Theor Appl Genet (1985) 70:620–627

Construction and mapping of safflower chloroplast DNA recombinants and location of selected gene markers

Cheng Ma [1] and M. A. Smith [2,*]

[1] Institute of Botany, Academia Sinica, Peking, China
[2] Graduate Section of Biochemistry, Brigham Young University, Provo, Utah 84602, USA

Received January 4, 1985; Accepted January 22, 1985
Communicated by D. von Wettstein

Summary. DNA was isolated and purified from chloroplasts of safflower (*Carthamus tinctorius* L.), digested with HindIII restriction endonuclease, and ligated into the HindIII site of the plasmid pUC9. Recombinant DNAs were isolated from ampicillin resistant white colonies which grew in the presence of the appropriate indicator, digested with HindIII, and then identified by comparison of agarose gel electrophoretic mobilities. HindIII digests of chloroplast DNA were used as a standard. Such recombinants were radiolabeled and hybridized with Southern blots of PstI, SalI, KpnI, and HindIII single and double digests of safflower chloroplast DNA. A physical map was subsequently generated showing the location of each recombinant on the circular plastid genome. Recombinants containing heterologous chloroplast gene markers from spinach or *Euglena* were also radiolabeled and mapped. The relative mapping positions of these genes are in good agreement with those which have previously been published for spinach and several other higher plants.

Key words: Safflower – Chloroplast – DNA – Clonebank – Mapping

Introduction

Higher plant and algae DNAs have been isolated and studied from a number of different species (Bohnert et al. 1982). Restriction endonuclease fragment analyses have revealed that chloroplast DNAs (ctDNA) are circular, consist of 124 to 180 kbp, and frequently contain an inverted repeat of 20–25 kbp. Hybridization studies indicate that this inverted repeat contains genes for rRNAs and tRNAs (Bohnert et al. 1982). The chloroplast genome of *Euglena gracilis* contains a triple tandem repeat of ribosomal cistrons and has an extra copy of the 16S rRNA gene. Leguminosae have only a single copy of the rRNA cistrons (Palmer and Thompson 1982). Numerous chloroplast tRNAs (Weil et al. 1983), and structural genes for several chloroplast polypeptides (Whitfeld and Bottomley 1983; Bohnert et al. 1982) have been mapped. Recombinant DNA clone banks have also been constructed. Typical examples are mung bean, pea, and spinach (Palmer and Thompson 1981); and tobacco (Fluhr et al. 1983). Tobacco and petunia ctDNA segments have also been cloned in YIp5 and shown to replicate autonomously in yeast (Uchimiya et al. 1983; Overbeeke et al. 1984).

The chloroplast DNA of *Carthamus tinctorius* (L) (safflower) is similar to that of several other higher plants (Bohnert et al. 1982; Edelman 1981) having a density of 1.700 g/cm^3 (G+C=40.8%), a T_m of 86 °C (G+C=40.7) and a genome size of approximately 10^8 daltons (Ma et al. 1984). Having a well characterized clone bank is an important asset in beginning studies on many different aspects of organelle biogenesis and gene expression (Hollingsworth et al. 1984; Uchimiya et al. 1983). Accordingly, we have generated a HindIII library of safflower chloroplast DNA by shotgun cloning in the bacterial plasmid pUC9. Individual recombinants in this clonebank have been radiolabeled by nick-translation and mapped by hybridization to Southern blots of single and double digests of ctDNA, using PstI, SalI, KpnI and HindIII restriction endonucleases.

Materials and methods

DNA isolation and characterization

Safflower chloroplast DNA was isolated and characterized as previously described (Ma et al. 1984).

* To whom correspondence should be sent

Restriction with endonuclease

Chloroplast DNA (ctDNA) (about 2 μg) was digested with 5–10 units endonuclease in TA Buffer containing 33 mM Tris-acetate, 66 mM Kacetate, 10 mM Mgacetate, 0.05 mM dithiothreitol, 100 μg/ml BSA (nuclease free), pH 7.9 at 37 °C for 2–6 h. The resulting fragments were separated by horizontal electrophoresis in 0.8% Agarose (Bethesda Research Laboratories, Inc.) in TAE buffer containing 0.04 M Tris-Acetate, 0.002 M EDTA, pH 8.0, and 0.5 μg/ml ethidium bromide. HindIII fragments of lambda DNA were used as molecular size standards. Electrophoresis was carried out at about 2 volts/cm at room temperature.

Southern blots

Bidirectional transfers were prepared as described by Smith and Summers (1980) using either nitrocellulose filters (Schleicher and Schuell) or GeneScreenPlus (New England Nuclear).

Isolation of restriction fragments from agarose gels

Restriction fragments were separated as above except in 1% LMP agarose gels (Bethesda Research Laboratories). Gels were cast at 4 °C and run at room temperature for about 25 h. Individual fragments were recovered as described by Maniatis et al. (1982) or by the freeze thaw method of Vedel and Mathieu (1983).

^{32}P-Radiolabeling

DNA was labeled by nick-translation using the method of Maniatis et al. (1982) with 1–2 μCi of ^{32}P-dCTP (1 μCi/1.26 μmoles) and ~0.1 μg of DNA per reaction. Unincorporated ^{32}P-dCTP was removed by Sephadex G-50 using either column or centrifugation techniques (Maniatis et al. 1982).

Hybridization with nick-translating probes

Hybridizations were carried out in sealed freezer bags at 41 °C for 48 h in the presence of 50% formamide as described by Maniatis et al. (1982). Post hybridization washes were carried out in 0.1×SSPE Buffer containing (for 1×) 0.18 M NaCl, 10 mM NaH_2PO_5, 1 mM EDTA, pH 7.4 and 0.1% SDS; three times at 53 °C with gentle agitation each time for 20 min. GeneScreenPlus was washed as described by the manufacturer with comparable results. Autoradiograms were generated using Kodak XAR-5 X-ray film in cassettes containing X-Omatix Regular intensifying screens at −70 °C from a few to several hours, depending on observed intensities.

Heterologous probes

These were a gift of Richard Hallick and are as follows. The first is a spinach probe and contains the α-subunit of ATPase, tRNA^{Arg} and the 3′-end of tRNA^{Gly} (SalI 10). The second is also from spinach and contains the 3′-end of psbA and tRNA^{His} and is a PstI-SalI 1.25 kbp fragment. Both of these are cloned in pUC8. The third is a *Euglena gracilis* chloroplast Eco RI 2.5 kbp fragment cloned in pMB9 (pPG50) and contains the rRNA 16S leader, all of the 16S sequence, tRNA^{Ile}, tRNA^{Ala}, and the 5′-end of the 23S gene. It also contains an aberrant 450 bp EcoRI fragment. The fourth is a *Euglena gracilis* EcoRI-PvuI fragment containing most of the coding region of the large subunit of ribulose-bis-phosphate carboxylase, rbcL, and is cloned in pBR325 (pEZC738).

Cloning of chloroplast DNA pUC9 (~1 μg) and safflower chloroplast (~1.5 μg) DNAs were digested with 10 units of HindIII restriction endonuclease at 37 °C for 5 h, treated with diethylpyrocarbonate (0.1%), mixed with ethanol (2 vol.), precipitated overnight (−20 °C), ligated with T_4 DNA ligase (2–4 units, 16 °C, overnight), and subsequently used to transform Ca^{++}-treated *E. coli* (K-12, strain JM83). Various amounts of such transformed cells were plated over agar in media (L-broth) containing X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) (2%), and ampicillin (0.05 mg/ml); and grown overnight at 37 °C. White colonies were selected, grown in fresh medium, and pUC9-ctDNA recombinant DNAs isolated (alkaline lysis method), restricted with HindIII, and compared with total HindIII digests of safflower chloroplast DNA by agarose gel electrophoresis. These procedures were essentially as described by Maniatis et al. (1982).

Results and discussion

HindIII restriction endonuclease digestion of safflower chloroplast DNA yields about 20 fragments ranging in size from 1–14 kbp. These have been shotgun cloned in *E. coli* (K-12, strain JM83) using the plasmid vector pUC9. Transformed cells were selectively multiplied by inclusion of ampicillin in the plating medium. White recombinant colonies were easily selected from a background of blue vector colonies when such transformed cells were grown in the presence of an appropriate indicator (X-Gal). Of the 323 colonies randomly selected on this basis about half (49%) were found to contain ctDNA fragments larger than about 1 kbp, as determined by digestion of isolated plasmid DNA with HindIII and comparison of agarose gel electrophoresis patterns with a similar digest of safflower ctDNA. The sizes and relative frequencies of these successfully cloned ctDNA fragments are summarized in Table 1. The maximum frequency of 27.6% (1.1 kbp fragment) is similar to that observed for PstI fragments of tobacco (Fluhr et al. 1983) and spinach (Palmer and Thompson 1981) using pBR322 as a vector in *E. coli* strain HB101. However, the restriction fragment size giving this frequency was smaller using the pUC9 vector, presumably due to the fact that an overnight culture of transformed cells was sometimes used to inoculate agarose plates in generating additional recombinants, and bacterial cells containing larger recombinants probably have a longer doubling time. Several attempts were made to clone the 14 and 11 kbp fragments directly using material that had been isolated from LMP agarose gels. Even though characteristic white colonies were obtained, they were apparently unstable since HindIII digests of plasmid DNAs were identical with control isolates containing only HindIII digested plasmid vector.

A comparison of electrophoretic mobilities of HindIII digested recombinant plasmid DNAs, in 0.8% agarose gels, is shown in Fig. 1. HindIII digests of total safflower ctDNA are included for reference. Linearized plasmid (pUC9) has an intermediate mobility and can be seen about halfway down each recombinant lane. Also observed are the individual ctDNA fragments excised by HindIII digestion. Most recombinants contained only a single ctDNA fragment; however, some contained two or even three. Lanes have been arranged in order of decreasing chloroplast fragment size.

Table 1. Frequency of safflower HindIII ctDNA-pUC9 recombinants (cloned in *E. coli* K12, strain JM 83)

ctDNA fragment size (kbp)	No. of clones	% of total recombinants
14	–	–
11	–	–
9.2	2	1.3
7.4	3	1.9
6.8	–	–
6.5	3	1.9
6.3	1	0.63
4.8	9	5.7
4.3	7	4.4
4.1	6	3.8
3.7	7	4.4
3.3	6	3.8
2.7	11	7.0
2.5	6	3.8
2.2	3	1.9
1.6	11	7.0
1.1	42	27.6
1.0	19	12.0
0.9	13	8.2
0.5	9	5.7

In order to better characterize individual recombinants, each was nick-translated with ^{32}P-dCTP and hybridized to Southern blots of ctDNA digested with various combinations of SalI, PstI, KpnI and HindIII restriction endonucleases. Typical autoradiograms are shown in Fig. 2, and a summary of all such hybridization data is listed in Table 2.

This hybridization data was used in the construction of a physical map showing the location of each recombinant on the circular plastid genome. Clone Nos. 141 and 317 (HindIII 4.1 and 6.5 kbp, respectively) are of particular interest in this regard, since they cross hybridize. In addition, both hybridize with Sal 46, and Pst 24 and 18.5 kbp. These observations are consistent with these fragments mapping at boundaries of repeated sequences and suggest that the safflower plastid genome is typical of many higher plants in this respect (Bohnert et al. 1982). This being the case clone Nos. 141 and 317 probably map from points in the single copy region symmetrically into to the repeated sequences. The map shown in Fig. 3 is consistent with this hypothesis, and the hybridization data shown in Table 2. The justification for the repeat being inverted

Table 2. Hybridization of ^{32}P-dCTP nick-translated, HindIII ctDNA-pUC9 recombinants to homologous KpnI, SalI, PstI, HindIII single and double digests of safflower chloroplast DNA

Clone no.	HindIII fragment cloned (kbp)	Restriction fragments hybridizing to specific safflower chloroplast DNA recombinants								
		KpnI	K/P	SalI	S/P	PstI	P/H	HindIII	K/H	S/H
	14	44	18.5	24, 16.5	12	18.5	14	14		
	11	44, 27, 3.5	12, 10, 4.5 3.5	24, 14.2, 11.5	11.5, 10, 2.4	18.5, 12, 10, 4.5	8.0, 5.7, 2.9	11		
168	9.2	44, 27	7.7, 4.5	46	24, 18.5, 1.8	24, 18.5, 4.5	7.7, 1.4	9.2		
139	7.4			16.5		18.5, 9.2	3.9, 3.5		6.8	7.4
256	6.5	6.8, 4.7		16.5, 14.2		14.2, 5.0	5.1, 1.5		4.6	3.3, 3.2
317	6.5	15, 10	15,10	46	24, 18.5	24, 18.5	6.5, 4.1	6.5, 4.1		
155	4.8	10		16.5		4.5, 1.0	4.1, 0.6		4.8	3.7, 0.6
162	4.3	10		16.5		14.2, 10	3.9, 0.4		4.3	4.3
259	4.1	6.8	6.8	16.5	12	14-2	4.1	4.1		
141	4.1			46		24, 18.5	6.5, 4.1		4.1	4.1
231	3.7	3.0		14.2		18.5, 6	2.5, 1.0		2.3	3.7
160	3.3	15		11.5		18.5	3.3		3.3	3.3
167	2.7	4.7	4.7	16.5	9.2	9.2	2.7	2.7		
106	2.5	10, 4.7	4.7, 1.3	16.5	9.2	9.2	2.5	2.5		
161	2.2 (1.6)	44, 27, 15, 10, 4.7	15, 12, 10, 4.7	46, 24, 14.2	24, 18.5 11.5, 10	24, 18.5, 12, 10	2.2, 1.6, 1.0	2.2, 1.6, 1.0		
208	1.6	44, 27	12, 10	24, 14.2	11.5, 10	12, 10	1.6, 0.6	1.6, 0.6		
200	1.1	15, 10, 0.6	15, 10, 0.6	46	24, 18.5	24, 18.5	1.1	1.1		
314	1.0	15	15	14.2, 7.1	7.1, 1.6	18.5	1.0	1.0		
209	0.6	44, 27	12, 10	24, 14.2	11.5, 10	12, 10	1.6, 0.6	1.6, 0.6		

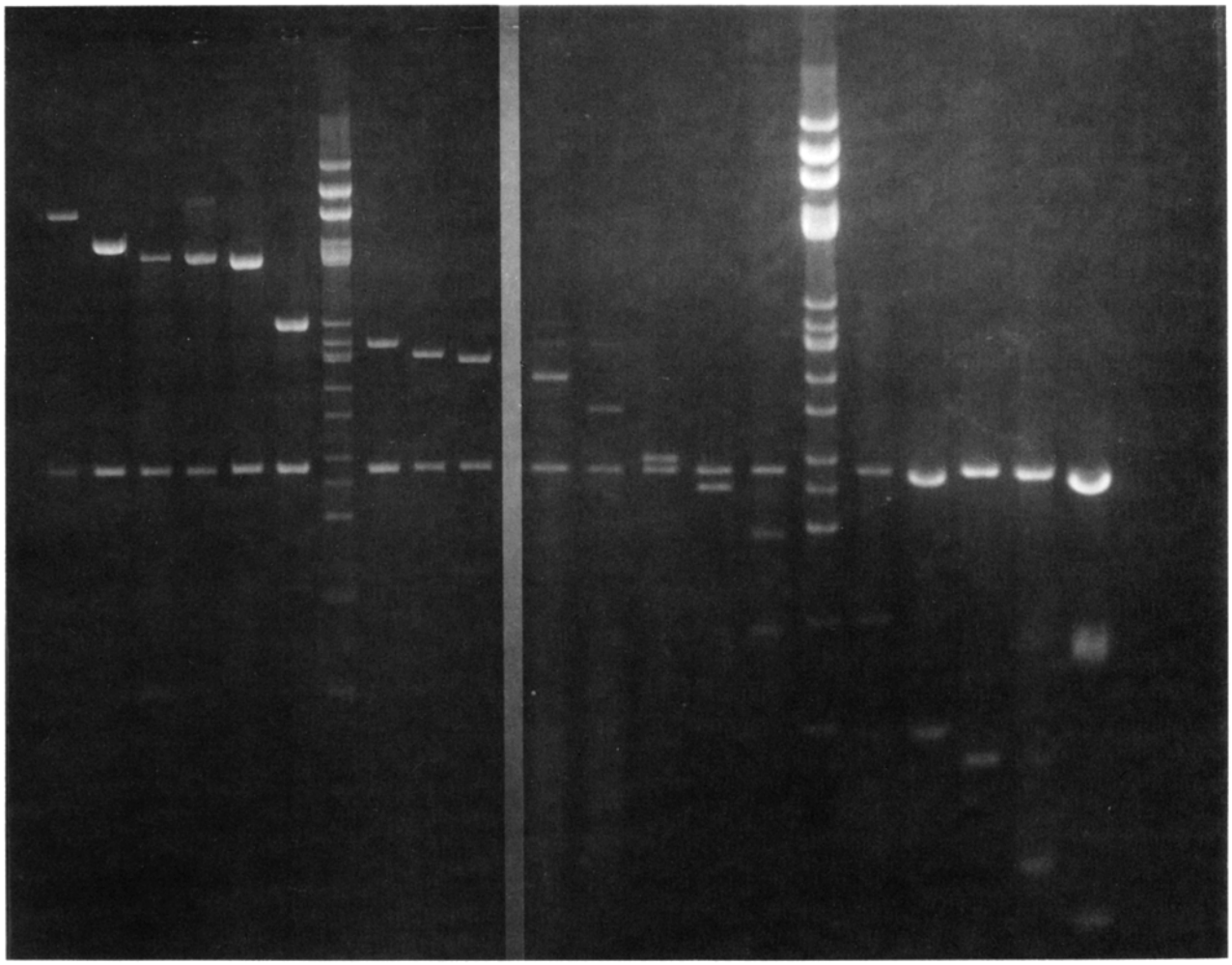

Fig. 1. Agarose gel electrophoresis of HindIII digested safflower-pUC9 recombinant plasmid DNAs. Recombinants are arranged according to clone number in order of decreasing ctDNA insert size as follows: Nos. 168, 247, 86, 256, 317, 169, HindIII digested ctDNA, Nos. 128, 259, 202 and 231, 160, 167, 106, 161, 208, HindIII digested ctDNA, Nos. 200, 314, 209, and 211. Electrophoresis was carried out in 0.8% agarose at 40 V for 15 h

is not obvious from these recombinants, but it is consistent with hybridization data described below. Mapping at the opposite ends of the repeated sequence are clone Nos. 208 (HindIII 1.6 kbp) and 209 (HindIII ~0.6 kbp). They also cross hybridize; and both of these recombinants hybridize with Sal 24 and 14.2 and other restriction fragments shown in Fig. 4. Thus, the SalI restriction fragment sequence 14.2-46-24 (kbp) is apparent. This sequence spans from one end of the large single copy region, across the inverted repeats and the entire small single copy region in between, to the other end of the large single copy region. It accounts for more than half (56%) of the entire chloroplast genome. HindIII clone Nos. 168 (9.2 kbp), 161 (2.2 kbp) and 200 (1.1 kbp) map entirely in the repeated sequences as shown in Fig. 5.

The chloroplast fragment in clone No. 155 (HindIII 4.8) overlaps two additional SalI fragments, and hybridizes with PstI fragments 4.5 and 1.0 (kbp), with PstI/SalI fragments 0.8, 3.5 and 1.0 (kbp), and with SalI/HindIII fragments 3.7 and 0.6 kbp. This locates a Sal site in both PstI 4.5, and in the HindIII 4.8 kbp insert used as a probe. The only SalI fragments which hybridize to clone No. 155 are the two 16.5 kbp fragments. This suggests that these two fragments of almost identical size are juxtapositioned. This observation is consistent with relative ethidium bromide fluorescence intensities of SalI and SalI/HindIII digests of ctDNA, separated by agarose gel electrophoresis, which show that both SalI 16.5 kbp fragments disappear upon addition of HindIII (SalI/HindII digest, data not shown). HindIII clone Nos. 139 (7.4 kpb), 167

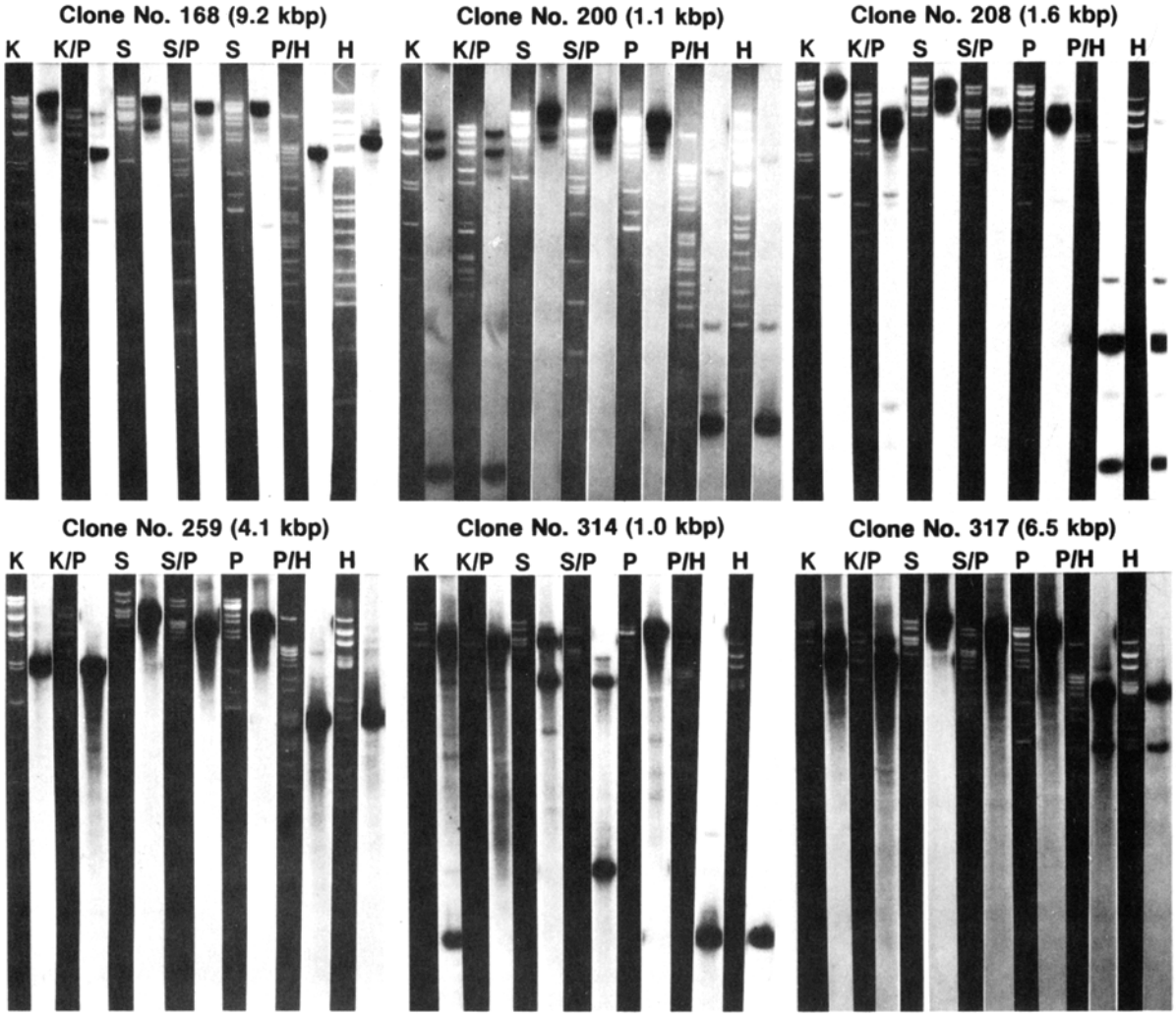

Fig. 2. Hybridization of typical HindIII ctDNA recombinants to Southern blots of fragments generated by various restriction enzymes. KpnI, KpnI/PstI, SalI, SalI/PstI, PstI, PstI/HindIII, and HindIII digests of Safflower ctDNA were separated in 0.8% agarose gels and transferred to nitrocellulose or GeneScreenPlus filters. On the left are photographs of the respective ethidium bromide stained gels, and on the right the corresponding autoradiograms obtained from each ^{32}P-dCTP nick-translated recombinant

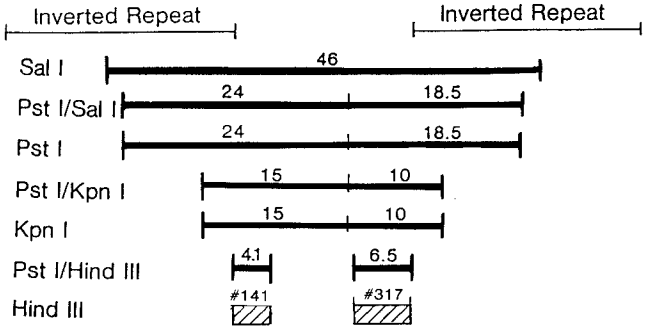

Fig. 3. Hybridization map of HindIII recombinant Nos. 141 and 317 to homologous safflower ctDNA restriction fragments. These two recombinants cross hybridize. Shaded horizontal bars represent ^{32}P-dCTP recombinant probes. Lines above each shaded bar represent PstI/HindIII, KpnI, PstI/KpnI, PstI, PstI/SalI and SalI fragments which hybridize to each probe. Numbers above these lines represent fragment sizes in kbp. See Table 2

(2.7 kbp), 106 (2.5 kbp), 162 (4.3 kbp), 259 (4.1 kbp) and 256 (6.5 kbp), also hybridize with these SalI 16.5 kbp fragments. Hybridization data from these recombinants provides sufficient information for mapping this region of the genome, Fig. 6.

From examination of the above data, it is obvious that the 44 kbp KpnI restriction fragment overlaps SalI fragments 46 (Fig. 5), 24 (Fig. 4) and 16.5 kbp (Fig. 6). This establishes the link between the SalI 14.2-46-24 kbp sequence mentioned above and the two SalI 16.5 kbp fragments.

Clone No. 256 (HindIII 6.5 kbp) overlaps SalI fragments 16.5 and 14.2 kbp as shown in Fig. 7, and clone No. 206 (HindIII 1.0 kbp) overlaps SalI fragments 14.2 and 7.1 (Fig. 8). Also shown is the hybridization of clone Nos. 231 and 160 in this region of the genome.

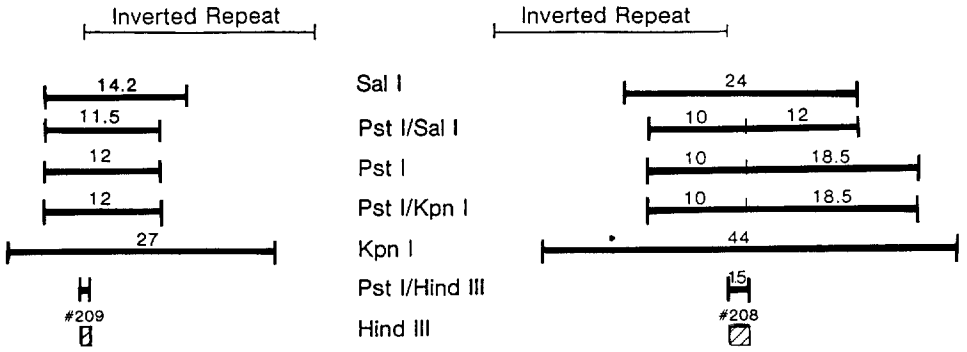

Fig. 4. Hybridization map of HindIII recombinant Nos. 208 and 209 to homologous safflower ctDNA restriction fragments. These recombinants cross hybridize. Symbols are as described in Fig. 3

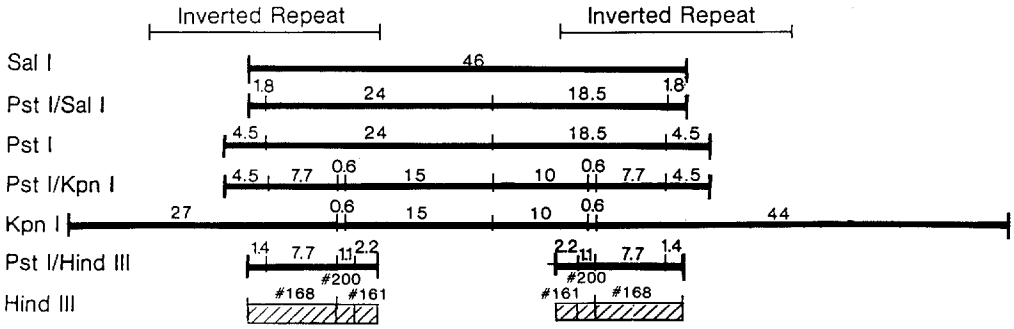

Fig. 5. Hybridization map of HindIII recombinant Nos. 168, 200 and 161 to homologous safflower ctDNA restriction fragments. These recombinants map in the inverted repeats as shown. Symbols are as described in Fig. 3

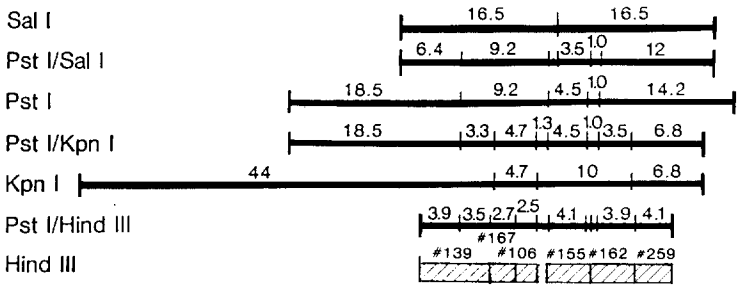

Fig. 6. Hybridization map of HindIII recombinant Nos. 139, 167, 106, 155, 162, and 259 to homologous safflower ctDNA restriction fragments. Symbols are as described in Fig. 3

This hybridization data establishes the SalI sequence 16.5-14.2-7.1-11.5 (kbp). Since there are two SalI fragments of about 14.2 kbp, the one mentioned in this sequence should not be confused with the other shown previously in Fig. 4.

The key to mapping the remainder of the genome lies in the fact that the KpnI 27 kbp fragment overlaps SalI fragments 11.5, 1.8 kbp (data not shown), 14.2 and 46 kbp (Figs. 4 and 5). This suggests the remaining SalI sequence, in order given, and provides evidence of the circular nature of the safflower chloroplast genome. A physical map of all characterized HindIII recombinants is shown in Fig. 9, where fragment sizes of KpnI, SalI, and PstI are given in kbp, and clone numbers are given for HindIII recombinants. Mapping of HindIII fragments which have not yet been successfully cloned was accomplished with nick-translated restriction fragments isolated directly from LMP agarose gels (see, for example, HindIII 14 and 11 kbp, Table 2).

Also shown in Fig. 9 are the approximate mapping positions of rrnA, rbcL, atpA and psbA. These were determined using heterologous recombinant DNA

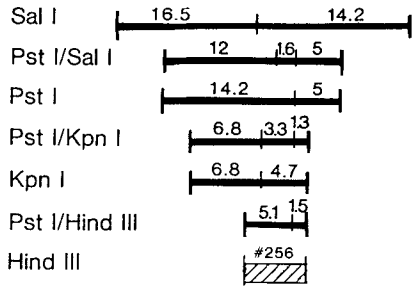

Fig. 7. Hybridization map of HindIII recombinant No. 256 to homologous safflower ctDNA restriction fragments. Symbols are as described in Fig. 3

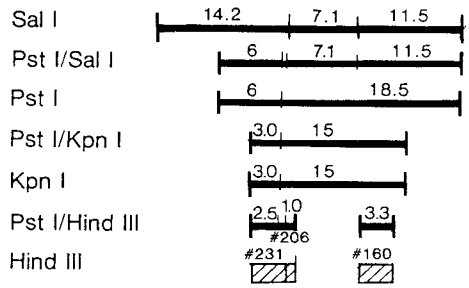

Fig. 8. Hybridization map of HindIII recombinant Nos. 231, 206, and 160 to homologous safflower ctDNA restriction fragments. Symbols are as described in Fig. 3

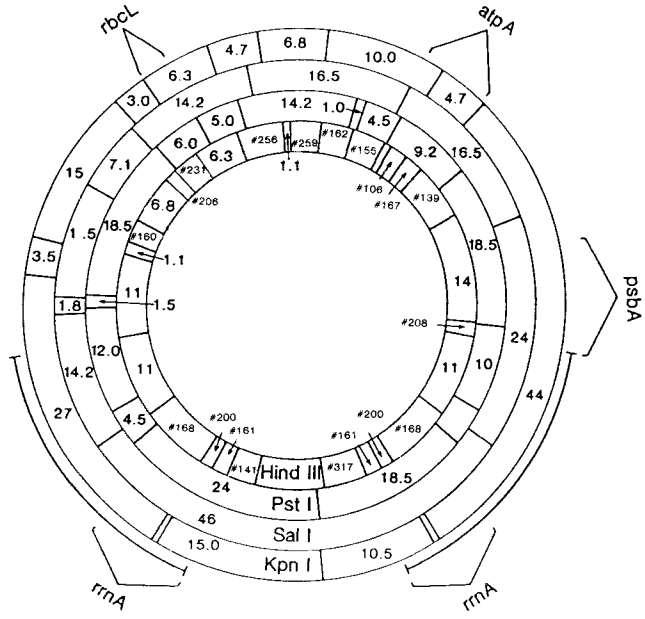

Fig. 9. Circular hybridization map of characterized HindIII recombinants to safflower ctDNA genome. Restriction fragment sizes of KpnI, SalI and PstI are in kbp; and HindIII recombinants are identified by clone number. HindIII fragments that have not been cloned are also given in kbp. These were mapped using probes isolated directly from LMP gels (e.g. see HindIII 14 and 11 kbp Table 2)

probes from spinach and *Euglena* graciously provided by Richard Hallick. Each of these probes was nick-translated and hybridized with Southern blots of ctDNA digested with various combinations of SalI, PstI, KpnI and HindIII as described for homologous HindIII recombinant ctDNA mapping above (details to be published elsewhere).

In summary, SalI, PstI, and KpnI restriction sites have been mapped in safflower ctDNA, relative to a homologous ^{32}P-dCTP nick-translated recombinant DNA library. This library was generated by inserting HindIII ctDNA fragments into the HindIII site of pUC9 and cloning in *E. coli* K-12 (strain JM83). Various SalI, KpnI, PstI and HindIII ctDNA probes, isolated from LMP agarose gels, were helpful in establishing correct restriction endonuclease sites from a number of similar alternatives (data not shown). We conclude that safflower chloroplasts have a circular genome of approximately 151 kbp with a single inverted repeat of approximately 25 kbp; and small and large single copy regions of approximately 20 and 81 kbp, respectively. In addition preliminary hybridization data with heterologous probes from spinach and *Euglena* provided the approximate mapping sites of selected gene markers. In general, safflower ctDNA appears to be similar to chloroplast DNAs of several other higher plants (Bohnert et al. 1982).

References

Bohnert HJ, Crouse EJ, Schmidt JM (1982) Nucleic acids and proteins in plants. In: Boulter D, Parthier D (eds) Encyclopedia of plant physiology, vol 14B. Springer, Berlin Heidelberg Stuttgart, pp 475–530

Edelman M (1981) Nucleic acids of chloroplasts and mitochondria. In: Stumf PK, Conn EE (eds) The biochemistry of plants, vol 6. Academic Press, New York, pp 249–301

Fluhr R, Fromm H, Edelman M (1983) Clone bank of *Nicotiana tobacum* chloroplast DNA: mapping of the alpha, beta and epsilon subunits of the ATPase coupling factor, the large subunit of ribulosebisphosphate carboxylase, and the 32-kDal membrane protein. Gene 25:271–280

Hollingsworth MJ, Johanningmeier U, Karabin GD, Stiegler GL, Hallick RB (1984) Detection of multiple, unspliced precursor mRNA transcripts for the M_r 32,000 thylakoid membrane protein from *Euglena gracilis* chloroplasts. Nucleic Acid Res 12:2001–2017

Ma C, Tyrawska D, Smith MA (1984) Isolation and characterization of safflower (*Carthamus tinctorius* L.) chloroplast DNA. Mol Cell Biochem 65:181–188

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a labroatory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Overbeeke N, Haring MA, John H, Nijkamp J, Kool AJ (1984) Cloning of *Petunia hybrida* chloroplast DNA sequences capable of autonomous replication in yeast. Plant Mol Biol 3:235–241

Palmer JD, Thompson WF (1981) Clone banks of the mung bean, pea and spinach chloroplast genomes. Gene 15: 21–26

Palmer JD, Thompson WF (1982) Chloroplast DNA rearrangements are more frequent when a large inverted repeat sequence is lost. Cell 29:537–550

Smith GE, Summers MD (1980) The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethol-paper. Anal Biochem 109:123–129

Uchimiya H, Ohtani T, Ohgawara T, Harada H, Sugita M, Suguira M (1983) Molecular cloning of tobacco chromosomal and chloroplast DNA segments capable of replication in yeast. Mol Gen Genet 192:1–4

Vedel F, Mathieu C (1983) Physical mapping of chloroplast DNA from normal and cytoplasmic male sterile (radish cytoplasm) lines of *Brassica napus*. Curr Genet 7:13–20

Weil JH, Mubumbila M, Kuntz M, Keller M, Steinmetz A, Crouse EJ, Burkard G, Gillemant P, Selden R, McIntosh L, Bogorad L, Loffelhardt W, Mucke H, Bohnert HJ (1983) Gene mapping studies and sequence determination of chloroplast transfer RNAs from various photosynthetic organisms. Mol Biol (Moskow) 17:1147–1153

Whitfeld PR, Bottomley W (1983) Organization and structure of chloroplast genes. Annu Rev Plant Physiol 34:279–310