum × *benavidesii*	+
tabacum × *cordifolia*	+
tabacum × *glutinosa*	+
tabacum × *knightiana*	+
tabacum × *longiflora*	+
tabacum × *megalosiphon*	+
tabacum × *nudicaulis*	+
tabacum × *plumbaginifolia*	+
tabacum × *sylvestris*	+

[a] A minus sign denotes leaf chlorosis; a plus sign denotes a normal appearance.

RESULTS AND DISCUSSION

Examination of 30 interspecific hybrids, of which 14 were reciprocal showed that in all cases the reaction of the female parent to tentoxin determined the reaction of the F_1 progeny (Table I). Without exception, all seedlings of any one genotype reacted identically. The Ca^{2+}-dependent ATPase activity of CF_1 isolated from the insensitive hybrid plants was not inhibited by tentoxin at 10 μg/ml.

These results show that the receptor site on CF_1 responsible for sensitivity to tentoxin is cytoplasmically inherited. Because it is not known whether the binding of tentoxin to this site involves both the α and β subunits or only

one of them, we can only conclude that at least one of these subunits is encoded in the cytoplasm.

Cytoplasmic inheritance of chloroplastic proteins has previously been reported (Sager, 1972). In the case of fraction 1 protein, the large subunits which contain ribulose diphosphate carboxylase activity are encoded within the chloroplast, while the small subunits are encoded within the nucleus (Kung, 1976). The results of Mendiola-Morgenthaler *et al.* (1976) suggest that a similar situation exists with regard to CF_1. Supporting this notion is the requirement of a cytoplasmic protein-synthesizing system, as reported by Horak and Hill (1972), which could mean that the synthesis of the γ and/or δ subunits is under nuclear control. However, it is also possible that the cytoplasmic system is involved in thylakoid protein synthesis with which CF_1 subunit synthesis is obligatorily coordinated. Furthermore, as Ohad (1975) has pointed out, the sites within the cell of transcription and translation may be different. Thus it is premature to conclude that the structural genes for the subunits of CF_1 reside in different organelles.

ACKNOWLEDGMENTS

The assistance of L. G. Burk and J. R. Stavely in providing seed of some of the *Nicotiana* species and interspecific hybrids is gratefully acknowledged.

REFERENCES

Durbin, R. D., and Uchytil, T. F. (1977) A survey of plant insensitivity to tentoxin. *Phytopathology* **67:** 602.

Eaglesham, A. R. J., and Ellis, R. J. (1974). Protein synthesis in chloroplasts. II. Light-driven synthesis of membrane proteins by isolated pea chloroplasts. *Biochim. Biophys. Acta* **335**:396.

Horak, A., and Hill, R. D. (1972). Adenosine triphosphatase of bean plastids. *Plant Physiol.* **49**:365.

Kung, S. D. (1976). Tobacco fraction 1 protein: A unique genetic marker. *Science* **191**:429.

Mendiola-Morgenthaler, L. R., Morganthaler, J. J., and Price, C. A. (1976). Synthesis of coupling factor CF_1 protein by isolated spinach chloroplasts. *FEBS Lett.* **62**:96.

Nelson, N., Deters, D. W., Nelson, H., and Racker, E. (1973). Partial resolution of the enzymes catalyzing photophosphorylation. XIII. Properties of isolated subunits of coupling factor 1 from spinach chloroplasts. *J. Biol. Chem.* **248**:2049.

Ohad, I. (1975). Biogenesis of chloroplast membranes. In Tzagoloff, A. (ed.), *Membrane Biogenesis*, Plenum, New York, pp. 279–350.

Sager, R. (1972). *Cytoplasmic Genes and Organelles*, Academic Press, New York.

Steele, J. A., Uchytil, T. F., Durbin, R. D., Bhatnagar, P., and Rich, D. H. (1976). Chloroplast factor 1: A species-specific receptor for tentoxin. *Proc. Natl. Acad. Sci.* **73**:2245.

Steele, J. A., Uchytil, T. F., and Durbin, R. D. (1977). The binding of tentoxin to a tryptic digest of chloroplast coupling factor 1. *Biochim. Biophys. Acta* **459**:347.