Volume 14 Number 14 1986 Nucleic Acids Research

Tripartite mitochondrial genome of spinach: physical structure, mitochondrial gene mapping, and locations of transposed chloroplast DNA sequences

David B.Stern[1]* and Jeffrey D.Palmer[2]

Carnegie Institution of Washington, Department of Plant Biology, 290 Panama Street, Stanford, CA 94305, [1]Department of Botany, University of California, Berkeley, CA 94720 and [2]Department of Biology, University of Michigan, Ann Arbor, MI 48109, USA

Received 19 May 1986; Accepted 20 June 1986

ABSTRACT

A complete physical map of the spinach mitochondrial genome has been established. The entire sequence content of 327 kilobase pairs (kb) is postulated to occur as a single circular molecule. Two directly repeated elements of approximately 6 kb, located on this "master chromosome", are proposed to participate in an intragenomic recombination event that reversibly generates two "subgenomic" circles of 93 kb and 234 kb. The positions of protein and ribosomal RNA-encoding genes, determined by heterologous filter hybridizations, are scattered throughout the genome, with duplicate 26S rRNA genes located partially or entirely within the 6 kb repeat elements. Filter hybridizations between spinach mitochondrial DNA and cloned segments of spinach chloroplast DNA reveal at least twelve dispersed regions of interorganellar sequence homology.

INTRODUCTION

Mitochondria contain their own DNA, which encodes parts of the organellar translational apparatus and components of the electron transport chain. Genes encoding 26S, 18S and 5S rRNA (1-3), subunits I and II of cytochrome oxidase (4, 5), subunits 6 (6), 9 (7) and alpha (8) of the mitochondrial ATPase, apocytochrome B (9) and a gene homologous to URF-1 (10), an unidentified reading frame found in animal (11) and fungal (12-14) mitochondria, and several tRNAs (15, 16) have been identified and sequenced for one or more plant mitochondrial genomes (for reviews of plant mitochondrial genes and genome structure, see refs. 17 and 18). Plant mitochondrial DNA (mtDNA) also contains substantial regions of homology to chloroplast DNA (ctDNA; 19-21). No functions have as yet been ascribed to this "promiscuous" (22) DNA. Plant mtDNAs are distinguished from their fungal and animal counterparts by their large size (23) and by the presence of repeated DNA elements, certain of which appear to be involved in inter- and intramolecular recombination events (24-27). In turnip mtDNA, intramolecular recombination is postulated to generate a tripartite genome structure (27), whereas the corn mitochondrial genome exhibits a multicircular organization that is proposed to result from recombinational activity at five major pairs of repeated elements (26).

In the present work, we describe the organization of the 327 kb spinach mitochondrial genome. Its proposed tripartite structure is very similar to

that of turnip mtDNA (22). Heterologous filter hybridizations reveal that both *bona fide* mitochondrial genes and regions homologous with ctDNA are scattered throughout the genome.

MATERIALS AND METHODS

Methods used for the purification, restriction endonuclease digestion, gel electrophoresis and filter hybridization of spinach (*Spinacia oleracea*) mtDNA have been described (20, 28, 29). Plasmid and cosmid DNAs were prepared by a modified alkaline lysis (30) procedure. Recombinant plasmid DNA clones of spinach mtDNA were generated by digestion of the mtDNA with Sal I, Pst I or Kpn I, followed by ligation to an appropriately cut pUC plasmid vector (31) and transformation (32) into *E. coli* strain JM83. A spinach mtDNA cosmid library, established in pHC79 (33), was screened and maintained as described (34).

Spinach ctDNA clones used in heterologous filter hybridizations were described in an earlier publication (35). Mitochondrial gene probes for subunits I and II of cytochrome c oxidase, the 26S, 18S and 5S rRNAs, the α subunit and subunit 6 of the mitochondrial ATPase, apocytochrome B and URF-1 are described in detail in the text and in Table 1.

RESULTS

Physical Map of Spinach mtDNA

In order to establish a restriction map of the spinach mitochondrial genome, plasmid DNA clones were randomly selected from mtDNA libraries generated with Sal I, Pst I and Kpn I. Small-scale plasmid DNA preparations were analysed by restriction endonuclease digestion and gel electrophoresis to establish a set of unique clones for each enzyme. These clones were subsequently labelled with ^{32}P and hybridized to filter blots containing single and double digests of spinach mtDNA.

An example of this mapping strategy is shown in Fig. 1. A ^{32}P-labelled cloned Sal I fragment of 6.45 kb is shown to be cleaved by Kpn I into fragments of 3.35 kb and 3.10 kb (Fig. 1B, lane SK). The clone, pJDP20, hybridizes with Kpn I fragments of 22 kb and 18.1 kb (Fig. 1B, lane K), demonstrating their physical linkage. The Sal I insert of pJDP20 is uncut by Pst I (Fig. 1B, lane SP), and the clone hybridizes with a single Pst I fragment of 13.5 kb (Fig. 1B, lane P). Thus, this 6.45 kb Sal I fragment overlaps both the 22 kb and 18.1 kb Kpn I fragments, but is internal to a 13.5 kb Pst I fragment. The physical map was extended by similar analyses of plasmid clones containing a total of 67% of the genome.

Since the restriction map produced by filter hybridizations of plasmid clones was incomplete, as judged by the number of Sal I fragments visible in an ethidium bromide-stained gel (Fig. 1A, lane S) but not present on the preliminary linkage map, a cosmid DNA library consisting of 384 clones with 35-45 kb inserts of partial Sau3A digestion products was constructed. Cosmids were selected from the library by their hybridization with ^{32}P-

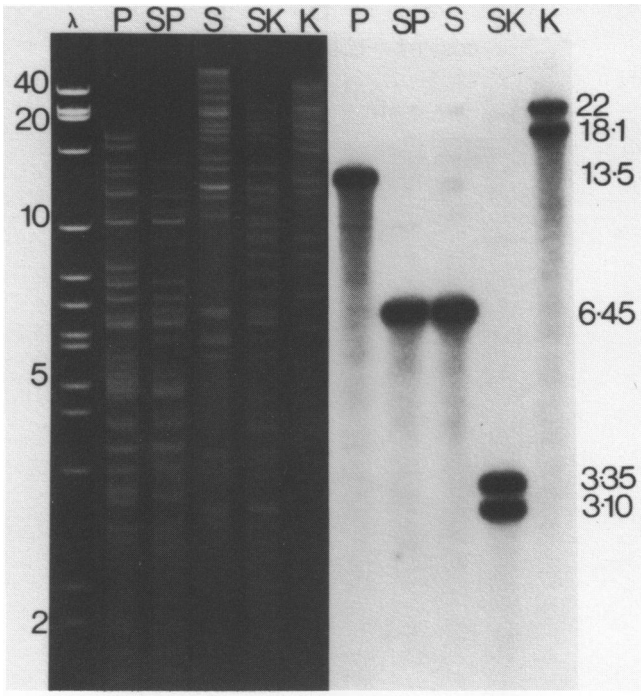


Figure 1. Mapping strategy for plasmid clones of spinach mtDNA. Spinach mtDNA was digested with Pst I (P), Sal I + Pst I (SP), Sal I (S), Sal I + Kpn I (SK) and Kpn I (K), electrophoresed in a 0.7% agarose gel, stained with ethidium bromide (left panel) and transferred to a GeneScreen filter. The filter was hybridized with the nick-translated clone pJDP20, which consists of pUC8 and a 6.45 kb Sal I fragment of spinach mtDNA (right panel). Size markers are in kb, and were determined from Sal I, Eco RI and Hind III digests of phage lambda (λ) DNA.

labelled restriction fragments from the ends of previously characterized linkage groups. Each cosmid was placed on the restriction map, and simultaneously verified for integrity by the strategy illustrated in Fig. 2. The cosmid clone cSIIE10 was digested with Sal I, Pst I or Kpn I, electrophoresed in an agarose gel next to a lane of spinach mtDNA digested with the same enzyme, transferred to nitrocellulose, and probed with the ^{32}P-labelled cosmid. Since the cosmid vector has one Sal I site and one Pst I site (33), each of these enzymes generates two vector-containing fragments which do not align with genomic DNA fragments (Fig. 2, lanes 1 and 3, marked "v"). Kpn I does not cleave the vector, so only a single vector-containing fragment is expected (Fig. 2, lane 5). The remaining DNA fragments, which are fully contained within the cosmid insert, both hybridize and align with the corresponding fragments in the mtDNA digest (e.g. 12.5 kb, 8.1 kb and 2.3 kb Kpn I fragments; Fig. 2, lanes 5 and 6). The cosmid also hybridizes

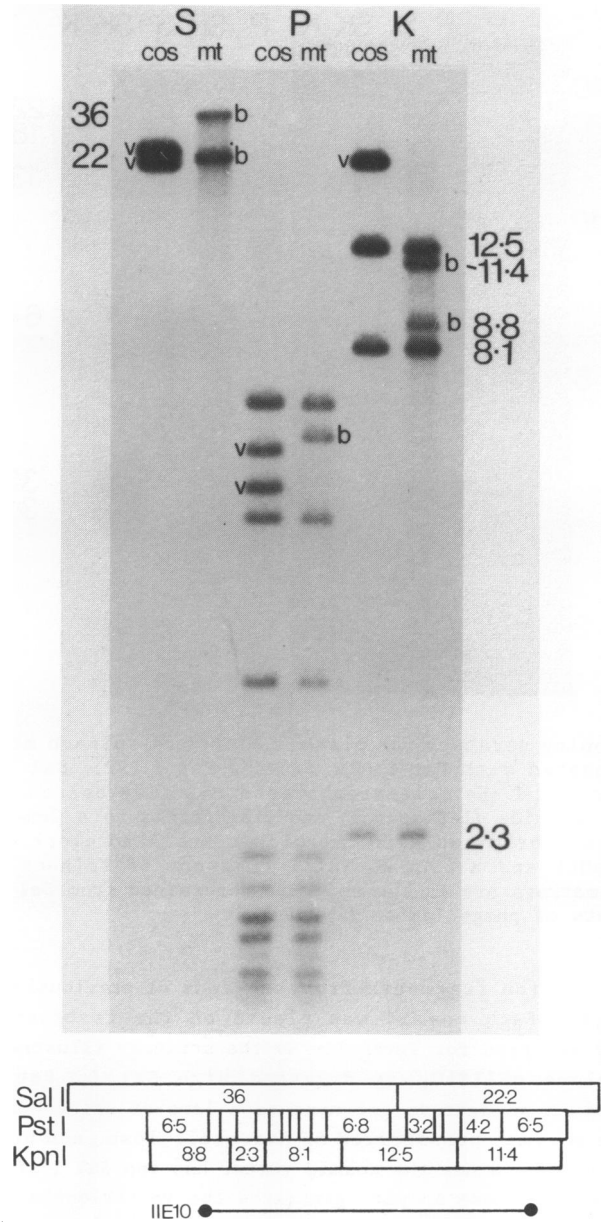


Figure 2. Mapping strategy for cosmid clones of spinach mtDNA. 0.2 μg of cosmid csIIE10 DNA (lanes marked "cos") and 1.5 μg of spinach mtDNA (lanes marked "mt") were digested with Sal I (S), Pst I (P) or Kpn I (K), electrophoresed in a 0.7% agarose gel, transferred to GeneScreen and hybridized with nick-translated csIIE10 DNA. Cosmid DNA fragments

consisting partly of vector sequences are indicated with a "v". Spinach fragments which are partially included within the cosmid insert ("border" fragments) are marked with a "b". The bottom of the figure shows a restriction map of the spinach mtDNA region cloned in csIIE10, and below it, the extent of cosmid csIIE10. Sizes of fragments are in kb, and were determined as in Fig. 1.

with two genomic DNA fragments not fully contained within its insert (e.g. 36 kb and 22 kb Sal I fragments; Fig. 2, lane 2, also lanes 4 and 6, marked "b"). The combined results of hybridizations with overlapping cosmid and plasmid clones led to construction of short restriction maps such as that shown at the bottom of Fig. 2, which could be linked together. Eventually, cosmid and plasmid clones covering over 96% of the genome were analysed, and the physical map was ascertained to be complete.

A restriction map of the spinach mitochondrial genome is shown in Fig. 3. The genome exists as a circular molecule approximately 327 kb in length. The map accounts for every Sal I and Kpn I fragment visible in a stained gel

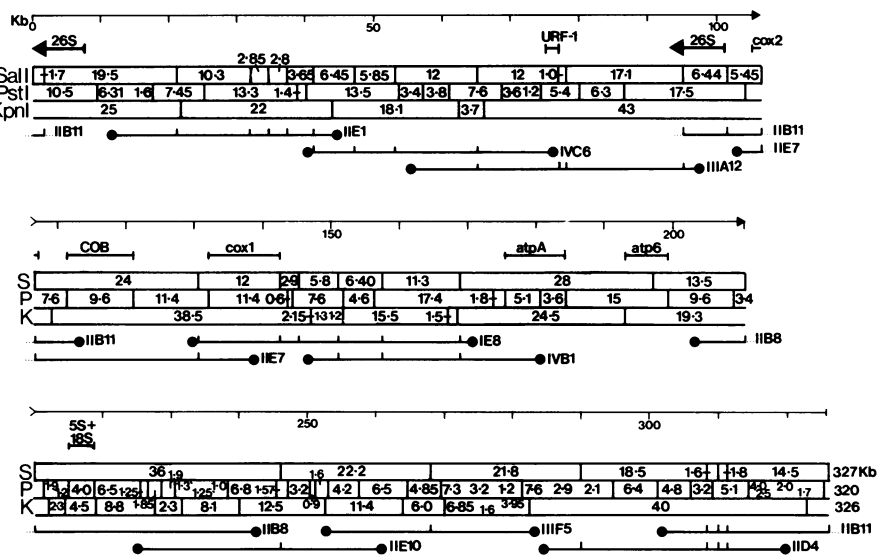
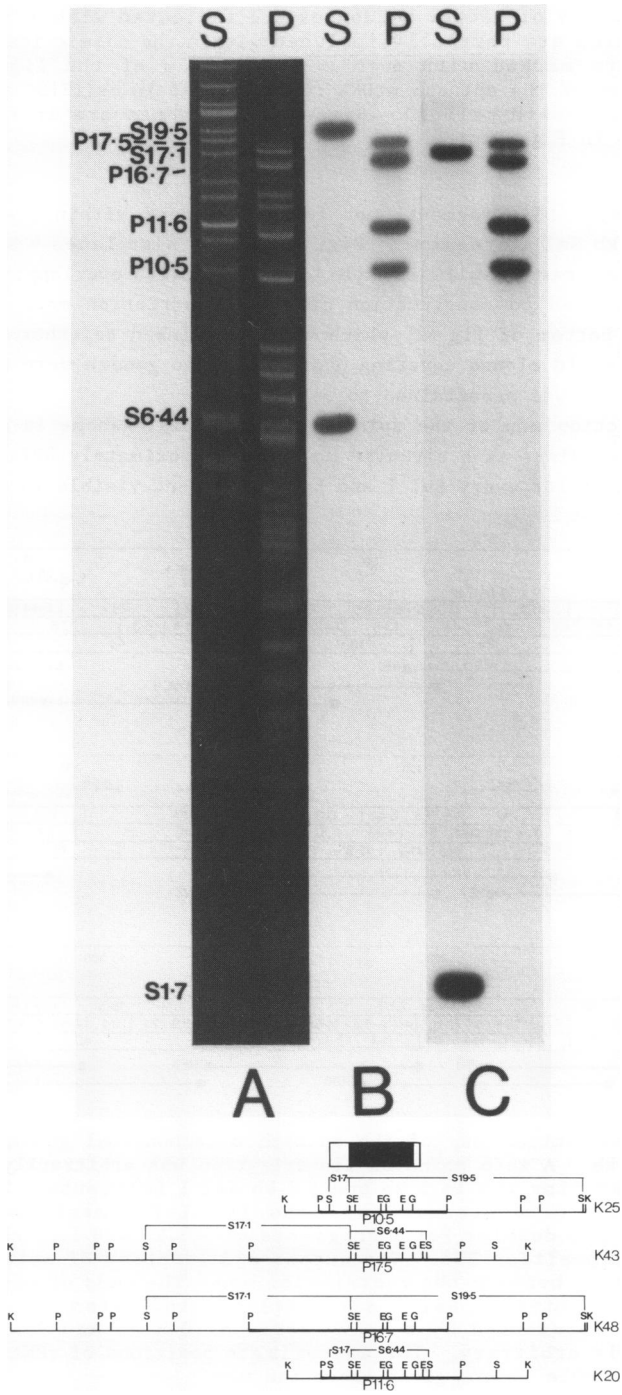


Figure 3. Restriction map of the spinach mitochondrial genome. Fragment sizes are in kb. A zero point of linearization was arbitrarily selected as the site separating the 14.5 kb and 1.7 kb Sal I fragments. The relative order of certain clustered Kpn I and Pst I fragments has not been determined. Two doublet Pst I fragments of 1.3 kb and 1.25 kb are marked with (·) near position 235. The extent and location of selected cosmid clones are shown beneath the restriction map. The ends of cosmid inserts (●) and their internal Sal I sites (|) are indicated. A 6 kb repeat sequence is represented by a heavy arrow at 0-6 kb and 93-100 kb. Its orientation is arbitrary. The approximate positions of rRNA and protein-coding genes (Table 1) are given above the map.


S P S P S P
P17·5
S19·5
S17·1
P16·7
P11·6
P10·5
S6·44
S1·7
A B C
S17
S19·5
K P S SE EG E G P P P SK
K25
P10·5
S17·1
S6·44
K P P P S P P SE EG E G ES P S K
K43
P17·5
S17·1
S19·5
K P P P S P P SE EG E G P P P SK
K48
P16·7
S17
S6·44
K P S SE EG E G ES P S K
K20
P11·6

(Fig. 1A, lanes S and K). Because of the large (>50) number of Pst I fragments, some ambiguities remain in their locations. A prominent structural feature of the genome is a two-copy direct repeat of approximately 6 kb that contains part or all of the 26S rRNA gene (Fig. 3, 0 and 93 kb; ref. 24). This is the only major repeated sequence that is detectable by filter hybridization. Evidence that the "6 kb repeat" is involved in the generation of a tripartite genome organization is given in the next section.

Intramolecular Recombination in Spinach mtDNA

The 6 kb repeat possess a single Sal I site, and is contained within Sal I fragments of 1.7 kb, 19.5 kb, 17.1 kb and 6.44 kb (Fig. 3). The 1.7 kb and 17.1 kb Sal I fragments and the 19.5 kb and 6.44 kb Sal I fragments cross-hybridize, since each pair shares common repeat sequences (Fig. 3 and Figs 4B and 4C, lanes S). Each fragment also cross-hybridizes with Pst I fragments of 17.5 kb, 16.7 kb, 11.6 kb and 10.5 kb (Figs. 4B and 4C, lanes P). The four repeat-containing Pst I fragments are substoichiometric, as judged by their reduced fluorescence relative to other Pst I fragments, in an ethidium bromide-stained gel (Fig. 4A, lane P). Of these submolar Pst I fragments, only those of 17.5 kb and 10.5 kb are accounted for by the restriction map shown in Fig. 3. The 16.7 kb and 11.6 kb Pst I fragments, however, can be generated by a single postulated recombination event between the repeat sequences embedded within the 17.5 kb and 10.5 kb fragments. The structural relationships of the four Pst I fragments, the larger, substoichiometric Kpn I fragments within which they lie, and the four repeat-containing Sal I fragments are shown in Fig. 4D. Each pair of Kpn I (or Pst I) fragments conserves genome size (i.e. for Kpn I, 25 + 43 kb = 48 + 20 kb), but contain different combinations of flanking sequences.

The filter hybridization data shown in Figs. 4B and 4C, the substoichiometric levels of the four repeat-containing Pst I fragments (Fig. 4A, lane P), and their physical relationships (Fig. 4D), are consistent with

Figure 4. Filter hybridization analysis of the 6 kb repeat. Spinach mtDNA was digested with Sal I (S) and Pst I (P), electrophoresed in a 0.7% agarose gel, stained with ethidium bromide (A), transferred bidirectionally (ref. 51) to GeneScreen and hybridized with ^{32}P-labelled pS6.44 (B) and pS1.7 (C). Sizes of fragments are in kb, and were determined as in Fig. 1. (D) Organization of the four repeat-containing Kpn I fragments. K25 and K43 are located on the "master genome" (Figs. 3 and 5A), and K48 and K20 are found on the 93 kb and 234 kb circles, respectively (Fig. 5C). The two pairs of fragments (K25/K43 and K48/K20) are postulated to interconvert via reciprocal recombination (Fig. 5B). The locations of the four repeat containing Pst I fragments (P10.5, P17.5, P16.7 and P11.6; Figs. 4A-C) are indicated by thickened lines. Sal I fragments discussed in the text are S1.7, S19.5, S17.1 and S6.44. Selected restriction sites are shown for Kpn I (K), Pst I (P), Sal I (S), Eco RI (E) and Bgl II (G). The maximum extent of the repeat is shown at the top by an open box; The filled portion represents its minimum size. The orders of the two Pst I fragments completely internal to S19.5, and of those to the left of S17.1 are unknown.

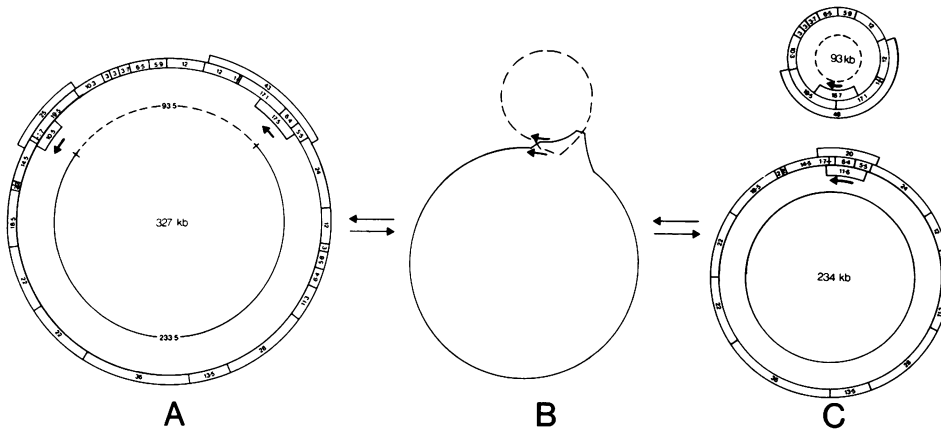


Figure 5. (A) Restriction map of the spinach mtDNA master genome. Pst I (inner circle), Sal I (middle circle) and Kpn I (outer circle) fragments are shown. The 6 kb repeat is indicated by heavy arrows. The portions of the master genome from which the 93 kb and 234 kb subgenomes are derived are shown as broken and solid lines, respectively, on the innermost circle. A postulated reversible recombination event is diagrammed in (B), leading to the formation of two subgenomes via dissolution of the master genome (C), or to cointegration of the two smaller circles to form the master genome (A).

the occurrence of intragenomic recombination. The 6 kb repeats present in direct orientation on the 327 kb "master genome" (Fig. 5A) are proposed to pair (Fig. 5B), leading to recombination and the resolution of two "subgenomes" of 93 kb and 234 kb (Fig. 5C). Presumably, this event is reversible via cointegration of the two smaller circles to form a "master" genome. Furthermore, either of the smaller circles could recombine with the master genome, leading to circles of 420 kb and 561 kb. These molecules could be involved in further events, *ad infinitum*. We hypothesize, however, that the major molecular forms of the genome are those shown in Figs. 5A and 5C. An exact evaluation of the relative proportions of the master genome and two subgenomes is difficult. Nonetheless, it is clear that the repeat-containing Sal I fragment of 1.7 kb gives a hybridization signal that is approximately two-fold stronger with the 16.7 kb Pst I fragment than with the 17.5 kb Pst I fragment, although it has an equal length of homology with each (Fig. 4D). There is a less marked, but correspondingly stronger signal with the 11.6 kb Pst I fragment compared to the 10.5 kb Pst I fragment (Fig. 4C, lane P). These results together suggest that the subgenomes, which contain 16.7 kb and 11.6 kb Pst I fragments (Fig. 5C), are present in higher abundance than the 327 kb master genome, which contains 17.5 kb and 10.5 kb Pst I fragments (Fig. 5A). We emphasize, however, that these estimates are hampered by differential degradation of fragments, and the difficulty of resolving the 17.5 kb and 16.7 kb Pst I fragments sufficiently to perform densitometric scans.

Table 1. Mapping of Spinach Mitochondrial Genes

Gene*	Clone	Source Species	Vector	Insert Size (kb)	Enzyme	Ref.	Hybridizing spinach mtDNA fragments (kb) Sal I	Pst I	Kpn I
26S	pS4.8	corn	pUC8	4.8	Sma I	1	19.5, 17.1, 6.44, 1.7	17.5, 16.7, 11.6, 10.5	48, 43, 25, 20
18S	pP3.2	corn	pUC8	3.2	Pst I	2	36	4.0	4.5
5S	206NX	corn	pUC12	0.085	XmnI-HpaII	**	36	4.0	4.5
cob	pE1.5	Oeno-thera	pBR322	1.5	EcoRI	50	24	9.6	38.5
cox1	pEB3.9	corn	pUC8	3.9	BamHI-EcoRI	4	12	11.4	38.5
cox2	pZmE1	corn	pBR322	2.4	EcoRI	5	24, 5.45	7.6	43, 20
urf-1	pH1.9	water-melon	pUC8	1.9	HindIII	10	12	5.4	48, 43
atpA	pH2.7	corn	pUC13	2.7	HindIII	8	28	5.1, 3.6	24.5
atp6	pH4.2	corn	pUC19	4.2	HindIII	6	28, 13.5	15	19.3

* Gene designations are: 26S, 18S, 5S; genes encoding RNA components of the mitochondrial ribosomes; cob, apocytochrome B; cox1 and cox2, subunits I and II of cytochrome oxidase; urf-1, gene homologous to unidentified reading frame-1 of animal and fungal mitochondria (11-14); atpA and atp6; alpha subunit of the F_1-ATPase and subunit 6 of the F_0-ATPase, respectively.

** A. Maloney, Department of Biological Sciences, Stanford University.

Mapping of Mitochondrial Genes

To place known mitochondrial genes on the physical map of spinach mtDNA, previously characterized gene-containing clones were used in heterologous filter hybridizations. A description of the clones, and a list of hybridizing fragments is given in Table 1. Sample filter hybridizations with gene-containing clones for 18S rRNA, apocytochrome B and subunit II of cytochrome oxidase are shown in Fig. 6. Most of the genes are scattered widely throughout much of the spinach mitochondrial genome (Fig. 3). A sole exception to this pattern is the close linkage of the 18S and 5S rRNA genes, which are also adjacent in other plant mitochondrial genomes (1, 36). All the genes are present only once per genome, except for the 26S rRNA gene, which, as previously noted (24), is coincident with the 6 kb repeat and exists in two copies per genome.

Regions Homologous with Chloroplast DNA

It has previously been established that substantial sequence homologies exist between ctDNA and mtDNA in flowering plants, including spinach (20, 37). To further our understanding of the nature of these interorganellar DNA homologies, regions of homology between spinach chloroplast and mitochondrial DNAs were mapped by filter hybridization. Pst I, Sal I and Kpn I restriction digests of mitochondrial and chloroplast DNAs were electrophoresed in agarose gels, transferred to GeneScreen, and probed with ^{32}P-labelled clones of spinach ctDNA. Typical results are shown in Fig. 7. A cloned spinach ctDNA Pst I fragment of 1.9 kb, P1.9, hybridizes as expected to a 1.9 kb fragment present both in the ctDNA lane and (as a

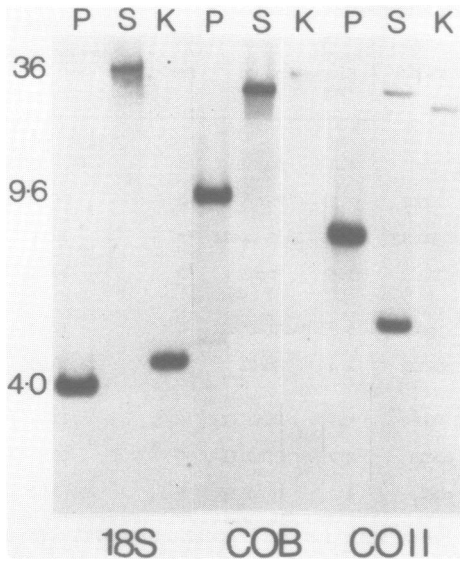


Figure 6. Mapping of rRNA and protein-coding genes. Spinach mtDNA digested with Pst I (P), Sal I (S) or Kpn I (K) was electrophoresed in 0.7% agarose gels, transferred to GeneScreen, and hybridized with the indicated gene clones (cf. Table 1).

contaminant) in the mtDNA preparation [Fig. 7, P1.9, lanes P(c) and P(m)]. The clone also hybridizes with a *bona fide* mtDNA Pst I fragment of 3.6 kb, which is not present in the ctDNA (38). Sequences present in P1.9 are therefore homologous to sequences that lie within the mitochondrial 3.6 kb Pst I fragment. Similar hybridizations with Sal I, Kpn I and doubly-digested mitochondrial and chloroplast DNAs (Fig. 7; double digests not shown), allow placement of the homologous region within a 3.6 kb Pst I fragment that lies within a 12 kb Sal I fragment (cf. Figs. 3 and 8). A more complex pattern is obtained with a cloned spinach ctDNA Xho I fragment of 15.3 kb (Fig. 7, X15.3). This fragment contains the chloroplast 16S rRNA gene (39); its weak hybridization with the mitochondrial 18S rRNA gene (Fig. 7, X15.3, open arrows) is therefore expected (40). X15.3 also hybridizes with two other distinct regions of the mitochondrial genome (Fig. 7, X15.3, filled arrows).

Fine mapping of these homologies was accomplished for clones spanning virtually the entire spinach chloroplast genome. The regions of cross-hybridization are indicated schematically in Fig. 8, and are summarised in Table 2. At least 14 regions of spinach mtDNA are homologous with chloroplast DNA. Two of these homologies result from the primordial common ancestry of the mitochondrial and chloroplast rRNA genes (Fig. 8, dotted lines). Whether any of the 12 other ctDNA-homologous mtDNA sequences contain functional genes is currently unknown.

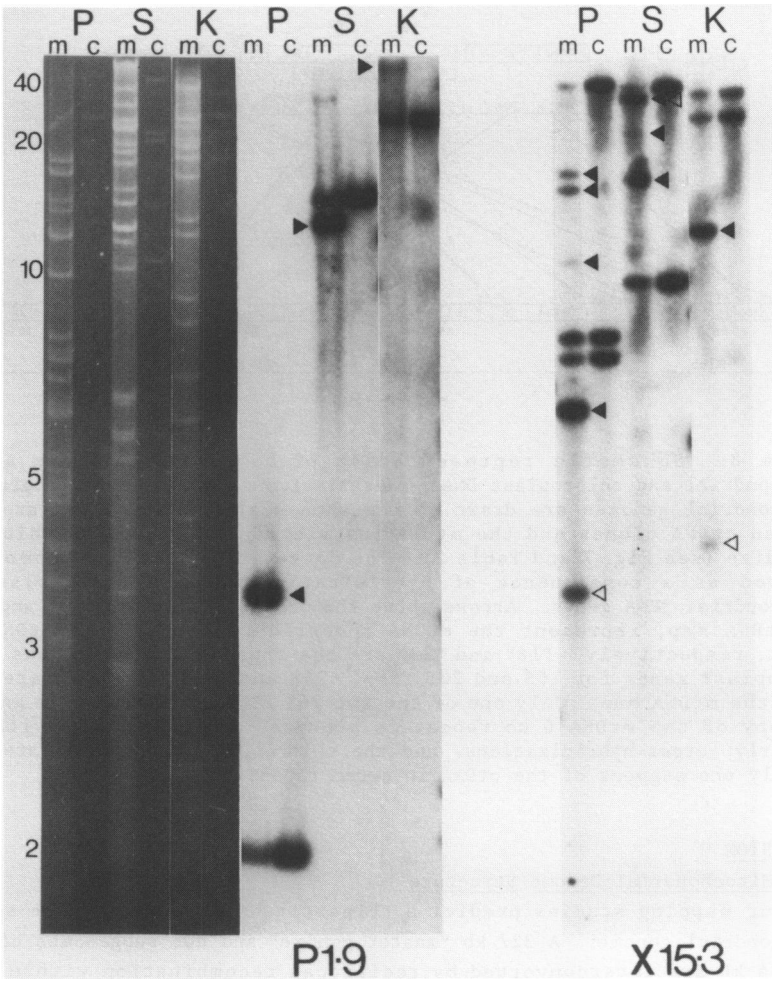


Figure 7. Homologies between spinach mitochondrial and chloroplast DNAs. Mitochondrial (m) and chloroplast (c) DNAs were digested with Pst I (P), Sal I (S) or Kpn I (K), electrophoresed in a 0.7% agarose gel, stained with ethidium bromide (left), transferred to GeneScreen, and hybridized with the nick-translated ctDNA clones indicated under each panel. MtDNA restriction fragments homologous with the ctDNA probes are marked with closed arrows (◀). Open arrows (◁) designate mtDNA fragments of 4.0 kb (Pst I), 4.5 kb (Kpn I) and 36 kb (Sal I) which contain the 18S rRNA gene, and are therefore presumed (40) to cross-hybridize with the 16S rRNA gene contained within X15.3. The filter probed with X15.3 was derived from a different gel than the one shown, and does not align precisely with the size markers on the left.

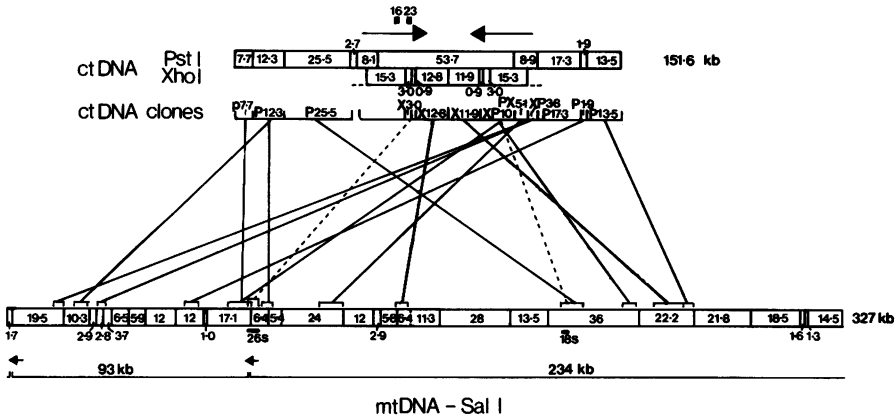


Figure 8. Schematic representation of homologies between spinach mitochondrial and chloroplast DNAs. Restriction maps of the chloroplast and mitochondrial genomes are drawn to the same scale. Solid lines are drawn between ctDNA clones and the mtDNA restriction fragments to which they hybridize (see Fig. 7 and Table 2). The dotted lines indicate homologies observed as a consequence of hybridization between chloroplast and mitochondrial rRNA genes. Arrows above the ctDNA restriction map, and below the mtDNA map, represent the ctDNA inverted repeat and the mtDNA 6 kb repeat, respectively. "16" and "23" are the approximate positions of the chloroplast genes for 16S and 23S rRNA. 26S and 18S rRNA genes are shown below the mtDNA map. Only one of the two 26S rRNA genes is shown and only one copy of the mtDNA 6 kb repeat is pictured as hybridizing with X3.0. Similarly, cross-hybridizations, and the chloroplast rRNA genes, are shown for only one segment of the ctDNA inverted repeat.

DISCUSSION

Plant Mitochondrial Genome Structure

Our mapping studies predict a tripartite structure for the spinach mitochondrial genome. A 327 kb "master genome" and two subgenomes of 93 kb and 234 kb are interconverted by reciprocal recombination within a 6 kb direct repeat. The tripartite organization of spinach mtDNA parallels that first reported for turnip mtDNA, which contains a 218 kb master genome and subgenomes of 135 kb and 83 kb (27). Furthermore, four species in *Brassica* and the closely related genus *Raphanus* feature a similar tripartite mtDNA organization (J. Palmer and L. Herbon, unpublished data). In each case, the recombination repeats are in direct orientation, despite the occurrence of multiple inversions throughout the genome.

The organization of spinach and several *Brassica* and *Raphanus* mitochondrial genomes contrasts with that of corn and wheat mtDNAs. Corn mtDNA is postulated to have a highly complex multipartite organization, in which five pairs of repeats are actively engaged in recombination (26). Similarly, wheat mtDNA possesses multiple pairs of repeated and recombining sequences (41), although a complete physical map is unknown.

Table 2. Cross-Homologies Between Spinach mtDNA and Cloned Spinach chloroplast DNA.

Clone*	Hybridizing ctDNA fragments**			Hybridizing mtDNA fragments***		
	Pst I	Sal I	Kpn I	Pst I	Sal I	Kpn I
P7.7	7.7	22.3 22.3	22.3 9.7, 1.9	17.5 16.7	17.1	48 43
P12.3	12.3	27.3 20.5	9.7 4.4, 1.6	17.5 13.3	10.3 6.44, 5.45	NT
P25.5	25.5	20.5, 5.2 6.0, 4.0	1.6, 18.4 3.2, 26.6	6.5	3.6	NT
X3.0	53.7	47.9	36.9 26.6, 0.80	17.5+, 11.6 16.7+, 10.5	19.5+, 6.44+	NT
X12.8	53.7	47.9	26.6	4.6	6.40	15.5
X11.9	53.7	47.9	26.6	4.2	22.2	11.4
XP10	53.7	10.6, 47.9	36.9	6.8, 17.5 4.0+, 16.7	3.7, 17.1	12.5, 4.8 4.5+, 43
PX5.1	8.9	10.6	36.9	11.4	24	38.5
XP3.8	8.9	10.6, 9.0	36.9	13.3, 7.45	2.80, 19.5	NT
P17.3	17.3	9.0 13.9, 2.4	36.9, 22.3	none	none	none
P1.9	1.9	13.9	22.3	3.6	12	43
P13.5	13.5	22.3, 13.9	22.3	4.85	22.2	6.0

* Clones (ref. 35) are designated by enzyme (P = Pst I, X = Xho I) and length of insert in kb. Fragments hybridizing with XP10, PX5.1 and XP3.8 were determined by comparing results using overlapping 15.3 kb Xho I and 8.9 kb Pst I clones.
** Fragment sizes are in kb. Mapping data are from refs. 38 and 39.
*** Fragment sizes are in kb. NT = not tested.
+ Hybridization resulting from homology between mitochondrial and chloroplast rDNAs.

Several trends are emerging in the structure of plant mtDNAs. Most genomes are characterised by the presence of large repeated sequences. Most of these repeats are involved in intra- and intergenomic recombination. Such "recombination repeats" have been reported for turnip (27), corn (26), wheat (24, 41) and pokeweed mtDNAs (24), in addition to spinach mtDNA. Where it has been determined, all recombination repeats are direct repeats. Accordingly, all fully mapped plant mitochondrial genomes have a multipartite organization, as opposed to the flip-flop dualism characteristic of inverted repeat-containing chloroplast genomes (42). Finally, the monocots examined thus far, corn and wheat, feature multiple pairs of repeated sequences, whereas spinach and five species of *Brassica* and *Raphanus* possess only a single major repeat. We note that all the above observations are based on an extremely limited sample size. For example, the much larger cucurbit mitochondrial genomes (43) may well have a more complex structure than the smaller dicot genomes.

Organization of Mitochondrial Genes

The map positions of six protein and three rRNA-encoding genes have

been determined by heterologous filter hybridizations (Figs. 3 and 6). These genes are scattered throughout the genome. Given the small (15-25, ref. 23) number of proteins thought to be encoded by plant mitochondria, and their large genome sizes, the possibility has been raised (23) that functional genes may be clustered, with the clusters separated by long regions of non-coding DNA. Yeast mtDNA, for example, contains long, presumably non-coding, (A+T)-rich regions (44). Our findings for spinach do not suggest any clustering, or "operon-like" regions, with the exception of the 18S and 5S rRNA genes, which are closely linked, as in all plant mitochondrial genomes examined (1, 36) (Fig. 3). Many more genes remain to be mapped, however, and it is still possible that clustering occurs for other loci.

Homology with Chloroplast DNA

It has been previously established that sequence homologies exist between chloroplast and mitochondrial DNAs in a wide variety of flowering plants (20, 21, 45). It was found that virtually every ctDNA clone tested hybridizes with one or more plant mtDNAs, and that in the case of spinach, five mung bean ctDNA clones hybridize with mtDNA (20). Cross-homology between a 7.7 kb spinach ctDNA Pst I fragment and spinach mtDNA has been more fully characterized (37). We have mapped this homology to a region near the end of the mtDNA 6 kb repeat (Fig. 8). Furthermore, we have extended these mapping studies by utilizing spinach ctDNA clones spanning most of the genome, and by locating the positions of homologous sequences on the mtDNA restriction map.

Eleven of the twelve ctDNA clones tested hybridize with mtDNA (Table 2). As in the case of *bona fide* mitochondrial genes (Fig. 3), regions homologous with ctDNA are highly dispersed in the mitochondrial genome (Fig. 8). In addition, the extent of homology, as judged by the relative strength of cross-hybridization signals, is quite variable from one region to another [e.g. Fig. 7, lane X15.3 P(m)]. Because both the length and degree of complementarity of these homologous sequences are not known, we cannot speculate on the timing of interorganellar DNA transfer. It is obvious, however, that the linear order of cross-hybridizing sequences is quite different in the two genomes (Fig. 8). This suggests either that the mitochondrial genome has undergone multiple rearrangements since interorganellar DNA transfer occurred, or that many separate events have led to dispersal of this "promiscuous" DNA. We feel it likely that both multiple transfer events and genome rearrangements have contributed towards the present situation. Further studies, including nucleotide sequence analysis, are required to refine our knowledge of the frequency, direction and mechanism of DNA exchange between organelles.

These ctDNA-homologous sequences, along with *bona fide* mitochondrial genes and repeated sequences engaged in recombination, comprise less than one third of the 327 kb spinach mitochondrial genome. The origin, function and composition of the remainder is currently unknown. One possibility, proven so far for corn (46) and spinach (37), as well as for animals (47,

48) and fungi (49) is that nuclear DNA is homologous with mtDNA. While the extent of these homologies is unclear, a physical map and clone bank of the mitochondrial genome will facilitate their analysis and also aid in unraveling the mode of plant mitochondrial gene expression.

ACKNOWLEDGMENTS

We thank William Thompson for the laboratory facilities used to perform this work. D.B.S. was supported by an N.I.H. training grant and a McKnight Interdisciplinary Award in Plant Biology. This is CIW-DPB Publication #924.

*To whom correspondence should be addressed

References

1. Stern, D.B., Dyer, T.A. and Lonsdale, D.M. (1982) Nucleic Acids Res. 10, 3333-3340.
2. Chao, S., Sederoff, R.R. and Levings, C.S. III (1983) Plant Physiol. 71, 190-193.
3. Dale, R.M.K., Mendu, N., Ginsburg, H. and Kridl, J.C. (1984) Plasmid 11, 141-150.
4. Isaac, P.G., Jones, V.P. and Leaver, C.J. (1985) EMBO J. 4, 1617-1623.
5. Fox, T.D. and Leaver, C.J. (1981) Cell 26, 315-323.
6. Dewey, R.E., Levings, C.S. III and Timothy, D.H. (1985) Plant Physiol. 79, 914-919.
7. Dewey, R.E., Schuster, A.M., Levings, C.S. III and Timothy, D.H. (1985) Proc. Natl. Acad. Sci. USA 82, 1015-1019.
8. Braun, C.J. and Levings, C.S. III (1985) Plant Physiol. 79, 571-577.
9. Dawson, A.J., Jones, V.P. and Leaver, C.J. (1984) EMBO J. 3, 2107-2113.
10. Stern, D.B., Bang, A.G. and Thompson, W.F. (1986) Curr. Genet., in press.
11. Anderson, S., Bankier, A.T., Barrell, B.G., deBruijn, M.H.L., Coulson, A.R., Drouin, J., Peron, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Stader, R. and Young, I.G. (1981) Nature 290, 457-465.
12. Brown, T.A., Davies, R.W., Ray, J.A., Waring, R.B. and Scazzocchio, C. (1983) EMBO J. 427-435.
13. Burger, G. and Werner, S. (1985) J. Mol. Biol. 186, 231-242.
14. Cummings, D.J., MacNeil, I.A., Domenico, J. and Matsuura, E.T. (1985) J. Mol. Biol. 185, 659-680.
15. Parks, T.D., Dougherty, W.G., Levings, C.S. III and Timothy, D.H. (1984) Plant Physiol. 76, 1079-1082.
16. Gray, M.W. and Spencer, D.F. (1983) FEBS Lett. 161, 323-327.
17. Quetier, F., Lejeune, B., Delorme, S. and Falconet, D. (1985) in Encyclopedia of Plant Physiology, New Series, Douce, R. and Day, D.A. Eds., Vol. 18, pp. 25-36, Springer-Verlag.
18. Palmer, J.D. (1985) in Monographs in Evolutionary Biology: Molecular Evolutionary Genetics, MacIntyre, R.J. Ed., pp. 131-240, Plenum, New York.
19. Stern, D.B. and Lonsdale, D.M. (1982) Nature 298, 698-702.
20. Stern, D.B. and Palmer, J.D. (1984) Proc. Natl. Acad. Sci. USA 81, 1946-1950.
21. Pring, D.R. and Lonsdale, D.M. (1985) Int. Rev. Cytology 97, 1-46.
22. Ellis, J. (1982) Nature 299, 678-679.

23. Leaver, C.J. and Gray, M.W. (1982) Ann. Rev. Plant Physiol. 33, 373-402.
24. Stern, D.B. and Palmer, J.D. (1984) Nucleic Acids Res. 12, 6141-6157.
25. Falconet, D., Lejeune, B., Quetier, F. and Gray, M.W. (1984) EMBO J. 3, 297-302.
26. Lonsdale, D.M., Hodge, T.P. and Fauron, C.M.-R. (1984) Nucleic Acids Res. 12, 9249-9261.
27. Palmer, J.D. and Shields, C.R. (1984) Nature 307, 437-440.
28. Kolodner, R. and Tewari, K.K. (1972) Proc. Natl. Acad. Sci. USA 69, 1830-1834.
29. Palmer, J.D. (1982) Nucleic Acids Res. 10, 1593-1605.
30. Birnboim, H.C. and Doly, J. (1979) Nucleic Acids Res. 7, 1513-1523.
31. Vieira, J. and Messing, J. (1982) Gene 19, 259-268.
32. Dagert, M. and Ehrlich, S.D. (1979) Gene 6, 23-28.
33. Hohn, B. and Collins, J. (1980) Gene 11, 291-298.
34. Lonsdale, D.M., Thompson, R.D. and Hodge, T.P. (1981) Nucleic Acids Res. 9, 3657-3669.
35. Palmer, J.D. and Thompson, W.F. (1981) Gene 15, 21-26.
36. Huh, T.Y. and Gray, M.W. (1982) Plant Molec. Biol. 1, 245-249.
37. Whisson, D.L. and Scott, N.S. (1985) Plant Molec. Biol. 4, 267-273.
38. Hermann, R.G., Whitfeld, P.R. and Bottomley, W. (1980) Gene 8, 179-191.
39. Westhoff, P., Alt, J., Widger, W.R., Cramer, W.A. and Herrmann, R.G. (1985) Plant Molec. Biol. 4, 103-110.
40. Stern, D.B., Hodge, T.P. and Lonsdale, D.M. (1984) Plant Molec. Biol. 3, 355-361.
41. Quetier, F., Lejeune, B., Delorme, S., Falconet, D. and Jubier, M.F. (1985) in Molecular Form and Function of The Plant Genome, Van Vloten-Doting, L, Groot, G.S.P. and Hall, T.C. Eds., pp. 413-420, Plenum Press, New York.
42. Palmer, J.D. (1985) Ann. Rev. Genet. 19, 325-354.
43. Ward, B.L., Anderson, R.S. and Bendich, A.J. (1981) Cell 25, 793-803.
44. Borst, P. and Grivell, L.A. (1978) Cell 15, 705-723.
45. Stern, D.B., Palmer, J.D., Thompson, W.F. and Lonsdale, D.M. (1983) UCLA Symp. Mol. Cell Biol., New Ser. 12, 467-477.
46. Kemble, R.J., Mans, R.J., Gabay-Laughnan, S. and Laughnan, J.R. (1983) Nature 304, 744-747.
47. Jacobs, H.T., Posakony, J.W., Grula, J.W., Roberts, J.W., Xin, J.-H., Britten, R.J. and Davidson, E.H. (1983) J. Mol. Biol. 165, 609-632.
48. Gellissen, G., Bradfield, J.Y., White, B.N. and Wyatt, G.R. (1983) Nature 301, 631-634.
49. Farrelly, F. and Butow, R.A. (1983) 301, 296-301.
50. Schuster, W. and Brennicke, A. (1985) Curr. Genet. 9, 157-163.
51. Smith, G.E. and Summers, M.D. (1980) Anal. Biochem. 109, 123-129.