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An Altered Chloroplast Ribosomal Protein in a Streptomycin Resistant Tobacco Mutant

N.P. Yurina and M.S. Odintsova
Bakh Institute of Biochemistry, Moscow (U.S.S.R.)

P. Maliga
Institute of Plant Physiology, Biological Research Center, Szeged (Hungary)

Summary. Ribosomal proteins from chloroplasts of *Nicotiana tabacum* L. (cv. Petit Havana) and of SR1, a mutant derived from it, with uniparentally inherited streptomycin resistance, were characterised by two-dimensional gel electrophoresis. From the 67 proteins identified, one has an altered electrophoretic mobility when isolated from the mutant. Streptomycin resistance of the SR1 mutant therefore seems to be the consequence of a mutation in the chloroplast DNA coding for a chloroplast ribosomal protein.

Key words: Chloroplast – Ribosomal protein – Streptomycin resistance – Tobacco

Introduction

One of the possible approaches to the identification and localisation of the structural genes of the organelle ribosomal proteins is the isolation of mutants defective in ribosomal functions. In *E. coli*, as well as in other bacteria, it has been shown that mutants selected for resistance to antibiotics inhibiting protein synthesis usually contain ribosomes with altered protein components (Chua and Luck 1974). Organelle ribosomes in plants are in many respects, antibiotic sensitivity being one, similar to bacterial ribosomes. Selection for resistance to the same antibiotics in plants, therefore may result in the isolation of mutants with altered organellar ribosomal proteins. Such mutants have been described for the unicellular green alga *Chlamydomonas* (Chua and Luck 1974; Gillham et al. 1976).

SR1, a streptomycin resistant tobacco (*Nicotiana tabacum* cv. Petit Havana) is the first drug-resistant mutant in flowering plants which has been shown to inherit the resistance as a uniparental, non-Mendelian trait (Maliga et al. 1973; 1975). Uniparental transmission of a trait in crosses indicated that the mutated gene is located in either the chloroplast or the mitochondrial DNA (Sager 1972). The chloroplast ribosomal proteins of the streptomycin-sensitive and resistant plants were studied by a sensitive method of analysis, two-dimensional gel electrophoresis, in order to reveal any differences that could be correlated with streptomycin resistance.

Materials and Methods

Chloroplast ribosomes were isolated from the leaves of greenhouse-grown streptomycin sensitive (*Nicotiana tabacum* cv. Petit Havana) and resistant SR1 plants.

Young leaves (5 to 9 cm in length) were harvested from 6-week-old plants. The midribs of the leaves were removed and the leaf tissue was homogenised in a medium (w/v, 1:2) containing 0.5 M sucrose, 0.025 M tris-HCl (pH 7.8) 0.01 M magnesium acetate, and 0.006 M 2-mercaptoethanol. The homogenate was filtered through cheese-cloth and centrifuged at 500 X g for 40 sec. The chloroplasts were isolated from the supernatant by centrifugation at 3000 X g for 15 min., and then washed once with the isolation medium. Chloroplasts were subsequently disrupted by the osmotic shock caused by suspending them in the isolation medium without sucrose.

The suspensions of the disrupted chloroplasts and the supernatant obtained after the 500 X g centrifugation step (the isolation of cytoplasmic ribosomes) were centrifuged at 17,000 X g for 20 min. Triton X100 was added to the supernatant to a final concentration of 0.5-1 % and the ribosomes were pelleted through a cushion of 1 M sucrose by centrifugation for 4 h at 160,000 X g.

The ribosomal proteins were solubilised by suspending the pellet in the isolation medium (without sucrose) and an equal volume of 6 M LiCl, 10 M urea, and 1 % 2-mercaptoethanol (Borque and Wildman 1972). All procedures were carried out at 0°C.

The proteins were separated by two dimensional (2-D) gel elec-

trophoresis as described by Kaltschmidt and Wittmann (1970). The proteins (200 to 300 mg per sample) were run first in tubes (inner diameter, 1.6 mm; height, 100 mm) for 9 h at 50 V using 8 % acrylamide gel at pH 8.6. Discs were then removed from the tubes and placed on top of 1 mm thick slabs. In the second dimension, proteins were separated in an 18 % acrylamide gel at pH 4.5 by a 15 h electrophoresis at 100 V. Proteins were stained by incubating the slabs for 20 min. in a solution containing Amido black 10B (0.15 %) and Cromassie-brilliant blue R-250 (0.05 %). The gels were destained in methanol-water-acetic acid (5:5:1).

Results and Discussion

In the chloroplasts of the streptomycin sensitive plants, 67 proteins were identified by 2-D electrophoresis (Fig. 1). Fifty-two of these appeared as dark spots, the remainder were less intensively stained. Under the conditions of this experiment, 59 proteins moved towards the cathode.

The number of chloroplast ribosomal proteins has been determined only in a few species. The numbers reported vary between 32 and 75. In wheat, 75 (Jones et al. 1972); in *Chlamydomonas reinhardi*, 32-59 (Hanson et al. 1974; Brügger and Boschetti 1975; Spiess and Arnold 1975); in *Euglena gracilis*, 56-58 (Freyssinet 1975); in pea, 71 (Odintsova and Yurina 1975) ribosomal proteins have been found. The contradictory results may be explained by species specific differences in the number of proteins and differences in the methodology. In order to find out the real numbers, isolation of the individual proteins and their subunits seems to be essential. This has been done with *E. coli* ribosomes (Garrett and Wittmann 1973).

The number of chloroplast ribosomal proteins was found to be identical in the streptomycin sensitive and resistant plants. Comparison of the electrophoretic mobility of the chloroplast ribosomal proteins, however, revealed that one intensively stained protein from the SR1 plants moved faster in the first direction (Figs. 1 and 2; arrows).

Under the conditions used, the proteins were separated in the first dimension mainly according to their charge, while in the second dimension mainly according to the molecular weights. The inference is that the mutation resulting in streptomycin resistance has lead to an alteration in the charge of this protein but not in its molecular weight.

Variations in the concentration of some acidic proteins (at pH 8.6) were also observed.

In the extracts from cytoplasmic ribosomes, 88 proteins were separated (Fig. 3) Fifty-seven appeared as dark spots. In the first dimension, at pH 8.6, 79 proteins migrated towards the cathode. Comparing several slabs of the cytoplasmic ribosomal proteins from resistant and sen-

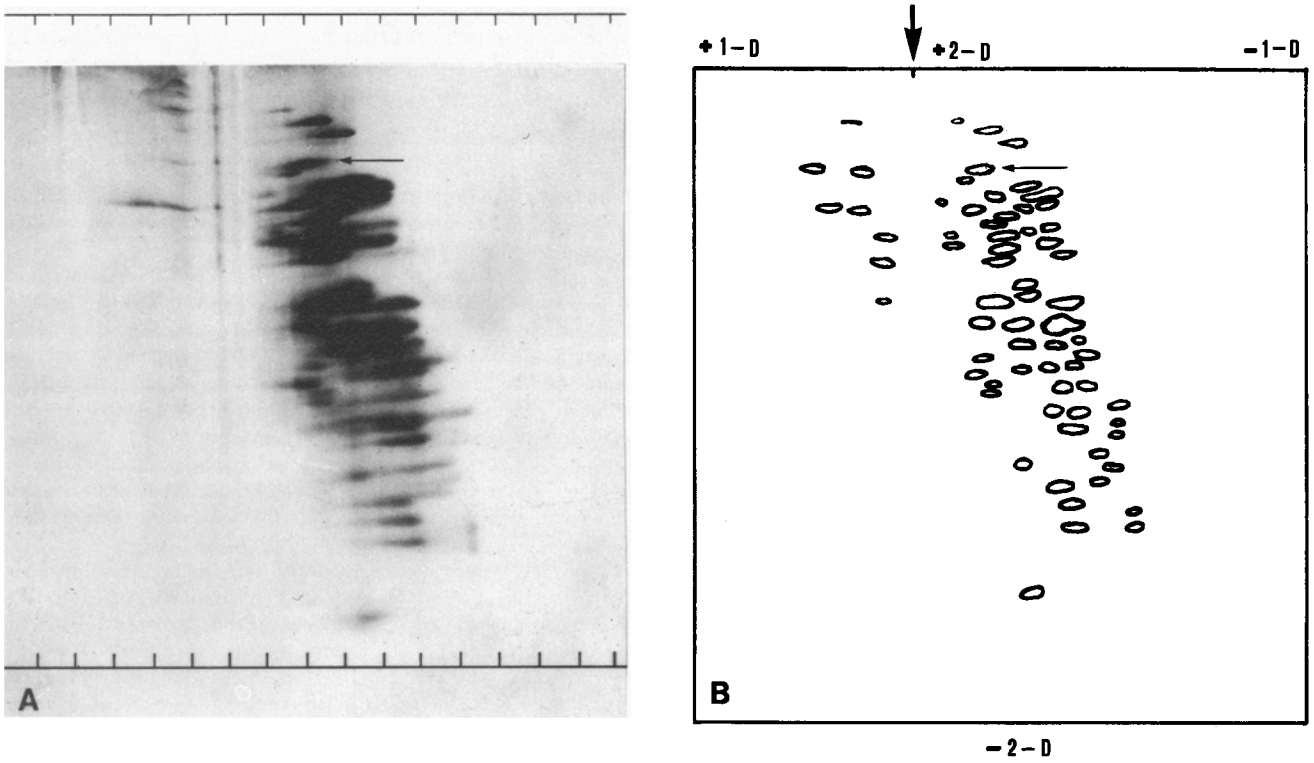

Fig. 1. Two-dimensional gel electrophoretic pattern (A) and schematic representation (B) of chloroplast ribosomal proteins from streptomycin sensitive tobacco. Arrow indicates origin

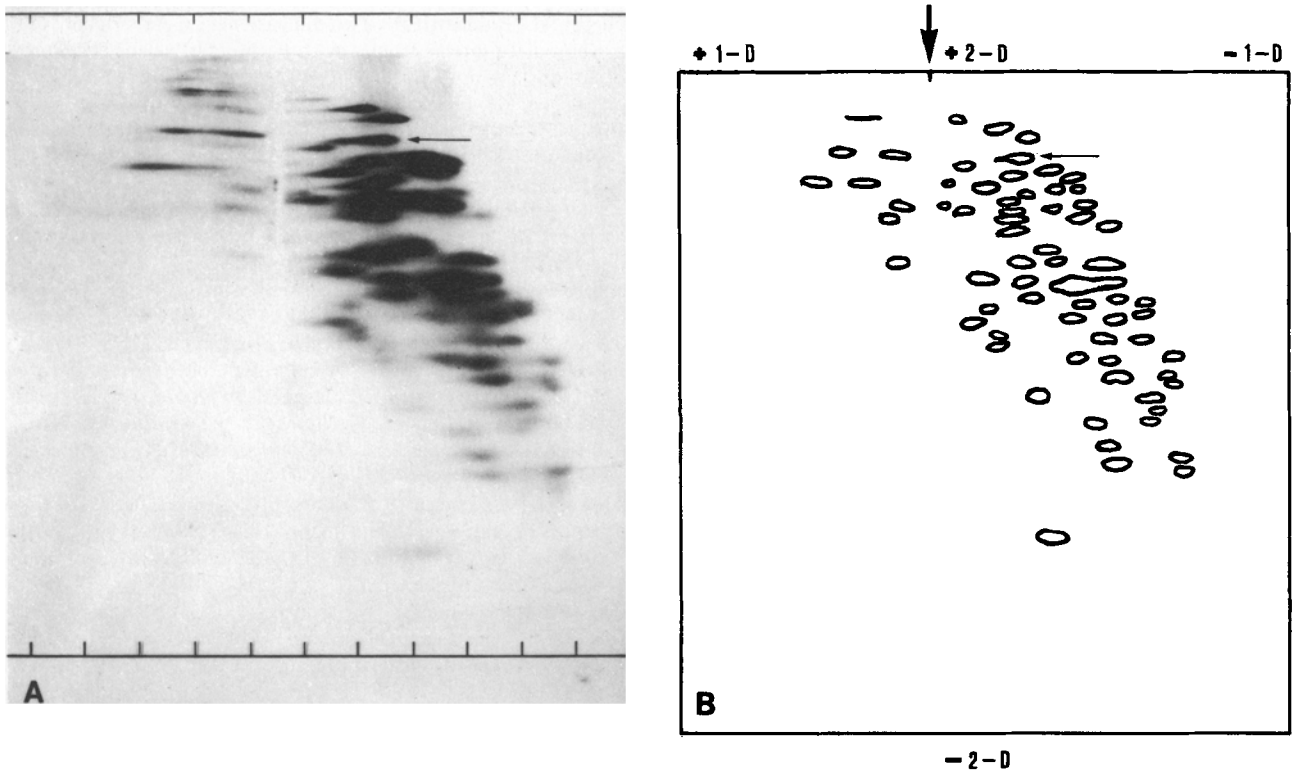

Fig. 2. Two-dimensional gel electrophoretic pattern (A) and schematic representation (B) of chloroplast ribosomal proteins from the SRI streptomycin resistant tobacco mutant. Arrow indicates origin

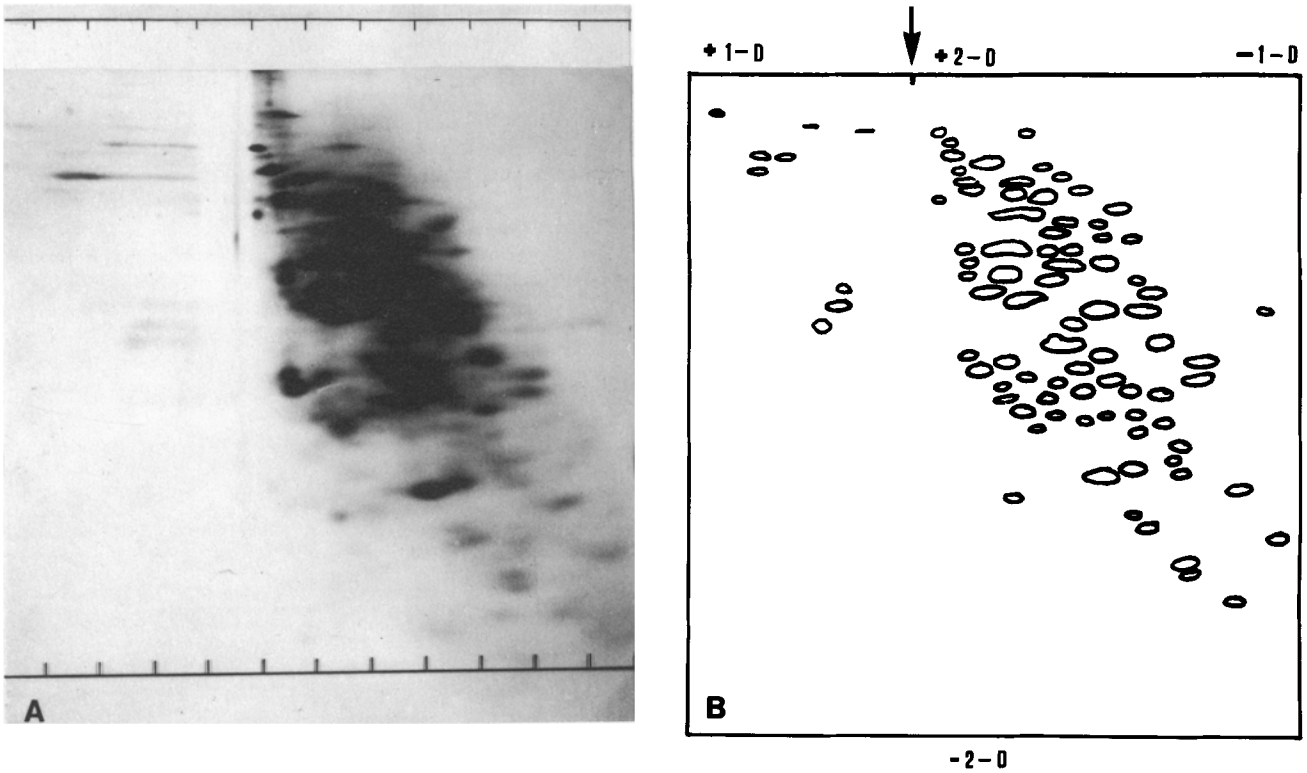

Fig. 3. Two-dimensional gel electrophoretic pattern (A) and schematic representation (B) of proteins from cytoplasmic ribosomes of the SRI mutant. Arrow indicates origin

sitive plants, no differences in the electrophoretic patterns were detected.

The dissimilar patterns of 70 S and 80 S ribosomal proteins indicated that there was no mutual contamination in the ribosomal preparations.

SR1 is the first mutant in flowering plants in which a correlation between a uniparental trait and a chloroplast ribosomal phenotype could be demonstrated. Similar changes in the chloroplast ribosomal proteins were described in the uniparental streptomycin resistant mutants of the green alga, *Chlamydomonas* (Ohta and Sager 1975; Brügger and Boschetti 1975).

Alteration of the ribosomal protein as the reason for streptomycin resistance could be accepted beyond doubt only if resistance of the ribosomes can be demonstrated in vitro, e.g. in a streptomycin binding assay. It cannot be excluded otherwise that resistance is brought about by a membrane alteration and that mutation in the chloroplast DNA, resulting in an altered protein, occurred independently. This possibility in the case of SR1, however, has been eliminated by experiments conducted in a different laboratory (Bourque et al. 1977).

While this manuscript was under preparation, the work of Bourque et al. (1977) came to our attention. They have arrived at similar conclusions independently, using the same tobacco mutant.

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Dr. P. Maliga
Institute of Plant Physiology
Biological Research Center
H-6701 Szeged, P.O.B. 521
(Hungary)

Dr. N.P. Yurina
Dr. M.S. Odintsova
Bakh Institute of Biochemistry
Moscow, V71, Leninskij prosp. 33
(U.S.S.R.)