Volume 9 Number 15 1981 Nucleic Acids Research

Cloning and mapping of the chloroplast DNA sequences for two messenger RNAs from mustard (Sinapis alba L.)

Gerhard Link

Biologisches Institut II der Universität Freiburg, Schänzlestr. 1, D-7800 Freiburg, GFR

Received 6 May 1981

ABSTRACT

Restriction fragments of chloroplast (cp)DNA from mustard leaves were cloned in E. coli using pBR 322 as the vehicle. Cloned fragments containing the structural sequences for two polypeptides of 56,000 and 35,000 daltons were selected by a mRNA hybridization-translation procedure. The cloned mustard genes are structurally related to chloroplast genes from maize. They hybridize to the maize mRNAs for the large subunit of ribulosebisphosphate carboxylase and for the 34,500 dalton precursor to a membrane protein. The coding sequence on mustard cpDNA for the 56,000 dalton polypeptide is colinear with a 1,500 base pair transcript, and the sequence for the 35,000 dalton polypeptide is colinear with a 1,220 base pair transcript.

INTRODUCTION

The chloroplasts of plants and algae contain multiple copies of circular double-stranded DNA molecules (1, 2). Among the genes identified so far on cpDNA, those for chloroplast rRNAs and tRNAs have been extensively characterized with regard to their map position and nucleotide sequence (3, 4, 5, 6, 7). Even though a number of genes for chloroplast proteins have been assigned to cpDNA (8), physical information on structural chloroplast genes is still comparatively rare. This is partly due to the difficulties in purifying specific mRNA probes. An approach that avoids mRNA purification was successfully used to map the genes for the large subunit (LS) of ribulosebisphosphate carboxylase in Zea mays (9, 10) and Chlamydomonas reinhardii (11), and for the precursor to a membrane polypeptide in maize (12, 13). This work involved random cloning of cpDNA fragments followed by transcription-translation analysis of the cloned fragments (14, 15). Further details of selected cloned cpDNA fragments were then

revealed by transcript mapping (16) and DNA sequencing (17).

In vitro transcription-translation is a powerful technique for relating structural genes to their polypeptide products. Its use as a screening technique is, however, restricted to DNA fragments that contain the entire uninterrupted coding sequence and the correct start and stop signals for transcription and translation. In the present report, hybrid-selection of mRNAs (18) by cloned cpDNA fragments was exploited as an alternative approach. This method has the advantage that DNA fragments that contain only portions of coding sequences can be detected. As a result, two structural sequences for chloroplast polypeptides were located on the physical map (19) of cpDNA from mustard (Sinapis alba L.). These two genes are equivalent to the polypeptide genes which had been previously mapped on maize cpDNA. This research is part of a program aimed to determine the molecular basis for developmentally regulated gene expression in mustard chloroplasts.

MATERIAL AND METHODS

Growth of mustard seedlings, the isolation procedure for cpDNA, and agarose gel electrophoresis were as described (19). Digestion of DNA with restriction endonucleases was carried out under conditions recommended by the suppliers (BRL and Boehringer, Mannheim).

RNA isolation

Mustard chloroplast rRNA was prepared from purified chloroplast ribosomes (20). Total chloroplast RNA from either mustard or maize seedlings was extracted from purified chloroplasts, and RNA larger than 5S was collected from linear 5 - 20 % sucrose gradients as described previously (12).

Radioactive labeling of RNA and DNA

RNA was labelled by exchanging 5'-OH groups with ^{32}P from γ-^{32}P [ATP] using T_4 polynucleotide kinase (Boehringer) following limited alkaline hydrolysis (12). ^{32}P-labelled copy RNA from purified DNA fragments was synthesized by using E. coli DNA-dependent RNA polymerase (21). Nick translation (22) was performed with α-^{32}P dATP] (350 Ci/nmol; Amersham) at a concentration of 1.8 µM and the three unlabelled nucleotides at

10 μM each.

DNA Cloning

Plasmid pBR 322 (23) was linearized with either Eco RI or Pst I, and 5'-phosphate groups were removed with calf intestine alkaline phosphatase (Boehringer) prior to ligation with cpDNA fragments as described (24). Three μg of the pretreated vehicle DNA and 15 μg mustard cpDNA fragments generated by Eco RI or Pst I were incubated with 20 units of T_4 DNA ligase (P-L Biochemicals) at 12°C for 18 hrs. The reaction products were used without further purification to transform calcium-treated (25) cells of E. coli, strain HB 101 (26). Antibiotic screening of the Pst I transformants showed that more than 80 % were $tet^r amp^s$. Individual transformants were isolated and plasmids from small bacterial cultures were analyzed for their sizes by gel electrophoresis. Selected plasmids were then transfered to nitrocellulose sheets (27) and hybridized with ^{32}P-labelled chloroplast RNA in 2 x SSC at 60°C. Plasmid DNA was prepared from bacterial lysates and purified on CsCl-ethidium bromide gradients (28). Chloroplast DNA inserts of plasmids were characterized by single and double digestion with restriction endonucleases and coelectrophoresis with fragments of total cpDNA.

mRNA selection by cloned cpDNA fragments (18)

Plasmid DNA was sonicated and bound to discs of diazobenzyloxymethyl (DBM) paper. Filters were incubated in 100 μl of 70 % formamide, 0.1 M PIPES-NaOH pH 6.8, 1 mM EDTA, 0.4 M NaCl with 30 μg of chloroplast RNA at 50°C for 4 hrs. The liquid was withdrawn and non-hybridized RNA recovered. Filters were washed 5 times in 20 mM NaCl, 8 mM trisodium citrate, 50 % formamide, and 0.2 % SDS at 34°C for 30 min each, and once in 2 x SSC at 34°C. Hybridized RNA was released by washing each filter twice with 100 μl 90 % formamide, 20 mM PIPES-NaOH pH 6.4, 1 mM EDTA, 0.5 % SDS at 50°C for 10 min. RNA was precipitated with ethanol, washed, and resuspended in sterile water. Chloroplast RNA and hybrid-selected mRNAs were translated in rabbit reticulocyte lysates, and polypeptides were analyzed on 10 - 15 % polyacrylamide gels as described (9, 29).

Linked transcription-translation (14)

Two μg of plasmid DNA digested with Eco RI or Pst I were

transcribed with E. coli DNA-dependent RNA polymerase, followed by RNA translation in the reticulocyte system (9).

Nuclease S1 analysis of cpDNA (30) has been described in detail (16). Briefly, cloned DNA fragments were hybridized with chloroplast RNA in solution under high formamide conditions (31). Single-stranded regions were removed from the hybrids by nuclease S1 (Boehringer), and the remaining duplex regions were sized on alkaline agarose gels. S1 protected DNA regions were detected by transfer to nitrocellulose (27) and hybridization with ^{32}P-copy RNA prepared from purified cpDNA fragments.

RNA electrophoresis (32) and transfer of RNA to DBM paper (33) were as described (16). Hybridization of RNA with nick translated cpDNA fragments was in the presence of dextran sulfate (34).

Containment

Experiments with live bacteria containing cpDNA clones were carried out under L2B1 conditions according to the "Richtlinien für den Umgang mit neukombinierten DNA-Molekülen" of the "Bundesministerium für Forschung und Technologie".

RESULTS

Detection of coding regions for abundant RNAs

DNA fragments with coding regions for chloroplast RNAs were located on the Sal I/Xho I restriction map of mustard cpDNA (19) by gel transfer hybridization (27) with ^{32}P-labelled RNA. In the experiment shown in Fig. 1, purified ribosomal RNA hybridizes to Xho fragments 2, 3b, 5b, and 10 (track b). Total high-molecular weight chloroplast RNA in addition hybridizes to Xho 6 and, less intensely, to several other fragments (track d). By extending this analysis to cpDNA fragments generated by Sal I, Sma I, and combinations of the three enzymes (data not shown), two regions for abundant non-ribosomal RNAs were located within Sal 6 (Xho 6) and Xho 1, respectively. In the experiment shown in Fig. 1 hybridization to Xho 1 was less pronounced than hybridization to Xho 6, due to the low transfer efficiency of the largest Xho fragment. The two coding regions were delimited further by using cloned cpDNA fragments (next section). A transcript map which summarizes this work is presented in Fig. 2.

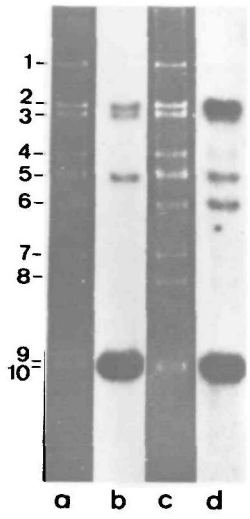

Fig. 1. Transcribed regions on mustard cpDNA. DNA fragments generated by Xho I (19) were separated in a 0.5 % agarose gel and stained with ethidium bromide (tracks a and c). Following transfer to nitrocellulose, DNA fragments were hybridized with ^{32}P-labelled chloroplast rRNA (b) or total chloroplast RNA (d).

Cloning and identification of two coding regions for mRNAs

Mustard cpDNA fragments generated by Eco RI or Pst I were ligated to pBR 322 (23) and cloned in *E. coli* as described in the METHODS section. Plasmids that hybridized strongly with total

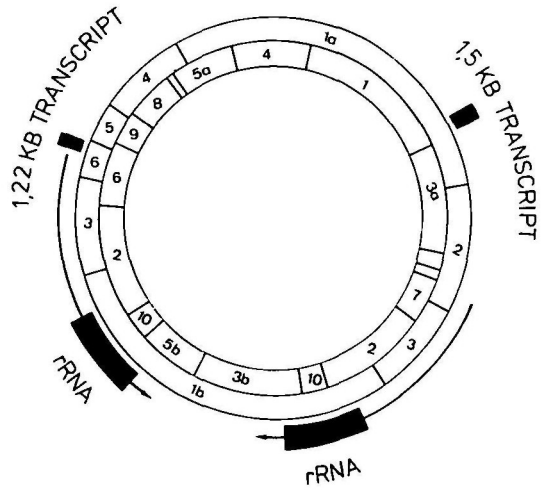

Fig. 2. Transcript map of mustard cpDNA. The positions of rRNA genes and coding sequences for the 1.5 KB and 1.22 KB transcripts (Fig. 7) are shown in relation to the recognition sites for Sal I (outer circle) and Xho I (inner circle) (19). Arrows: Inverted repeat regions.

chloroplast RNA but not with rRNA, as revealed by Southern analysis (27), were further screened for the presence of sequences for mRNAs. Plasmid DNA was bound to DBM filters (18) and hybridized with total chloroplast RNA. Hybrid-selected mRNAs were released from the filters and translated in rabbit reticulocyte lysates. The studies reported here focussed on the cloned cpDNA fragments which cover the transcribed cpDNA regions within Xho 1 (plasmids pSA 204 with the 4.2 KBP Eco RI fragment 6; pSA 530 with the 30 KBP Pst I fragment 1) and within Sal 6 (plasmids pSA 364 with the 13 KBP Pst I fragment 5; pSA 452 with the 2.15 KBP Pst I fragment 9). Fig. 3 shows that plasmid pSA 204 directs the synthesis of three polypeptides, 56, 40, and 18 kilodalton (KD) in size (track g), the largest of which is the same size as a major product directed by total chloroplast RNA (track c). The three polypeptides are found neither among the products of endogeneous mRNAs in the reticulocyte lysate (track a) nor do they represent products of mRNAs selected by

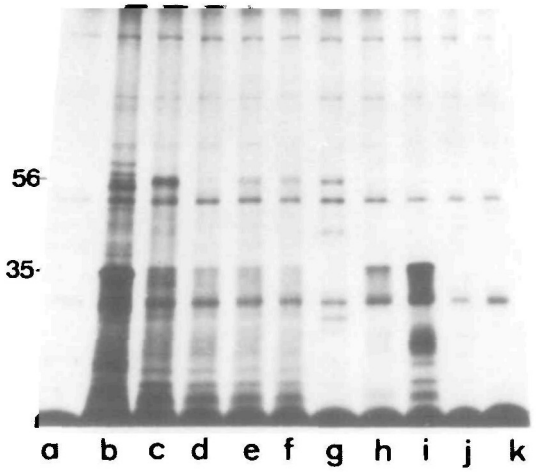

Fig. 3. Translation products of hybrid-selected mustard chloroplast mRNAs. Products of: endogeneous mRNAs in the reticulocyte system (track a); maize chloroplast RNA (b); mustard chloroplast RNA (c); mustard RNAs not hybrid-selected by plasmids pSA 204, pSA 364, and pSA 452, respectively (d - f); mustard mRNAs hybrid-selected by pSA 204, pSA 364, and pSA 452 (g - i); RNA selected by pBR 322 (j), and by a DBM filter without DNA (k). Molecular sizes (KD) of translation products by selected mRNAs are marked.

vehicle sequences (track j) or by the DBM paper itself (track k). RNA that did not hybridize to pSA 204 (track d) contains greatly reduced amounts of translatable mRNA for the 56 KD polypeptide as compared to total chloroplast RNA (track c). The synthesis of a 56 KD polypeptide was also directed by a mRNA selected by plasmid pSA 530 (data not shown). Plasmids pSA 364 (track h) and pSA 452 (track i) each select mRNAs for a 35 KD polypeptide and for several smaller translation products, all of which appear to be represented among the products directed by total chloroplast RNA (track c).

The 56 KD and 35 KD polypeptides directed by mustard chloroplast mRNAs are similar in size to the 52 - 53 KD and 34.5 KD products of chloroplast RNA from maize (track b), which have previously been identified as ribulosebisphosphate carboxylase (LS) (9, 10) and the precursor for a 32 KD membrane polypeptide (12, 13), respectively. Fig. 4 shows that the mustard cpDNA clones share sequence homology with the maize genes for these

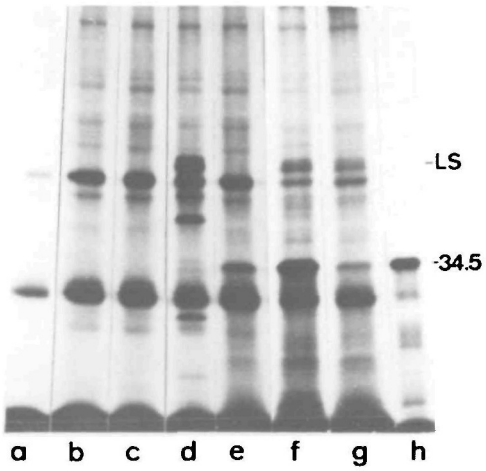

Fig. 4. Selection of maize mRNAs by mustard cpDNA clones. Products of: endogeneous mRNAs in the reticulocyte system (track a); RNA selected by a DBM filter without DNA (b), and by pBR 322 (c); RNA selected by pSA 204 (d); RNA not selected by pSA 204 (e); total maize chloroplast RNA (f); RNA not selected by pSA 452 (g); RNA selected by pSA 452 (h). The known positions of ribulosebisphosphate carboxylase (LS) (9, 10) and the 34.5 KD precursor for a membrane protein (12, 13) are marked.

two polypeptides. Following hybridization with total maize chloroplast RNA (track f) a mRNA is selected by pSA 204 which directs the synthesis of polypeptides equal in size to LS (track d and e). Likewise, pSA 452 selects the mRNA for a polypeptide equal in size to the 34.5 KD precursor protein (tracks g and h).

Mapping of the two coding regions and their transcripts

Plasmids pSA 204, pSA 364, pSA 452, and pSA 530 were tested for their capacity to direct the synthesis of polypeptides in a linked transcription-translation system (9, 14). Plasmid pSA 204 (Fig. 5, track d) directs the synthesis of several products in addition to those of the Eco RI linearized vehicle pBR 322 itself (track g). The two largest of these additional polypeptides are 57 KD and 54 KD in size, i.e. slightly larger and smaller, respectively, than the RNA-directed 56 KD product (track a) assigned to this plasmid (Fig. 3, track g). Synthesis of the 54 KD polypeptide is also directed by pSA 530, which in addition accounts for two smaller products (track e) not found with Pst I

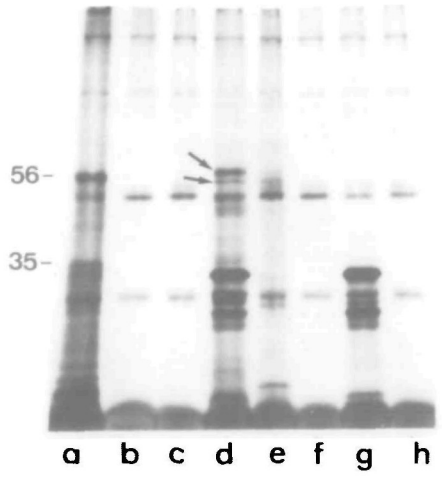

Fig. 5. Products of linked transcription-translation (9, 14) of mustard cpDNA clones. Track a: Products of translation, directed by mustard chloroplast RNA. Tracks b - h: Products of linked transcription-translation, directed by Pst I-digested plasmids pSA 364 (b), pSA 452 (c), pSA 530 (d), pBR 322 (e), Eco RI-digested plasmids pSA 204 (d) and pBR 322 (g), and without added DNA (f). The positions of the 56 KD and 35 KD RNA-directed products are marked. Arrows: 57 KD and 54 KD products directed by pSA 204.

linearized pBR 322 itself (track h). The 4.2 KBP Eco RI insert of pSA 204 maps within the 30 KBP Pst I insert of pSA 530 (Results shown in Fig. 7). Since both plasmids direct the synthesis of the 54 KD polypeptide, it is concluded that the entire coding sequence is represented in pSA 204. Plasmids pSA 364 (track b) and pSA 452 (track c) did not direct the synthesis of polypeptides in the linked transcription-translation system. All products formed in the presence of these plasmids can be assigned to endogeneous mRNAs in the reticulocyte lysate (track f).

To determine the sizes of their transcripts, cloned cpDNA fragments Eco 6 (pSA 204) and Pst 9 (pSA 452) were hybridized to electrophoretically separated chloroplast RNAs, which had been transfered to DBM paper (33). As is evident from Fig. 6, Eco 6 hybridizes to a single RNA species of 1.5 KB (track d). Likewise, Pst 9 hybridizes to a 1.2 KB RNA (track c). These two RNAs comigrate with 16S rRNA and a breakdown product of 23S rRNA, respectively, which form major bands on the stained gel (track b).

In Fig.7 physical maps of the four cloned cpDNA fragments are presented, which relate to the positions of these fragments on the mustard cpDNA molecule. Fragment Pst 1 (pSA 530) overlaps Sal 1a and Sal 2 , and it ends in Xho 1 and Xho 7, respectively. Fragment Eco 6 (pSA 204) maps within a 9.0 KBP Xho/Pst subfragment of Pst 1, which is equivalent to a position within Xho 1 near the

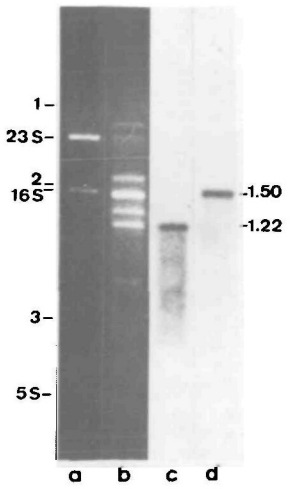

Fig. 6. Sizes of transcripts. Mustard chloroplast RNAs were separated by electrophoresis (32) (track b). Following transfer to DBM paper (33) RNAs were hybridized with cpDNA fragments Pst 9 (c) or Eco 6 (d). For size comparison *E. coli* rRNAs are shown (a). 1 - 3: pBR 322 fragments (1: 4,362 BP; 2: 1,631 BP; 3: 517 BP) (40).

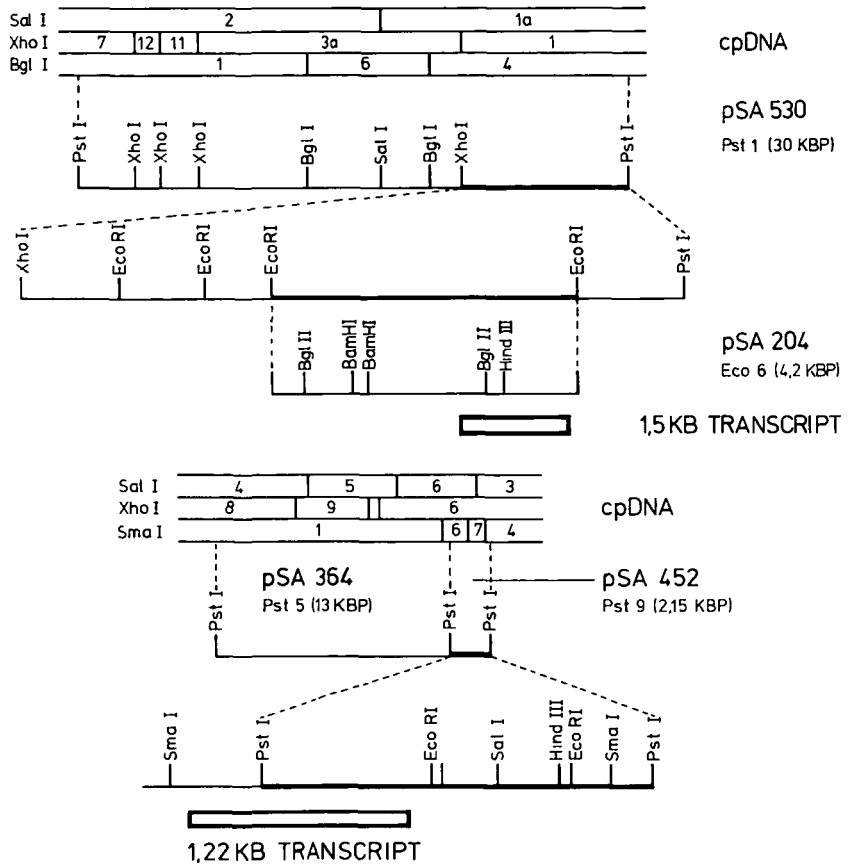

Fig. 7. Physical maps of cloned cpDNA regions and map positions of two transcripts. Upper panel: CpDNA region covered by Pst 1 (pSA 530). A 9.0 KBP Pst/Xho subfragment that contains Eco 6 (pSA 204) is shown on an expanded scale. The map position of the 1.5 KB transcript (mRNA for a 56 KD polypeptide) is marked by the framed box. Lower panel: CpDNA region covered by Pst 5 (pSA 364) and Pst 9 (pSA 452). A fine map of Pst 9 and the right-hand border of Pst 5 is shown on an expanded scale. The box marks the map position of the 1.22 KB transcript (mRNA for the 35 KD translation product).

junction with Xho 3a (Fig. 2). Fragments Pst 5 (pSA 364) and Pst 9 (pSA 452) are adjacent. They together cover a region on the cpDNA molecule that is defined by Sal fragments 4, 5, 6, and 3.

The sizes of the 1.5 KB and 1.2 KB transcripts were confirmed,

and the precise positions of their coding sequences on mustard cpDNA were determined by nuclease S1 mapping (16, 30). The 1.5 KB transcript is colinear with a coding sequence entirely within Eco 6. This sequence extends 0.6 KB into the large (3.2 KBP) Eco/Hind subfragment and covers most of the small (1.0 KBP) Eco/Hind subfragment except for a short region near the Eco RI site (Fig. 8A). The 1.22 KB transcript is colinear with an uninterrupted DNA sequence over its entire length. A 0.82 KB S1-protected fragment represents the portion of this coding sequence within Pst 9, and a 0.4 KB S1-protected fragment is generated from the region of Pst 5 at the border with Pst 9 (Fig. 8B).

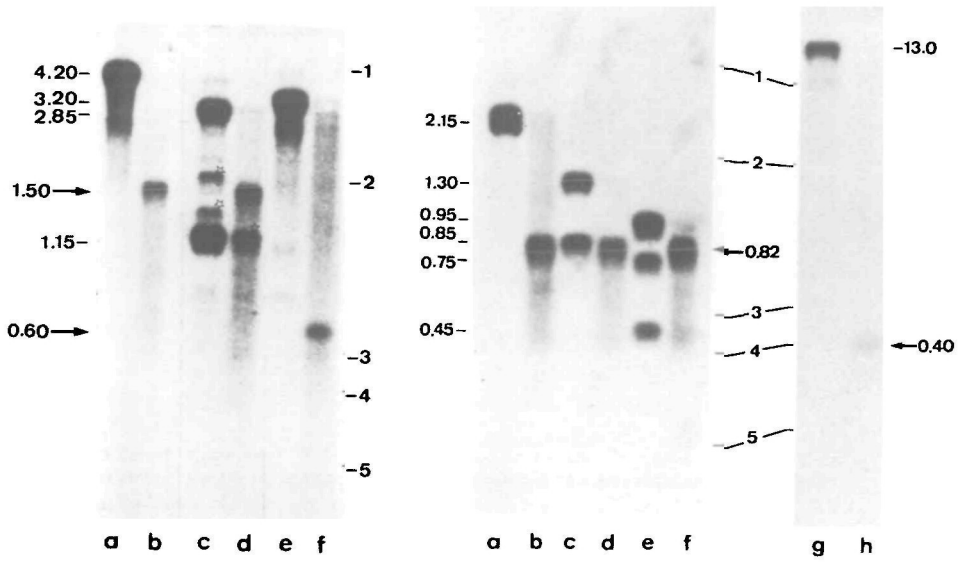

Fig. 8. Nuclease S1 mapping of cloned cpDNA regions.
A. Plasmid pSA 204 was digested with Eco RI (tracks a and b), Eco RI plus Bam HI (c and d), or Eco RI plus Hind III (e and f). Tracks a, c, e: minus S1 (sizes in KBP); b, d, f: plus S1 (sizes in KB). CpDNA fragments were detected with copy RNA from the larger Eco/Hind subfragment. Asterisks: Partial digest bands. B. Plasmid pSA 452 was digested with Pst I (tracks a and b), Pst I plus Sal I (c and d), or Pst I plus Eco RI (e and f). Plasmid pSA 364 was digested with Pst I (g and h). Track a, c, e, g: minus S1; b, d, f, h: plus S1. CpDNA fragments were detected with copy RNA from Pst 9 (a - f) or the 0.5 KBP Pst/Sma subfragment of Pst 5 (g, h). 1 - 5: pBR 322 marker fragments (1: 4,362 BP; 2: 1,361 BP; 3: 517 BP; 4: 396 BP; 5: 221 BP) (40).

DISCUSSION

Fig. 2 shows the positions of transcribed regions which have been identified on the physical map of mustard cpDNA. The DNA molecule contains two copies of a large inverted repeat sequence, which are separated by unique regions of different sizes (19). Though exceptions have been reported (35, 36), this structural organization is typical for cpDNA of many plants, e.g. maize (3), petunia (37), Oenothera (2), spinach (38), and tobacco (39). Comparison of mustard cpDNA with other plant species shows that, despite differences in the distribution of restriction sites, the map positions of chloroplast genes appear to be highly conserved. Each copy of the inverted repeat contains one set of genes for rRNAs (1). The single gene for the 34.5 KD precurser of a membrane polypeptide in maize was mapped in the large unique cpDNA region close to one end of the inverted repeat (12, 13), i.e. in a position equivalent to the single 1.22 KB gene for the 35 KD polypeptide in mustard plants. Likewise, the position of the gene for ribulosebisphosphate carboxylase (LS) on maize cpDNA (9, 10) is equivalent to the position on mustard cpDNA of the 1.5 KB gene for the 56 KD polypeptide. Although definite proof awaits comparison of the nucleotide sequences, there is evidence that the two polypeptide genes of mustard and maize are equivalent. The coding sequences, mRNAs, and RNA-directed translation products are of similar size in both plant species, and the cloned mustard cpDNA sequences selectively hybridize the two mRNAs from maize.

The LS gene of maize cpDNA maps at a distance of 330 base pairs from an adjacent gene for a 2.2 KB transcript (16), the coding function of which is not known. On the 4.2 KBP Eco fragment 6 of mustard cpDNA no transcribed regions other than the 1.5 KB transcript were detected. Since this sequence maps close to one end of Eco 6, it is possible that a transcribed region is present on the adjacent portion of Pst 1. Application of the procedures described in this report to this region and other cloned fragments of cpDNA will provide more detailed information on the arrangement of chloroplast genes. This information is a prerequisite for studying the mechanisms which control the expression of single and clustered genes during chloroplast development.

ACKNOWLEDGEMENTS

I thank Professor H. Mohr for providing the research facilities, Professors K. Hahlbrock and H. Kössel for critical reading of the manuscript, Miss S.E. Chambers and Miss M. Krenz for expert assistance, and Mrs. B. Link for preparing the typescript. This research was supported with a fellowship (Habilitanden-Stipendium) and a grant (SFB 46) from the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Bedbrook, J.R. and Kolodner, R. (1979) Annu. Rev. Plant Physiol. 30, 593-620.
2. Herrmann, R.G. and Possingham, J.V. (1980) in Results and Problems in Cell Differentiation, Reinert, J. Ed., Vol. 10, pp. 45-96, Springer, Berlin.
3. Bedbrook, J.R., Kolodner, R. and Bogorad, L. (1977) Cell 11, 739-749.
4. Rochaix, J.D. and Malnoe, P. (1978) Cell 15, 661-670.
5. Schwarz, Z. and Kössel, H. (1980) Nature 283, 739-742.
6. Orozco, E.M., Gray, P.W. and Hallick, R.B. (1980) J. Biol. Chem. 255, 10991-10996.
7. Driesel, A.J., Crouse, E.J., Gordon, K., Bohnert, H.J., Herrmann, R.G., Steinmetz, A., Mubumbila, M., Keller, M., Burkard, G. and Weil, J.H. (1979) Gene 6, 285-306.
8. Kung, S.D. (1977) Annu. Rev. Plant Physiol. 28, 401-437.
9. Coen, D.M., Bedbrook, J.R., Bogorad, L. and Rich, A. (1977) Proc. Natl. Acad. Sci. USA 74, 5487-5491.
10. Bedbrook, J.R., Coen, D.M., Beaton, A.R., Bogorad, L. and Rich, A. (1979) J. Biol. Chem. 254, 905-910.
11. Malnoe, P., Rochaix, J.D., Chua, N.H. and Spahr, P.F. (1979) J. Mol. Biol. 133, 417-434.
12. Bedbrook, J.R., Link, G., Coen, D.M., Bogorad, L. and Rich,A. (1978) Proc. Natl. Acad. Sci. USA 75, 3060-3064.
13. Bogorad, L., Jolly, S.O., Kidd, G., Link, G., McIntosh, L. (1980) in Genome Organization and Expression in Plants, Leaver, C.J. Ed., Plenum Press, N.Y.
14. Roberts, B.E., Gorecki, M., Mulligan, R.C., Danna, K.J., Rozenblatt, S. and Rich, A. (1975) Proc. Natl. Acad. Sci. USA 72, 1922-1926.
15. Zubay, G., Chambers, D.A., and Cheong, L.C. (1970) in The Lactose Operon, Beckwith, J.R. and Zipser, D. Eds., pp. 375-392, Cold Spring Harbor Laboratory Press, N.Y.
16. Link, G. and Bogorad, L. (1980) Proc. Natl. Acad. Sci. USA 77, 1832-1836.
17. McIntosh, L., Poulson, C. and Bogorad, L. (1980) Nature 288, 556-560.
18. Williams, J.G., Lloyd, M.M. and Devine, J.M. (1979) Cell 17, 903-913.
19. Link, G., Chambers, S.E., Thompson, J.A. and Falk, H. (1981) Mol. Gen. Genet. 181, 454-457.
20. Vasconcelos, A.C.L. and Bogorad, L. (1970) Biochim. Biophys. Acta 228, 492-502.

21. Burgess, R.R. and Jendrisak, J.J. (1975) Biochemistry 14, 4634-4638.
22. Rigby, P.W., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
23. Bolivar, F., Rodriguez, R.L., Green, P.J., Betlach, M.C., Heyneker, H.L., Boyer, H.W., Crosa, J.H. and Falkow, S. (1977) Gene 2, 95-113.
24. Ullrich, A., Shine, J., Chirgwin, J., Pictet, R., Tischer, E., Rutter, W.J. and Goodman, H.M. (1977) Science 196, 1313-1317.
25. Cohen, S.N., Chang, A.C.Y. and Hsu, L. (1972) Proc. Natl. Acad. Sci. USA 69, 2110-2114.
26. Boyer, H.W. and Roulland-Dussoix, D. (1969) J. Mol. Biol. 41, 459-472.
27. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
28. Clewell, D.D. and Helinski, D.R. (1969) Proc. Natl. Acad. Sci. USA 62, 1159-1166.
29. Link, G., Coen, D.M. and Bogorad, L. (1978) Cell 15, 725-731.
30. Berk, A.J. and Sharp, P.A. (1977) Cell 12, 721-732.
31. Casey,J. and Davidson,N. (1977) Nucl. Acids Res.4, 1539-1552.
32. McMaster, G.K. and Carmichael, G.G. (1977) Proc. Natl. Acad. Sci. USA 74, 4835-4839.
33. Alwine, J.C., Kemp, D.J. and Stark, G.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5350-5354.
34. Wahl, G.M., Stern, M. and Stark, G.R. (1979) Proc. Natl. Acad. Sci. USA 76, 3683-3687.
35. Jenni, B. and Stutz, E. (1978) Eur. J. Biochem. 88, 127-134.
36. Koller,B. and Delius,H. (1980) Mol. Gen. Genet. 178, 261-269.
37. Bovenberg, W.A., Kool, A.J. and Nijkamp, H.J.J. (1981) Nucl. Acids Res. 9, 503-517.
38. Herrmann, R.G., Whitfeld, P.R. and Bottomley, W. (1980) Gene 8, 179-191.
39. Jurgenson, J.E. and Bourque, D.P. (1980) Nucl. Acids Res. 8, 3505-3516.
40. Sutcliffe, J.G. (1978) Nucl. Acids Res. 5, 2721-2728.