Curr Genet (1990) 17:359–364

Current Genetics

Chloroplast DNA from *Adiantum capillus-veneris* L., a fern species (Adiantaceae); clone bank, physical map and unusual gene localization in comparison with angiosperm chloroplast DNA

Mitsuyasu Hasebe and Kunio Iwatsuki

Botanical Gardens, Faculty of Science, University of Tokyo, Hakusan 3-7-1, Bunkyo-ku, Tokyo 112, Japan

Received November 7, 1989/January 15, 1990

Summary. We cloned all of *Adiantum capillus-veneris* chloroplast DNA *Pst*I fragments longer than 1.0 kb, which cover 98% of the genome. These cloned fragments were used to construct a physical map for five restriction enzymes. The genome of *A. capillus-veneris* is approximately 153 kb long and contains a 24 kb inverted repeat. Mapping of 12 chloroplast DNA genes and heterologous hybridization, involving *A. capillus-veneris* chloroplast DNA and angiosperm chloroplast DNA probes, demonstrated that chloroplast DNA of *A. capillus-veneris* has a different gene order from typical angiosperm cpDNA (e.g., tobacco) in the inverted repeat region and the flanking segment of the large single copy region.

Key words: Chloroplast DNA – Gene order – Inverted repeat – *Adiantum*

Introduction

Chloroplast DNAs (cpDNAs) from many angiosperms have been extensively analyzed and characterized (reviewed in Palmer 1985a, b; Birky 1988). The high level of conservation of their genome size, organization and gene order has been well documented. All angiosperm cpDNAs are circular and most range in size from 120–217 kb. The most noteworthy feature of their organization is the presence of a large inverted repeat (IR) separated by a large and a small single copy region (LSC and SSC) except for some legumes (Kolodner and Tewari 1979; Koller and Delius 1980; Palmer and Thompson 1981; Chu and Tewari 1982). The size of the IR is nearly always about 25 kb in most angiosperms, but is significantly larger or smaller in a limited number of groups (Palmer 1985a; Palmer et al. 1987). Gene mapping and cross hybridization studies have shown that gene order is identical in most angiosperms, although several species from six families exhibit varying gene orders; the alterations appear to have been caused by inversions (reviewed in Palmer 1985a, b).

In contrast with our rich knowledge regarding angiosperm cpDNA, however, little is known about fern cpDNA. The only fern group in which genome organization and the gene order of cpDNA has been studied is *Osmunda,* in which gene order is reported to be identical with that of most angiosperms (Palmer and Stein 1982, 1986). A characteristic of *Osmunda* cpDNA is the reduced size (only 10 kb) of its IR.

It has long been clear that further studies on cpDNAs in ferns are necessary. To begin addressing this deficiency, we characterized *Adiantum capillus-veneris* cpDNA. We show that *A. capillus-veneris* has an IR of about 24 kb, almost as long as that of angiosperms and significantly longer than that of *Osmunda.* Based on data from heterologous hybridization, involving *A. capillus-veneris* cpDNA and typical angiosperm cpDNA probes, we report that *A. capillus-veneris* cpDNA has a different gene order in the IR than do most angiosperm cpDNAs and *Osmunda* cpDNA.

Materials and methods

Plants of the cosmopolitan species *Adiantum capillus-veneris* L., cultivated in a greenhouse of the Botanical Gardens, Faculty of Science, University of Tokyo, were used as material. CpDNA was isolated by essentially the same method used by Hirai et al. (1985) and Palmer (1986). Ten grams of young leaves with petioles removed were blended in liquid nitrogen. After the liquid nitrogen had completely evaporated, the powder obtained was suspended in 500 ml of buffer A (50 mM Tris-HCl, pH 8.0; 0.35 M sucrose; 7 mM EDTA-2Na; 5 mM 2-mercaptoethanol) containing 0.1% bovine serum albumin. This liquid suspension was filtered through two layers of cheesecloth and then two layers of miracloth, followed by centrifugation at 1,000 g for 10 min at 4°C. The pellet was resuspended in 2 ml of buffer A. The resuspended pellet was loaded on the top of a stepwise 20–55–70% sucrose gradient made in 50 mM Tris-HCl, pH 8.0; 0.3 M sorbitol; 7 mM EDTA-2Na, and centrifuged at 7,000 g for 20 min at 4°C. The green band at the 20–55% interface was collected and diluted with five volumes of buffer B (50mM Tris-

Offprint requests to: M. Hasebe

Table 1. Sources of gene probes. Style of designation follows Jansen and Palmer (1987); "−X" indicates that the gene probe starts from X bp upstream from initiation codon; "+X" indicates that the gene probe starts or ends X bp downstream from initiation codon; and "++X" indicates that the gene probe ends X bp downstream from the termination codon

Gene name	Fragment	Gene location
psbA	1,691 bp *Dra*I	−158 trnK3′ exon/++223 psbA
atpF–atpA	1,798 bp *Pvu*II-*Bgl*I	+186 atpF/+679 atpA
rpoB	1,073 bp *Bam*HI	+1,301/+2,373
psbC	997 bp *Pst*I	+3,703/+1,366
psaA–psaB	2,446 bp *Bam*HI	+4 psaA/+280 psaB
atpB	773 bp *Pvu*II-*Bam*HI	+849/++125
rbcL	989 bp *Pst*I-*Bam*HI	+732/++285
psbE–rpl33	5,471 bp *Sal*I	++1,569 PsbE/++450 rpl33
petD-rps11-rps8	2,954 bp *Xho*I	+676 petD/rps11/+337 rps8
rp12–rps19	1,573 bp *Pst*I	+487 rp12/++229 rps19
16SrDNA	1,905 bp *Pvu*II-*Bam*HI	+140/++555
23SrDNA	3,979 bp *Pvu*II	−999/++171

HCl, pH 8.0; 20 mM EDTA-2Na). Chloroplasts were pelleted by centrifugation at 3,000 g for 5 min at 4°C. The chloroplast pellet was resuspended in 1 ml of distilled buffer B, and then added to 1/10 volume of 10 mg/ml pronase E (Kaken-seiyaku, Tokyo, Japan) and 1/5 volume of lysis buffer [5% (w/v) sodium sarcosinate; 50 mM Tris-HCl, pH 8.0; 25 mM EDTA-2Na]. Chloroplast DNA was prepared from the purified chloroplasts by CsCl equilibrium density gradient centrifugation with ethidium bromide, as described by Palmer (1986). Restriction enzymes purchased from the Takara Shuzo Co Ltd (Kyoto, Japan) and BRL (Gaitherburg, USA) were used. CpDNAs were digested by the enzymes according to the supplier's instructions. Agarose gel electrophoresis followed Maniatis et al. (1982). DNA fragments, digested with restriction enzymes and fractionated on agarose gel, were transferred onto a Gene Screen Plus membrane (NEN, Boston, USA) by the bidirectional blotting technique (Smith and Summers 1980). Hybridization probes were prepared by the oligolabelling method (Feinberg and Vogelstein 1983, 1984). Homologous hybridization of cloned *A. capillus-veneris* fragments was performed at 42°C for 12–18 h in hybridization buffer (1 M NaCl, 10% dextran sulfate, 1% SDS, 100 μg/ml denatured salmon sperm DNA) containing 50% formamide. The filters were washed in 2 × SSC and 1% SDS at 65°C, prior to autoradiography. Heterologous hybridization of angiosperm cpDNA cloned fragments and restriction enzyme digested *A. capillus-veneris* cpDNA was performed at 55°C for 12–18 h in a hybridization buffer. The filters were washed in 2 × SSC and 1% SDS at 55°C, which did not contain formamide. The twelve gene probes used are listed in Table 1. The indicated restriction fragments from a tobacco cpDNA clone bank (clones supplied by the courtesy of Dr. M. Sugiura, Nagoya University, Nagoya, Japan) were sliced out of a 0.7% agarose gel and purified with DNA PREP (Asahi Glass Co Ltd, Tokyo, Japan). As heterologous probes of angiosperm cpDNAs, cloned cpDNA fragments of tobacco, lettuce and petunia were used (lettuce and petunia clones supplied by the courtesy of Dr. J. D. Palmer, Universiy of Michigan, Ann Arbor, USA). Those clones cover cpDNA completely and arrange into the same organizational pattern as the general genome organization seen in most other angiosperms. Generation of a clone bank of *A. capillus-veneris* cpDNA *Pst*I fragments was performed as follows. Nineteen *Pst*I fragments of *A. capillus-veneris* cpDNA longer than 1.0 kb were inserted into plasmid pUC12 (Yanisch-Perron et al. 1985). Because no recombinant pUC12 clone with the 11.4 kb *Pst*I fragment was recovered, this fragment was inserted into pUC18 (Yanisch-Perron et al. 1985). 1.2 μg of the *Pst*I fragments of *A. capillus-veneris* cpDNA were ligated with 10–100 ng of *Pst*I-digested vector which had been dephosphorylated with calf intestinal alkaline phosphatase (Böhringer Mannheim, Penzberg, West Germany). The ligated DNA was introduced into *E. coli* strain JM109 (Yanisch-Perron et al. 1985) and recombinant white colonies were selected on ampicillin/X-gal plates. About 400 recombinant white colonies were analyzed using the alkaline lysis method (Maniatis et al. 1982). Restriction-digested fragments sliced out of a gel were purified with RDP mini column (BIO-RAD, California, USA), or DNA PREP, before the ligation assay.

Results

Isolation of Adiantum capillus-veneris chloroplast DNA

After CsCl equilibrium density centrifugation, two bands became visible under UV light. DNA fractions from the two bands were digested by *Bam*HI and hybridized with tobacco cpDNA clones. The fragment from the lower DNA band hybridized to the tobacco cpDNA clones. Because cpDNA sequence is known to be very conservative among vascular plants (Palmer and Stein 1986), the lower band is considered to contain cpDNA, while DNA in the upper band is thought to be nuclear DNA which contaminated the chloroplasts at the step of the sucrose density gradient. The lower band was, for this reason, isolated as the cpDNA fraction.

Clone bank of Adiantum capillus-veneris chloroplast DNA

The shotgun cloning strategy yielded clones containing 17 of the 20 *Pst*I restriction fragments longer than 1.0 kb. Subsequently, the uncloned fragments (Nos. 1, 4 and 11 in Table 2) were cloned after gel-isolation. The clone bank of *A. capillus-veneris* covers 98% of the entire chloroplast genome. This library represents the first nearly complete clone bank obtained from fern cpDNAs (Fig. 1).

Physical mapping and gene mapping of Adiantum capillus-veneris chloroplast DNA

Chloroplast DNA from *A. capillus-veneris* was digested with the restriction endonucleases *Eco*RV, *Hin*dIII, *Pst*I, *Stu*I and *Xho*I and separated by agarose gel electrophore-

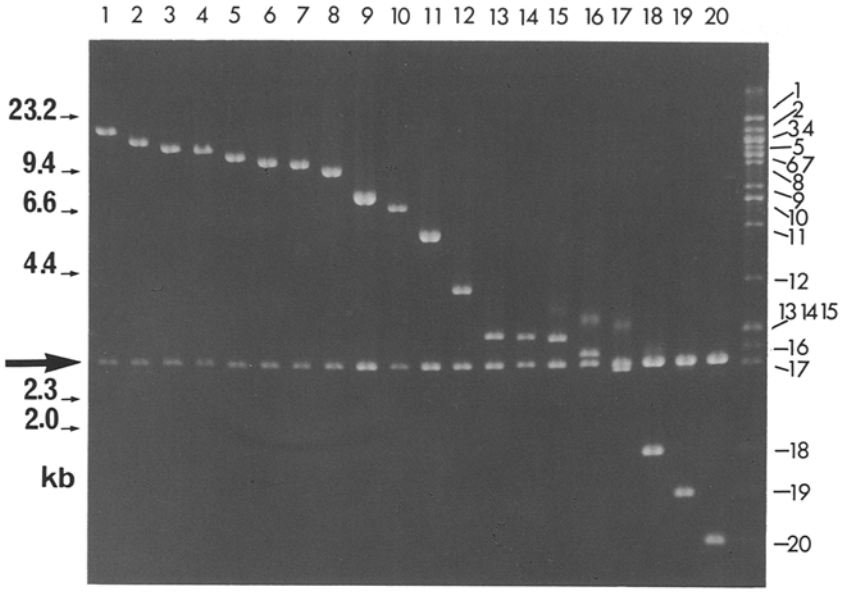

Fig. 1. Clone bank of *Adiantum capillus-veneris* cpDNA. *Pst*I clones (*1–20*) and the cpDNA (*lane furthest right*) were digested with *Pst*I and separated on a 0.7% agarose gel. *Arrow* indicates the vector. Restriction fragment numbers correspond to those given in Table 2. Sizes of marker DNA fragments are indicated on the left

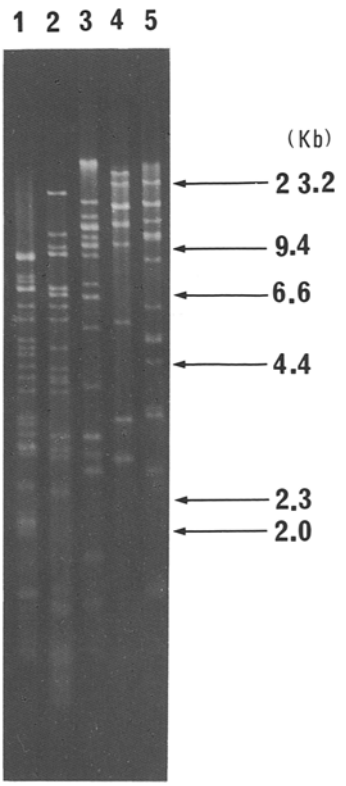

Fig. 2. Restriction patterns of *Adiantum capillus-veneris* cpDNA generated by digestion with *Eco*RV (*1*), *Hin*dIII (*2*), *Pst*I (*3*), *Stu*I (*4*) and *Xho*I (*5*)

sis (Fig. 2). Summation of the restriction fragment sizes from these five enzymes (Table 2) indicates that *A. capillus-veneris* has a genome of approximately 153 kb.

A combination of identifying overlapping fragments by hybridization, and of digestion of the cloned *Pst*I fragments with the above-mentioned five restriction endonucleases (singly and in combination) was performed in order to construct physical maps. For some ambiguous bands, combinations of additional enzymes (*Bst*EII, *Xba*I, *Sac*I) were used for digestion. The results of these hybridizations and digestions enabled us to construct complete physical maps for the five enzymes (Fig. 3). The data indicate that *A. capillus-veneris* cpDNA is a circular molecule with an IR. This IR is estimated to range in size from a maximum of 24.7 kb to a minimum of 23.2 kb.

Hybridizations of tobacco chloroplast gene probes (Table 1) and restriction endonuclease-digested fragments of *A. capillus-veneris* cpDNA clarified the locations of these genes (Fig. 3). Since hybridization and membrane washing at 65°C was too stringent to bring out the hybridized bands, these procedures were performed at 55° C. These gene mapping studies revealed that, within the LSC, the genes are arranged in the same order among *A. capillus-veneris,* tobacco (Shinozaki et al. 1986), and *Osmunda* (Palmer and Stein 1986). Considerable differences among the three species were, however, observed in the IR and at the border region between the LSC and IR. In tobacco and *Osmunda,* 23S rDNA is located closer to the SSC than is 16S rDNA, while the two genes are arranged in the reverse order in *A. capillus-veneris.* PsbA, located in LSC in tobacco and *Osmunda,* was found in the IR in *A. capillus-veneris.* Moreover, psbA of *A. capillus-veneris* is located closer to the SSC than are 23S and 16S rDNAs. These results suggest that the gene order within the IR of *A. capilus-veneris* is significantly different from that of tobacco and *Osmunda.*

Rp12 is a single copy gene located in the LSC of *A. capillus-veneris.* This is the same as in representatives of the gymnosperms, pteridophytes and bryophytes, such as *Ginkgo* (Palmer and Stein 1986), *Osmunda* (Palmer and Stein 1982, 1986), *Marchantia* (Ohyama et al. 1986), and *Physcomitrella* (Calie and Hughes 1987), whereas rpl2 is a duplicated gene located at the terminus of the IR in tobacco (Fig. 4) and many other angiosperms (Palmer 1985a, b).

Table 2. Sizes (in kb) of restriction fragments from *Adiantum capillus-veneris* chloroplast DNA

Fragment number	Fragment size				
	*Eco*RV	*Hin*dIII	*Pst*I	*Stu*I	*Xho*I
1	8.7 × 4	19.5	15.9	32.1	25.0
2	7.7	10.5	12.5	22.0	15.2 × 2
3	7.3	9.4	11.5	15.0 × 3	12.5
4	7.1 × 3	9.0 × 2	11.4 × 2	11.5 × 2	10.5 × 2
5	6.2	7.3 × 2	9.6	9.9	10.0
6	5.7	7.0 × 2	9.4	5.7	8.7
7	5.0	6.2	9.2	3.3 × 2	6.2
8	4.8	5.7	8.3	2.8 × 2	5.2
9	4.5	4.8	7.0	1.2	5.0 × 2
10	4.4	4.3	6.2 × 2		4.5
11	4.2	4.1	5.3		3.7
12	3.7	4.0	3.9		3.6 × 2
13	3.2	3.8	3.0		2.7
14	3.15	3.1	3.0		1.5
15	3.1	2.9	3.0		1.0
16	2.9 × 3	2.8	2.7		0.9
17	2.8	2.4	2.6 × 2		0.7
18	2.5	1.7 × 2	1.8		
19	2.2	1.5 × 2	1.5		
20	2.1 × 3	1.3 × 2	1.2		
21	2.0 × 2	1.2 × 4	0.8 × 2		
22	1.7 × 2	1.0 × 2	0.7		
23	1.4	0.8 × 4	0.6		
24	1.2 × 2	0.7 × 3	0.5		
25	0.5 × 2	0.6	0.2		
26		0.5			
Sum	153.25	151.4	152.8	151.1	151.2

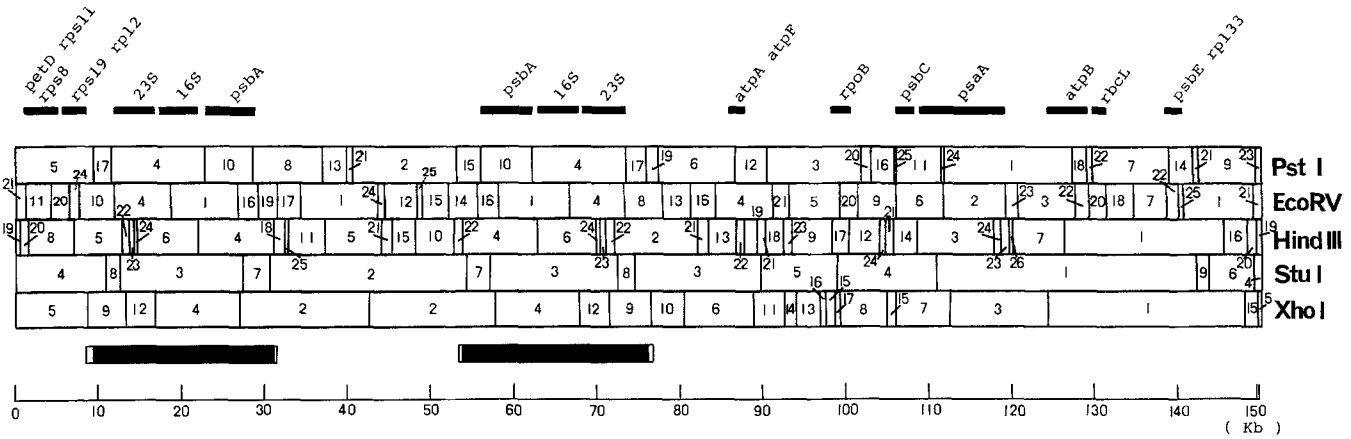

Fig. 3. Restriction maps of *Adiantum capillus-veneris* cpDNA. Circular maps were linealized at the *Pst*I site separating the 0.6 kb (*number 23*) and 9.6 kb (*number 5*) fragments. Restriction fragment designations are as in Table 2. The two *long, solid lines* below the maps represent the minimum extent of the inverted repeat (IR), and the *open extensions of these lines* its maximum extent. The minimum boundaries are defined by *Pst*I (*fragment number 17*) and *Hin*dIII (*4*), while the maximum boundaries are defined by *Eco*RV (*19*) and *Eco*RV (*10*). The *short solid lines* above maps show the positioning of the 12 gene probes listed in Table 1

Comparative organization of Adiantum capillus-veneris chloroplast DNA and typical angiosperm chloroplast DNA

The gene mapping hybridization described above reveals a highly conserved arrangement of genes in the LSC, and a significantly different arrangement of genes in the IR of *A. capillus-veneris,* tobacco and *Osmunda.* However, the strength of this conclusion is limited by the small size of the gene probes used, which do not cover much more than 15% of a typical angiosperm cpDNA genome of 150 kb.

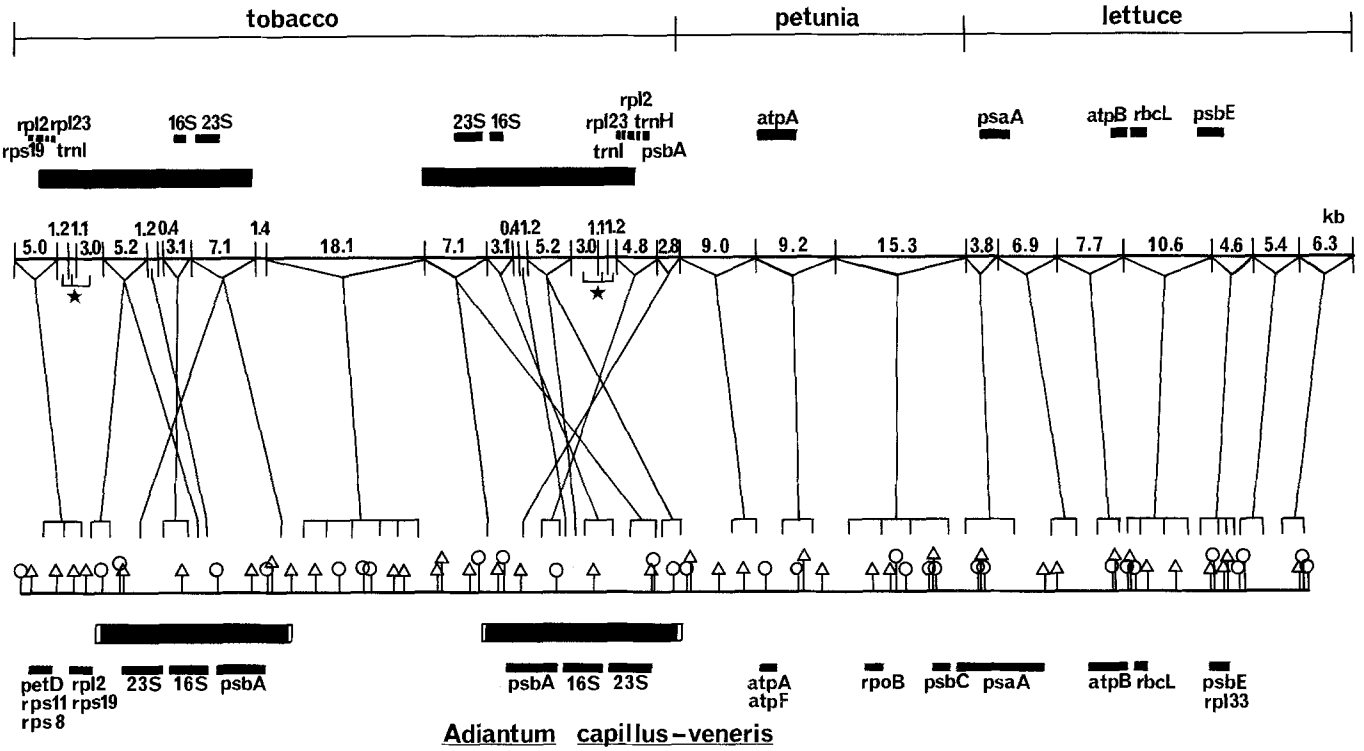

Fig. 4. Arrangement of homologous sequences between *Adiantum capillus-veneris* and angiosperm cpDNAs. The hybridization data are diagrammed in terms of *A. capillus-veneris Pst*I and *Eco*RV, tobacco *Bam*HI, petunia *Pst*I and lettuce *Sac*I clones with physical and gene maps. The angiosperm cpDNA fragments are indicted by the *upper line* and those of *A. capillus-veneris* by the *lower line.* Hybridizatin of angiosperm probes to *A. capillus-veneris* fragments is indicated by *vertical lines between the two horizontal lines.* Restriction sites in *A. capillus-veneris* cpDNA are: *Pst*I (♀), *Eco*RV (♁). *Stars* indicate tobacco fragments that do not hybridize to *A. capillus-veneris* fragments

To provide a more complete coverage of the genome, we examined the arrangement of homologous sequences using cloned fragments covering an entire angiosperm chloroplast genome as heterologous hybridization probes to *A. capillus-veneris Pst*I and *Eco*RV fragments. Cloned cpDNA fragments of tobacco, lettuce and petunia were used as probes. For the LSC, we used lettuce *Sac*I (Jansen and Palmer 1987) and petunia *Pst*I fragments (Palmer et al. 1983; Bovenberg et al. 1984a, b). To obtain better resolution in the IR and SSC regions, we used tobacco *Bam*HI clones (Sugiura et al. 1986) as probes. Hybridization and membrane washing were performed at 55°C as mentioned in regard to the gene mapping experiments. The results are shown in Fig. 4.

Tobacco 3.0, 1.1 and 1.2 kb *Bam*HI fragments, which contain ORF581 and ORF1708, did not hybridize to *A. capillus-veneris* cpDNA (see stars in Fig. 4), to which all other angiosperm probes hybridized.

Within the LSC and the SSC, cross hybridization experiments revealed conserved sequence order in *A. capillus-veneris* cpDNA and the typical angiosperm cpDNA. The 5.0 kb *Bam*HI tobacco fragment, which contained trnI, rp123 and rp12 genes all located in the IR as duplicated genes, hybridized not to the IR but to a single portion of the LSC near the petD gene of *A. capillus-veneris* cpDNA (Fig. 4). This suggests that these genes may be regarded as single genes located in the LSC in *A. capillus-veneris.*

Furthermore, within the IR, the results of cross hybridization (Fig. 4) clearly demonstrate that significant structural differences exist between *A. capillus-veneris* cpDNA and typical angiosperm cpDNA.

Discussion

Inverted repeat length variation within ferns and between angiosperms and ferns

Our study addressed IR length variation in ferns. The IR of *A. capillus-veneris* (about 24 kb) is longer than that of *Osmunda* (about 10 kb; Palmer and Stein 1986), and is almost as long as that of tobacco. This is associated with variation in the position and copy number of genes. The psbA gene and some neighboring genes located on the tobacco 4.8 and 2.8 kb *Bam*HI fragments shown in Fig. 4 (such as trnK, rps16) are located in the portion of the LSC flanked by the IR in *Osmunda* (Palmer and Stein 1986), but within the IR in *A. capillus-veneris.* This is probably one of the reasons why *A. capillus-veneris* has a longer IR than does *Osmunda.* There may be other reasons for the IR length difference (reviewed by Palmer 1985a, b) since the translocated region, corresponding to the *Pst*I 10 fragment of *A. capillus-veneris* cpDNA (shown in Figs. 3 and 4), is approximately 6 kb and too short to explain the total difference of about 14 kb.

Palmer and Stein (1986) reported that the IR length difference in cpDNAs between angiosperms and the fern genus *Osmunda* was associated with the translocation of a border region between the IR and the LSC. Our study revealed that *A. capillus-veneris* does not hybridize to the approximately 7 kb region containing ORF581 and ORF1708 in the tobacco chloroplast genome. We initially hybridized a tobacco cpDNA fragment containing ORF581 and ORF1708 to *O. cinnamomea* cpDNA and some angiosperm cpDNAs (data not shown). The fragment hybridized to angiosperm (maple tree, lettuce and spinach) chloroplast restriction fragments which belong to the IR, but not to *O. cinnamomea.* We speculated that the chloroplast genomes of these angiosperms have homologous regions to ORF581 and ORF1708 in the IRs, but that the cpDNA of *O. cinnamomea* does not. The absence of ORF581 and ORF1708 may be one reason that *Osmunda* has a shorter IR than typical angiosperms. Alternatively, the tobacco probes containing these ORFs may not hybridize to *A. capillus-veneris* and *O. cinnamomea* cpDNA because the ORFs are highly variable, with homology being too low to detect them by Southern hybridization in the stringency of our experiment. Sequence data in this region are necessary to critically rule out the presence of these ORFs.

An IR shorter than that of angiosperms has been reported in a liverwort (10 kb in *Marchantia polymorpha,* Ohyama et al. 1986) and a moss (9.4 kb in *Physcomitrella patens,* Calie and Hughes 1987). *M. polymorpha* does not contain ORF581 and ORF1708 in its IR, but rather these reading frames are fused in LSC as ORF2136 (Zhou et al. 1988). Absence of ORF581 and ORF1708 has recently been reported in rice, *Oryza sativa* (Hiratsuka et al. 1989).

Comparison of gene order between Adiantum capillus-veneris chloroplast DNA and typical angiosperm chloroplast DNA

Cross hybridization revealed differences of gene order in the IR of *A. capillus-veneris* cpDNA and typical angiosperm cpDNA. Although a more detailed gene mapping study is needed, the occurrence of some inversions or translocations of genes in the IR between typical angiosperm cpDNAs and *A. capillus-veneris* cpDNA can be inferred. As is illustrated in Fig. 4, both the 7.1 and 5.2 kb *Bam*HI fragments of tobacco hybridized not to neighboring but to separate fragments of *A. capillus-veneris* in both *Eco*RV and *Pst*I digests. Even though the tobacco 2.8 kb *Bam*HI fragments and the petunia 9.0 kb *Pst*I fragment are in close physical proximity, they do not hybridize to neighboring fragments of *A. capillus-veneris.* This suggests that there are at least two inversions or translocations between typical angiosperm cpDNAs and *A. capillus-veneris* cpDNA, and that their end points are located on the 7.1 and 5.2 kb *Bam*HI fragment in tobacco and the portion between tobacco 2.8 kb *Bam*HI and petunia 9.0 kb *Pst*I fragments. More detailed work on gene mapping is in progress.

Acknowledgements. We would like to thank Drs. K. Kutsukake, T. Ishibashi and H. Hirano for their invaluable help and advice regarding laboratory techniques, and M. Sugiura and J. D. Palmer for their generosity in providing the clones used in this study. We also are indebted to Drs. M. Kato, T. Yahara, J. Murata and N. Murakami for their instructive suggestions during the preparation of this manuscript and Miss Y. Iwashita for her continuous encouragement. We would also like to thank Mr. K. Hirai for his careful cultivation of plant materials. All our experiments using radioisotope were conducted at the Radioisotope Center, University of Tokyo. Thanks are also due Drs. K. Ijiri, H. Nakamoto and everyone on the center staff. This work was partially supported by a grant from the Ministry of Education, Science and Culture 62440004 to K. I.

References

Birky CW Jr (1988) Evolution and variation in plant chloroplast and mitochondrial genomes. In: Gottlieb LD, Jain SK (eds) Evolutionary biology. Chapman and Hall, London New York, pp 23–53

Bovenberg WA, Howe CH, Kool AJ, Kijkamp HJJ (1984a) Curr Genet 8:283–290

Bovenberg WA, Koes RE, Kool AJ, Nijkamp HJJ (1984b) Curr Genet 8:231–241

Calie PJ, Hughes KW (1987) Mol Gen Genet 208:335–341

Chu NM, Tewari KK (1982) Mol Gen Genet 186:23–32

Feinberg AP, Vogelstein B (1983) Anal Biochem 132:6–13

Feinberg AP, Vogelstein B (1984) Addendum Anal Biochem 137:266–267

Hirai A, Ishibashi T, Morikami A, Iwatsuki N, Shinozaki K, Sugiura M (1985) Theor Appl Genet 70:117–122

Hiratsuka J, Shimada H, Whittier R, Ishibashi T, Sakamoto M, Mori M, Kondo C, Honji Y, Sun CR, Meng BY, Li YQ, Kanno A, Nishizawa Y, Hirai A, Shinozaki K, Sugiura M (1989) Mol Gen Genet 217:185–194

Jansen RK, Palmer JD (1987) Curr Genet 11:553–564

Koller B, Delius H (1980) Mol Gen Genet 178:261–269

Kolodner R, Tewari KK (1979) Proc Natl Acad Sci USA 76:41–45

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

Ohyama K, Fukuzawa H, Kohch T, Shirai H, Sano T, Sano S, Umesono K, Shiki Y, Takeuchi M, Chang Z, Aota SI, Inokuchi H, Ozeki H (1986) Nature 322:572–574

Palmer JD (1985a) Annu Rev Genet 19:325–354

Palmer JD (1985b) Evolution of chloroplast and mitochondrial DNA in plants and algae. In: MacIntyre RJ (eds) Molecular evolutionary biology. Plenum, New York, pp 131–240

Palmer JD (1986) Methods Enzymol 118:167–186

Palmer JD, Stein DB (1982) Curr Genet 5:165–170

Palmer JD, Stein DB (1986) Curr Genet 10:823–833

Palmer JD, Thompson WF (1981) Proc Natl Acad Sci USA 78:5533–5537

Palmer JD, Shields CR, Cohen DB, Orton TJ (1983) Theor Appl Genet 65:181–189

Palmer JD, Nugent JM, Herbon LA (1987) Proc Natl Acad Sci USA 84:769–773

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986) EMBO J 5:2043–2049

Smith GE, Summers MD (1980) Anal Biochem 109:123–129

Sugiura M, Shinozaki K, Zaita N, Kusuda M, Kumano M (1986) Plant Science 44:211–216

Yanisch-Perron C, Vieira J, Messing J (1985) Gene 33:103–119

Zhou DX, Massenet O, Quingley F, Marison MJ, Moneger F, Huber P, Mache R (1988) Curr Genet 13:433–439

Communicated by B. B. Sears