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Restriction and gene maps of plastid DNA from *Capsicum annuum*

Comparison of chloroplast and chromoplast DNA

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Summary. Chloroplasts and chromoplasts were isolated from green and red fruits, respectively, of the bell pepper, *Capsicum annuum* var. Emerald giant. A comparison of the restriction patterns of DNAs isolated from these plastids was made using single and double digests by *Sac*I, *Pvu*II, *Pst*I, and *Sal*I and found to be indistinguishable. It is inferred therefore that the conversion of chloroplasts to chromoplasts in *Capsicum annuum* does not involve any large rearrangements of the plastid chromosome. A restriction map of *Capsicum annuum* plastid DNA was constructed using the same restriction enzymes in single digests and in all possible pair combinations. Overlapping restriction fragments were identified by digesting each product of a single digest with each of the other three enzymes. The resulting restriction map is similar to that of chloroplast DNA from other members of the *Solanaceae* with respect to most restriction sites. The genome size corresponds to 143 kbp. The locations of 24 genes, coding for ribosomal RNAs and for proteins of Photosystem I (PSI), Photosystem II (PSII), ATP synthase, cytochromes, the large subunit of ribulose-1,5-bisphosphate carboxylase-oxygenase (E.C. 4.1.1.29) (RuBPC), and ribosomal proteins were determined by probing Southern blots of *Capsicum* chloroplast DNA with probes of genes from spinach and tobacco. The gene locations are completely conserved with respect to those of other members of the *Solanaceae* and the majority of higher land plants.

Key words: Pepper – Plastid DNA – Restriction map – Gene map

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Abbreviations and notations: Gene names follow the convention of Hallick and Bottomley (1983): *atp*A, *atp*B, *atp*E, and *atp*F, genes for the α, β, ϵ, and I subunit, respectively, of ATP synthase; cpDNA, chloroplast DNA; *pet*A, *pet*B, *pet*D, genes for cytochrome *f*, cytochrome b_6, and subunit IV of cytochrome b_6/f complex, respectively; *psa*A, *psa*B, *psa*C, genes for the P_{700} apoproteins; *psb*A, gene for Q_B; *psb*B and *psb*C, genes for the 51-kDa and 44-kDa proteins, respectively, of PSII; *psb*D, gene for the Q_B-like polypeptide of PSII; *psb*E, gene for cytochrome b_{559}; *rbc*L, gene for the large subunit of RuBPC; *rpl*2, gene for ribosomal protein L2; *rpo*A, gene for the α subunit of RNA polymerase; *rps*11, *rps*12 and *rps*19, genes for ribosomal proteins S11, S12, and S19, respectively; *rps*19′, open reading frame for a protein with N terminus similar to that of S19; RuBPC, ribulose-1,5-bisphosphate carboxylase-oxygenase (E.C. 4.1.1.29); *trn*H, gene for histidine transfer RNA; *URF*39 and *URF*509, unidentified reading frames for polypeptides of 39 and 509 amino acids, respectively

Introduction

The development of chromoplasts from chloroplasts involves ultrastructural and physiological alterations including the breakdown of thylakoid membranes and of chlorophyll, the appearance of invaginations of the internal plastid envelope, increase in amounts of some carotenoids, and the formation of new species of carotenoids, mainly epoxidated xanthophylls (Rosso 1968; Harris and Spurr 1969; Mohr 1969; Simpson et al. 1977; Khudairi 1979; Camara and Brangeon 1981; Camara et al. 1982; Iwatsuki et al. 1984).

The phenomenon is a controlled process rather than degenerative (Thompson et al. 1967) and it is under genetic control, as indicated by mutations affecting the process (Simpson et al. 1977; Darby 1978). Nuclear genes are involved at least in the case of carotenoid biosynthesis (Simpson et al. 1977; Hurtado-Hernandez and Smith 1985).

The chloroplast genome appears to be concerned mainly with the synthesis of the photosynthetic ap-

paratus and of components required for transcription and translation. As judged by restriction patterns, chromoplast DNA appears to be identical to chloroplast DNA in *Narcissus pseudonarcissus* (Thompson 1980) and tomato (Iwatsuki et al. 1985). Since chromoplast development corresponds to a disappearance of the photosynthetic apparatus, the most likely role of the plastid genome in chromoplast development is passive; i.e., it ceases to be expressed. The data in the literature, however, do not exclude the possibility of an active role for the plastid. Northern blots of plastid RNAs from *Oenothera* petals probed with fragments of chloroplast DNA revealed the existence of both chloroplast- and chromoplast-specific transcripts (Bisanz-Seyer 1985). Bisanz-Seyer pointed out, however, that her data did not distinguish between chromoplast-specific transcripts that represented the transcription of previously silent genes or differential maturation of transcripts. Similar experiments in tomato fruits using sequences of specific genes as probes indicated the disappearance or decreases in the levels of transcripts for subunits of ATP synthase, apoproteins of the active centers of PSI and PSII, the large subunit of RuBPC, and in the amount of ribosomal RNAs (Piechulla et al. 1985).

The existence of a variety of mutants of the pepper, *Capsicum annuum* (Simpson et al. 1977; Hurtado-Hernandez and Smith 1985; R. Morrison, R. Whitaker, and D. Evans, personal communication), and its suitability for mutagenesis (Sadanandam et al. 1981) make pepper well suited to studies of the role of the plastid genome in chromoplast development.

We have initiated a study on chromoplast development in pepper by the development of methods for the isolation of pure, intact plastids from the fruit and the construction of restriction and gene maps of its plastid DNA.

Materials and methods

Plastid isolation. Stalk, seeds, and other non-carpal tissues were removed from 300 g of green or red fruits of *Capsicum annuum* var. Emerald giant and the fruit tissues blended with 500 ml ice-cold homogenization buffer, consisting of 0.35 M sucrose, 50 mM tris, 10 mM Na_4EDTA, 30 mM β-mercaptoethanol, and 0.1% (w/v) bovine serum albumin, all adjusted to pH 7.5. The tissue brei was filtered through four layers of Miracloth, centrifuged at 800 g for 3 min to remove unbroken cells, nuclei, and other cellular debris, and the plastid pelleted from the supernatant at 1,000 g for 10 min. Plastids were resuspended in 1 to 2 ml of the homogenization buffer and, in the case of chloroplasts, layered over a 30–60% w/v gradient of sucrose or, in the case of chromoplasts, 25–50% w/v gradient of sucrose, each containing the other components of the homogenization medium at the same concentrations. The gradients were centrifuged at 25,000 rpm in a SW-27 rotor at 4° for 1 h. The chloroplast or chromoplast bands were collected, diluted with an equal volume of ice-cold 10 mM Tris-1 mM EDTA buffer, pH 8, and recovered by sedimentation at 2,500 g for 5 to 7 min.

Isolation of plastid DNA. The plastid pellet was resuspended in 1 to 2 ml of the homogenization medium, 1/10 volume of 1 mg/ml ribonuclease A, and then an equal volume of 4% w/v sodium sarkosinate in deionized water. After standing 1 h at room temperature, the preparation was extracted once with phenol, twice with phenol/chloroform, and once with chloroform/2-butanol (4:1, v/v). DNA was precipitated from the upper aqueous phase by the addition of 1/10 volume of N NaCl and 2.5 volumes of ethanol and storage in dry ice for 5 to 10 min, pelleted, washed once in 100% ethanol at −20°, dried under nitrogen, and allowed to dissolve in 100–200 μl of sterile deionized water. The final yield of both chloroplast and chromoplast DNA was about 30 μg.

Digestion of plastid DNA with restriction endonucleases. 4 to 5 μg of DNA were digested for 2 to 3 h using approximately 10 units of *Pst*I, *Sac*I, *Pvu*II, or *Sal*I in a reaction volume of 50 μl and salt concentration and buffer standards as suggested by the supplier. Restriction fragments were resolved at 20 to 30 V in 25-cm gels of 0.4 or 0.5% regular or low-melting agarose and 90 mM Tris-borate, 90 mM boric acid, 2 mM EDTA, pH 8.0 buffer (TBE).

Sequential digestion. Chloroplast DNA bands obtained by digestion with a single endonuclease were excised from low-melting agarose gels, melted at 71° for 4 to 5 min, and subjected to digestion with a second enzyme (Seyer et al. 1981; Herrmann and Whitfeld 1982). This method avoids losses of DNA that occur during extraction from agarose gels and was suitable for detecting small fragments of DNA. Alternatively the DNA fragments were extracted from slices of agarose gel by centrifugation through Millipore GVWP filters (Zhu et al. 1985). The fragments were then recovered by ethanol precipitation. After the second digestion, subfragments of each primary fragment were resolved in 15-cm, 0.5% w/v agarose gels in TBE for 10 to 12 h at 30 to 40 V.

DNA transfer. Single digests of 0.2 μg of chloroplast DNA by *Sac*I, *Pvu*II, *Sal*I, and *Pst*I were resolved in 0.4% w/v agarose gels. After soaking the gels in distilled water for 1 h, in 0.5 M NaOH-1.5 M NaCl for 15 min, and in 0.5 M Tris-3 M NaCl, pH 7.5 for 15 min, the DNA bands were transferred overnight by capillary action to 0.45-μm pore nitrocellulose filters (Schleicher and Schüll) with 20 x SSC. The filters were baked at 85° under vacuum for 2 h.

Labelling of probes. [^{32}P]-labelled probes were prepared from spinach and tobacco chloroplast fragments by nick translation to a specific activity of ca. 5×10^8 cpm/μg DNA. The reaction was stopped by addition of EDTA to 25 mM. Unincorporated nucleotides were removed by passing the sample through a column of G50 Sephadex.

Prehybridization and hybridization. The filters were prehybridized at 37° for 4 to 10 h in sealed plastic bags containing 4 to 5 ml of prehybridization solution (5 x SSC, 10 x Denhardt's, 0.05 M sodium phosphate buffer, pH 6.7, 500 μg/ml denatured salmon sperm DNA, 5% w/v dextran sulfate, and 50% v/v formamide). Hybridization of each 4-lane filter (0.8 μg DNA) to 0.2 μg of labelled probe was done at 37° for 12 h in sealed plastic bags containing the probe and 4 to 5 ml of hybridization buffer (5 x SSC, 1 x Denhardt's solution, 0.02 M sodium phos-

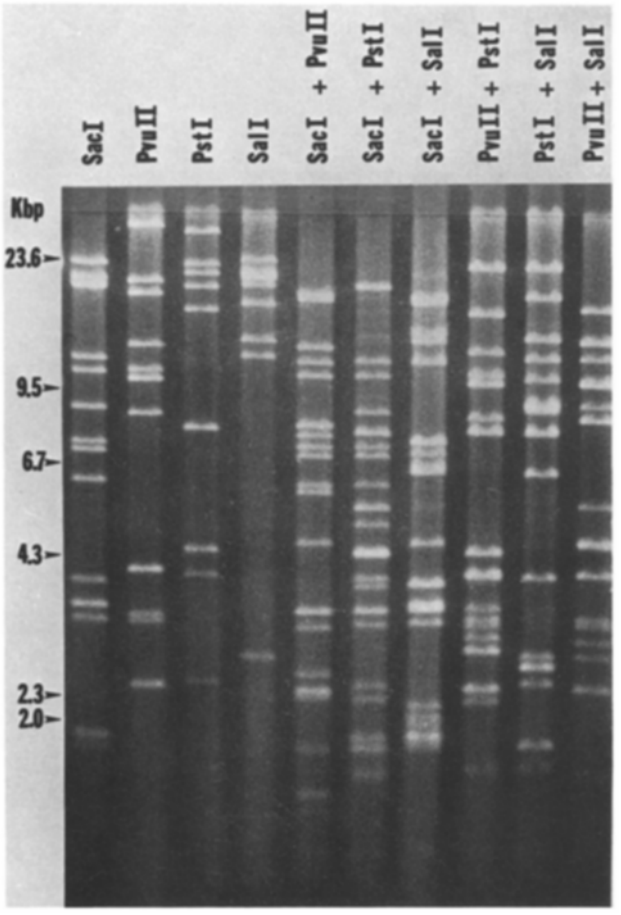

Fig. 1. Restriction fragments of cpDNA from *Capsicum annuum*. Chloroplast DNA was digested with *Sac*I, *Pvu*II, *Pst*I, and *Sal*I singly and in pairs

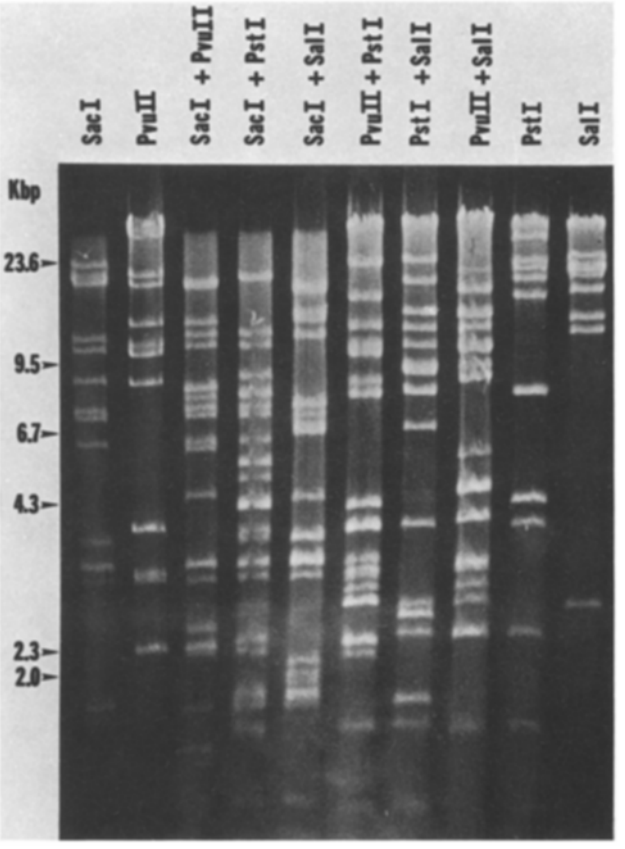

Fig. 2. Restriction fragments of chromoplast DNA from *Capsicum annuum*. Chromoplast DNA was digested with *Sac*I, *Pvu*II, *Pst*I, and *Sal*I under the same conditions as in Fig. 1

phate buffer, pH 6.7, 100 μg/ml salmon sperm DNA, 10% w/v dextran sulfate, and 50% v/v formamide). Immediately before use, the probe and hybridization solutions were denatured at 85° for 15 min and cooled quickly in ice. After washing the filters in 50% v/v formamide, 3 x SSC, and 1% SDS for 30 min at 37°, and with 2 x SSC and 0.1% SDS at room temperature for 1 to 2 h to remove unspecifically bound probe, they were exposed to Kodak SB-5 X-ray film for 20 min to 4 h.

Results and discussion

Chloroplast and chromoplast DNA

The methods for the isolation of DNA from pepper plastids are simple and rapid and yield 6 to 15 μg of plastid DNA per 100 g of fruit tissue. In order to obtain plastid DNA suitable for cutting with restriction endonucleases, extraction with phenol and phenol/chloroform was necessary. SDS used alone or in combination with sodium sarkosinate to solubilize plastids interfered with subsequent phenol extraction.

Single and double digests of cpDNA resolved on agarose gels are shown in Fig. 1. The single digests by the enzymes *Sac*I, *Pvu*II, *Pst*I, and *Sal*I produce 14, 10, 11, and 8 bands, respectively. Three bands of *Sac*I, four bands of *Pvu*II, two bands of *Pst*I, and one band of *Sal*I are doublets, as revealed by their relative fluorescence intensity after staining with ethidium bromide and subsequent digestion of the fragments with a second endonuclease (see below). Double digests with *Pst*I/*Pvu*II, *Sal*I/*Pvu*II, *Sac*I/*Pst*I, *Sal*I/*Pst*I, *Pvu*II/*Sac*I, and *Sac*I/*Sal*I produced 18, 14, 22, 16, 24, and 19 discrete bands, respectively (Table 1). Several doublets were resolved only after repeated electrophoresis for three days in 25-cm gels using different concentrations of agarose.

Restriction patterns were also obtained with chromoplast DNA (Fig. 2). The similarity of chloroplast and chromoplast restriction pattern is evidence against the occurrence of large deletions, insertions, or inversions during the formation of chromoplasts. Small rearrangements, however, might not have been detected.

Table 1. Sizes and stoichiometries of restriction fragments from double digestion of *Capsicum annuum* cpDNA. The numbers of multiple bands are shown in parentheses. Poorly resolved bands are shown as a range

Fragment	Fragment size (kbp)					
No.	*Sac*I + *Pvu*II	*Sac*I + *Pst*I	*Sac*I + *Sal*I	*Pst*I + *Pvu*II	*Pst*I + *Sal*I	*Pvu*II + *Sal*
1	16.6	17.9	15.8	21.6	22.1	14.4
2	16.2	10.5	15.7	14.4	16.3	11.5 (2x)
3	11.5	9.6	11.7	10.9	11.6	10.6
4	10.2	8.0	11.5	9.7	10.6	9.5 (3x)
5	9.6	7.2 (2x)	10.2	9.5 (2x)	9.6	8.2
6	7.7 (2x)	6.8	6.9 (2x)	7.9	7.9–8.4 (4x)	7.6 (2x)
7	7.0	6.7	6.7	7.2 (2x)	7.2 (2x)	5.1
8	6.8	5.8	6.2–6.3 (3x)	4.3 (2x)	6.2	4.3–4.5 (3x)
9	6.7	5.1 (2x)	4.5	3.7–3.8 (3x)	3.7	3.8 (2x)
10	5.8	4.7	3.5–3.6 (2x)	3.2	2.6	3.0 (2x)
11	5.4	4.3 (2x)	3.3 (2x)	3.0 (2x)	2.5 (2x)	2.8
12	4.4	3.6	3.2	2.8	2.3	2.6
13	3.3 (2x)	3.5	3.0	2.7 (2x)	1.8 (2x)	2.3 (2x)
14	3.0	3.3 (2x)	2.2	2.3 (2x)	1.5	1.4
15	2.4	3.0	2.1	2.2	1.0	
16	2.3 (2x)	2.3	2.0	1.5	0.5	
17	2.2	2.2	1.8 (3x)	1.0		
18	1.7	1.8 (2x)	1.7	<0.1		
19	1.4 (2x)	1.7 (2x)	0.8			
20	1.3	1.5				
21	1.2	1.3				
22	1.0 (2x)	1.0				
23	0.4 (2x)					
24	<0.1 (2x)					

Restriction map

We employed a method of sequential digestion for the analysis of restriction patterns (Seyer et al. 1981; Herrmann and Whitfeld 1982) based on the identification of those restriction fragments of chloroplast DNA (cpDNA) produced by two different restriction endonucleases which partially or wholly overlap. Two overlapping restriction fragments produced by a pair of endonucleases should yield the overlapping region as an identical subfragment when either fragment is subjected to digestion by the other enzyme of the pair. This method locates the correct position of each such fragment along the genome.

An example of the way we used the sequential digestion method to construct the restriction map of pepper cpDNA is given in Fig. 3. In this example, each fragment of cpDNA produced by *Pvu*II or *Pst*I alone (*primary* fragments) was digested by the other enzyme (producing *secondary* fragments). Having both the primary and secondary fragments resolved on the same gel permits one to identify bands that are the results of incomplete digestion and to resolve ambiguities resulting from contamination by closely adjacent bands. A summary of the results of sequential restriction is presented in Tables 2 through 5. Lengths of individual primary restriction fragments were calculated by averaging the sums of subfragments produced from each primary fragment by the action of the other three endonucleases.

Because of the large number of primary and secondary fragments of the same size, data from all six pair combinations were required for the construction of the restriction map (Fig. 4). The principal features of the map were confirmed and some ambiguities resolved through the use of gene probes (below).

Gene map

Samples of cpDNA were digested with *Sac*I, *Pvu*II, *Pst*I, and *Sal*I, transferred to nitrocellulose filter, and hybridized with cloned and nick-translated fragments of spinach and tobacco chloroplast genes. The origins of the probes are described in Table 6. An example of a hybridization pattern obtained with a probe for *rpo*A (Sijben-Müller et al. 1986) is shown in Fig. 5 and the full results summarized in Table 7.

We observed an inverted repeat of at least 22.7 kbp, representing approx. 16% of the DNA. The position and extent of the inverted repeats were estimated from the

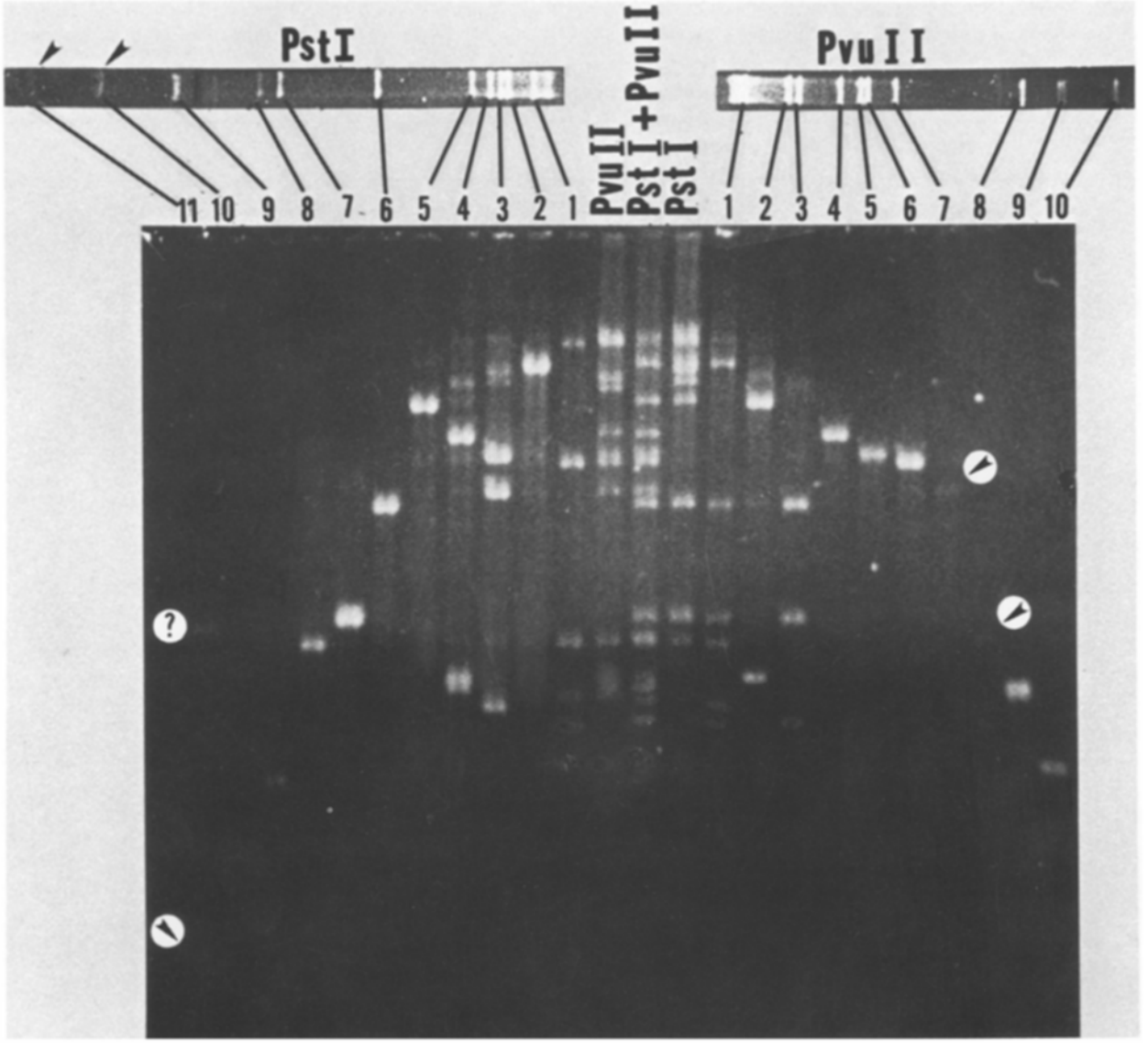

Fig. 3. Sequential restriction of cpDNA of *Capsicum annuum* with *Pst*I and *Pvu*II. *Center set of lanes*, cpDNA was digested with *Pvu*II alone, *Pvu*II + *Pst*I, and *Pst*I alone. *Left set of lanes*, fragments produced by *Pst*I were excised from the gel and digested with *Pvu*II. *Right set of lanes*, fragments produced by *Pvu*II were excised from the gel and digested with *Pst*I. The locations of faint bands are indicated by *arrows*

pattern of hybridization with the rDNA, *rps*19, and *rps*19′/*trn*H probes; in spinach and *N. debneyi* (Zurawski et al. 1984) *rps*19 and *rps*19′/*trn*H have been shown to mark the junctions of the inverted repeats and the large single-copy region. Three groups of genes could not be ordered unambiguously: a single probe was used for 23S, 4.5S, and 5S rDNA; *pet*B, *pet*D, and *rpo*A mapped on the same single fragment in each of the four digests; and *psb*E and *pet*A hybridized to the same restriction fragment in each of the four digests.

Under the hybridization conditions employed, the 23S/4.5S/5S rDNA probe showed some secondary hybridization to other regions of the genome, e.g., the 6.6-kbp fragment of *Sac*I. Such false hybridization may be due to the high GC content of those genes and to relatively low stringency.

The hybridization pattern of *psb*A improved the restriction map. The probe hybridized to the 11.1-kbp *Pvu*II fragment and to the 17.3- and 2.3-kbp *Pst*I fragments. These data unambiguously positioned those *Pst*I fragments and also showed a small (< 100-bp) overlap between the 11.1-kbp *Pvu*II fragment and the 2.3-kbp *Pst*I fragment, which could not be detected in stained gels.

Although the restriction sites for *Sac*I and *Pvu*II are clearly defined, the relative positions of the 24- and

Table 2. Sizes and stoichiometries of subfragments of *Sac*I fragments of *Capsicum annuum* cpDNA produced by a second digestion with *Pst*I, *Pvu*II, or *Sal*I. The size of each primary fragment was calculated as the average of the sums of the three sets of subfragments

Primary *Sac*I fragment		Size of secondary fragment (kbp)		
No.	Size (kbp)	*Pst*I	*Pvu*II	*Sal*I
1	22.9	7.2, 5.1, 4.3, 4.2, 2.3	16.2, 4.4, 2.3, 0.1	15.8, 6.9
2	19.2	7.2, 5.1, 4.3, 1.5, 1.3	16.6, 2.3, 0.1	11.5, 6.9, 0.8
3	18.1 (2x)	17.9, 10.5, 6.7, 1.0	11.5, 7.7 (2x), 6.7, 3.0	15.7, 11.7, 6.3, 2.1
4	10.1	4.7, 3.6, 1.7	10.2	10.2
5	9.5	9.6	9.6	6.2, 3.2
6	8.0	8.0	7.0, 1.3	6.3, 2.0
7	6.6	6.8	6.8	4.5, 1.7
8	6.5	4.3, 2.2	5.4, 1.0	6.7
9	5.8	5.8	5.8	3.6, 2.1
10	3.5	3.5	2.4, 1.2	3.5
11	3.3 (2x)	3.3 (2x)	3.3 (2x)	3.3 (2x)
12	3.0	3.0	2.2, 1.0	3.0
13	1.8 (2x)	1.8 (2x)	1.4 (2x), 0.4 (2x)	1.8 (2x)
14	1.7	1.7	1.7	1.7

Sum 143.2 kbp

Table 3. Sizes and stoichiometries of subfragments of *Sal*I fragments of *Capsicum annuum* cpDNA produced by a second digestion with *Pst*I, *Pvu*II, or *Sac*I

Primary *Sal*I fragment		Size of secondary fragment (kbp)		
No.	Size (kbp)	*Pst*I	*Pvu*II	*Sac*I
1	24.0	22.1, 1.8	9.5, 4.5, 3.8, 3.0, 2.3, 1.4	11.7, 6.9, 3.3, 1.8
2	21.2	11.6, 8.4, 1.0	9.5, 7.6, 4.3	15.7, 3.5, 2.0
3	19.6 (2x)	7.9 (3x), 7.2, 3.7, 2.5, 2.3	14.4, 11.5, 8.2, 5.0	15.8, 10.2, 6.3, 3.6, 3.2
4	18.2	16.3, 1.8	7.6, 4.5, 3.8, 2.3	6.9, 6.3, 3.3, 1.8
5	15.6	9.6, 6.2	9.5, 3.0, 2.8	6.7, 3.0, 2.2, 2.1, 1.7
6	11.6	7.2, 2.5, 1.5, 0.5	11.5	11.5
7	10.6	10.6	10.6	6.2, 4.5
8	2.6	2.6	2.6	1.8, 0.8

Sum 143.0 kbp

Table 4. Sizes and stoichiometries of subfragments of *Pvu*II fragments of *Capsicum annuum* cpDNA produced by a second digestion with *Pst*I, *Sal*I, or *Sac*I

Primary *Pvu*II fragment		Size of secondary fragment (kbp)		
No.	Size (kbp)	*Pst*I	*Sal*I	*Sac*I
1	43.9	21.6, 7.2, 4.3, 3.7, 2.8, 2.7, 1.5	14.4, 11.5, 10.6, 4.5, 2.6	16.6, 10.2, 9.6, 6.8, 1.3
2	18.7	14.4, 3.2, 1.0	9.5 (2x)	11.5, 5.4, 1.7
3	16.2	7.2, 4.3, 2.7, 2.2	11.5, 4.5	16.2
4	11.1	10.9, 0.1	8.2, 2.8	5.8, 4.4, 1.0
5	9.5	9.7	5.1, 4.3	7.0, 2.4
6	9.3 (2x)	9.5 (2x)	9.5, 7.6, 1.4	7.7 (2x), 1.4 (2x)
7	7.8	7.9	7.6	6.7, 1.2
8	3.8 (2x)	3.8 (2x)	3.8 (2x)	3.3 (2x), 0.4 (2x), 0.1 (2x)
9	3.0 (2x)	3.0 (2x)	3.0 (2x)	3.0, 2.2, 1.0
10	2.3 (2x)	2.3 (2x)	2.3 (2x)	2.3 (2x)

Sum 142.0 kbp

Table 5. Sizes and stoichiometries of subfragments of *Pst*I fragments of *Capsicum annuum* cpDNA produced by a second digestion with *Pvu*II, *Sac*I, or *Sal*I

Primary *Pst*I fragment		Size of secondary fragment (kbp)		
No.	Size (kbp)	*Pvu*II	*Sac*I	*Sal*I
1	38.8	9.5 (2x), 3.8 (2x), 3.0, 2.7 (2x), 2.3 (2x)	17.9, 5.1 (2x), 3.3 (2x), 1.8 (2x)	22.1, 16.3
2	21.9	21.6	9.6, 6.8, 4.7, 1.3	10.6, 7.9, 2.6, 0.5
3	19.9	9.7, 7.9, 2.8	8.0, 6.7, 3.5, 1.7	11.6, 7.9
4	17.3	10.9, 3.2, 3.0	5.8, 4.3 (2x), 3.0	9.6, 7.9
5	14.5	14.4	10.5, 2.2, 1.7	8.4, 6.2
6	7.2 (2x)	7.2 (2x)	7.2 (2x)	7.2 (2x)
7	4.3 (2x)	4.3 (2x)	4.3 (2x)	2.5 (2x), 1.8 (2x)
8	3.7	3.7	3.6	3.7
9	2.3	2.2, 0.1	2.3	2.3
10	1.5	1.5	1.5	1.5
11	1.0	1.0	1.0	1.0

Sum 143.9 kbp

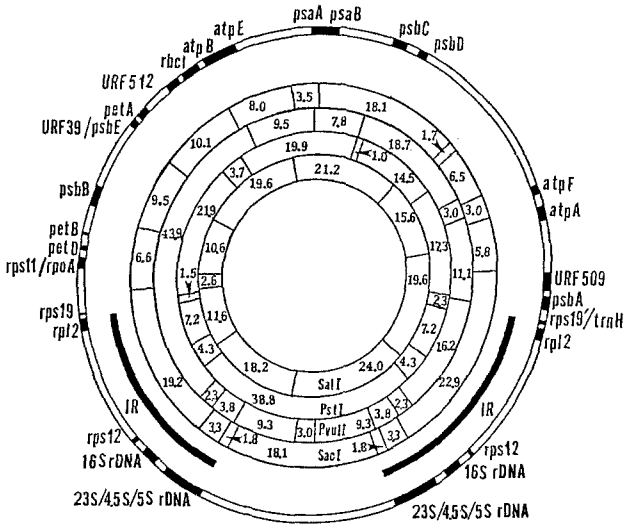

Fig. 4. Restriction and gene map of cpDNA of *Capsicum annuum*. The *inner circles* represent the restriction patterns with *Sal*I, *Pst*I, *Pvu*II, and *Sac*I. Sizes of the restriction fragments are marked in kbp and the regions of the inverted repeat are indicated by the solid segments labelled *IR*. The *outer circle* represents the locations of genes as inferred from the hybridization of restriction fragments of *Capsicum annuum* cpDNA with gene fragments from spinach and tobacco cpDNA. The order of the 23S, 4.5S, and 5S rDNAs; the *pet*A and *psb*E/*URF*39; and of *pet*B, *pet*D, and *rpo*A is assumed from the order determined for these in spinach

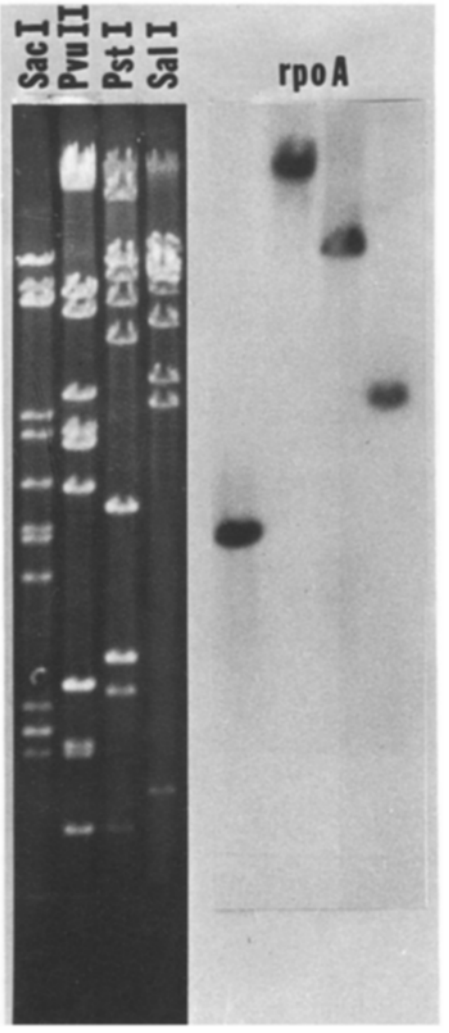

Fig. 5. Southern blot of cpDNA of *Capsicum annuum* probed with *rpo*A. Chloroplast DNA was digested with *Sac*I, *Pvu*II, *Pst*I, and *Sal*I, blotted to nitrocellulose, and hybridized with the *Sal*I/*Xho*I fragment of spinach, which contains portions of *rpo*A and *rps*11

Table 6. Probes from spinach and tobacco chloroplast genes used for hybridization with restriction fragments of cpDNA from *Capsicum annuum*. The convention for naming chloroplast genes is that recommended by Hallick and Bottomley (1983)

Gene symbol	Gene product	Fragment	Size (bp)	Reference
*atp*A	α subunit of ATP synthase	*Hind*III/*Sma*I	1,200	Deno et al. 1983
*atp*B	β subunit of ATP synthase	*Cla*I/*Eco*RI	1,627	Zurawski et al. 1982
*atp*E	ϵ subunit of ATP synthase	*Eco*RI/*Xba*I	431	Zurawski et al. 1982
*atp*F	subunit I of ATP synthase	*Sal*I/*Sac*I	500	Henning and Herrmann 1986
*pet*A	cytochrome *f*	*Bam*HI/*Eco*RI	1,200	Alt et al. 1983
*pet*B	cytochrome b_6	*Xho*I coding region	300	Alt et al. 1983 Heinemeyer et al. 1984
*pet*D	subunit IV of cytochrome b_6/f complex	*Bam*HI coding region	400	Alt et al. 1983 Heinemeyer et al. 1984
*psa*A	P_{700}-apoprotein	*Bam*HI/*Kpn*I	1,100	Westhoff et al. 1983 Alt et al. 1984
*psa*B	P_{700}-apoprotein	*Bam*HI fragment 28	350	Westhoff et al. 1983 Alt et al. 1984
*URF*512	P_{700}-apoprotein (?)[a]	*Bam*HI/*Eco*RI	1,100	Smith and Gray 1984
*psb*A	Q_B	*Nla*III/*Hind*III	500	Zurawski et al. 1982
*psb*B	55-kDa subunit of PSII	*Bam*HI/*Sal*I	1,700	Morris and Herrmann 1984 Westhoff et al. 1983
*psb*C	44-kDa subunit of PSII	*Pvu*II	1,400	Westhoff et al. 1983 Holschuh et al. 1984
*psb*D	D_2 = Q_B-like protein	*Pvu*II/*Eco*RI	950	Alt et al. 1984
*psb*E/*URF*39	cytochrome b_{559}/unknown	*Eco*RI fragment 4	550	Herrmann et al. 1984 Westhoff et al. 1985
*rbc*L	RuBPC, large subunit	*Eco*RI	1,750	Zurawski et al. 1981 Whitfeld and Bottomley 1980
16S rDNA		*Bam*HI fragment 10	3,600	Briat et al. 1982 Bohnert et al. 1980
23S/4.5S/5S rDNA		*Bam*HI fragment 6	4,900	Bohnert et al. 1979, 1980
*rpl*2	ribosomal protein L2	*Xho*I/*Sal*I	800	Zurawski et al. 1984
*rpo*A/*rps*11	α subunit of RNA polymerase/ribosomal protein S11	*Sal*I/*Xho*I	1,200	Sijben-Müller et al. 1986
*rps*12	ribosomal protein S12	*Sau*3A/*Eco*RI	285	Fromm et al. 1986
*rps*19	ribosomal protein S19	*Hind*III/*Hpa*III	178	Zurawski et al. 1984
*rps*19′/*trn*H	ribosomal protein S19/$tRNA^{HIS}$	*Bal*I/*Xba*I	280	Zurawski et al. 1984
*URF*509	unknown	*Eco*RI	1,930	Sugita et al. 1985

[a] Smith and Gray (1984) identified this reading frame as coding for a P_{700} subunit. It was subsequently referred to as *psa*C. J.C. Gray has recently advised us, however, that the coding function of this region needs to be reinvestigated

18.2-kbp *Sal*I and the 7.2- and 1.5-kbp *Pst*I fragments remain ambiguous. It is possible, of course, that recombination could occur inside the two inverted repeats leading to the existence of two tautomeric populations of cpDNA, as has been shown for several DNAs (cf. Palmer et al. 1984). If this occurred in pepper, both arrangements of the 24- and 18.2-kbp fragments of *Sac*I could exist on separate DNA molecules.

We compared the restriction map of *Capsicum annuum* cpDNA with restriction maps of other members of the *Solanaceae*: PvuII of *Petunia parodii* and of *Atropa belladona* (Fluhr and Edelman 1981); *Pst*I, *Pvu*II, and *Sal*I of tomato (Phillips 1985; Piechulla et al. 1985); and *Sal*I and *Pvu*I of tobacco (Seyer et al. 1981; Hildebrandt et al. 1985). Conservation of the restriction sites occurs inside the inverted repeats and in the right half of the large single-copy region; differences occur in the left half of the large single-copy region and in the small single-copy region. The region near *psb*A, in which Kung's group reported a "hot spot" among a number of *Nicotiana* species (Kung et al. 1982; Tassopulu and Kung 1984), lies within a fully conserved region for *Capsicum annuum*.

Table 7. Hybridization of heterologous cpDNA gene probes from spinach and tobacco to restriction fragments of *Capsicum annuum* cpDNA. Fragments are identified by their sizes in kbp (cf. Tables 2 to 5)

Probe	Probe hybridized to			
	*Sac*I	*Pvu*II	*Pst*I	*Sal*I
*atp*A	7.3	5.8	11.1	16.6
*atp*B	8.0	43.9	19.9	19.6
*atp*E	8.0	9.5	19.9	19.6
*atp*F	3.0	3.0	17.3	15.6
*pet*A	10.1	43.9	21.9	19.6
*pet*B	6.6	43.9	21.9	10.6
*pet*D	6.6	43.9	21.9	10.6
*psa*A	3.5	9.5	19.9	21.2
*psa*B	18.1	7.8	19.9	21.2
*URF*512	10.1	43.9	3.7	19.6
*psb*A	22.9	11.1	17.3	19.6
*psb*B	9.5	43.9	21.9	10.6
*psb*C	18.1	18.7	14.5, 1.0	21.4
*psb*D	18.1	18.8	14.5	21.4
*psb*E/*URF*39	10.1	43.9	21.9	19.6
*rbc*L	10.1	43.9	19.3, 3.7	19.6
16S rDNA	22.9, 19.2	43.9, 16.2, 2.3	38.8	24.0, 18.2
23S/4.5S/5S rDNA	22.9, 18.1, 6.6, 3.3, 1.8	43.9, 9.3, 3.8, 2.3	38.8, 21.9	24.0, 18.2, 10.6
*rpl*2	22.9, 19.2	43.9, 16.2	7.2, 2.3, 1.5	19.6, 11.6
*rps*11/*rpo*A	6.6	43.9	21.9	10.6
*rps*12	22.9, 19.2	43.9, 16.2	38.8	24.0, 18.2
*rps*19	22.9, 19.2	43.9	1.5	11.6
*rps*19′/*trn*H	22.9, 19.2	43.9, 16.2	2.3, 1.5	19.6, 11.6
*URF*509	22.9	11.1	17.3	19.6

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