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Some physicochemical properties of rice mitochondrial DNA

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Summary. Certain physicochemical properties of rice mitochondrial DNA (mtDNA) were determined. Certain low-molecular-weight mtDNA bands were found in addition to the major mtDNA band. Rice mtDNA appeared in the electron microscope as a collection of linear molecules with heterogeneous length in the range of 1–156 kb. The major distribution area was 60–105 kb. A small fraction (less than 5%) of rice mtDNA was found in the form of a circular molecule. Some molecules had the appearance of being supercoiled. Replication fork structures were found in both circular and linear mtDNA molecules. In one rice species, Jin Nante, 15 different circular molecules were found. Rice mtDNA was digested with different restriction enzymes. The total molecular weight of rice mtDNA was calculated to be about 300 kb according to the data of restriction enzyme digestion and electron microscopy.

Key words: Mitochondrial DNA – Plasmids – Rice-electron microscopy – CMS

Introduction

MtDNAs of higher plants appear to be substantially larger and more complex than those of animals or microbes. Plant mtDNAs range from 100–2,500 kb in size (Evans 1983; Levings 1983). Study of mtDNA preparations from different plants reveals a broad size range of molecules, primarily linear with a low proportion of circles (Sederoff 1984). There is evidence that these mtDNAs may occur in vivo as a large circular molecule, together with some smaller molecules derived from the large circular molecule by recombination (Lonsdale et al. 1984; Palmer and Shields 1984). Studies have been made on mtDNA, leading to the understanding of the role that this organellar genome plays in maize cytoplasmic male-sterility (CMS) (Kemble et al. 1980; Pring et al. 1982; Schardl et al. 1985).

As is known, rice is the most important food crop in China. CMS hybrid rice is widely grown in China and great economic benifits have been achieved. In order to known if mtDNA plays an important role in rice CMS, some physicochemical properties of rice mtDNA were studied by electrophoretic analysis and electron microscopic analysis.

Materials and methods

Plant materials

Rice seeds were obtained from the Agricultural Academy of Beijing City and the Agricultural Research Institute of Neijing City, Sichuan Province, respectively. Rice seeds were sterilized with 10% sodium chlorite (commercial name: Antiformine) for 10 min, then washed with sterilized water five times and set in a 25 °C darkroom for germination.

DNA modifying enzymes and molecular weight markers

Restriction enzymes, DNase, Proteinase K and DNA molecular weight markers were purchased from Boehringer Mannheim. All enzymes were used according to the supplier's recommendations.

Isolation of mtDNA

Rice mtDNA was isolated from 7-day-old dark grown seedlings by the procedure of Chase (Chase and Pring 1986).

Gel electrophoresis

Electrophoresis was performed on horizontal slab gels of 1% or 0.8% agarose gel (Sigma) at 70 V, 40 mA for 10–12 h or 70 V,

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30 mA for 2 h (for minigel) at room temperature (RT). Gels were stained with ethidium bromide (0.5 μg/ml) for 30 min, then washed with water for another 30 min. A photograph was taken under illumination of short wave UV light.

Electron microscopy

Electron microscopy was carried out according to the method of Kleinschmidt (Kleinschmidt 1968).

Results

Electrophoretic analysis

Rice mtDNA was fractionated by 1% agarose gel electrophoresis. Yamaguchi has reported that rice mtDNA from sterile line of BT type possessed two low-molecular-weight (LMW) DNA bands in addition to the high-molecular-weight (HMW) DNA band (Yamaguchi and Kakiuchi 1983). We found LMW DNA bands in all of the examined plants. (Forty lines belong to different types of cytoplasm. Their differences will be reported in another paper.)

Generally speaking, rice mtDNA separated into a heavy major band at the top of the gel with HMW and some weak bands with LMW in front of the gel (Fig. 1). The mtDNAs in these LMW bands are certain so-called plasmid-like mtDNAs. They are named: pLMR 1, pLMR 2, pLMR 3, pLMR 4 and pLMR 5 (pLMR: plasmid-like mtDNA of rice). Their molecular weights are 19.0, 4.2, 3.1, 1.6 and 1.1 kb, respectively. Their molecular weights were obtained by comparing their mobilities after extensive S1 nuclease treatment with the mobility of linear DNA size marker on the same gel. The number of plasmid-like mtDNA bands varied from three to five, depending on different species.

These plasmid-like mtDNAs were examined according to their enzymological properties. All of these plasmid-like mtDNA bands disappeared after nuclease and DNase digestion, but they were resistant to RNase treatment (Fig. 2). These results confirm that they are DNA rather than RNA.

Electron microscopic analysis

In the electron microscope, rice mtDNA preparation appeared as a mixture of linear and circular molecules. Rice mtDNA comprised mainly linear molecules. Their molecular weights were heterogeneous and ranged from 1 to 156 kb. Some of the linear molecules are shown in Fig. 3A. The major distribution area was in the size range of 60–105 kb (Table 1). Of 900 measured linear molecules from the species Jin Nante, 453 were within the range of 60–105 kb, 294 were within the range smaller than 60 kb, and 153 were in the range larger than 105 kb. This results was similar to those from the other three examined species (results were not shown). Only 4.7% of

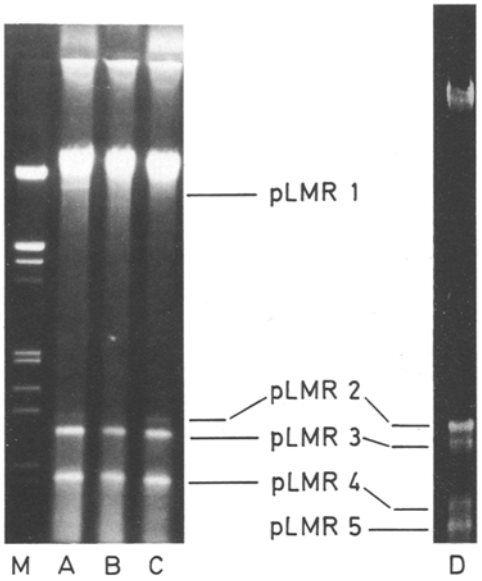

Fig. 1. Rice mitochondrial DNA from different species. *M:* Molecular weight marker III. *A, B and C* were three different species run in the same gel. *D* was another species run in a separate gel in a different experiment

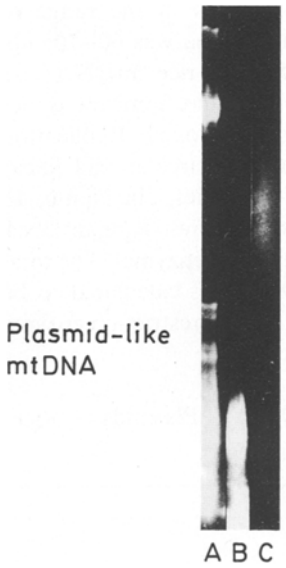

Fig. 2. Identification of rice plasmid-like mtDNAs. *A:* Rice mtDNA. *B:* Rice mtDNA treated with DNase and RNasinR. *C:* Rice mtDNA treated with nuclease

Table 1. Size distribution of linear molecules in rice mtDNA

Size (kb)	No. of molecules	%
<60	294	32.7
60–105	453	50.3
>105	153	17.0
Total	900	100.0

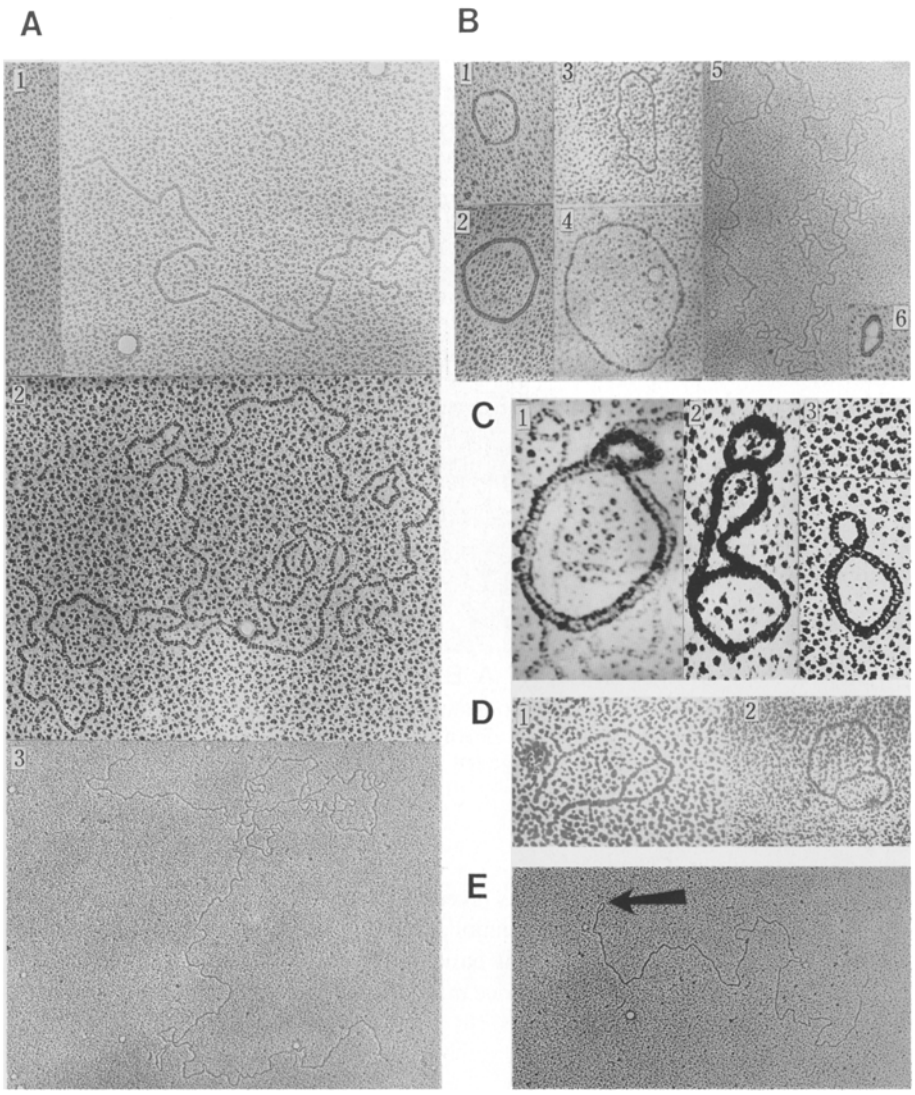

Fig. 3A–E. Electron micrographs of rice mtDNA. **A** Linear molecules. *1* – 16.3 kb; *2* – 36 kb; *3* – 54.5 kb. **B** Circular molecules. *1* – 3 kb; *2* – 6.5 kb; *3* – 8.3 kb; *4* – 9.0 kb; *5* – 64 kb; *6* – 1.6 kb. **C** Supercoiled molecules. **D** Replication fork structures. **E** Lariat structure

Table 2. Configuration of rice mtDNA molecules in the species of Jin Nante

Configuration	No. of molecules	%
Linear	928	95.3
Circular	46	4.7
Total	974	100.0

Table 3. Size distribution of circular molecules in rice mtDNA

Contour length (kb)	No. of molecules	%
1–3	29	60.4
3–19	13	27.1
19–60	4	8.3
60	2	4.2
Total	48	100.0

rice mtDNA was found in the form of circular molecules (Table 2). The range of their contour length was 1–96 kb. In the species Jin Nante, 15 different circular molecules were found. Their contour lengths were calculated as follows: 96, 64.2, 41.6, 28.8, 24.5, 17.4, 15.0, 9.0, 8.3, 6.4, 4.2, 3.8, 3.0, 1.6 and 1.1 kb, respectively. Some circular molecules are shown in Fig. 3B. The contour length distribution of the circular molecules in one of the examined mtDNA (Jin Nante) is given in Table 3. The major distribution area was 1–19 kb. Of 48 examined circular molecules from the mtDNA of Jin Nante, 29 (about 60%) were within the range of 1–3 kb, and 13 (27.1%) were within the range of 3–19 kb. Only 12.5% of the molecules had a size larger than 19 kb. The results of the other three species examined agreed with the result from Jin Nante. Some circular molecules had the appearance of being supercoiled (Fig. 3C). The replication fork structures were found in both circular and linear mole-

Table 4. Molecular weights of PstI-digested major fragments of rice mtDNA

Band no.	Molecular weight (kb)
1	25 × 2
2	22
3	19
4	16
5	13.5 × 2
6	12.5
7	12
8	10.5
9	9.8
10	7.8 × 2
11	7.5
12	6.5 × 2
13	6.3
14	5.9
15	5.4
16	5.2
17	4.9
18	4.4
19	4.2
20	3.6 × 2
21	3.4 × 2
22	3.0 × 2
23	2.7
24	2.3
25	2.0
26	1.6 × 2
27	1.4
28	1.3
29	1.2
30	1.1
Total	286.4

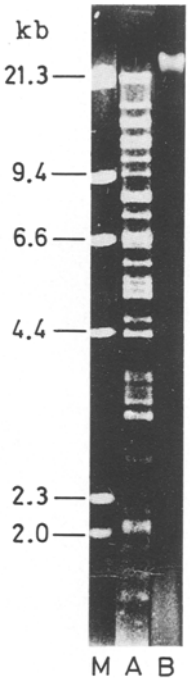

Fig. 4. Restriction fragment pattern of rice mtDNA from the species Qui Guang. *M:* Molecular weight marker II. *A:* Rice mtDNA digested with Pst I. *B:* Rice mtDNA

cules (Fig. 3D). Some lariat structures were found in linear molecular also (Fig. 3E). They may be the replicating intermediates.

Molecular weight determination of the total mtDNA

Plant mtDNA genomes range in size from approximately 100 to 2,500 kb. The molecular weight of mtDNA from many plants has been reported. Regarding rice mtDNA, Yamaguchi et al. (1986) reported that the total molecular weight of the mtDNA genome was larger than 150 kb. In May 1987, at the Third National Congress of the Chinese Genetics Society, we reported that the total molecular weight of rice mtDNA was about 300 kb. When rice mtDNA was digested with restriction enzymes EcoRI, HindIII or PstI in the electrophoretic pattern, rice mtDNA gave 25–30 major fragment bands and about 8–10 faint bands (in the low-molecular-weight area), depending on different enzymes. The restriction fragment pattern of rice mtDNA (from the species Qiu Guang) digested with PstI is shown in Fig. 4. The major fragment size data is given in Table 4. The total molecular weight of the 30 major fragments was calculated to be 286.4 kb. If the faint bands were considered, the total molecular weight of rice mtDNA genome should be about 300 kb.

Discussion

In the study of rice mtDNA, we found that in agarose gel there were some LMW bands in addition to the HMW band, and that in electron microscopy there were many linear and circular molecules in different sizes. This result is in agreement with the results from maize (Kemble and Bedbrook 1980; Weissinger et al. 1982), brassica (Palmer et al. 1983), sorghum (Dixon and Leaver 1982; Pring et al. 1982), faba bean (Boutry and Briquet 1982; Negruk et al. 1982), sugarbeet (Powling 1981), wheat (Handa et al. 1984), sunflower (Leroy et al. 1985) and rice (Mignouna et al. 1987; Yamaguchi and Kakiuchi 1983). Although there are considerable variations among plants with regard to the number, size and configuration of the LMW mtDNAs, the existence of such LMW mtDNAs seems to be a common feature in plants. The LMW mtDNAs are called plasmid-like mtDNAs (including minicircular and minilinear molecules).

Electron microscopy of the DNAs in these LMW bands in different plants demonstrated that certain

bands comprised mainly circular molecules (Bailey-Serres et al. 1987; Handa et al. 1984), but some bands comprised mainly linear molecules (Kembel and Bedbrook 1980; Pring et al. 1982; Weissinger et al. 1982). In rice, we also found that some bands consisted mainly of linear molecules, but some bands consisted mainly of circular molecules (the results will be reported in another paper on plasmid-like mtDNA and CMS in rice). The electron microscopy of the total rice mtDNA revealed 15 different circular molecules and many linear molecules. This number is much higher than the band number found in agarose gel. That is because even though some molecules can be seen in the electron microscope, their density is not high enough to be seen in agarose gel.

The origin of these LMW mtDNAs in higher plants is not clear yet. Many scientists suggested that they might be the products of recombination events in mtDNAs (Chase and Pring 1985; Chase and Pring 1986; Bailey-Serres et al. 1987, Handa et al. 1984; Weissinger et al. 1982). The evidence from the extensive study of maize plasmid-like mtDNA, S-1 and S-2, strongly supports this hypothesis. According to this theory, the recombination should take place in some homologous sequences. For the formation of circular molecules, the recombination should take place within direct repeat sequences. In rice mtDNA, 15 different circular molecules were found. This number is higher than that in maize (7) and soybean (8). This suggests that there are more direct repeat sequences in rice mtDNA than in maize and soybean mtDNAs.

The statistical data from about 1,000 scope-fields in 100 slides indicated that rice mtDNA comprised mainly linear molecules (more than 95%) and a small number of circular molecules (less than 5%). This proportion is similar to that of maize and soybean, but much lower than that (20%–45%) of tobacco. Considering the existence of more direct repeat sequences, the copy-number of the repeat sequences in rice mtDNA could not be very high.

Whether or not the plasmid-like mtDNAs are capable of self replication is a question that needs to be answered. In rice mtDNA, we have found replication fork structures in both circular and linear molecules. Some lariat structures were found in linear molecules also (Fig. 3 E). Some scientists named this structure lasso-like structure (Goddark and Cummings 1975; Kim et al. 1982). They may be the replicating intermediates. These results provide fresh insight into the replication of plant mtDNA.

There is evidence in maize and cabbage that these mtDNA genomes may occur in vivo as a large circular molecule (called master circular molecules) together with some smaller circular molecules derived from the master circular molecule by recombination at specific sequences. The master circular molecules have been found in maize and cabbage. They were 570 and 218 kb, respectively (Grierson and Covey 1984). In rice, 15 different circular mtDNA molecules were found; the largest one was about 96 kb. Many linear molecules in different sizes were also found. The largest linear molecule which we found in rice mtDNA was about 156 kb. However, owing to the technological difficulty and mechanical damage during the isolation process, we did not find the master circular molecule in rice mtDNA. According to the restriction enzyme digesting experiment, the total molecular weight of rice mtDNA genome was calculated to be about 300 kb. It is slightly larger than that of faba bean and petunia, but much smaller than that of maize. Compared to that (2,560 kb) of muskmelon, rice mtDNA is a small one in higher plant mtDNAs. Now, pulsed field gel electrophoresis (PFGE) is used to separate the large mtDNA fragments and the intact mtDNA molecules in our laboratory. PFGE enables DNA fragments up to several million base pairs to be isolated, therefore, it may be useful in the isolation and molecular weight determination of the intact molecules of rice mtDNA.

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