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Homologous recombination mediated by two palindromic repeated sequences in the mitochondrial genome of *Oryza*

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Abstract Palindromic repeated sequences (PRSs) are distributed in at least ten regions of the mitochondrial (mt) genome of rice and are, apparently, mobile. In the present study, we examined the possibility of homologous recombination via some PRSs during the course of evolution of *Oryza*. We first performed Southern hybridization of the DNA from 11 species (18 strains) of *Oryza* in order to identify the distribution of PRSs in the mitochondrial genome of *Oryza*. The hybridization patterns revealed genome type-specific and/or species-specific variations. We speculated that homologous recombination via some PRSs might have made a contribution to such variations. After subsequent polymerase chain reaction, Southern hybridization and sequencing, we concluded that homologous recombination mediated by two PRSs occurred in the mtDNA of *Oryza* after divergence of the BB genome type and the other genome types of *Oryza*. Evidence was obtained that some PRSs were involved in both insertion and recombination events during the evolution of *Oryza*. Our results indicate, therefore, that PRSs have contributed considerably to the polymorphism of *Oryza* mtDNAs.

Key words *Oryza* · Mitochondrial DNA · Palindromic repeated sequences · Polymorphism · Homologous recombination

Introduction

The mitochondrial (mt.) genomes of higher plants are much larger and more complicated than those of other eukaryotic organisms. Their complexity is mainly due to recombination via large repeated sequences and to the transfer of DNA fragments from the chloroplast or the nucleus (reviewed by Lonsdale et al. 1988; Schuster and Brennicke 1988). Inter- and/or intramolecular recombination via recombination repeats seems to create multipartite structures and to occur reversibly (Lonsdale et al. 1984). In addition to such active recombination repeats, mtDNAs of higher plants have a number of small repeated sequences (reviewed by André et al. 1992). These are not involved in the frequent recombination events mentioned above. However, several analyses of recombination events have indicated that the small repeated sequences may be related to rearrangements of mtDNA that have been postulated to have occurred during evolution or that are seen in tissue culture (Brears et al. 1989; Small et al. 1989; Shirzadegan et al. 1991). Many chimeric genes, produced by such infrequent recombination via the small repeated sequences, are found in the mtDNA of several plants (Dewey et al. 1986; Young and Hanson 1987; Kadowaki et al. 1990.) Some of the chimeric genes are associated with the male-sterile phenotype (Dewey et al. 1986; Young and Hanson 1987). Therefore, the small repeated sequences appear to have contributed to the variation in mitochondrial genomes and to the phenotypes of plants.

We reported previously that small repeated sequences of 60–66 basepairs (bp) are present in the mitochondrial genome of rice (*Oryza sativa*; Nakazono et al. 1994). These sequences are highly conserved with respect to one another and are widely distributed in at least ten regions of rice mtDNA. We designated these sequences PRSs (*P*alindromic *R*epeated *S*equences) because they are potentially capable of forming a stem-and-loop structure. We noted that some of the PRSs had been inserted into the intron of the gene for ribosomal protein S3 (*rps3*) and into the flanking sequence of the gene for chloroplast-like $tRNA^{Asn}$ (*trnN*) of the *Oryza* mtDNA during the course of evolution. In this study, we found that at least one PRS might have been used as a site of homologous recombination during the evolution

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of the genus *Oryza*. This report provides the first evidence, to our knowledge, that small, mobile repeated sequences have been involved in recombination events in plant mtDNA.

Materials and methods

Plant materials and isolation of total DNA

Eleven species (18 strains) of *Oryza* were used in this study (Table 1). They consisted of 4 (5 strains), 1 (2 strains), 2 (3 strains), 2 (4 strains), 2 (2 strains) and 1 (2 strains) species of the AA, BB, BBCC, CC, CCDD and EE genome types, respectively. Plants were grown in a greenhouse, and total DNA was isolated from each by the method described by Honda and Hirai (1990).

Southern hybridization analysis

Total DNAs were digested with *Eco*RI (Takara Shuzo, Kyoto, Japan) at 37 °C for 2 h, fractionated by electrophoresis on a 0.7% agarose gel and transferred to a nylon membranes (Micron Separations, Westborough, Mass.). Polymerase chain reaction-(PCR) generated fragments of DNA were labeled at their 5′-ends with [^{32}P] by the procedure of Maniatis et al. (1982). Prehybridization and hybridization were carried out in 5 × Denhardt's reagent (0.1% Ficol 400, 0.1% polyvinylpyrrolidone and 0.1% BSA), 5 × SSPE (50 m*M* NaH_2PO_4, pH 7.4, 0.75 *M* NaCl and 5 m*M* EDTA), 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA at 54 °C. Filters were washed twice for 5 min in 6 × SSC (0.9 *M* NaCl and 90 m*M* sodium citrate) and 0.1% SDS at 54 °C. The hybridization signals were detected with a Bio-image analyzer (BAS-2000; Fuji Photo Film, Tokyo, Japan).

Cloning of DNA fragments that contained PRS-min and PRS-off

Total DNA from W1319 (BBCC) and W1252 (CC), digested by *Eco*RI and *Sph*I, respectively, was dephosphorylated with CIAP (alkaline phosphatase from calf intestine; Boehringer Mannheim, Germany). The dephosphorylated DNA fragments were ligated with pUC19 vectors that had been digested by *Eco*RI/*Sal*I or *Sph*I/*Hin*dIII using a DNA ligation kit (Takara Shuzo). The ligated DNA fragments were used for PCR as templates. Oligonucleotides **a**, **d** and **M13** (250 ng each; see below) were used as primers. PCR was performed using oligonucleotides **a** and **M13** and the ligated DNA fragments from W1319 and using oligonucleotides **d** and **M13** and the ligated DNA fragments from W1252, respectively, with a DNA Amplification System (Perkin Elmer Cetus, USA). *Taq* DNA polymerase (2.5 U; Pharmacia, Sweden) and Bind-Aid™ Amplification Enhancer (United States Biochemical, Cleveland, Ohio) were added after the annealing step (94 °C, 5 min and then 45 °C, 5 min). Amplification was then performed for 35 cycles (94 °C, 1 min; 45 °C, 2 min; 72 °C, 2 min). PCR-generated fragments were cloned in pUC19.

Table 1 The species and strains of *Oryza* as sources of total DNA

Species	Strain	Genome type
O. sativa	Nipponbare	AA
O. rufipogon (annual type)	W0107	AA
O. rufipogon (perennial type)	W0120	AA
O. meridionalis	W1298	AA
O. glaberrima	W0025	AA
O. punctata	W1514	BB
	W1515	BB
O. punctata	W1564	BBCC
O. minuta	W1319	BBCC
	Y0022	BBCC
O. officinalis	W0002	CC
	W0012	CC
	W1252	CC
O. eichingeri	W1521	CC
O. latifolia	W0542	CCDD
O. alta	W0017	CCDD
O. australiensis	W0008	EE
	W1538	EE

Polymerase chain reaction, DNA sequencing and synthesis of oligonucleotides

PCR and DNA sequencing were performed as described previously (Nakazono et al. 1994). DNA sequencing data were analyzed with GENETYX software (SDC, Tokyo, Japan).

Oligonucleotides were synthesized with a DNA synthesizer (Applied Biosystems, Foster City, USA). The following oligonucleotides were used as primers for amplification by PCR: **a**, 5′-gcggaTCCTAATTAAGGGCTTTCCAAT-3′; **b**, 5′-ccggatccTAGTAGTGAACGGTTCTAA-3′; **c**, 5′-CCTTCCCACAACATGAGACT-3′; **d** 5′-GGGAGCAAATACGATCAACTG-3′; **e**, 5′-CTAGCGCGAAACGTTAGCAC-3′; **f**, 5′-TGCTAACGTTTCGCGCTAGT-3′; and **M13**, 5′-GTTTTCCCAGTCACGAC-3′.

Results

Distribution of the palindromic repeated sequences (PRSs) in 11 species of *Oryza*

At least ten copies of PRSs are dispersed throughtout rice mitochondrial DNA (Nakazono et al. 1994). In maize and wheat mtDNAs, there are also many sequences homologous to the PRS (Gualberto et al. 1988; Nakazono et al. unpublished data). Moreover, there have been many reports of some small repeated sequences being involved in rearrangements of plant mtDNAs (reviewed by André et al. 1992). It has been proposed that homologous recombination mediated by some of PRSs might have occurred during the course of evolution of the genus *Oryza*. To examine this possibility, we first examined whether PRSs are widely distributed in the mitochondrial genomes of other species in the genus *Oryza*. Total DNA was prepared from each of 11 species (18 strains) of *Oryza* (Table 1). The DNA from each strain was digested with *Eco*RI and subjected to electrophoresis on a 0.7% agarose gel. Southern hybridization was performed using a 5′-end-labeled 61-bp fragment of DNA that contained only a PRS. As shown in Fig. 1, many hybridization signals were detected. These signals were assumed to be due to mtDNA since the patterns of hybridization obtained with rice total DNA were exactly the same as those obtained with rice mtDNA (data not shown).

The hybridization patterns showed the genome type- and/or species-specific variations. We suspected that homologous recombination, mediated by some of the PRSs, might have contributed to these variations. From 10 to 13 *Eco*RI fragments were homologous to PRS from each strain (Fig. 1). This result indicates that the number

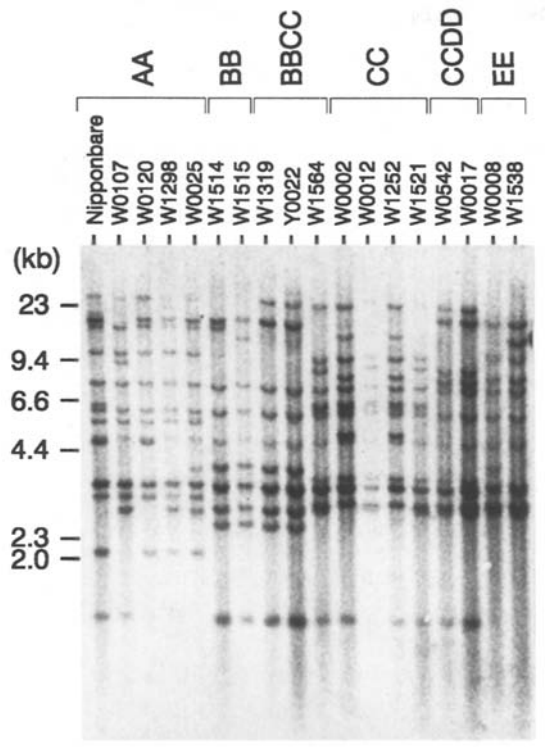


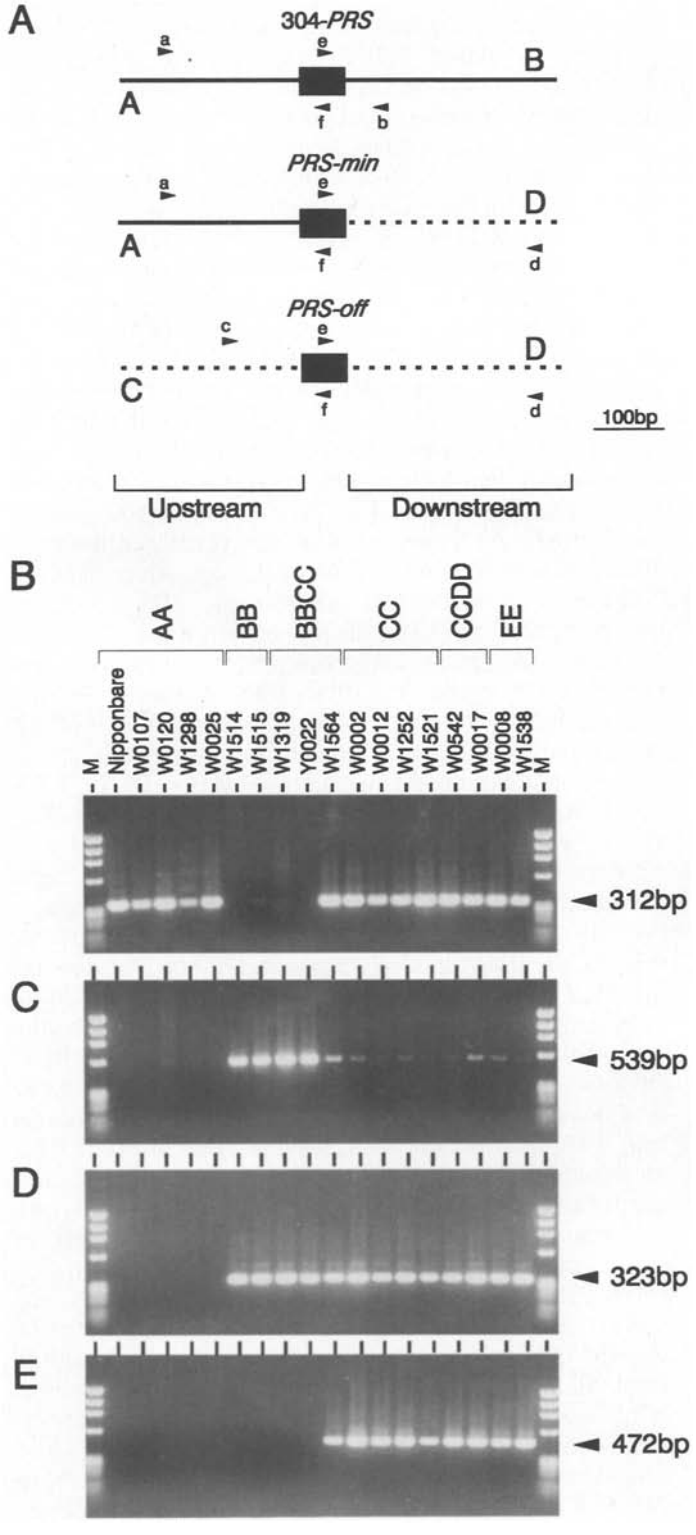
Fig. 1 Southern hybridization analysis of total DNAs from various strains of *Oryza* digested with *Eco*RI. Hybridization was performed with a 5′-end-labeled 61-bp DNA fragment that contained only a PRS. *AA*, *BB*, *BBCC*, *CC*, *CCDD* and *EE* indicate each nuclear genome type of *Oryza*. The *numbers* given on the *left* indicate sizes of fragments in kilobasepairs (kb).

of copies of PRSs was roughly the same in each of the species of *Oryza* examined. There were also uniquely sized hybridization signals for individual species. In the case of 3 strains of the genome type BBCC, namely, *O. minuta* (W1319 and Y0022) and *O. punctata* (W1564), the hybridization patterns were similar to those of the BB and CC genome types, respectively (see Discussion). This result was consistent with the results from comparative studies of the chloroplast DNA (ctDNA; Dally and Second 1990; Kanno and Hirai 1992) of *Oryza*.

Homologous recombination mediated by two PRSs

To examine the possibility of homologous recombination via PRSs, we amplified DNA fragments that contained a PRS by the PCR using total DNA from the 11 species (18 strains) of *Oryza* listed in Table 1 as templates. Two primers corresponded to the unique regions in the upstream and the downstream sequences of the individual PRSs. If homologous recombination had occurred between two PRSs, each original flanking sequences ought to be located in the separated regions. Therefore, we expected that no amplified fragments would be obtained from regions that contained PRSs that had been involved in homologous recombination. The anticipated fragments of six of the ten regions of PRSs were amplified in all of the species of *Oryza* examined, while no fragments of the other regions were amplified from DNA from several species (data not shown). We postulated that the sequences of the various regions that were not amplified, with the exception of the region designated 304-PRS (Nakazono et al. 1994), had been lost. More complicated recombination may have been occurred in the mtDNAs of these species. Therefore, we carried out further analysis of 304-PRS. Some fragments (312 bp) of the region of 304-PRS were not amplified using primers **a** and **b** of Fig. 2A from 4 strains of the BB (*O. punctata*, W1514 and W1515) and BBCC (*O. minuta*, W1319 and Y0022) genome types (Fig. 2B). We synthesized oligonucleotides **e** and **f**, which correspond to the internal sequences of PRS (Fig. 2A). Amplification by PCR was performed using primers **a** and **f** and primers **b** and **e**. Total DNAs from W1514, W1515 and W1319 were used as templates. Amplified DNA fragments were obtained using only primers **a** and **f** from each of 3 strains (data not shown). This result indicates that the downstream sequence (**D**) of the PRS in W1514, W1515 and W1319 mtDNAs differs from the downstream sequence (**B**) of rice 304-PRS (Fig. 2A). Furthermore, we cloned the DNA fragment that contained this PRS from W1319 (*O. minuta*) by the method described in the Materials and methods and determined its nucleotide sequence (Fig. 3). We designated this PRS "PRS-min". As expected, the upstream sequence of PRS-min was the same as the upstream sequence (**A**) of 304-PRS, but the downstream sequence (**D**) was different from the downstream sequence (**B**) of 304-PRS (Fig. 2A). This result indicates that a homologous recombination event via this PRS occurred in the mitochondrial genomes of *O. punctata* (BB) and *O. minuta* (BBCC). We synthesized oligonucleotide **d** that corresponded to the downstream sequence (**D**) (Figs. 2A and 3). Amplification by PCR from the total DNAs of 18 strains was carried out using primers **a** and **d** and primers **d** and **e**. Amplified fragments (539 bp) were obtained using primers **a** and **d** from only the 4 strains (W1514, W1515, W1319 and Y0022) mentioned above (Fig. 2C), while amplified fragments (323 bp) were found for all of the strains except for those of the AA genome type using primers **d** and **e** (Fig. 2D). Several weak bands were obtained using primers **a** and **d** from the BBCC (W1564), CC, CCDD and EE genome types (Fig. 2C). It was possible that such weak bands might be artifacts that was annealed between 304-PRS and another PRS (designated "PRS-off" below) and then amplified in the test tubes during PCR. To eliminate this possibility, we performed PCR using primers **a** and **d** and the mixture of two plasmid DNAs that contained 304-PRS or another PRS (PRS-off) as template. The DNA fragment was not amplified under these PCR conditions (data not shown). Therefore, we assumed that the weak bands originated from molecules present at substoichiometric levels and obtained as a result of rare recombination events (Small et al. 1987). Thus, it appeared that the downstream sequences of this PRS in *O. punctata* (W1564) with the BBCC genome type and in the species

Fig. 2A Organization of 304-PRS, PRS-min and PRS-off. The *closed boses* indicate the locations of PRSs. *Arrowheads* indicate the positions of primers *a–f* that were used for PCR. *A–D* indicate each of the flanking sequences. **B–E** Staining with ethidium bromide after electrophoresis of DNA fragments obtained by amplification by PCR from total DNAs of various strains of *Oryza* using oligonucleotides *a* and *b* (**B**), *a* and *d* (**C**), *d* and *e* (**D**) and *c* and *d* (**E**), respectively, as primers. *M* size markers (a *Hae*III digest of ϕX174 RF-DNA)



with CC, CCDD and EE genome types were the same as the downstream sequence (**D**) of PRS-min, while the upstream sequence (**C**) was different from the upstream sequence (**A**) of PRS-min. To confirm this possibility, we cloned the fragment that contained this region from the total DNA of W1252 (*O. officinalis*; CC genome type) (as described in Materials and methods) and determined its nucleotide sequence (Fig. 3). This PRS was designated "PRS-off". We confirmed the possibility described above from the nucleotide sequencing data. When we compared the nucleotide sequences that contained 304-PRS with PRS-off, only the sequences of the PRSs exhibited homology to one another (Fig. 3). Furthermore, we synthesized oligonucleotide **c**, which corresponded to the upstream sequence (**C**), which exhibited no homology to the upstream sequence (**A**) of PRS-min or 304-PRS, of PRS-off (Fig. 2A,3). A PCR experiment was performed with primers **c** and **d** and total DNAs from 18 strains as templates. Fragments (472 bp) were amplified from W1564 of the BBCC genome types and from all of the strains of the CC, CCDD and EE genome types (Fig. 2E). These results indicated that both 304-PRS and PRS-off were present in the mitochondrial genomes of *O. punctata* (W1564) with the BBCC genome type and in the CC, CCDD and EE genome types, but that only 304-PRS was present in the AA genome type. The sequence including PRS-min of the BB genome type and *O. minuta* (W1319 and Y0022) of the BBCC genome type appears to have been generated as a result of homologous recombination mediated by two PRSs (304-PRS and PRS-off). In order to confirm the evidence of homologous recombination via the two PRSs, 304-PRS and PRS-off, we performed Southern hybridization analysis. Total DNAs digested by *Eco*RI were hybridized with 5′-end-labeled DNA fragments that corresponded to the individual flanking sequences of 304-PRS and PRS-off (probes **1–4**; as shown in Fig. 4A). Using probes **1** and **4**, as expected, we detected the same hybridization signals for the BB genome type and for *O. minuta* (W1319 and Y0022) with the BBCC genome type (Fig. 4B, C). In *O. punctata* (W1564) with the BBCC genome type and the AA, CC, CCDD and EE genome types, the same hybridization signals were obtained using probes **1** and **2** and probes **3** and **4**, respectively (data not shown). No hybridization signal was obtained using probes **3** and **4** from any strains of the AA genome type, indicating that mtDNA of the AA genome type does not contain the sequence that includes PRS-off and its flanking sequences [the upstream sequence (**C**) and the downstream sequence (**D**); Fig. 4C].

Discussion

Mitochondrial DNA polymorphism among the species of *Oryza*

To determine the copy numbers and to identify the distribution of PRSs in the mtDNA of *Oryza*, Southern

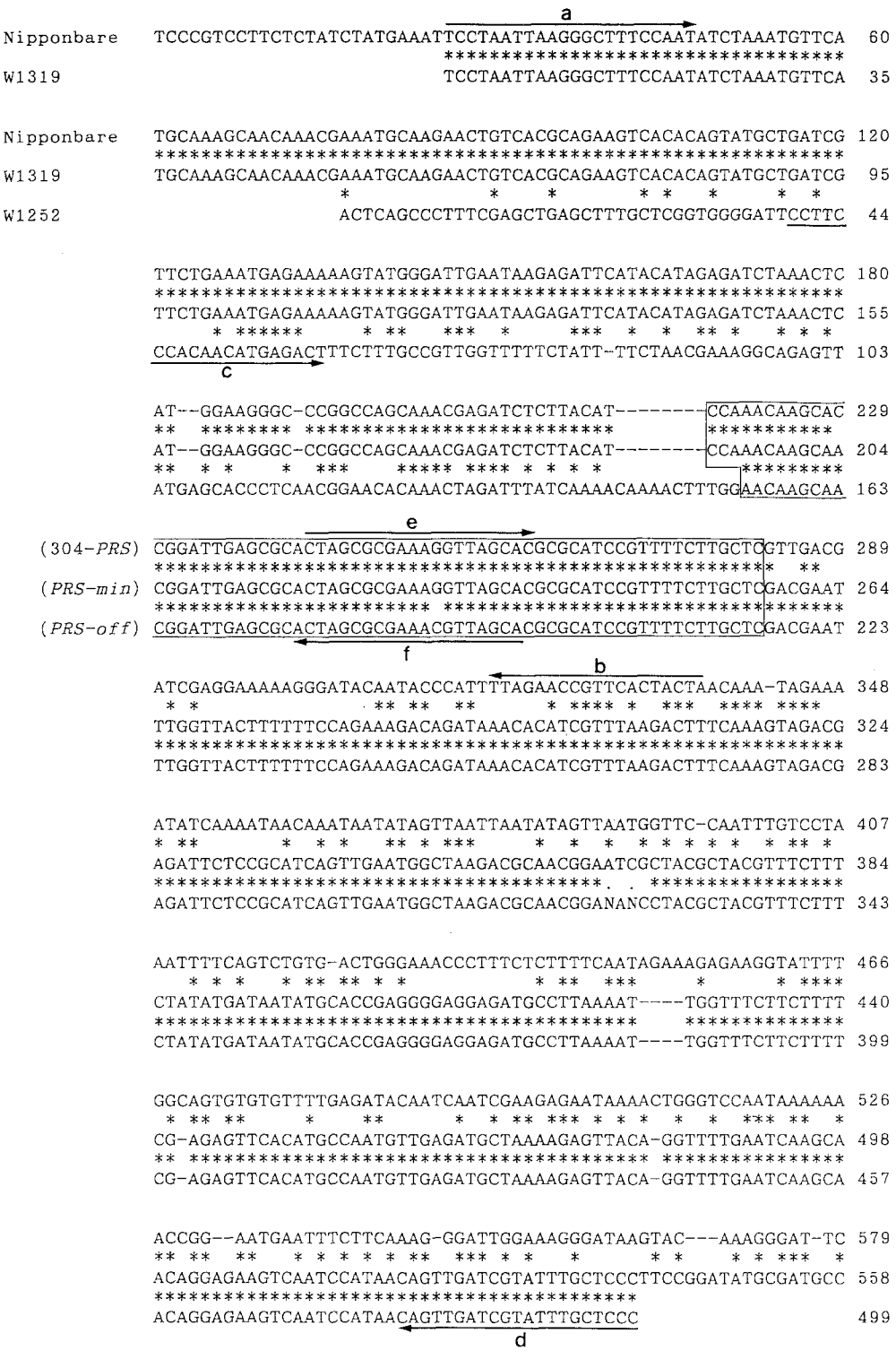


Fig. 3 Alignment of nucleotide sequences of fragments cloned from the DNAs of *O. sativa* ('Nipponbare'), *O. minuta* (W1319) and *O. officinalis* (W1252). An *asterisk* indicates that a nucleotide is identical to that in the sequence from W1319. A *dash* indicates a nucleotide that is absent from a sequence. The nucleotide sequence of PRSs is *boxed*. *Arrows* indicate the primers (*a–f*) used for amplification by PCR. The sequences reported in this figure have been deposited in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the following accession numbers D13097 ('Nipponbare'), D29790 (W1319) and D29789 (W1252)

hybridization of total DNAs from 11 species (18 strains; see Table 1) was performed using a 61-bp DNA fragment that contained only a PRS as probe. As shown in Fig. 1, the number of hybridization signals was roughly the same among all 18 strains of *Oryza*. The hybridization patterns represented genome type- and/or species-specific variations. Among 3 strains with the BBCC genome type, the hybridization patterns of *O. minuta* (W1319 and Y0022) and *O. punctata* (W1564) were similar to those of strains with the BB and CC genome types, respectively. In comparative studies of the structure of the ctDNA of *Oryza*, *O. minuta* (BBCC) and *O. punctata* (BBCC) also gave profiles specific to BB and CC genome types, respectively (Dally and Second 1990; Kanno and Hirai 1992). It is believed that the BBCC genome type originated as a result of crossing between

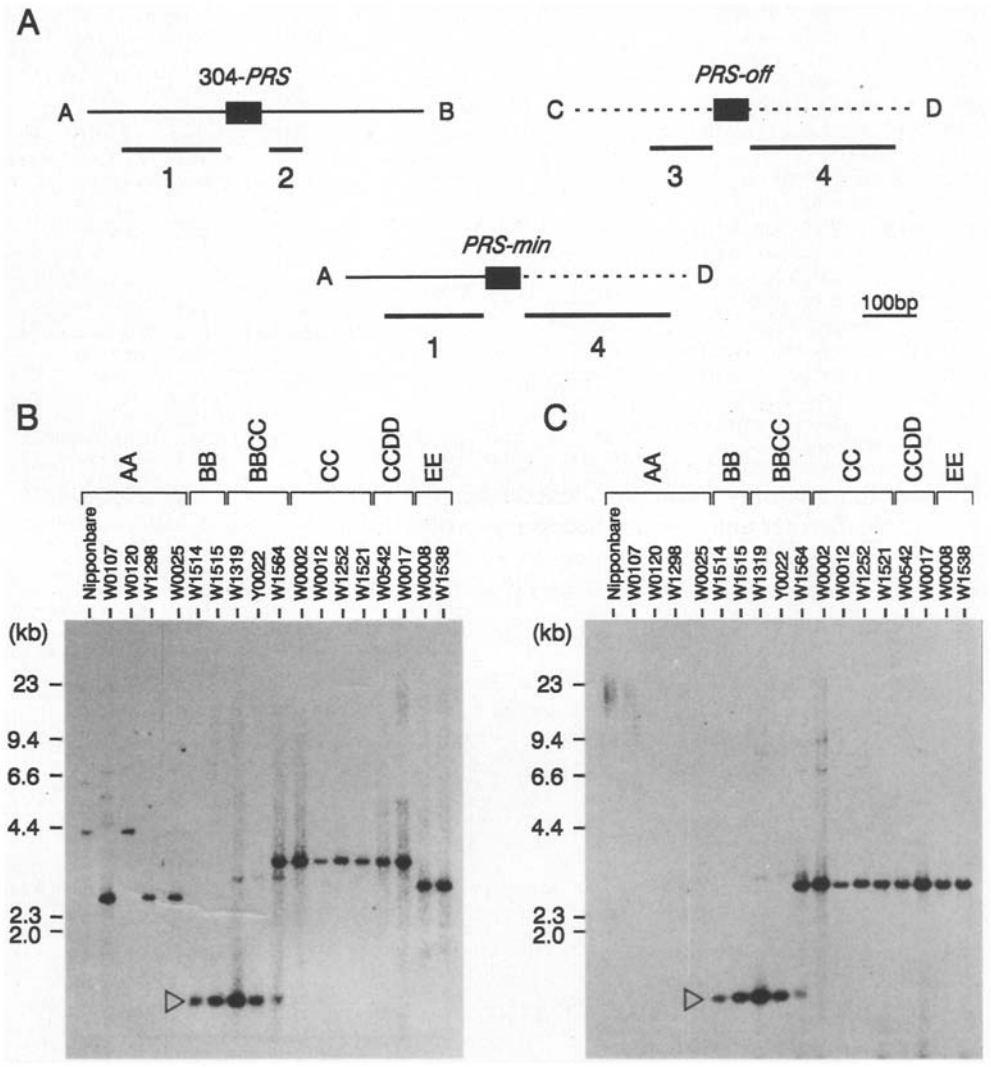


Fig. 4A Positions of probes (*1–4*) for Southern hybridization of total DNAs digested with *Eco*RI. **B–C** Autoradiograms after Southern hybridization with probe *1* (**B**) and probe *4* (**C**). *Open triangles* indicate hybridization signals from W1514, W1515, W1319 and Y0022 obtained with probes *1* and *4*. The *numbers* given on the *left* indicate sizes of fragments in kb

the BB and CC genome types. Our results support the diphyletic origin of the BBCC genome type. The cytoplasm of *O. minuta* (BBCC) and *O. punctata* (BBCC) may have originated from those of BB and CC genome types, respectively.

Phylogenetic relationships between closely related species or genera have been examined by means of comparative studies of the structure of ctDNA in many groups of plants (Ogihara and Tsunewaki 1982; Kung et al. 1982; Palmer et al. 1983). In the genus *Oryza*, the relationships between a wider range of species has been analyzed by Ichikawa et al. (1986) and Dally and Second (1990). There are also several published analyses of the genetic differentiation of mtDNA among cultivated rices (Kadowaki et al. 1988; Kanazawa et al. 1992; Ishii et al. 1993). There is, to our knowledge, little comparative information about mtDNAs of other species of *Oryza*. In general, highly interspersed repetitive sequences are suitable for studies of genetic differentiation and phylogenetic relationship between related taxa. Because PRSs are distributed in at least ten regions of rice mtDNA, a PRS may be a good tool for the study of genetic differentiation of mtDNA among species of *Oryza*.

Homologous recombination via two PRSs is specific to the BB genome type

Experiments by PCR and Southern hybridization analysis indicated that the mitochondrial genome of *O. punctata* (W1564) with the BBCC genome type and those of the CC, CCDD and EE genome types contain both 304-PRS and PRS-off, while the AA genome type contains only 304-PRS. For the BB genome type and *O. minuta* (W1319 and Y0022) with the BBCC genome type, PRS-min instead of 304-PRS and PRS-off was found in the mitochondrial genome. These results are consistent with the hypothesis that the cytoplasms of *O. minuta* and *O. punctata* (BBCC) originated from those of the BB and CC genome types, respectively. If it is assumed that mutational events

occurred at a minimal number of times and that recombination via two PRSs is irreversible, the most likely hypothesis is that the common ancestor of *Oryza* contained both 304-PRS and PRS-off and that PRS-min was then generated as a result of homologous recombination mediated by two PRSs (304-PRS and PRS-off) in the mtDNA of the BB genome type after divergence of the BB genome type and the other genome types. Independently, the sequence containing PRS-off in the mtDNA of the AA genome type was lost by another deletion event after divergence of the AA genome type from the other genome types. At present, it is unknown which events occurred earlier. Our hypothesis is consistent with the results of a phylogenetic study of another cytoplasmic genome, the chloroplast genome, of *Oryza*, reported by Dally and Second (1990). Because there is only limited evidence to support this hypothesis, further analyses are necessary to confirm it. However, it is clear that homologous recombination mediated by two PRSs occurred in the mitochondrial genome during the course of the evolution of *Oryza*.

Homologous recombination via small repeated sequences in mtDNA occurs in animal (Schon et al. 1989; Zeviani et al. 1989), fungal (Almasan and Mishra 1991; Weiller et al. 1991) and plant cells (reviewed by André et al. 1992). It has been reported that 7-bp repeats are involved in homologous recombination in rice mtDNA (Kadowaki et al. 1990). In yeast mtDNA, it has been suggested that some GC-rich repeated sequences, namely GC-clusters, which are presumed to be mobile genetic elements, were involved in macro-deletion or recombination (Weiller et al. 1991). In the case of plant mtDNA, the present report is, to our knowledge, the first example of small, mobile repeated sequences being identified as sites of homologous recombination. PRSs appear to have caused both insertion and recombination events in the mtDNA of *Oryza* during evolution. It is clear that they have contributed both to the complexity of and to the variations in the mitochondrial genome of *Oryza*.

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References

Almasan A, Mishra NC (1991) Recombination by sequence repeats with formation of suppressive or residual mitochondrial DNA in *Neurospora*. Proc Natl Acad Sci USA 88:7684–7688

André C, Levy A, Walbot V (1992) Small repeated sequences and the structure of plant mitochondrial genomes. Trends Genet 8:128–132

Brears T, Curtis GJ, Lonsdale DM (1989) A specific rearrangement of mitochondrial DNA induced by tissue culture. Theor Appl Genet 77:620–624

Dally AM, Second G (1990) Chloroplast DNA diversity in wild and cultivated species of rice (Genus *Oryza*, section Oryza). Cladistic-mutation and genetic-distance analysis. Theor Appl Genet 80:209–222

Dewey RE, Levings CS III, Timothy DH (1986) Novel recombinations in the maize mitochondrial genome produce a unique transcriptional unit in the Texas male-sterile cytoplasm. Cell 44:439–449

Gualberto JM, Wintz H, Weil J-H, Grienenberger J-M (1988) The genes coding for subunit 3 of NADH dehydrogenase and for ribosomal protein S12 are present in the wheat and maize mitochondrial genomes and are co-transcribed. Mol Gen Genet 215:118–127

Honda H, Hirai A (1990) A simple and efficient method for identification of hybrids using nonradioactive rDNA as probe. Jpn J Breed 40:339–348

Ichikawa H, Hirai A, Katayama T (1986) Genetic analyses of *Oryza* species by molecular markers for chloroplast genomes. Theor Appl Genet 72:353–358

Ishii T, Terachi T, Mori N, Tsunewaki K (1993) Comparative study on the chloroplast, mitochondrial and nuclear genome differentiation in two cultivated rice species, *Oryza sativa* and *O. glaberrima*, by RFLP analyses. Theor Appl Genet 86: 88–96

Kadowaki K, Yazaki K, Osumi T, Harada K, Katsuta M, Nakagahra M (1988) Distribution of mitochondrial plasmid-like DNA in cultivated rice (*Oryza sativa* L.) and its relationship with varietal groups. Theor Appl Genet 76:809–814

Kadowaki K, Suzuki T, Kazama S (1990) A chimeric gene containing the 5′ portion of *atp6* is associated with cytoplasmic male-sterility of rice. Mol Gen Genet 224:10–16

Kanazawa A, Sakamoto W, Nakagahra M, Kadowaki K, Tsutsumi N, Tano S (1992) Distribution and quantitative variation of mitochondrial plasmid-like DNAs in cultivated rice (*Oryza sativa* L.) Jpn J Genet 67:309–319

Kanno A, Hirai A (1992) Comparative studies of the structure of chloroplast DNA from four species of *Oryza*: cloning and physical maps. Theor Appl Genet 83:791–798

Kung SD, Zhu SY, Shen GF (1982) *Nicotiana* chloroplast genome. 3. Chloroplast DNA evolution. Theor Appl Genet 61:73–79

Lonsdale DM, Hodge TP, Fauron CM-R (1984) The physical map and organisation of the mitochondrial genome from the fertile cytoplasm of maize. Nucleic Acids Res 12:9249–9261

Lonsdale DM, Brears T, Hodge TP, Melville SE, Rottmann WH (1988) The plant mitochondrial genome: homologous recombination as a mechanism for generating heterogeneity. Philos Trans R Soc London Ser B 319:149–163

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Nakazono M, Kanno A, Tsutsumi N, Hirai A (1994) Palindromic repeated sequences (PRSs) in the mitochondrial genome of rice: evidence for their insertion after divergence of the genus *Oryza* from the other Gramineae. Plant Mol Biol 24: 273–281

Ogihara Y, Tsunewaki K (1982) Molecular basis of the genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. I. Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ct-DNAs. Jpn J Genet 57:371–396

Palmer JD, Shields CR, Cohen EB, Orton TJ (1983) Chloroplast DNA evolution and the origin of amphidiploid *Brassica* species. Theor Appl Genet 65:181–189

Schon EA, Rizzuto R, Moraes CT, Nakase H, Zeviani M, DiMauro S (1989) A direct repeat is a hotspot for large-scale deletion of human mitochondrial DNA. Science 244:346–349

Schuster W, Brennicke A (1988) Interorganellar sequence transfer: plant mitochondrial DNA is nuclear, is plastid, is mitochondrial. Plant Sci 54:1–10

Shirzadegan M, Palmer JD, Christey M, Earle ED (1991) Patterns of mitochondrial DNA instability in *Brassica campestris* cultured cells. Plant Mol Biol 16:21–37

Small ID, Isaac PG, Leaver CJ (1987) Stoichiometric differences in DNA molecules containing the *atpA* gene suggest mechanisms for

the generation of mitochondrial genome diversity in maize. EMBO J 6:865–869

Small I, Suffolk R, Leaver CJ (1989) Evolution of plant mitochodrial genomes via substoichiometric intermediates. Cell 58:69–76

Weiller GF, Bruckner H, Kim SH, Pratje E, Schweyen RJ (1991) A GC cluster repeat is a hotspot for mit$^-$ macro-deletions in yeast mitochondril DNA. Mol Gen Genet 226:233–240

Young EG, Hanson MR (1987) A fused mitochondrial gene associated with cytoplasmic male sterility is developmentally regulated. Cell 50:41–49

Zeviani M, Servidei S, Gellera C, Bertini E, DiMauro S, DiDonato S (1989) An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. Nature 339:309–311