# ISOLATION AND CHARACTERIZATION OF MINICIRCULAR DNAs FOUND IN MITOCHONDRIAL FRACTION OF *VICIA FABA*

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## 1. Introduction

First detailed analysis of small DNA species from higher plant mitochondria was done on *Zea mays* [1,2]. It was found that normal and 3 types of male-sterile *Zea mays* plants contained several types of minicircular DNA molecules. Some of these DNA species were, apparently, associated with the male sterility phenomenon while the biological role of minicircular DNA molecules found in normal cells remained unknown. We observed 2 small DNA bands in mitochondrial preparations of normal *Vicia faba* etiolated seedlings [3]. Here, we describe the purification, electron microscopy and restriction analysis of DNA in these 2 bands. A study of small DNA species from different plant tissues and several varieties of *Vicia faba*, from different legumes and different species of *Vicia* genus will be published elsewhere.

## 2. Materials and methods

Mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) were extracted as in [3]. mtDNA was isolated from 6-day-etiolated seedlings. Seedlings were grown in dark room at 20°C. cpDNA was extracted from leaves of 14–18-day-old plants. Plants were grown in phytotron with 16 h light period and 8 h dark period at 20°C. The procedure of mtDNA and cpDNA isolation combined the alkaline method of prokaryotic plasmid DNA isolation [4] and following phenol treatment. The minicircular DNAs were eluted from 0.6% agarose gel by freeze–squeeze procedure [5] with following phenol treatment and passing through a Sephadex G-50 column. Electron microscopy was done according to [6].

## 3. Results and discussion

All preparations of mtDNA obtained by laurylsarcosine/phenol/RNase/proteinase K treatment of mitochondria from *Vicia faba* contained 2 homogenous bands migrating with the mobility of linear DNA fragments $M_r$-values of ~0.75 and $0.85 \times 10^6$ $M_r$ [3]. To clarify the nature of DNA in these bands we obtained mtDNA and cpDNA by alkaline procedure developed for isolation of bacterial plasmids [4] and modified in accordance with our purposes [3]. As shown in fig.1b,

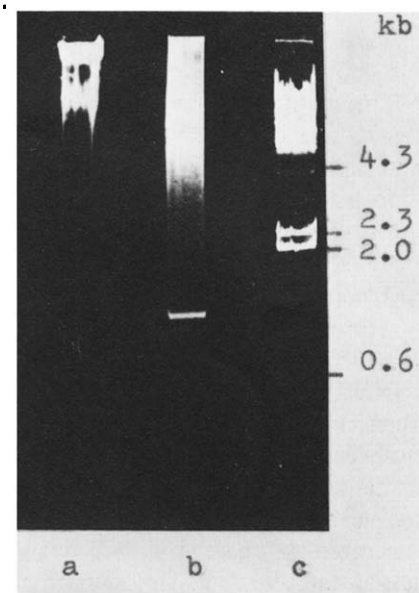


Fig.1. Fractionation of DNA preparations from chloroplasts (a) and mitochondria (b) of *Vicia faba* cv. Russian Black by the electrophoresis in 1% agarose slab gel. Numbers at right indicate lengths of phage *Hin*dIII fragments (c) in kilobase. Size values are given in [7].

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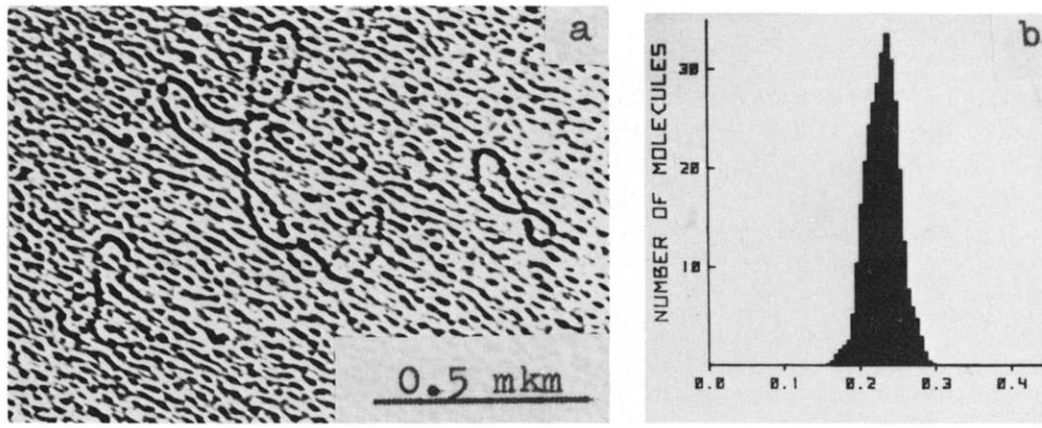


Fig.2. Electron microscopic analysis of minicircular DNA molecules (small circles) with ColE1 plasmid DNA used as a length marker (large circle) (a) and the length distribution analysis of minicircular DNA molecules (b). The lengths are expressed in units of ColE1 length. The number of molecules used was 237 for minicircular molecules and 70 for ColE1. The average lengths of plasmid-like molecules was 1560 ± 147 basepairs and for ColE1, 6700 ± 315 basepairs.

using the procedure of minicircular DNA isolation, we also extracted mtDNA preparations containing 2 homogenous DNA bands with the same electrophoretical mobility. Since DNA in these 2 bands survived alkaline treatment without changing of electrophoretical mobility we suggested that it was covalently closed circular DNA. We did not find any small DNA species in cpDNA preparations (fig.1a). This fact and the fact that DNA from mitochondrial preparations after the treatment with pancreatic DNase also contained supposed minicircular DNAs suggest that these DNA species are located within mitochondria.

To clarify the supposition about circular structure of the small DNA species found, we eluted 2 DNA bands together from an agarose gel and visualized them by electron microscopy. Electron microscopic analysis showed that preparations contained mainly circular molecules (fig.2a). A statistic analysis of length measurements revealed one class of circular DNA molecules with a size of ~1.6 kilobases (kb) (fig.2b), that corresponded to a major, slower migrating in agarose gel, DNA band (fig.1b). The minor band was not resolved by the electron microscopy.

Results of restriction analysis of minicircular DNAs eluted from an agarose gel are shown in fig.3. CCC1 and CCC2 are covalently closed circular DNAs. OC1 are relaxed CCC1 molecules. The CCC2 DNA band was resistant to *Eco*RI and *Hin*dIII (fig.3b–d). The CCC1 DNA band, apparently, contains 2 types of circular molecules. We came to this conclusion based on the fact that the *Hin*dIII treatment induced an appearance of 3 DNA bands with sizes of ~1.35, 1.15 and 0.45 kb instead of CCC1 and OC1 (fig.3a,c). So the sum of lengths of these fragments was of ~3 kb which was more than twice the length of CCC1 (fig.2). After the treatment of eluted CCC1 and CCC2 DNAs with

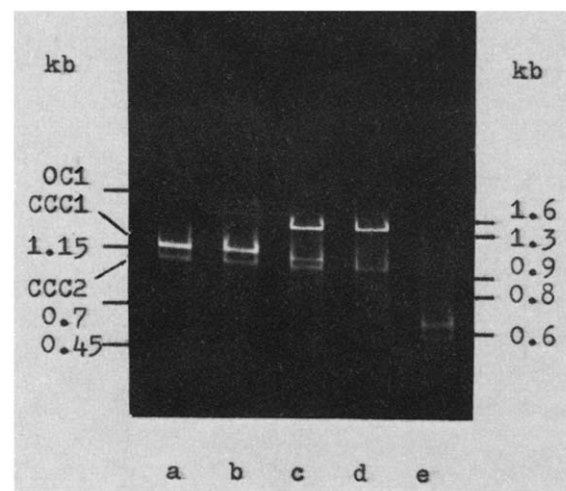


Fig.3. Fractionation of restriction fragments of minicircular DNAs in 1.5% agarose gel: (a) untreated minicircular DNAs; (b) minicircular DNAs digested with *Eco*RI enzyme; (c) with *Hin*dIII; (d) with *Eco*RI + *Hin*dIII; (e) with *Hae*III. Numbers on the right indicate lengths of phage *Hin*dIII + *Eco*RI fragments (size values given in [7]). Numbers on the left indicate lengths of restriction fragments of minicircular DNAs.

*Eco*RI one additional weak 1.6 kb DNA band appeared (fig.3a,b). The joint digestion of minicircular DNAs by *Eco*RI and *Hin*dIII produced 1.15, 0.7 and 0.45 kb DNA fragments (fig.3a,d). Analysing these results we suppose that the first type of CCC1 minicircular DNAs, CCC1A, contained one restriction site for *Eco*RI and 2 sites for *Hin*dIII. The second type of minicircular DNAs, CCC1B, was not cut by *Eco*RI, but *Hin*dIII digested CCC1B to 1.35 kb and smaller fragment(s) (fig.3b–d). *Hae*III cut all plasmid-like DNAs to short fragments with sizes of $\lesssim$0.6 kb (fig.3e).

Thus at least 3 types of ~1.6 kb minicircular DNA molecules were isolated from the normal fertile plants of *Vicia faba*. The hybridization of cloned plasmid-like DNAs with different parts of *Vicia faba* genome and with different types of *Vicia faba* RNAs are in progress.

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## References

[1] Kemble, R. J. and Bedbrook, J. R. (1980) Nature 284, 565–566.
[2] Kemble, R. J., Gunn, R. E. and Flavell, R. B. (1980) Genetics 95, 451–458.
[3] Negruk, V. I., Nikiforova, I. D. and Butenko, R. G. (1981) Dokl. Akad. Nauk SSSR 260, 1018–1021.
[4] Birnboim, H. C. and Doly, J. (1979) Nucleic Acids Res. 7, 1513–1523.
[5] Smith, H. O. (1980) Methods Enzymol. 65, 371–380.
[6] Davis, R. W., Simon, M. and Davidson, N. (1971) Methods Enzymol. 21, 413–428.
[7] Southern, E. (1980) Methods Enzymol. 68, 152–176.