EVIDENCE THAT NUCLEAR GENES CODE FOR SEVERAL CHLOROPLAST RIBOSOMAL PROTEINS

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Summary: Electrophoresis of the proteins of the 50S chloroplast ribosome subunit reveals that there are at least two detectable differences between Nicotiana tabacum and N. glauca. Nuclear genes contain the information for these two polypeptides since they are transmitted to F_1 interspecific hybrids independently of the maternal parent.

Introduction: Plastids and the genetic information they may contain are transmitted to zygotes only by the maternal parent and not by pollen in Nicotiana as well as in other plant genera (1). Since the kinetic complexity of chloroplast DNA is about $1.1\text{-}1.8 \times 10^8$ daltons (2), its information content is well in excess of that needed to code for a full complement of RNA and 50 or so proteins as found in bacterial 70S ribosomes, which have similar properties to chloroplast ribosomes (3). Thus, one possible function of chloroplast DNA could be to carry the information for chloroplast ribosomal proteins. However, there has been no direct genetic evidence showing whether chloroplast or nuclear DNA codes for chloroplast ribosomal proteins.

This report presents data which indicate that (a) there are at least two electrophoretically distinguishable differences among the basic proteins of the 50S chloroplast ribosome subunits of Nicotiana tabacum and N. glauca, and that (b) these proteins are coded by nuclear genes since neither polypeptide exhibits maternal inheritance in interspecific hybrids.

Methods: Chloroplasts were isolated from about 1 kg. of deribbed leaves by chopping 50 g. batches in 100 ml buffer (0.5 M sucrose, 50 mM Tris, 25 mM KCl, 25 mM $MgCl_2$, 0.4% β-mercaptoethanol, pH 8.0).

Centrifugation of the filtered homogenate (1100 g for 10 min.) yielded a chloroplast pellet. The 70S chloroplast ribosomes were osmotically released by resuspending the chloroplasts in solution A (25 mM Tris, 25 mM KCl, 2.5 mM $MgCl_2$, 8 mM β-mercaptoethanol, pH 7.5) in the ratio of 10 ml per 50 g. leaves and dialyzing overnight against 2 liters of solution A. The dialyzed chloroplast preparation was brought to 25 mM $MgCl_2$ to reaggregate the thylakoid membranes which were removed by centrifuging at 17,000 g for 20 minutes. Ribosomes were pelleted from the supernatant by centrifugation at 105,000 g. for 4 hours. The pellets were resuspended in 0.2 ml Solution A per 50 g. leaves and dialyzed against two changes of solution A to dissociate the 70S chloroplast ribosomes. After purification on sucrose gradients (Fig. 1), chloroplast ribosome subunits were precipitated overnight by adding solid ammonium sulfate to saturation. The collected precipitates were stored at 0°C.

Ribosomes were dissolved in 4 M LiCl-8M urea containing 1% β-mercaptoethanol. The precipitated RNA was removed by centrifugation after 24 hours in the cold. The RNA-free ribosomal proteins were desalted by dialysis against two changes of 8M urea-1% β-mercaptoethanol and subjected to disc electrophoresis (4). Gels were stained for 15 minutes with 1% Amido Black in methanol:acetic acid:water (5:1:5) followed by diffusion destaining. The K12 strain-specific ribosomal protein of E. coli was routinely resolved on our gels and the patterns were identical to those of Leboy, et al. (5). Therefore, we judged our methods to be sufficiently sensitive for comparative analysis of ribosomal proteins of different Nicotiana species and their hybrids.

Results and Discussion: After dialysis in buffer containing 2.5 mM $MgCl_2$, most of the chloroplast 70S ribosomes had dissociated into 30S and 50S subunits (Fig. 1A), whereas 80S cytoplasmic ribosomes remained

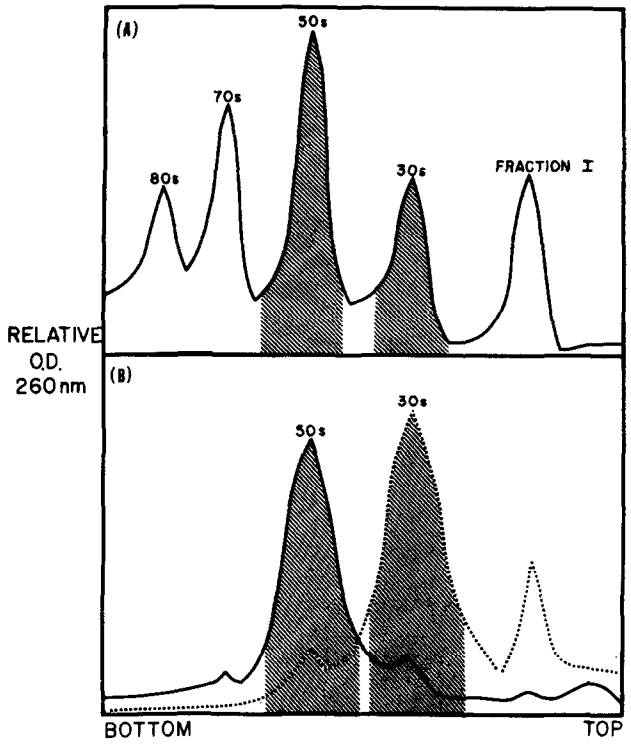

Figure 1. (A) Absorbance profiles of chloroplast ribosomes after centrifugation for 15 hours at 24,000 rpm in 15-34% exponential sucrose gradients containing 2.5 mM magnesium acetate, 25 mM KCl, 25 mM Tris, pH 7.5 and 8 mM β-mercaptoethanol. Sedimentation rates of 70S monosomes and 30S and 50S subunits of chloroplast ribosomes were indistinguishable from E. coli monosomes and subunits on similar gradients. Fractions within the hatched areas were pooled for repurification of subunits. The pooled samples were dialyzed to remove sucrose. Ribosomes were collected by centrifugation, dissolved in 0.1- 0.5 ml solution A (see text) and repurified on sucrose gradients. (B) Absorbance profiles of chloroplast ribosome subunits repurified from A. In some cases narrower gradient cuts were made to guarantee purity.

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intact under these conditions (6). These results are typical of those obtained with N. tabacum, N. glauca, and their hybrids. Fraction 1 protein (Fig. 1A) was always present in these ribosome preparations as were intact 80S cytoplasmic ribosomes in amounts which vary among preparations from 10-30% of the total ribosomal particles. Fractions containing the chloroplast ribosome subunits were pooled and repurified on a second gradient as shown in Figure 1B. The twice-purified 30S and 50S subunits were virtually free of cross contamination.

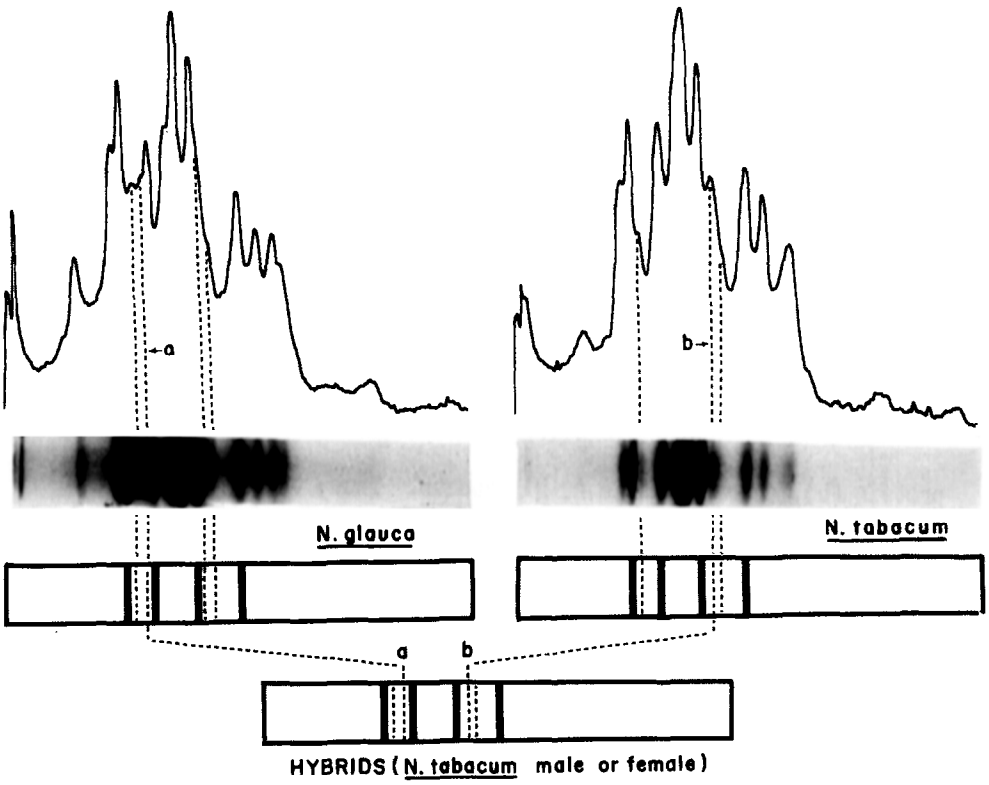

Figure 2. Electrophoretic patterns of basic ribosomal proteins of 50S chloroplast ribosome subunits from Nicotiana glauca and N. tabacum. The proteins labeled (a) and (b) can be followed (from the top of the figure) through the densitometer tracing, the gel photograph, a schematic representation of selected bands of the gel patterns, and a diagram of the inheritance pattern of these two proteins in the reciprocal interspecific hybrids between N. glauca and N. tabacum. The unlabeled dotted lines in the figure are provided for comparison as reference aids, as are the solid bands in the schematic patterns which correspond to the nearest intense band on either side of the region of the gel containing bands (a) and (b). The direction of electrophoretic migration is from left (cathode) to right (anode).

Electrophoresis was performed using an Ortec pulsed power supply (400v, 400cps, 1.0μfarad). Gels were run until a marker dye had reached the end of the gel (4-5 hours). All samples were run in duplicate and comparisons were made only between gels run at the same time and between protein samples prepared under identical conditions. No measureable variation in band migration was found in replicate samples. The stained gels were photographed and the negatives were scanned with a Joyce-Loebl recording microdensitometer.

The protein composition of the 50S chloroplast ribosome subunits tained from N. tabacum and N. glauca is shown in Figure 2. About 25 electrophoretically distinct bands were visually detectable.

The protein labeled (a) is present in N. glauca and absent in the corresponding position in N. tabacum. In N. tabacum, the protein labeled (b) has a greater mobility than the corresponding band in the N. glauca pattern. When the protein patterns of the reciprocal, interspecific hybrids were compared, markers unique to each of the parent species were found in both hybrids (Fig. 2). Protein (a), characteristic of N. glauca, was carried by both hybrid combinations. Both hybrids also contained a band whose mobility was the same as protein (b), characteristic of N. tabacum. Thus, the hybrids inherited a chloroplast ribosomal protein phenotype from each of the parent species irrespective of the parental sex.

It was previously reported (7) that both subunits of spinach chloroplast ribosomes contain about 20 different basic proteins. Our results show that Nicotiana chloroplast 50S ribosome subunits probably contain at least 25 basic proteins which is closer in number to conservative estimates (8) for E. coli 50S ribosome subunits. To date, we have detected no differences among the basic proteins of 30S chloroplast ribosome subunits or among the proteins of the 80S cytoplasmic ribosomes in comparisons of several species of Nicotiana. Extensive homologies in the 80S cytoplasmic ribosomal proteins of other plant species have been reported (9).

Since the inheritance in interspecific F_1 hybrids of the two 50S chloroplast ribosomal proteins reported in this study was independent of the maternal parent, we conclude that these proteins must be coded by DNA in the nucleus, and not by chloroplast DNA. It is possible that other constitutive proteins of the chloroplast 50S subunit may be carried by nuclear genes and that these genes may be closely linked. The close mapping of ribosomal protein mutants in E. coli (10, 11) and B. subtilis (12) engenders this hypothesis. The question of whether any proteins are shared between the cytoplasmic and chloroplast ribosomes is under investigation.

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