Planta (1981) 153:547–555

Planta

Synthesis of ribosomal RNA in ribosome-deficient plastids of the mutant "albostrians" of *Hordeum vulgare* L.

A. Siemenroth[1], R. Wollgiehn[2], D. Neumann[2], and Th. Börner[1]

[1] Wissenschaftsbereich Genetik, Sektion Biowissenschaften, Martin-Luther-Universität, Domplatz 1, DDR-401 Halle and
[2] Forschungszentrum für Molekularbiologie und Medizin der Akademie der Wissenschaften, Institut für Biochemie der Pflanzen, Weinberg, DDR-402 Halle, German Democratic Republic

Abstract. The nuclear gene-induced plastome mutant "albostrians" of *Hordeum vulgare* L. is characterized by a plastid ribosome-deficiency. This ribosome deficiency could be caused by the lack of or a defect in chloroplast RNA polymerase. However in our investigations we found an activity of chloroplast RNA polymerase in wild-type and mutant leaves of "albostrians" barley by (1) electron microscopic autoradiography after in vivo labelling of RNA, (2) determination of RNA polymerase activity in isolated plastids, and (3) characterization of the newly synthesized RNA by electrophoresis in polyacrylamide gels. The genes of 23S and 16S rRNA are transcribed in mutant plastids and the RNAs are processed. From these results we conclude that the enzymes involved in transcription and processing of chloroplast rRNA are synthesized on cytoplasmic ribosomes and that the plastid ribosome deficiency in albostrians barley is probably not caused by a defective chloroplast DNA dependent RNA polymerase or by a mutation in the genes of 23S and 16S chloroplast rRNA.

Key words: *Hordeum* – Plastid ribosome-deficiency – Plastid rRNA – RNA polymerase (CDNA dependent).

Introduction

In higher plants several nuclear genes are known which are able to affect chloroplast development by inducing either multiple types of plastome mutations or only one type (cf. Tilney-Bassett 1978). A nuclear gene in *Hordeum vulgare* L. belongs to the latter group. A recessive allele of this gene, called "albostrians", causes mutations of the plastid DNA resulting in the appearance of entirely white or green-white striped seedlings (Hagemann and Scholz 1962). Electron microscopic observations demonstrated that the mutant plastids of the white tissue are much smaller than wild-type barley chloroplasts. No ribosomes could be detected in the mutant plastids (Knoth and Hagemann 1977). The lack of ribosomes and therefore the inability of the mutant plastids to perform protein synthesis were confirmed by the analysis of rRNA and proteins: No plastid rRNA bands could be observed in polyacrylamide gels stained with methylene blue after electrophoretic separation of total nucleic acids of white leaves, and the two chloroplast proteins, ribulose-1,5-bisphosphate carboxylase/oxygenase and coupling factor CF_1 were not detected (Börner et al. 1976, 1979; Reichenbächer et al. 1978). Subunits of both proteins are made on chloroplast ribosomes (cf. Ellis 1977; Nelson et al. 1980). Therefore, their absence provides further evidence for the plastid ribosome-deficiency. Similar results have been obtained with the nuclear gene induced plastid ribosome deficient "iojap" mutant of *Zea mays* L. (Walbot and Coe 1979; Siemenroth et al. 1980). How a nuclear gene is able to induce plastome mutations is a mechanism which is not yet known. Furthermore, the way in which such a gene or the mutation which it induces leads to a lack of plastid ribosomes is also an open question.

The present study was undertaken to answer the following questions:

1. Do the mutant plastids contain DNA-dependent RNA polymerase? Several studies indicate a nucleo-cytoplasmic origin of the chloroplast RNA polymerase (Surzycki 1969; Ellis and Hartley 1971; Bünger and Feierabend 1980). Therefore one possible explanation for the plastid ribosome-deficiency is that the nuclear gene "albostrians" controls the synthesis of chloroplast RNA polymerase. The absence of RNA polymerase would result in the disappearance of plastid ribosomes, a defect which should be inherited like a genuine mutation of plastid DNA (Börner 1979; Walbot and Coe 1979).

0032-0935/81/0153/0547/$01.80

2. If there is RNA polymerase activity within the mutant plastids, is rRNA detectable among the products of transcription? A mutation of the rRNA genes which are located on plastid DNA (cf. Herrmann and Possingham 1980) or a defective processing of rRNA induced by the albostrians gene could also prevent chloroplast ribosome formation.

Material and methods

Material. The mutant line "albostrians" of *Hordeum vulgare* L. cv. 'Haisa' was grown in soil at 20–24° C in a greenhouse. Leaves of 8–10-d-old seedlings were harvested for all investigations. Green leaves with a homozygous (*As As*) or heterozygous (*As as*) "albostrians" genotype served as wild-type, while entirely white leaves having the homozygous mutant composition (*as as*) represented the mutant (cf. Hagemann and Scholz 1962). Green-white striped leaves (as as) were used in certain studies as indicated below.

Electron microscopic autoradiography. Pieces of white, 1 · 1 mm, green, or green-white striped leaves were incubated in small volumes of a solution of uridine-5-[^{3}H] (37 mBq ml^{-1}, specific activity 740 MBq mmol^{-1}, Institute of Research Production and Uses of Radioisotopes, Prague, Czechoslovakia) in daylight for 2 h. The samples were fixed with OsO_4 according to Kellenberger et al. (1958). The controls were treated with RNase (0.5 mg ml^{-1}, Boehringer, Mannheim, FRG) after 15 min prefixation at 37° C for 2 h. After dehydration in a graded series of acetone the samples were embedded in Durcupan ACM (FLUKA, Buchs). The sections were covered with Ilford L4 emulsion by the loop method (Caro and van Tubergen 1962), exposed in dry air at 4° C for 4 months, and developed in Kodak D 19b (5 min). After staining with basic lead citrate the sections were viewed with a transmission electron microscope (SEM 2/2, Werk für Fernsehelektronik, Berlin) at 80 keV acceleration voltage.

In vivo labelling with ^{32}P. Washed leaves were sterilized by submersion for 1.5 min in an aqueous solution of 1% sodium hypochloride and 0.1% Tween 60. After washing in sterile distilled water, the leaves were incubated for 20 min in a solution of 15 μg ml^{-1} chloramphenicol, 100 μg ml^{-1} penicillin, and 15 μg ml^{-1} rifampicin to inhibit the growth of bacteria on the leaf surface. The leaves were blotted dry and cut into small pieces with a razor blade. The leaf pieces were incubated overnight at room temperature under light with carrier-free $Na_2H^{32}PO_4$ (55–75 MBq g^{-1} leaf material; Zentrum für Kernforschung, Dresden-Rossendorf, GDR) in a solution containing the three antibiotics with the concentrations mentioned before.

Cell fractionation and isolation of nucleic acids. All steps of preparation were carried out at 4° C. Samples of 7 g leaf material were homogenized in 25 ml 50 mM Tris – HCl, pH 8.0, containing 0.5 M sucrose (RNase-free), 10 mM $MgCl_2$, 10 mM KCl, and 4 mM β-mercaptoethanol according to a method described previously (Munsche and Wollgiehn 1973). The homogenate was filtered through four layers of Miracloth and fractionated according to the scheme shown in Fig. 1. For the first fractionation step, the homogenate was centrifuged for 5 min at 100 *g* to remove cell residues and intact nuclei. The chloroplasts of green leaves were sedimented from the supernatant by centrifugation at 2,500 *g* for 50 s, while the mutant plastids of white leaves were sedimented by centrifugation at 2,500 *g* for 5 min. For isolation of cytoplasmic

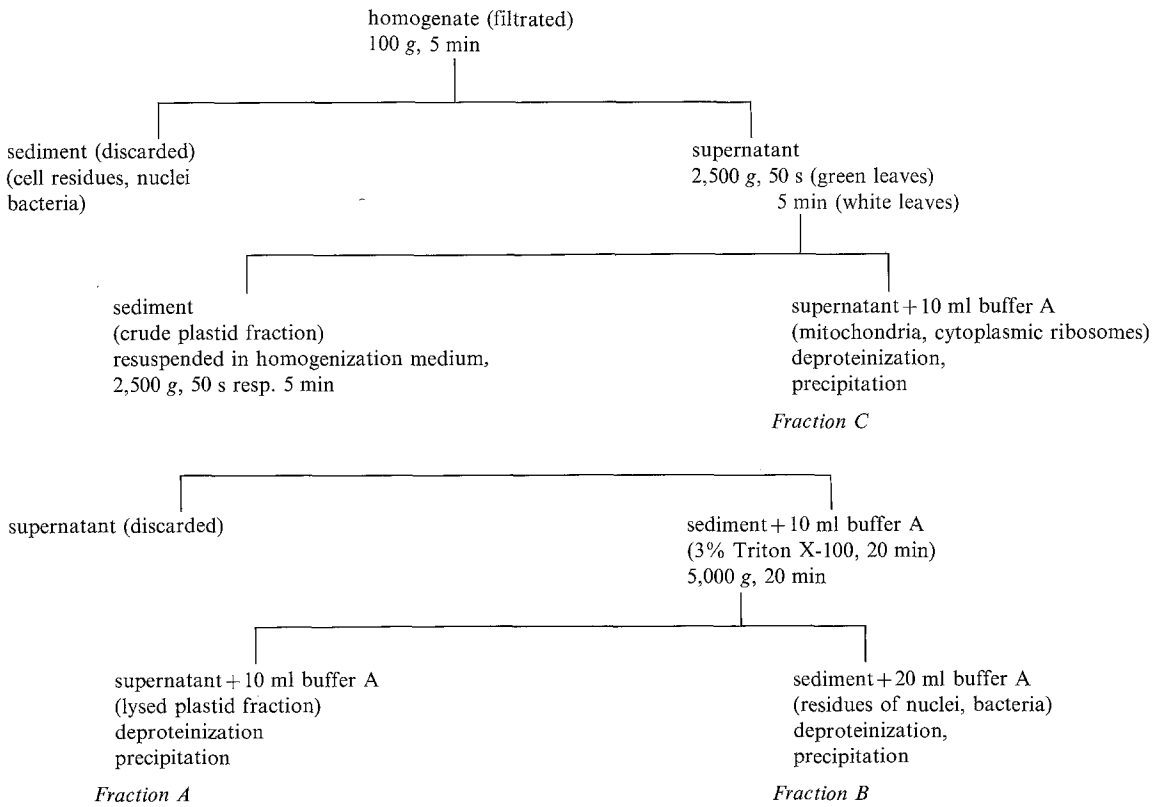


Fig. 1. Cell fractionation and isolation of total nucleic acids from leaf material and their partition into three different fractions

RNA, 10 ml supernatant was mixed with 10 ml homogenization medium (without sucrose) and deproteinized by phenol treatment (6% p-amino salicylic acid). After phase separation by centrifugation the aqueous layer was reextrated. The nucleic acids were precipitated by addition of 2.5 volumes ethanol and stored at −17° C for 12 h (fraction C). The crude plastid pellet was washed with homogenization medium and lysed by treatment with 10 ml 3% Triton X-100 in homogenization medium without sucrose for 20 min at 0° C. Nuclear residues and contaminating bacteria (fraction B) were sedimented by centrifugation at 5,000 *g* for 20 min. The supernatant (fraction A) contained the lysed chloroplasts and mutant plastids, respectively. Fractions A and B were deproteinized as described. Before precipitation of nucleic acids 50 µg of carrier RNA (total RNA from tobacco) per sample were added to the aqueous phase. The precipitated nucleic acids were dissolved in 100 µl 1 × SSC and were fractionated by electrophoretic separation in 2.4% polyacrylamide gels (Loening 1967). Determination of RNA synthesis in vitro by incorporation of [^{14}C]UTP was described earlier (Wollgiehn and Parthier (1979). Transcriptional activities of normal and mutant plastids were compared by using equal numbers of plastids per sample.

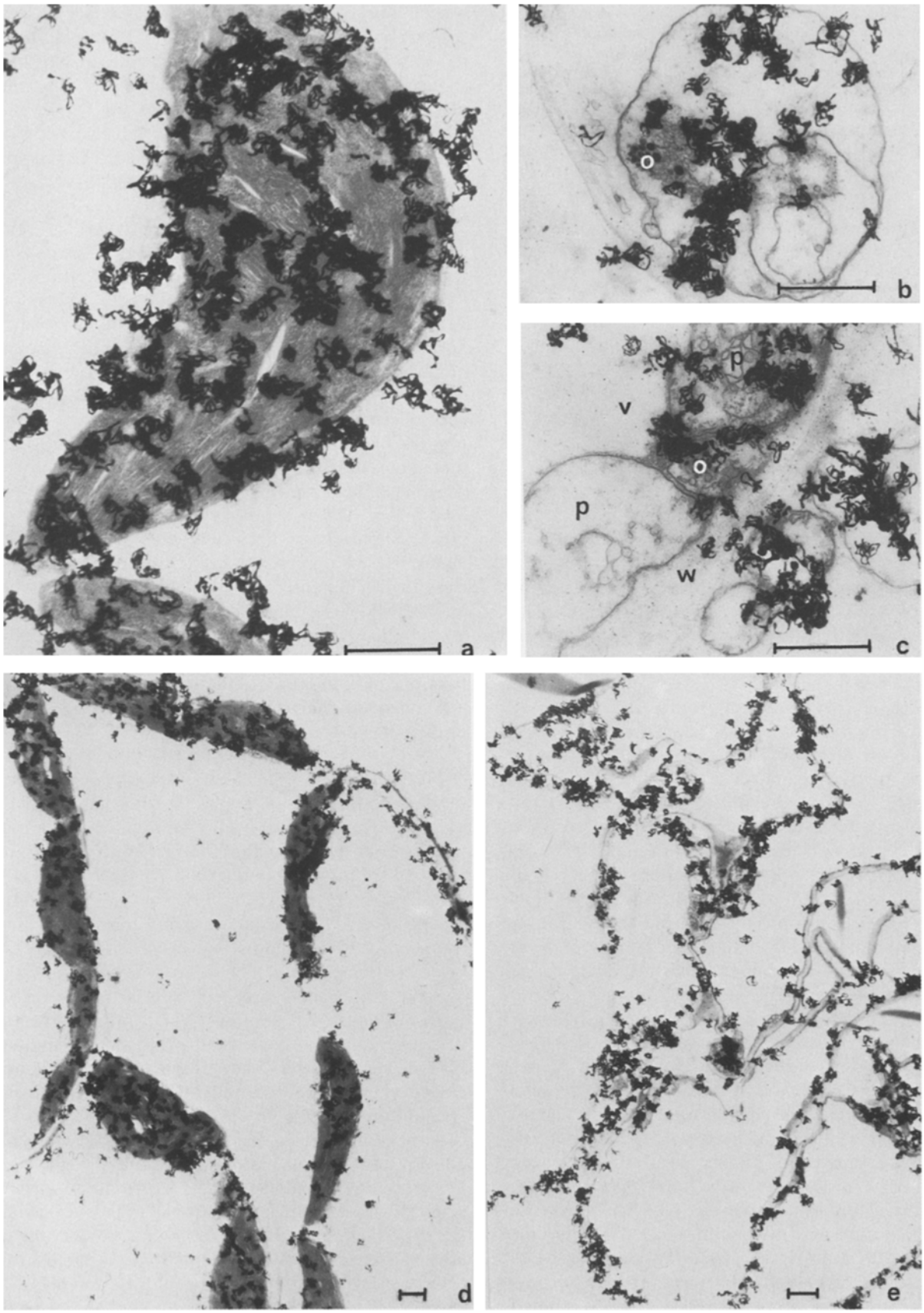
Results

Three methods were used to investigate RNA polymerase activity in plastids of the mutant line "albostrians" of barley: (1) Electron microscopic autoradiography after in vivo labelling of the RNA, (2) determination of RNA polymerase activity in isolated plastids and (3) characterization of the RNA labeled in vivo.

1. Autoradiography. Pieces of green and white barley leaves of the mutant line "albostrians" were submersed in a solution of [^{3}H]uridine. Specific incorporation of radioactivity in the RNA of chloroplasts and mutant plastids as an expression of plastid RNA-polymerase activity was detected by electron microscopic autoradiography. Under the conditions used an intense incorporation of [^{3}H]uridine into the RNA of green chloroplasts can be observed (Fig. 2a). All normal structured chloroplasts are found to be strongly labeled. As concluded from the lower number of silver grains observed over mutant plastids, [^{3}H]uridine is also definitely incorporated into mutant plastids (Fig. 2b, c) but the rate of incorporation is distinctly lower than in the case of green chloroplasts. Furthermore, the amount of incorporated [^{3}H]uridine varies among mutant plastids and for about 20% of them no label was detectable (Fig. 2c). However, it cannot be excluded that these unlabeled plastids do not incorporate any radioactivity. In this case the incorporated amount of [^{3}H]uridine might be lower than necessary for the indication by autoradiography. The difference between normal and mutant plastids in [^{3}H]uridine incorporation is clearly seen in studies on green-white striped leaves. We found a strong labeling of cells in the green parts of these leaves (Fig. 2d) and a weaker label over the cells of the white parts (Fig. 2e). Treatment of the preparations with RNase entirely removes the label from normal and mutant plastids, showing that [^{3}H]uridine was incorporated into the plastid RNA (Fig. 2f, g). Therefore we regard the label observed in mutant plastids as evidence for transcriptional activity within these plastids and for the existence of an active plastid RNA polymerase.

2. RNA polymerase activity in isolated plastids. Transcriptional activity in isolated plastids from green and white leaves was detected by determination of [^{14}C]UTP incorporation into RNA. A comparison of the kinetics of RNA polymerase activity in isolated normal and mutant plastids is shown by Fig. 3. Equal numbers of plastids per sample were used. Incorporation of [^{14}C]UTP into RNA (precipitated by 5% trichloroacetic acid) is much less intensive in mutant plastids than in normal chloroplasts. In contrast to mutant plastids the rate of RNA synthesis is nearly constant in wild-type chloroplasts for 30 min. No inhibition of RNA synthesis by α-amanitin (30 µg ml^{-1}), suppressing specifically nuclear RNA polymerase II, was observed in green chloroplasts in contrast to the mutant plastids in which incorporation of [^{14}C]UTP was inhibited by about 50% (data not shown). This might be due to nuclear contamination of the isolated mutant plastids. However, nuclear fractions of wild-type or mutant leaves did not show any transcriptional activity under the conditions used in case of the plastids.

3. Characterization of the plastid RNA labeled in vivo. In the special case of a plastid ribosome-deficient mutant, it is of particular importance to know whether the genes coding for rRNA can be transcribed. To answer this question we analyzed RNA labeled with ^{32}P in vivo by means of electrophoretic separation in polyacrylamide gels. Green and white leaves of the barley mutant line "albostrians" were labeled in vivo with ^{32}P. The leaves were homogenized and the homogenate was separated into three fractions, as described previously (cf. Fig. 1). Fraction A contains the nucleic acids of wild-type and mutant plastids, respectively. Contaminating bacteria and nuclear residues were removed by centrifugation after lysis of the plastids by 3% Triton X-100. At this concentration the detergent does not disrupt bacteria and nuclei (Spencer and Wildman 1964; Nelles and Parthier 1969; Wollgiehn et al. 1974). Their nucleic acids are represented by fraction B. Figures 4a and b show the results of electrophoretic separation of labeled nucleic acids of these fractions prepared from leaves washed with sodium hypochloride and incubated with ^{32}P in the presence of chloramphenicol, peni-


a
o
b
p
v
o
p
w
c
d
e

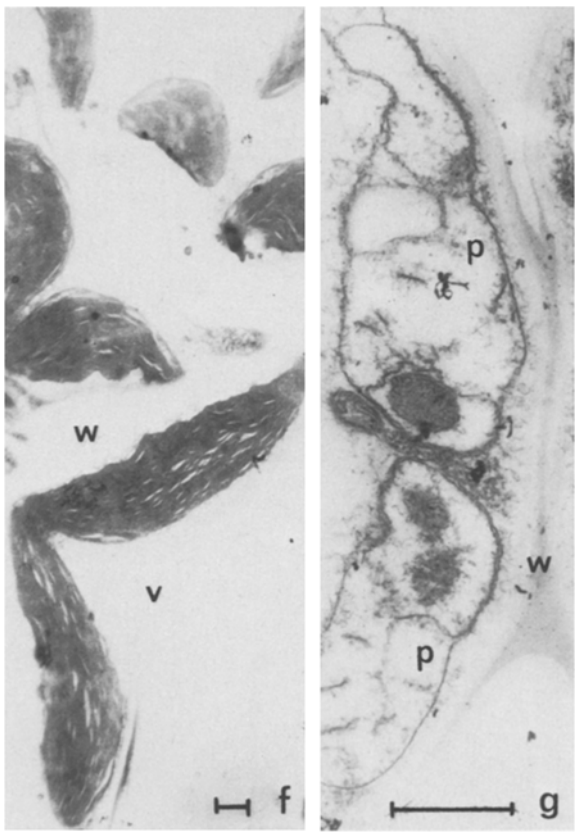


Fig. 2a–g. Electron microscopic autoradiographs of barley after 2 h incorporation of [^{3}H]uridine: plastid (*p*), osmiophilic globuli (*o*), cell wall (*w*), vacuole (*v*). Bar = 1 μm.
a Heavily labeled normal structured green chloroplasts. 16,000 ×
b Mutant plastid of white leaf tissue. 16,000 ×
c Mutant plastids of white leaf tissue. One of these plastids shows no label. 16,000 ×
d Low magnification picture of the green part of a green-white striped leaf. 4,000 ×
e Low magnification picture of the white part of the green-white striped leaf shown in Fig. 2d. 4,000 ×
f Normal green chloroplast treated by RNase after 2 h incorporation of [^{3}H]uridine. 16,000 ×
g Mutant plastid of white leaf tissue treated by RNase after 2 h incorporation of [^{3}H]uridine. 16,000 ×

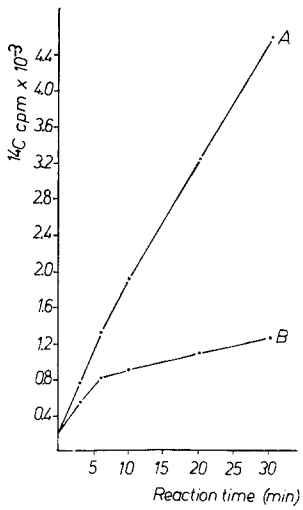


Fig. 3. Kinetics of [^{14}C]UTP incorporation in isolated plastids of green (**A**) and white (**B**) leaves of "albostrians" barley. The same number of plastids per sample were used. Chlorophyll content of green samples was 1.0 mg ml^{-1}

cillin, and rifampicin. The absorbance tracings (260 nm) of the gels with RNA of cytoplasmic ribosomes (M_r $1.3 \cdot 10^6 = 25$ S and $0.7 \cdot 10^6 = 18$ S) and of chloroplasts ribosomes (M_r $1.1 \cdot 10^6 = 23$ S and $0.56 \cdot 10^6 = 16$ S) can only serve to mark the position of the respective RNA, because these peaks represent mainly the carrier RNA (total RNA from green tobacco leaves) added to fractions A and B. To compare rRNA synthesis in green and in white barley leaves, newly synthesized RNA was visualized by autoradiography. With RNA from green barley leaves we found distinct peaks of radioactivity in the bands of mature chloroplast rRNA ($1.1 \cdot 10^6$ and $0.56 \cdot 10^6$; Fig. 4a). Fraction A from white mutant leaves also contains substantial amounts of labeled mature plastid rRNA, demonstrating the capability of white plastids to synthesize and to process 23 S and 16 S rRNA (Fig. 4b).

It was possible to observe distinct absorbance bands of plastid rRNA with molecular weights of $1.1 \cdot 10^6$ and $0.56 \cdot 10^6$ when total RNA was isolated from as much as 30 g mutant leaves and electrophoretically separated in one gel (Fig. 5). Despite of the obviously very low RNA content in the white plastids in comparison to normal chloroplasts the ^{32}P incorporation into the rRNA seems to be relatively high. According to an approximative quantitative calculation the incorporation of ^{32}P into the 1.1 and $0.56 \cdot 10^6$ RNA the synthetic capacity of white plastids is about half of what is found in green chloroplasts. In fraction A from green and white leaves we also found labeled, i.e., newly transcribed RNA which migrates during electrophoresis to the position of mature

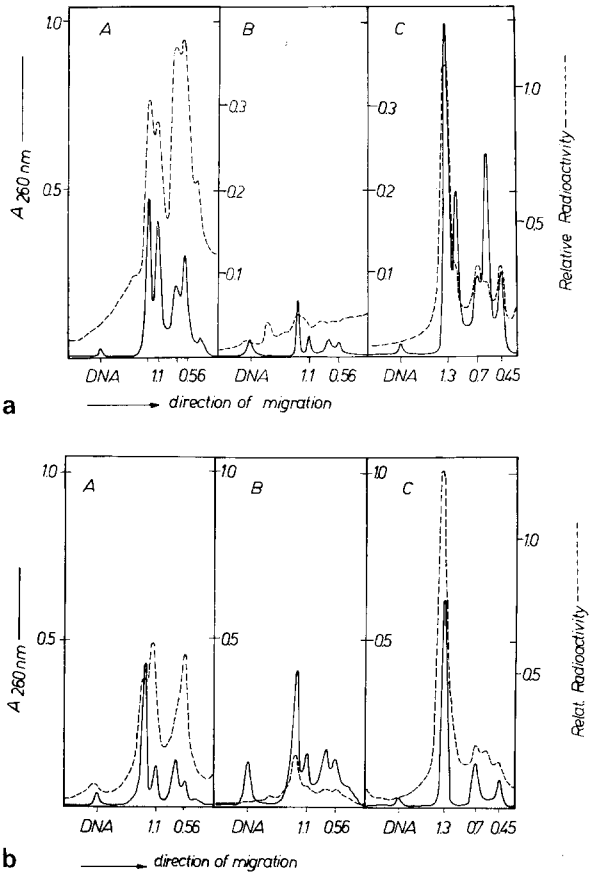


Fig. 4a, b. RNA synthesis in green (**a**) and white (**b**) leaves of "albostrians" barley washed in 1% sodium hypochloride and incubated in a solution of bacteriostatic antibiotics. Analysis of nucleic acids of fractions *A*, *B* and *C* isolated from 7 g leaf material by electrophoresis in 2.4% polyacrylamide gels. Low molecular weight RNAs are not included. Precipitation of nucleic acids of fractions *A* and *B* was carried out in presence of 50 µg total RNA from tobacco leaves. The numbers on the abscissa indicate molecular weights $\times 10^6$

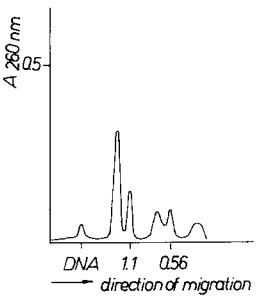


Fig. 5. Electrophoresis of total nucleic acids from plastids isolated from 30 g white leaves of "albostrians" barley in 2.4% polyacrylamide gels. Low molecular RNAs are not included. Precipitation of nucleic acids was carried out without addition of carrier RNA. The numbers on the abscissa are molecular weights $\times 10^6$

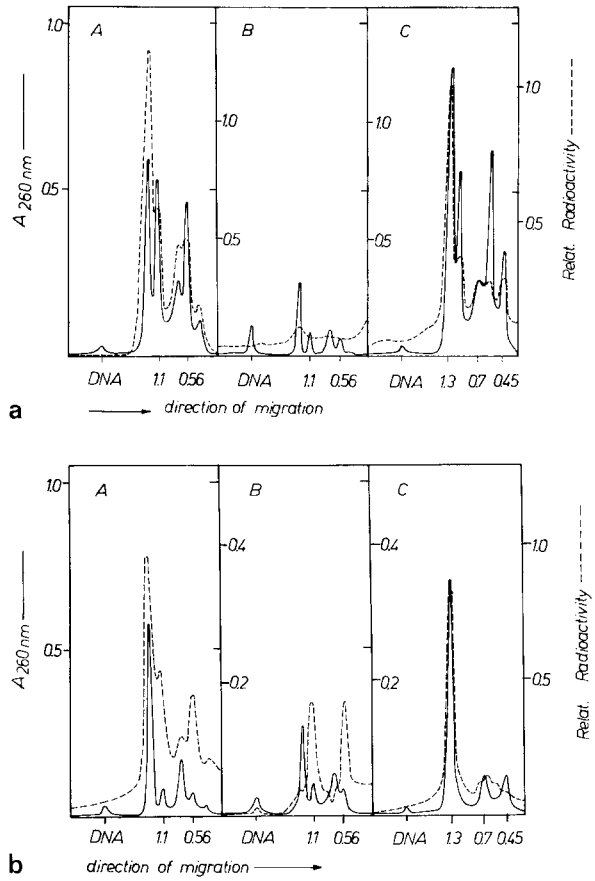


Fig. 6a, b. RNA synthesis in green (**a**) and white (**b**) leaves of "albostrians" barley untreated by sodium hypochloride and bacteriostatic antibiotics. Analysis of the nucleic acids of fractions *A*, *B* and *C* isolated from 7 g leaf material by electrophoresis in 2.4% polyacrylamide gels. Low molecular weight RNAs are not included. Precipitation of nucleic acids of fractions *A* and *B* was carried out in the presence of 50 µg total RNA from tobacco leaves. The numbers on the abscissa indicate molecular weights $\times 10^6$

cytoplasmic rRNA ($1.3 \cdot 10^6$ and $0.7 \cdot 10^6$) (Figs. 4 and 6). These bands probably contain not only contaminating cytoplasmic rRNA, but also precursor molecules of plastid rRNA which cannot be separated from the mature cytoplasmic rRNA because of their similar molecular weight (Munsche and Wollgiehn 1973). There is no distinct activity in the area of plastid rRNA when nucleic acids from fractions B and C were analyzed. Nevertheless, the nucleic acids from fraction C seem to be occasionally contaminated by plastid RNA lost from broken plastids during the preparation procedure (Figs. 4 and 6). This supposition is confirmed by appearance of the peak at the molecular weight of $0.45 \cdot 10^6$ RNA, probably due to cleaved $1.1 \cdot 10^6$ rRNA (Munsche and Wollgiehn 1974). The importance of the sterilization of leaves,

especially the white ones, with sodium hypochloride and antibiotics becomes evident from a comparison of the results shown in Figs. 4b and 6b. Mutant plastids of unsterile white leaves incorporate more radioactivity into nucleic acids than mutant plastids from leaves pretreated with antibiotics. This difference is obviously caused by bacterial contamination because the Triton insoluble fraction B isolated from unsterile white leaves is characterized by significant amounts of newly synthesized 1.1 and $0.56 \cdot 10^6$ RNA, in contrast to fraction B of pretreated white leaves. These results show at the same time that the sterilization of the leaves and incubation with antibiotics efficiently prevents bacterial contamination. The rate of incorporation of ^{32}P into cytoplasmic and plastid rRNA seems not to be affected by the concentrations chosen for the antibiotics (cf. Wollgiehn et al. 1974).

Discussion

In the barley mutant line "albostrians" a nuclear gene mutation leads to a plastid ribosome-deficiency which is maternally inherited (Hagemann and Scholz 1962; Börner et al. 1976). Among other possibilities, such a deficiency could be caused by the absence of chloroplast rRNA due to the absence of activity of chloroplast RNA polymerase or mutation of the rRNA genes or incorrect RNA processing. The results of the studies described in this paper show that the mutant "albostrians" plastids possess an RNA polymerase which is able to transcribe the genes of the 23 S and 16 S chloroplast rRNA and that the RNA is processed to its mature size. The possibility that contaminating bacteria and mitochondria have influenced our results can be excluded on the following grounds:

1. To avoid bacterial contamination, leaves were pretreated with sodium hypochloride and bacteriostatic antibiotics. The efficiency of this method could be demonstrated by comparing samples of pretreated leaf material with samples of leaves not incubated with sodium hypochloride and antibiotics (Figs. 4 and 6). In addition, the plastids were lysed with Triton X-100 at a concentration which leaves the bacteria intact, allowing their separation from plastid components by centrifugation. It could be more difficult to remove mitochondria from preparations of mutant plastids than bacteria. Very close contacts between mitochondria and mutant "albostrians" plastids have been observed (Knoth and Hagemann 1977, Wellburn and Wellburn 1979). Nevertheless it is highly improbable that the rRNA we found in samples of mutant leaves with molecular weights of $1.1 \cdot 10^6$ (23 S) and $0.56 \cdot 10^6$ (16 S) is of mitochondrial origin. The molecular weights of mitochondrial rRNA are not yet known, but the mitochondrial rRNA of closely related monocots can be easily distinguished from chloroplast rRNA by their molecular weights. Mitochondrial rRNA of maize has molecular weights of $1.26 \cdot 10^6$ and $0.75 \cdot 10^6$ (Pring 1974); the respective values for wheat mitochondrial rRNA are $1.26 \cdot 10^6$ and $0.26 \cdot 10^6$ (Cunningham et al. 1976).

In addition, a postmaturation of plastid 23 S rRNA was observed in the mutant (Rozier and Börner, unpubl.). The cleavage pattern is characteristic for cereals and rules out the possibility of bacterial or mitochondrial contamination with high probability.

2. Furthermore it is highly improbable that quantitative detection of 16 S and 23 S RNA by absorbance maxima at 260 nm in the polyacrylamide gel after separation of the RNA extract of plastids isolated from 30 g white leaves pretreated with antibiotics is caused by bacterial contamination.

3. Incorporation of [^{3}H]uridine into RNA of wild-type and mutant plastids was visualized by electron microscopic autoradiography. This label can be removed by RNase treatment and is clearly not due to bacterial or mitochondrial contamination.

4. Isolated normal and mutant plastids were shown to possess RNA polymerase activity.

The studies on the incorporation of [^{3}H]uridine into plastid RNA by electron microscopy revealed a substantial synthesis of RNA within the mutant plastids. The amount of incorporated activity varies from plastid to plastid and several mutant plastids show no transcriptional activity. These observations correlate with results of studies on the incorporation of [^{3}H]thymidine into the DNA of mutant "albostrians" plastids (Krahnert 1980). Calculated from the radioactivity of rRNA separated in polyacrylamide gels up to 50% rRNA is transcribed in mutant plastids as compared with normal chloroplasts. The lower rate of RNA synthesis in mutant plastids (also evident from the electron microscopic studies) may find its explanation in the fact that the mutant contains a lower amount of DNA per plastid (Börner et al. 1979; Börner and Heizmann, unpubl.).

The newly synthesized rRNA is obviously rapidly degraded within the mutant plastids and therefore only traces of RNA are found by optical density.

According to electron microscopic studies, the "albostrians" plastids have neither intact ribosomes nor unassembled ribosomal subunits (Knoth and Hagemann 1977). Thus, this differs from mutants in *Chlamydomonas* which have impaired plastid protein synthesis, too; but may assemble non-functional ribosomes or may produce ribosomal subunits not capable of assembling (Boynton et al. 1970; Gyurjan et al. 1979, 1980). From the observation of mature 23 S RNA and mature 16 S rRNA in the ribosome-defi-

cient plastids of "albostrians" barley, we conclude that the enzymes involved in transcription (DNA-dependent RNA polymerase) and processing of plastid rRNA are synthesized outside the plastids on cytoplasmic ribosomes and are afterwards transported across the plastid envelope into the plastid. A nucleo-cytoplasmic site of transcription and translation of the genetic information for chloroplast RNA polymerase was already suggested by studies using inhibitors of RNA and protein synthesis (Surzycki 1969; Ellis and Hartley 1971) and by studies on heat-bleached 70 S ribosome-deficient rye plants (Bünger and Feierabend 1980). The chloroplast RNA polymerase of higher plants consists of several different subunits (Smith and Bogorad 1974; Briat and Mache 1980). All the subunits necessary for correct transcription of the chloroplast rRNA cistrons seem to be made on cytoplasmic ribosomes, because we found the rRNA of the mutant plastids to be of normal size. The RNA polymerase differs in this respect from the other multisubunit enzymes of the chloroplast studied so far, ribulose-1,5-bisphosphate carboxylase/oxygenase and coupling factor CF_1. The formation of these proteins is known to be under the control of two genetic systems, that of the chloroplast itself and that of the nucleo-cytoplasm (cf. Barraclough and Ellis 1979; Nelson et al. 1980).

In summary, the results presented in this paper strongly suggest that the plastid ribosome deficiency of "albostrians" barley is not caused by a defective chloroplast RNA polymerase nor by mutation of the genes for 23 S and 16 S rRNA. Therefore other reasons for the plastid ribosome deficiency such as the lack of certain ribosomal protein(s) have to be taken into account. But the possibility still exists that the normal chloroplasts contain more than one RNA polymerase and that in the mutant plastid an enzyme is missing which is involved in the transcription of mRNA or tRNA. Furthermore, the observation of correctly processed rRNA alone is not sufficient to rule out mutations leading only to minor changes in the polynucleotide chain. Studies are in progress to investigate these possibilities.

References

Barraclough, R.G., Ellis, R.J. (1979) The biosynthesis of ribulose bisphosphate carboxylase. Eur. J. Biochem. **94**, 165–177

Börner, Th., Jahn, G., Hagemann, R. (1976) Electrophoretic mobility of the subunits of fraction I protein of higher plant species. Biochem. Physiol. Pflanzen **169**, 179–181

Börner, Th., Manteuffel, R., Wellburn, A.R. (1979) Enzymes of plastid ribosome-deficient mutants. Chloroplast ATPase (CF_1). Protoplasma **98**, 153–161

Börner, Th. (1979) Untersuchungen zur Chloroplastenbiogenese an Ribosomen-defizienten Plastomutanten von *Hordeum vulgare* L. und *Pelargonium zonale* hort. Dissertation B, Martin-Luther-Universität, Halle-Wittenberg

Boynton, J.E., Gillham, N.W., Burkholder, B. (1970) Mutations altering chloroplast ribosome phenotype in *Chlamydomonas*. II. A new Mendelian mutation. Proc. Natl. Acad. Sci. USA **67**, 1505–1512

Briat, J.F., Mache, R. (1980) Properties and characterization of a spinach chloroplast RNA polymerase isolated from a transcriptionally active DNA-protein complex. Eur. J. Biochem. **111**, 503–509

Bünger, W., Feierabend, J. (1980) Capacity for RNA synthesis in 70 S ribosome-deficient plastids of heat-bleached rye leaves. Planta **149**, 163–169

Caro, L.G., van Tubergen, R.P. (1962) High resolution autoradiography. J. Cell Biol. **15**, 173–179

Cunningham, R.S., Bonen, L., Doolittle, W.F., Gray, M.W. (1976) Unique species of 5 S, 18 S and 26 S ribosomal RNA in wheat mitochondria. FEBS Lett. **69**, 116–122

Ellis, R.J., Hartley, M.R. (1971) Sites of synthesis of chloroplast proteins. Nature New Biol. **233**, 193–196

Ellis, R.J. (1977) Protein synthesis by isolated chloroplasts. Biochim. Biophys. Acta **463**, 185–215

Gyurjan, I., Yurina, N.P., Turischewa, M.S., Odintsova, M.S. (1979) Altered chloroplast ribosomal proteins in a yellow mutant of *Chlamydomonas reinhardii*. Mol. Gen. Genet. **170**, 205–211

Gyurjan, I., Yurina, N.P., Turischewa, M.S., Odintsova, M.S., Alexandrova, N.N. (1980) Protein deficient chloroplast ribosomes in a yellow mutant of *Chlamydomonas reinhardi*. Planta **147**, 287–294

Hagemann, R., Scholz, F. (1962) Ein Fall Gen-induzierter Mutationen des Plasmotyps bei Gerste. Züchter **32**, 50–59

Herrmann, R.G., Possingham, J.V. (1980) Plastid DNA – The plastome. In: Chloroplasts, results and problems in cell differentiation, vol. 10, pp. 45–96, Reinert, J., ed. Springer, Berlin Heidelberg New York,

Kellenberger, E., Ryter, A., Sechaud, J. (1958) Electron Microscope Study of DNA-Containing Plasms. II. Vegetative and Mature Phage DNA as Compared with Normal Bacterial Nucleoids in Different Physiological States. J. Biophys. Biochem. Cytol. **4**, 671–678

Knoth, R., Hagemann, R. (1977) Struktur und Funktion der genetischen Information in den Plastiden. XVI. Die Feinstruktur der Plastiden und der elektronenmikroskopischen Nachweis echter Mischzellen in Blättern der Plastommutationen auslösenden Genmutante albostrians von *Hordeum vulgare* L., Biol. Zentralbl. **96**, 141–150

Krahnert, U. (1980) Biochemisch-genetische und elektronenmikroskopische Untersuchungen an Plastiden höherer Pflanzen. Dissertation A. Martin-Luther-Universität, Halle-Wittenberg

Loehning, U.E. (1967) The fractionation of high-molecular-weight ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem. J. **102**, 251–257

Munsche, D., Wollgiehn, R. (1973) Die Synthese von ribosomaler RNA in Chloroplasten von *Nicotiana rustica*. Biochim. Biophys. Acta **249**, 106–117

Munsche, D., Wollgiehn, R. (1974) Altersabhängige Labilität der rRNA aus Chloroplasten von *Nicotiana rustica*. Biochim. Biophys. Acta **340**, 437–445

Nelles, S., Parthier, B. (1969) Protein synthesis in sterile chloroplasts from *Lemna minor* L. A contribution to the role of bacterial contamination. Exp. Cell Res. **58**, 225–233

Nelson, N., Nelson, H., Schatz, G. (1980) Biosynthesis and assembly of the proton-translocating adenosine triphosphatase com-

plex from chloroplasts. Proc. Natl. Acad. Sci. USA **77**, 1361–1364

Pring, D.R. (1974) Maize mitochondria purification and characterization of ribosomes and ribonucleic acid. Plant Physiol. **53**, 677–683

Reichenbächer, D., Börner, Th., Richter, J. (1978) Untersuchungen am Fraktion-I-Protein der Gerste mit Hilfe quantitativer Immunelektrophoresen. Biochem. Physiol. Pflanzen **172**, 53–64

Siemenroth, A., Börner, Th., Metzger, U. (1980) Biochemical studies on the iojap mutant of maize. Plant Physiol. **65**, 1108–1110

Smith, H.J., Bogorad, L. (1974) The polypeptide subunit structure of the DNA-dependent RNA polymerase of *Zea mays* chloroplasts. Proc. Natl. Acad. Sci. USA **71**, 4839–4842

Spencer, D., Wildman, S.G. (1964) The incorporation of amino acids into protein by cell-free extracts from tobacco leaves. Biochemistry **3**, 954–959

Surzycki, S.J. (1969) Genetic function of the chloroplast in *Chlamydomonas reinhardi*: Effect of rifampin in chloroplast DNA-dependent RNA-polymerase. Proc. Natl. Acad. Sci. USA **63**, 1327–1334

Tilney-Bassett, R.A.E. (1978) Gene-induced plastid mutations. In: The plastids, pp. 378–388, Kirk, J.T.O., Tilney-Bassett, R.A.E., eds., Elsevier North Holland, Amsterdam

Walbot, V., Coe, E.H. (1979) Nuclear gene iojap conditions a programmed change to ribosome-less plastids in *Zea mays*. Proc. Natl. Acad. Sci. USA **76**, 2760–2764

Wellburn, F.A.M., Wellburn, A.R. (1979) Conjoined mitochondria and plastids in the barley mutant 'albostrians'. Planta **147**, 178–179

Wollgiehn, R., Munsche, D., Mikulowitsch, T.P. (1974) ^{32}P-Inkorporation in die Chloroplasten-RNA von Tabakblättern. Ausschluß der Beteiligung von Bakterien-Verunreinigungen. Biochem. Physiol. Pflanzen **165**, 37–48

Wollgiehn, R., Parthier, B. (1979) RNA synthesis in isolated chloroplasts of *Euglena gracilis*. Plant Sci. Lett. **16**, 203–210

Received 24 June; accepted 16 September 1981