Mitochondrial plasmid-like DNAs of the B1 family in the genus *Oryza*: sequence heterogeneity and evolution

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(Received 28 August 1995)

ABSTRACT

Four kinds of circular plasmid-like DNA, designated B1, B2, B3 and B4, have been found in the mitochondria of *Oryza sativa* L. with an AA genome. Three novel B1-homologous mitochondrial plasmid-like DNAs, designated, M1, M2 and M3, were isolated in the present study from strains with CC and CCDD genomes in the genus *Oryza*. We cloned and sequenced these DNAs and found that the sequences of these molecules have wide regions of homology. B1, M2 and M3 each lack about 300 bp of a region that is present in M1 and small repeats were found at the sites of deleted sequences. Therefore, we propose the hypothesis that the B1 family differentiated from a common ancient molecule that was similar to M1 via, probably, slipped mispairing during DNA replication at several stages in the evolution in the genus *Oryza*.

1. INTRODUCTION

The mitochondria of some higher plants contain one or more kinds of small circular or linear DNA molecule in addition to the main genome (for review, see Pring and Lonsdale, 1985). These small DNAs are referred to as plasmid-like DNAs.

Four kinds of circular plasmid-like DNA were originally detected in the mitochondria of cultivated rice (*Oryza sativa* L.) and they were named B1, B2, B3 and B4. They have been cloned and their nucleotide sequences have been determined (Yamaguchi and Kakiuchi, 1983; Shikanai et al., 1987, 1989; Shikanai and Yamada, 1988). B1, B2, B3 and B4 are 2,135, 1,546, 1,514 and 969 bp in length, respectively. Most of the known circular plasmid-like DNAs exhibit no obvious homology to the main mitochondrial genome (Chase and Pring, 1985, 1986; Kemble and Bedbrook, 1980). In *O. sativa*, the four kinds of plasmid-like DNA also exhibit no obvious homology to the main mitochondrial genome. Therefore,

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it appears that the circular plasmid-like DNAs must be transmitted to progeny independently of the main mitochondrial genome.

Several kinds of homologous plasmid-like DNA have been detected in the mitochondria of sugarbeet (Thomas, 1986) and *Vicia fava* (Wahleithner and Wolstenholme, 1987; Flamand et al., 1993) and the mechanisms of their generation have been explained simply in terms of recombination or slipped mispairing during DNA replication. In *O. sativa*, no extensive homology exists among the four kinds of plasmid-like DNA and, therefore, the origin or the mechanism of generation of B1, B2, B3 and B4 remains elusive.

The four kinds of plasmid-like DNA were found in many strains of *O. sativa* and wild species with the AA genome in the genus *Oryza*, and their distribution was investigated (Kanazawa et al., 1992; Miyata et al., 1995). However, in strains in the genus *Oryza* with BB, BBCC, CC, CCDD and EE genomes, no plasmid-like DNA identical to B1, B2, B3 or B4 has been detected with the exception of B1- and B2-homologous molecules in the strains with CC and CCDD genomes. We designated these B1- and B2-homologous DNA as B1 and B2 family of plasmid-like DNA, respectively, because they were different from B1 and B2 in their molecular sizes and restriction maps (Miyata et al., 1995).

Here we report the identification and characterization of three novel plasmid-like DNAs of the B1 family, which we designated M1, M2 and M3. These DNAs were isolated from mitochondria of strains in the genus *Oryza* with CC and CCDD genomes and we present their complete nucleotide sequences. From a comparison of the sequences of B1, M1, M2 and M3, we present a hypothesis about the generation of the B1 family. Furthermore, the relationships between these DNA molecules and the diversification of the genus *Oryza* is discussed.

2. MATERIALS AND METHODS

Plant materials and the preparation of total and mitochondrial DNA

Seven perennial strains in the genus *Oryza* with CC and CCDD genomes, as listed in Table 1, were obtained from the National Institute of Genetics, Japan. These strains were maintained as plants. *O. sativa* cv. Chinsurah Boro II, which has four kinds of plasmid-like DNAs, was used as the standard. Total DNA was extracted from mature leaves of each strain by the method of Honda et al. (1990). As for *O. officinalis* W0065, sufficient amount of material was obtained, thus mitochondrial DNA was isolated from mature leaves by the method of Iwahashi et al. (1992).

Southern hybridization with a non-radioactive probe

Total DNA and mitochondrial DNA were digested with various restriction endonucleases and Southern hybridization analysis was performed as described previously (Iwahashi et al., 1992). Cloned B1 DNA (Sakamoto et al., 1989) was

Table 1. Strains examined and the plasmid-like DNAs detected in this study

Genome	Species	Strain	B1 family
AA	<i>O. sativa</i>	Chinsurah Boro II	B1
CC	<i>O. officinalis</i>	W0012	M1
		W0564	M1
		W0065	M2
CCDD	<i>O. latifolia</i>	W0542	M3
		W1175	M3
	<i>O. alta</i>	W0017	M3
	<i>O. grandiglumis</i>	W1194	M3

labeled with a DIG DNA labeling and detection kit (Boehringer Mannheim, Germany) and used for the probe.

Cloning and sequencing of M1, M2 and M3

M1, M2 and M3 were cloned into the λ gt11 vector with a Cloning kit (Stratagene, USA) and were then sub-cloned into pBluescript II (Stratagene). Total DNA from *O. officinalis* W0564 and *O. latifolia* W0542 was used for the cloning of M1 and M3, respectively. Mitochondrial DNA of *O. officinalis* W0065 was used for the cloning of M2. Each DNA was digested first with *Eco*RI, then fractions of DNA containing fragments of M1, M2 and M3 were recovered from agarose gels after electrophoresis and cloned into the *Eco*RI site of λ gt11. B1 was used as the probe for screening the clones from each phage clone bank. M1 and M2 were digested into large and small fragments by treatment with *Eco*RI, respectively, for cloning. Nucleotide sequences were determined with a DNA sequencer (Perkin Elmer ABD, USA).

Polymerase chain reaction (PCR)

The synthesis of oligonucleotides and PCR were performed with a DNA synthesizer (Perkin Elmer ABD) and a DNA Amplification system (Perkin Elmer Cetus, USA), respectively. The sequences of the primers were as follows:

- primer 1, 5'-AGTCACATTTAATGGCTAGA-3';
- primer 2, 5'-GTCAACTAGTTTATTGAATG-3';
- primer 3, 5'-CATACCGGACAGAAATACAG-3'.

The locations and orientations of primers are indicated by small arrows in Figure 4. Primers were designed for amplification of the partial sequences of M1, M2 and M3, which contained *Eco*RI sites. For each amplification, 250 ng of primers and 100 ng of template DNA were added to the reaction mixture. The conditions for denaturation, annealing and extension were 1 min., 2 min. and 2 min., at 94°C, 45°C and 72°C, respectively. Amplified fragments of DNA were cloned into

pBluescript and sequenced.

3. RESULTS

Detection of members of the B1 family in the strains with CC and CCDD genomes

We performed Southern hybridization with the B1 probe using total DNA from the strains with CC and CCDD genomes, as listed in Table 1, and we detected B1-homologous plasmid-like DNAs that we named members of the B1 family (Miyata et al., 1995). Chinsurah Boro II was used as the standard strain because its mitochondria contains four kinds of plasmid-like DNA. When total DNA was treated with only RNase A, circular mitochondrial plasmid-like DNAs that hybridized to B1 were detected (in lanes 1 and 2 in Fig. 1) as the signals that corresponded to open circular (oc) and covalently closed circular (ccc) forms. As shown in lane 3 in Figure 1, B1 was linearized by *Eco*RI and converted into a single signal of 2.1 kb. In lane 4, two bands, corresponding to 1.5 kb and 0.9 kb, were detected in the case of *O. officinalis* W0564. Therefore, we deduced that this molecule was a circular plasmid-like DNA with homology to B1, and we named it M1. M1 was detected in *O. officinalis* W0564 and W0012, each of which has a CC genome. When total DNA of *O. officinalis* W0065, with a CC genome, was

Fig. 1. Southern hybridization of mitochondrial plasmid-like DNA B1 to total DNA. Total DNA was subjected to electrophoresis, transferred to a filter and allowed to hybridize with digoxigenin-labeled B1. Lanes 1 and 3, *O. sativa* cv. Chinsurah Boro II; lanes 2 and 4, *O. officinalis* W0564. Samples of total DNA were not (lane 1 and 2) and were treated (lane 3 and 4) with *Eco*RI. The positions of the open circular (oc) and the covalently closed circular (ccc) forms of DNA are indicated on the both sides of lanes 1 and 2.

treated with *Eco*RI, the B1 probe hybridized to fragments of 1.1 kb and 0.9 kb (data not shown). We named this circular DNA M2. In the case of *O. latifolia* W0542, one fragment of 2.1 kb was detected. This fragment gave a different restriction pattern from that of B1 when it was treated with other several restriction enzymes, so we named it M3 (data not shown). M3 was detected in four strains of three species with a CCDD genome (Table 1).

Nucleotide sequences and properties of M1, M2 and M3

We cloned M1, M2 and M3 into a phage vector with an *Eco*RI site (see MATERIALS AND METHODS). If there had been some *Eco*RI sites close together, small *Eco*RI fragments would not have been detected and cloned by the present method. In order to determine whether any closely adjacent *Eco*RI sites were present, we amplified the partial sequences of M1, M2 and M3 that contained *Eco*RI sites by PCR, and then we cloned and sequenced the amplified fragments. The complete nucleotide sequences of M1 (2,430 bp), M2 (2,034 bp) and M3 (2,096 bp) were determined and alignment of the nucleotide sequences of M1, M2, M3 and B1 is shown in Figure 2. From comparisons of sequences, we found that the B1 family also exhibits homology to B2 and B3 of rice and to a 1.9-kb plasmid-like DNA of Black Mexican Sweet maize, as indicated by several open boxes. The presence of these homologous regions was reported previously (Shikanai et al., 1989). We also identified a new region of M1 with homology to B2 (as indicated by a1 in Fig. 2).

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M1 1 GAATTCGAGGATTGGCTAAGGCTCTAGCCATTAAATGTGACTGGGGTTGATCCAAGGGCTGGTCTCATGGTT-----TTGACTAGGTGTTTCTCAA
M2 1 *****
M3 1 T*CCGAGGT*AC*TATCT*TCGG*AT*G*TC*-*TT*A*T******A*****G*T**C*ACCGCAT**C**TACCC*AGA*TC*
B1 1 *****

M1 94 TTTCGTGATCATATTCTCTGCTGTATTAACCCCAAAATACGCTAAGTTCGCAACTCGAAG-TTCCCAGTTGCA-TGG-----GCAACCC-----TACAT
M2 94 *****
M3 100 *CCAC*G*TA*CC*G***--A*A*AA*TA*CT*ATG*****T**C*A**AA*CT*T**AAC*****AACT---TCG***-
B1 92 *****G******A*-----TACTACTA*****

M1 180 AGGGCG-TGAAGAGACGGACGA-----CAATAGTCTTTCTCTGCTTTCACCTCGAGCCAAGTAAAGTCTTCTAGGATTGGCCCCA
M2 180 *****
M3 194 -----T******TCTA**G*TGCCTCGGGGTACCTACTA**G*--*AA*GC*--*GA***A*****A*****C***CC***--T
B1 187 ****T*-----A*-----*****A*****T*****

M1 260 AAGCCATACATATGGTCACACGGTAACCCCGTCGATTTGATTTATCCGATCAATCGAGAGGCAGCATCTCTATCAATTACTCGGATCGGCAACCTCGGTT
M2 260 *****
M3 283 TT*****G**A*****T*****C*****A*****A*****TCT**GAG*****T*C*A*T*-----
B1 267 *****AG*****A*****

M1 360 CTGGAATGTGGACTTCATGACCAGCGC-CCTCTTAGTCCACCCAACTTGAAGTGTATTGATTGCG-----TTAGTCGCCTTC-GCCATCCTAATA
M2 360 *****
M3 375 ---*--AAA**CG**ACT*G*TA*AT*A**G***TCA*T*****C**G*T**AT*T**C**ATGAAC*A*A*T**A*---G*A*C*
B1 367 *****C*****-----*****AA*****

M1 452 CCCGA-GACTGTATTAGACTTTTATAGGGGTGCCGTCGTCCTGGGATGCCCGGCTGGTCGAGCAAGTATTAG-TGAACGGCAACGGCAATCAATA
M2 453 *****G*****
M3 468 *****-*****G*A**A**TTGC*****G**CG*G*-----**C*
B1 459 *****G*TT*AAG*TGTTAA******G*****G*****TC*****

M1 550 ACTACCCTGCTTAGGTATTAGCTCCAAAGTC--CCGAATCCT----TTGTCTCAAGTCTGCTTTGGTAATGTGACCACACATCCTTTGATGATGGAAG
M2 552 *****G*****
M3 552 -*T*A*T*A*CAA*****T*TTTG*G*AT**C*GC**GAGCA*****C*****T**TC*****GT***G**
B1 557 *****G**T*-----*****C*****

M1 643 TGTGTGTTCCCAACAATCCATCTCGGAATCCT-AGTCGGAGTAATGTTCCCAACATACTATCGCGCTAATGTAATCGGGTTCTCAGATCATACTCAATG
M2 645 *****-*****
M3 651 *-----GAT*****GG*****TTAT*GT*G*--T**T*GG*GT*--*TTC**
B1 650 *****C*C*-----*T*****A***T*****

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M1 742 AGGGGTGGCTAGTATCCCGAA---ACTTGG-AAG--AACATGAGTTAAGTAGTTCACCTTGGTTTCGCCACCAGTCAAAACGCCCTAAACCACGAT
M2 743 *****
M3 739 *TT*****-A*C*T**CCAT*C**C**CA*G**AA*AC*---*T*G**AGG*-*****CG*-A*TG*GC*AT*AA*-----A*
B1 749 *****

b1

M1 835 AGGTTTCTCGATCACCATTCTACGATTTTATTTCCACCAATTGATTGGTGTGAATCATAAAAGTGGATCATTATAATAATGTTGTTATTATTACC
M2 836 *****
M3 833 G**G*****T*****T*****TC**AA*AT*---*G*G***-
B1 842 *****

M1 935 TATTAAAGTGGAAATTAGCCCCCTTAGCAGTTCTAAACCTGGTTGTATGCGTTCATTTGAGGGATAGAAGTATTGTTTGGTATACCCCTAATGGTAGTTT
M2 -----
M3 -----
B1 -----

a1

M1 1035 CAATCTAAAGTGTGAACCTGCGTTGGTAGCGCATTTCAATTAAGAAAACAACCTCGCTCAGATGCGTCTATTCTCAGGTTTGGAGTAGTCCCTTTAT
M2 -----
M3 -----
B1 -----

M1 1135 GATTCACCTTGTGTTTGAAGAACAAACAGTGCCTTCTATCGAGCAATATAACAGCGGTTACCTGTTTGTAGACACATATATGTTTACCCTTAAGAGT
M2 -----
M3 -----
B1 878 -----*****A*****A*****GC*****

a2

M1 1235 TTATCACTAAATCAATAAGTTTATGAATGCACGAAAGTTGCGCTCATAA--AGTAGATGTTAGTACCCGACTAAAGGAGGGAATCAATAAAGGTA
M2 884 -----
M3 899 -----*****T*****T*****T*****A*****A*
B1 918 *****G*C**TT*AC*****T*****A*****G*****

M1 1334 AAG-----CTATC-----CTTTGGTAAAGGTATATTGCCGTAGCGAGGTTAAG--TCGGATCCTTAACCGAGTGAAGG--GCTTA
M2 946 -----
M3 989 -----*****C**C*A*****G*-AA*C**G*-----*A***
B1 1017 **CTCTCAATTAATAAATAAAG**GTTTA**T**G*****G*-AA*C**G*****CGAGGCC

M1 1407 GGTAAAGCACTAAAGTAAAGCACTAAAGTAGTGG---TTCTACTTCATTAGCTATCAAGTGTGTTGTATCAATTACTTTATTGTCAACTAGTT
M2 1019 *****-***GT*TTGG*---*C---*G*****T*****C*****
M3 1061 *****GTGAAT**GT**T*A*CTATC*G*****
B1 1116 *****GT*TTGG*---*C---*G*****T*****A*****C*****

M1 1502 TATTGAATGAAAGGGAATTCCTCGATAATGGATTTCACCTTTAGGAAAGATAATTAGTTATCCTCCCTCCCTCGAAGTGAAGTATTCTCTGTGTTAG
M2 1106 *****-*****A*****A*****G*****
M3 1161 *****
B1 1203 *****C*****T*****T*****A*T*A*C*A*****G*****C*****T*****G*****T*****C

c1

M1 1602 AATTGTATACACGAGGGGCTGCTCTTCCTCTGCTCAATCCCGGACAGGTATGAAAGTCATAATCTTTT--GGTTTGTGTCAATGTAGTGAGCGGAG
M2 1205 **C*****G*****
M3 1261 **C*****
B1 1303 **C**G*****C*****T*****G*****GG*T*****A*****G*A*****

M1 1700 GGAGATACATCCTCCATCC-AAAGG-GTGATGTCCCGTAGCGAGTGAATGGCTCCT-CCGGTTTAGTGGTATAGAAGCGGCTGAGTTCTTTTATGG
M2 1303 *****
M3 1360 *****A*****T*TT*G*AA*A*****T**C**G*****CT*
B1 1403 *****-*****T**G*****GG*-T**C*****CT*

M1 1797 AGTCCTTTCGAGGCTGAAGTGTCTTCTACAGTTTCTCGAAAGGGAAGTCAAGTAAAGGGTTAAAGGCTAGTTAATTC-TCCTTAAGTTTCTCC
M2 1400 *****
M3 1459 *****AG*****-*****TTTA*****A*
B1 1501 *****-*****TA*A*CTAG*G*C*A*G*C*AAGAAG**GGG*GAA

M1 1896 AAGCGAAGAAAG-ACTGTTCCCTTAAAGAGTAAGATAGTTCAGTCCAGTCTTCGAAGCGTTAGTAGTGCTCGGAAGGGGTGGGGATTCCAAAGGGGA
M2 1499 *****
M3 1558 C*****G*****C*****
B1 1598 **T*A*G*GG*CGA*TAG**CG*TCC*CT*G**GGAGGA**G**TTC**C*CT*CTAA**CCAAT*AT**TTCC*ATTC*---TA*T*AAATTC

M1 1898 AGGGGAACCCGACCCCTTGTTCATCAACCAATTTAAGTAAAAAAGTTCGACTTTCTATTCCGTCGCAATCTTCTGTCCGTTCAACCAATTG-
M2 1598 *****T*****
M3 1657 *****A*****T*****T*GG*****T*****
B1 1695 *TATTT*TTT*G*AAAA*A*-AA*CT*TT*C*TTA*CT*GTT*C*T*AAACC*-*****C*****AC*****T

c2

M1 2094 TCCTTGCCAAAGATTGTGACGAAGACTTTCGTACCTGTATTCTGTCCGGTATGACGTGCGCTTTCGTCTGACGATAGCGTCCCGAGTTGCCACAGTTGC
M2 1697 *****
M3 1756 *****
B1 1793 *GGA*****G*****

M1 2194 TAGGCTGTTAGAG-CCTCTGGACCCCGGCTAGGCATCTCCCGGTAATGCCAGGGGCTGCTTCAAGAAACGGGTGATGC---GGGTTCACAT--CATT
M2 1797 *****
M3 1856 *****T**T**A*****GA*G*TT*T*TA**CT**CTT*****T*G*GCT**
B1 1893 *****T*****T-----

Fig. 2. Alignment of the complete nucleotide sequences of M1, M2, M3 and B1. The nucleotides that are the same as those in M1 are indicated by asterisks and several gaps, introduced to maximize alignment, are indicated by dashes. Single and double underlining indicates the short repeats that might be involved in deletions via slipped mispairing during DNA replication. Open boxes a1 and a2 indicate B2-homologous regions; b1 and b2 indicate B3-homologous region; and c1, c2 and c3 indicate regions homologous to the 1.9-kb plasmid-like DNA of Black Mexican Sweet maize. The nucleotide sequences of M1, M2 and M3 has been deposited in the DDBJ, EMBL, NCBI and GSDB databases under the accession number D50618, D50619 and D50620, respectively.

With respect to functional aspects of the sequences, only one open reading frame (ORF) of 399 bp (beginning at position 2,430 and ending at position 2,032 on the opposite strand) was found in M1; two ORFs, of 309 bp (beginning at 1,748 and ending at 2,056) and 264 bp (beginning at 2,034 and ending at 1,771 on the opposite strand), respectively, were found in M2; and no ORF of longer than 200 bp was found in M3. B1 also has ORFs of 243 bp and 288 bp, (Shikanai et al., 1987). These ORFs are not conserved in B1, M1 and M2 and, therefore, these ORFs seem not to encode any essential proteins.

Many short repeated sequences (less than 25 bp) were found, but no long repeat and stem and loop structure was found in the sequence of M1, M2 and M3, in contrast to the long direct or inverted repeat and hairpin structure, respectively, of the mitochondrial plasmid-like DNAs of *Vicia faba* (Wahleithner and Wolstenholme, 1987) and the pO plasmid of *Beta vulgaris* (Hansen and Macker, 1984).

Comparison of sequences of M1, M2, M3 and B1

We compared the sequences of M1, M2, M3 and B1 and diagrams of homologous sequences are shown in Figure 3. Hatched boxes indicate M1 or regions that are more than 90% homologous to M1; open boxes indicate the unique regions of B1 and M3 with no homology to M1. Over 95% of the nucleotide sequence of M2 exhibits homology to M1, but M2 lacks 388 bp of M1 (beginning at 884 and ending at 1,271). Incomplete repeats of 15 bp of (T/A) GTTG (A/C) (A/C) TCATAAAA are present at the both borders of this deleted region in M1, and this element is present as one copy only in M2 in the corresponding region (indicated by double underlining in Figure 2 and by black arrowheads in Figures 3 and 4). About 90% of the sequence of B1 is homologous to M1, and B1 lacks 323 bp of M1 (beginning at 871 and ending at 1,193). Small repeats of the ACC triplet are also present at both borders of this deleted region in M1 and this element is present as a single copy in B1 at the corresponding position (indicated by single underlining in Figure

Fig. 3. A comparison of the sequences of members of the B1 family. Homologous and unique regions are indicated as boxes. The scale indicates nucleotide positions of M1 (see Figure 2). Hatched boxes indicate regions homologous to M1 and open boxes indicate the regions with no homology to M1. The unique region of M1 that is absent in B1, M2 and M3 is indicated as a closed box. White and black arrowheads indicate the small repeats of 3 bp and 15 bp that are indicated by single and double underlining in Figure 2.

2 and white arrowheads in Figures 3 and 4). In contrast to B1 and M2, only about 60% of M3 is homologous to M1. M3 also lacks 343 bp of M1 (beginning at 902 and ending at 1,244), but no repeat is found around this deleted region in M1 or at a corresponding site in M3. Thus, 291 bp of the sequence of M1 is absent from B1, M2 and M3 (indicated by a filled box in Figure 3).

4. DISCUSSION

We cloned and sequenced these novel mitochondrial plasmid-like DNAs, namely, M1, M2 and M3, from strains in the genus *Oryza* with CC and CCDD genomes. A comparison of sequences revealed that these molecules have a highly conserved sequence over a wide region (Fig. 3). In B1, 322 bp of M1, located between small triplet repeats, is deleted. In M2, as in B1, 388 bp of M1, located between imperfect repeats of 15 bp, is deleted. In B1 and M2, these 3-bp or 15-bp repeats are present as single copies at the position that corresponds to the deleted region in M1. In M3, no similar repeats are found. From our present results, it is suggested that B1 and M2 arose from M1 by recombination or slipped mispairing during DNA replication. Therefore, we propose the following reasonable hypothesis about the generation and diversification of the B1 family in the genus *Oryza* (Fig. 4). First, an original plasmid-like DNA, probably similar to M1, was present in mitochondria of ancient species in the genus *Oryza*. During evolution, parts of this original plasmid-like DNA were deleted by intra-molecular recombination or by slipped mispairing during DNA replication, and prototypes of B1, M2 and M3 were generated. During further evolution and diversification of the genus *Oryza*, several mutations, such as deletions, insertions, or base substitutions, and recombination with other plasmid-like DNAs occurred and the B1 family was generated as a result. M3 exhibits less homology to M1 than B1 does and repeats are not retained in M3. Thus, M3 seems to have differentiated at the earliest stage of differentiation of B1 family. B1 also exhibits less homology to M1 than M2 does and retained repeats are only 3 bp in length. Therefore, we suggest that the differentiation occurred in order M1 to M3 and then to B1 and M2.

Slipped mispairing between repeated sequences during DNA replication has been verified *in vivo* and *in vitro* as a mechanism that might be responsible for the generation of the polymorphic regions and large-scale deletions found in mam-

Fig. 4. The schematic representation of the explanation for the generation of members of the B1 family of mitochondrial plasmid-like DNAs during the diversification of the genus *Oryza*. Large arrows indicate the differentiation of the B1 family, as described in the text, and small arrows, labeled P1, P2 and P3, indicate primers 1, 2 and 3, respectively, that were used for amplification of partial sequences of M1, M2 and M3 by PCR. The dashed lines of A, B and C indicate the deleted regions of M1 and the corresponding sites in B1, M2 and M3, respectively. White and black arrowheads indicate the same sites as in Figure 3 (indicated as single and double underlining in Figure 2).

malian mitochondrial genomes (Madsen et al., 1993). In mitochondria of higher plant, recombination across large and small repeated sequences is thought to provide an explanation for the large and complex genomes (for review, see André et al., 1992). Slipped mispairing during DNA replication, rather than intra-molecule recombination, might result in the deletion of the parts of plasmid-like DNAs because the unique region of M1 that is deleted in B1, M2 and M3 seems to be too small to make the loop structures that are postulated to be intermediates during recombination.

To date, the genus *Oryza* has been studied on their relationships among the genome types of AA, BB, BBCC, CC, CCDD, EE and FF. Dally and Second (1990) and Second and Wang (1992) studied RFLPs of the chloroplast and mitochondrial DNAs in the genus *Oryza*, and they showed the phylogenetic relationships among each genome type regarding to cytoplasms. They also suggested that the results of their analyses are consistent with results of previous evaluations of genetic diversity and phylogeny based on isozymic or RFLP analyses of nuclear DNAs within the genus *Oryza* (Second, 1982, 1985; Glaszmann, 1987; Wang et al., 1992). From our present results, we suppose the presence of an original plasmid-like DNA which is similar to M1 in the ancient species of the genus *Oryza* before diversified into several genome types. The presence of this original plasmid-like DNA and our hypothesis of the generation of B1 family are consistent with previous studies about the phylogenetic relationships among the genome types in the genus *Oryza*.

The authors express their appreciation to A. Nakazono of The University of Tokyo for his valuable suggestions, and to A. Kanno of Tohoku University for his guidance during the experiments. This research was supported by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

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