PLASTID RIBOSOME DEFICIENT MUTANTS OF *PELARGONIUM ZONALE**

Th. BÖRNER, F. HERRMANN, and R. HAGEMANN
Wissenschaftsbereich Genetik, Sektion Biowissenschaften Martin-Luther-Universität, 402 Halle/S., Domplatz 1, DDR

Received 19 September 1973

1. Introduction

Most information about the function of chloroplast ribosomes is derived from studies with antibiotics inhibiting chloroplast or cytoplasmic protein synthesis. In spite of the great number of papers dealing with the site of synthesis of chloroplast components published in the last years, our knowledge on this topic is still rather limited [1]. Recently, several mutants of higher plants [2–6] and of *Chlamydomonas* [7, 8] have been described in which the plastid ribosomes are either entirely lacking or only present in a low frequency. As a result of the deficiency no proteins (or only a very low amount of proteins) can be synthesized within the plastids. Therefore, these mutants are useful objects for the investigation of the function of plastid ribosomes in the biogenesis of chloroplasts.

In *Pelargonium zonale* the nucleic acids were isolated from white and green leaves of 5 chimerical varieties and from green leaves of an entirely green variety. Plastid rRNA was separated from cytoplasmic rRNA by electrophoresis on polyacrylamide gels. The white leaves of all chimerical varieties, studied, were found to contain no plastid rRNA. The results reported in this communication suggest that plastid ribosome deficient mutants are more common than hitherto assumed.

2. Material and methods

Nucleic acids have been isolated from the following varieties and mutants of *Pelargonium zonale*: from the entirely green variety 'Trautlieb' (as control); from pure white shoots of F_1-plants containing the mutant plastids of the variety 'Flower of Spring'; from white leaves of the chimerical varieties 'Madame Salleron', 'Freak of Nature', and 'Greifswald' [9–11].

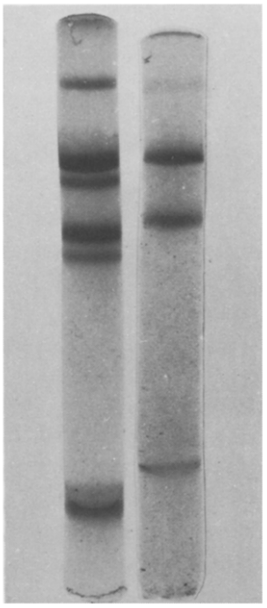
The nucleic acids were isolated as already described [12] modified for small quantities (0.5^4g) of leaf material.

The high molecular rRNA was electrophoretically separated on polyacrylamide gels in the semi-micro scale. The gels were polymerized in glass tubes with a length of 45 mm and an inside diameter of 2 mm. The gel solution was similar to that of Bishop et al. [13] modified for gels containing 3% acrylamide. About 2 μg of nucleic acids in 0.15 M NaCl–0.015 M Na-citrate were applied to each gel. Electrophoresis was carried out for 1 hr at room temperature at 0.5 mA per gel. After staining with 0.2% methylene blue [14] for 1–2 min and destaining in distilled water for 24 hr the gels were scanned at 578 nm with a Chromoscan (Joyce–Loebl, England).

3. Results and discussion

In green wild type plants of *Pelargonium zonale* ('Trautlieb') 4 bands of high mol. wt. rRNA are detectable in the gels: the 25 S and 18 S RNA of cytoplasmic ribosomes, and the 23 S and 16 S RNA of plastid ribosomes (fig. 1a). The upper band containing DNA is unstable and often disappears during the process of destaining. The rRNA of all white mutant forms of *Pelargonium zonale*, studied, shows the following pattern: the 25 S and 18 S RNA of cytoplasmic ribosomes are found in normal amounts; but the 23 S and 16 S RNA of plastid ribosomes are ab-

* This is part VIII of a series on: Structure and Function of the Genetic Information in Plastids.



a b

Fig. 1. Electrophoretic separation of RNA from wild-type (a) and mutant (b). Gels.

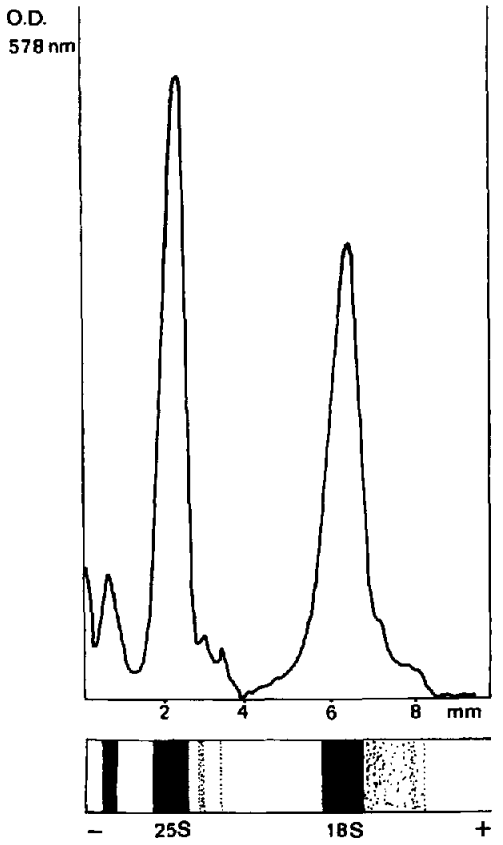


Fig. 2. Electrophoretic separation of mutant RNA. Densitogram and scheme of gel.

sent or strongly reduced quantitatively (fig. 1b). In the area, in which the 23 S rRNA is found in gels from green cells, often a weak band can be seen which as a rule is accompanied by a second thin band (approx. 22 S). In the area of the 16 S rRNA we could not find any band with certainty; sometimes a diffuse stain appeared (fig. 2). The origin of the thin bands in gels from white mutants is uncertain at the moment. Studies are in progress to elucidate whether these bands contain residues of plastid rRNA.

These findings are in agreement with the results of our analysis of the white plastom mutant 'Mrs. Parker' of *Pelargonium zonale*, in which the 23 S and the 16 S rRNA are absent, too, and in which the absence of plastid rRNA is connected with the loss of plastid ribosomes [4].

The loss of plastid rRNA in the white leaves of the varieties 'Mrs. Parker', 'Flower of Spring', 'Freak of Nature' and probably also in the varieties 'Madame Salleron', 'Gnom', and 'Greifswald' is caused by mutations in the plastid DNA [4, 10, 11]. It is of interest that the same defect was found in mutants with mutations in the nuclear DNA [5, 6]. Obviously, both plastid and nuclear DNA contain genetic information necessary for normal biogenesis of plastid ribosomes. The information for the plastid rRNA is encoded in the plastid DNA (for a recent summary see [1]. Therefore, the absence of plastid rRNA may be, in the case of our plastom mutants, the primary effect of the mutations. But it is also possible that a defect in the ribosomal proteins or in a regulator component causes the observed loss of RNA.

The absence of the plastid rRNA in these mutants, and the loss of the plastid ribosomes, respectively, should greatly impair the protein synthesis in the white plastids. In accordance with this expectation we found that in the different mutant *Pelargonium* forms the white leaves contain no (or only very small amounts of) ribulose-1, 5-diphosphate carboxylase (Börner, unpublished); the results of many investigators indicate that this enzyme – at least its large subunit – is synthesized on plastid ribosomes (for a survey

see [1]. Thus, the absence of RuDP-carboxylase demonstrates the impairment of plastid ribosome functions.

Plastid ribosome deficient mutants are of great value in studying the functions of plastid ribosomes. Plastid components which are present in such mutants must be synthesized outside the plastids, whereas absent proteins should be synthesized on the plastid ribosomes. At least in *Pelargonium*, this type of mutant does not seem to be so rare as was hitherto expected. Thus, it is imaginable that a number of entirely white mutants, not only of *Pelargonium* but also of other species, will show comparable plastid ribosome deficiencies.

References

[1] Börner, Th. (1973) Biol. Zbl. 92, 545.
[2] Shumway, L.K. and Weier, T.E. (1967) Amer. J. Bot. 54, 773.
[3] Dyer, T.A., Miller, R.H. and Greenwood, A.D. (1971) J. Exptl. Bot. 22, 125.
[4] Börner, Th., Knoth, R., Herrmann, F. and Hagemann, R. (1972) Theor. Appl. Genetics 42, 3.
[5] Sprey, B. (1972) Z. Pflanzenphysiol. 67, 223.
[6] Toyama, S. (1972) Bot. Mag. Tokyo 85, 1.
[7] Surzycki, S.J., Goodenough, U.W., Levine, R.P. and Armstrong, J.J. (1970) Symp. Soc. Exptl. Biol. 24, 13.
[8] Boynton, J.E., Gillham, N.W. and Chabot, J.F. (1972) J. Cell Sci. 10, 267.
[9] Bergann, F. and Bergann, L. (1959) Züchter 29, 361.
[10] Tilney-Bassett, R.A.E. (1963) Heredity 18, 485.
[11] Hagemann, R. and Scholze, M. Biochem. Physiol. Protozoa, in press.
[12] Herrmann, F. (1969) Dr. rer. nat. Thesis, University Halle.
[13] Bishop, D.H.L., Claybrook, V.R. and Spiegelman, S. (1967) J. Mol. Biol. 24, 373.
[14] Peacock, A.C. and Dingman, C.M. (1967) Biochemistry 6, 1818.