Linear and 'lasso-like' structures of mitochondrial DNA from *Pennisetum typhoides*

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Previously unidentified structures of plant mitochondrial DNA, namely intact linear molecules and 'lasso-like' structures, are described. The genomic-size circular DNA is concluded to be an end product of the progression of the 'lasso' structure. These findings give insight into the heterogeneity of mitochondrial DNA unique to higher plants.

Mitochondrial DNA (Pennisetum typhoides) *DNA–protein complex* *Linear DNA structure* *Lasso-like DNA structure* *Circular DNA*

1. INTRODUCTION

Heterogeneity of mitochondrial DNA (mtDNA) molecules appears to be unique to higher plants [1,2]. The suggested source of heterogeneity is many fold:

(1) Different sizes of circular genomic DNAs [3,4];
(2) Mixed population of the different classes of mtDNA; i.e., chromosomal and plasmid-like DNA molecules in linear, open-circle and supercoil conformations [5–9];
(3) Complexity of the restriction endonuclease digest patterns [10,11].

Furthermore, in each reported case of plant mtDNA, circles only constituted a minor proportion ($\sim$ 5%), with the majority of the population being linear molecules. The linear mtDNAs have been generally considered to be produced by breakage of circles during isolation [5]. During an electron microscopic survey intended to identify size and conformation of the pearl millet mtDNA molecules, we observed previously unidentified conformations, namely, intact linear molecules and 'lasso-like' structures. The genomic-size circular DNA may be an end product of the progression of the 'lasso' structure.

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2. MATERIALS AND METHODS

Etiolated shoots of pearl millet (*Pennisetum typhoides*, cv. Gehi) were grown, mitochondria were purified, any extra-mitochondrial DNA was digested with bovine pancreas DNase I and mtDNA was isolated by phenol–chloroform extraction and ethanol precipitation as in [12]. The DNA molecules were spread using the formamide procedure in [13]. The spreading solution contained 40% formamide, 0.1 M Tris (pH 7.5); the hypophase contained 10% formamide, 0.01 M Tris (pH 8.5); and the spreading was performed at 23°C. Grids were examined and photographed in a Philips 200 electron microscope. Measurements of molecules were made on an enlarged photocopy using a computerized graphics program.

3. RESULTS AND DISCUSSION

Table 1 shows the distribution of size and conformation of the mtDNA molecules. Among a random population of 207 molecules, 53% were linear, 42% were of 'lasso' structure, and only 3% were circular. The other 2% were highly twisted, irregular supercoil molecules. Typical circular duplex DNAs of genome size (two 16.7 μm and two 12.7 μm) and of plasmid-like DNA size (one 2.1 μm and one 0.5 μm) were identified in this population. Linear molecules and 'lasso' structures

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Table 1

Distribution of size and conformation of the mtDNA

DNA conformation	Size		Sum	% Total
	< 5 μm	> 5 μm		
Linear	38	72	110	53
'Lasso'	50	36	86	42
Circle	2	4	6	3
Other	5	0	5	2
Sum	95	112	207	100
Protein complex	46	23	69	33

Molecules are grouped into two classes, small-size DNA under 5 μm (see fig.1) and larger-genome-size DNA over 5 μm (see fig.2)

were present in both the small-size (under 5 μm) and the larger-genome-size mtDNAs as shown in fig.1 and fig.2, respectively. Both linear and 'lasso' structures were considered to have been isolated intact, as shown by the protein complexes attached to one end. Close examinations of the DNA–protein complex under higher magnification revealed tertiary DNA structures such as nucleosome-like knobs and rabbit ears. The terminal protein complex was not as highly structured as capsids of adeno 2 virus and T7 bacteriophage [14,15]. It was rather amorphous and varied in size, indicating that it might be the membrane-bound protein complex. The nature of the link between proteins and DNA is not yet known, but it is resistant to our conditions of extraction (2% Sarkosyl, 100 μg pronase/ml for 60 min at 37°C) and to 3 repeats of

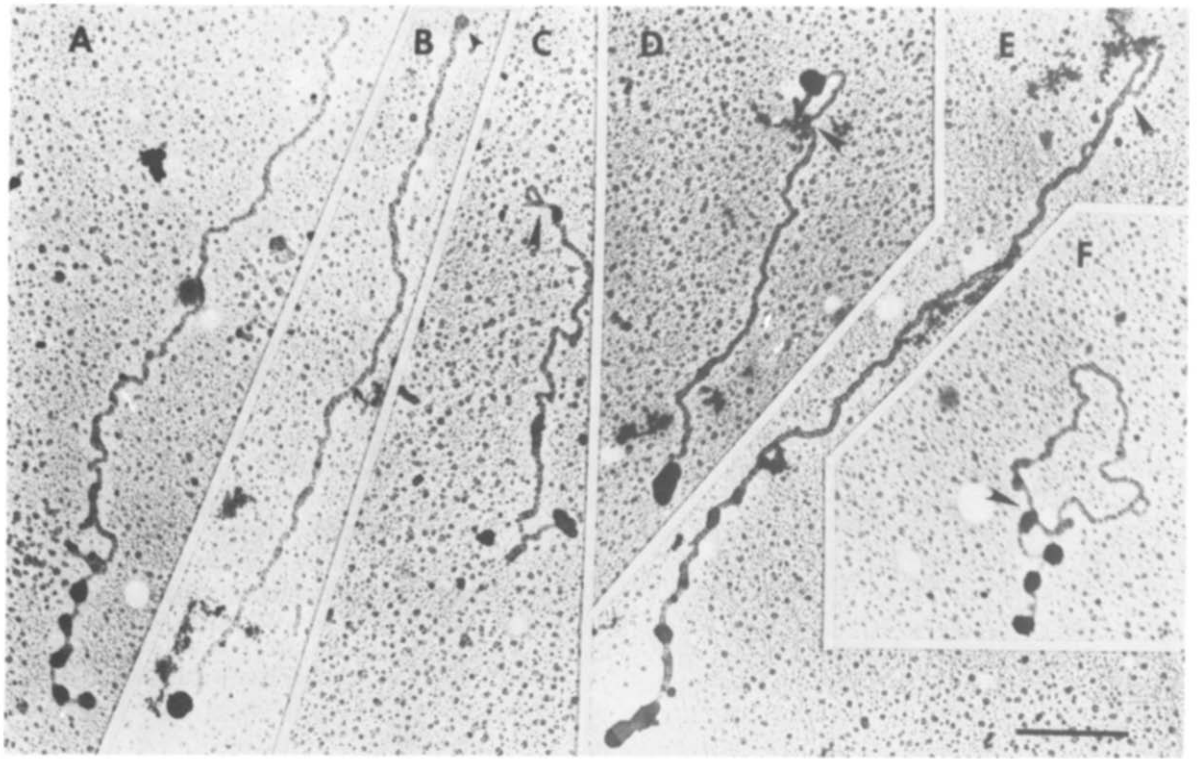

Fig.1. Electron micrograph of linear and 'lasso' structures of small-size mtDNA: (A) a linear molecule with 5 protein complexes on one end; (B) a linear molecule with a terminal protein complex and a swollen tip on the other end; (C,D) 'lasso' structures with different sizes of circle; (E) a 'lasso' structure with a small locally unwound strand below the circle; (F) a 'lasso' structure with 80% circularization. Molecular sizes, measured as circle plus tail, are: (A) 4.1 μm; (B) 3.6 μm; (C) 2.9 μm; (D) 2.7 μm; (E) 4.7 μm; (F) 3.6 μm. Arrowheads indicate the forks. Bar is 0.5 μm.

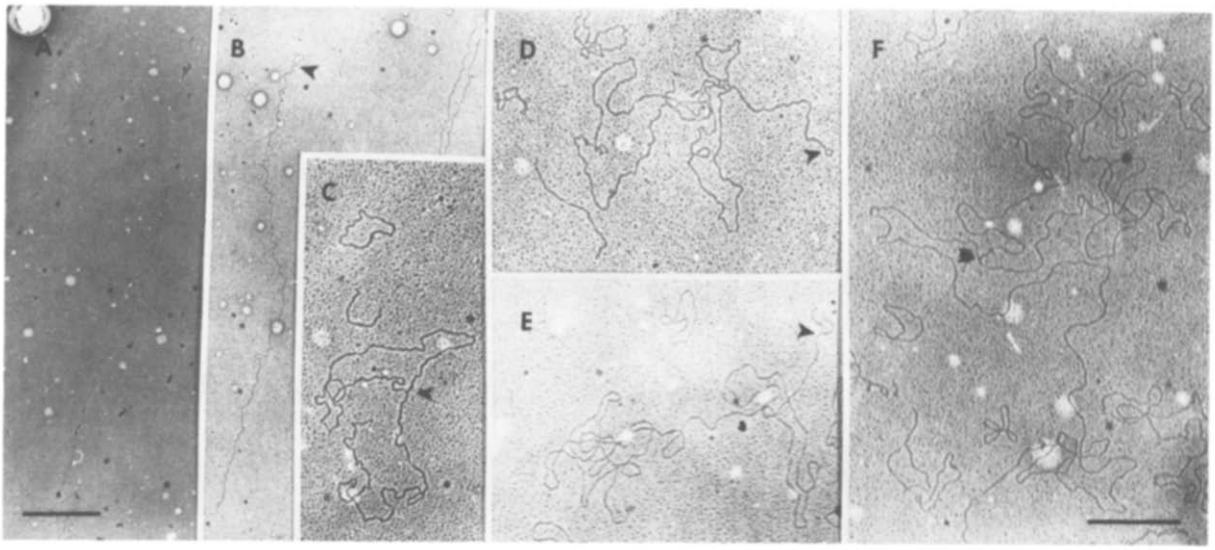

Fig.2. Electron micrograph of linear and 'lasso' structures of large-genome-size mtDNA: (A) intact linear molecule with a terminal protein complex; (B–F) 'lasso' structures with varying degrees of circularization. The small circular DNA molecules are pBR322, included in each spread as an internal size marker (4362 basepairs or 1.48 μm). Molecular sizes, measured as circle plus tail, are: (A) 7.3 μm; (B) 7.0 μm; (C) 5.7 μm; (D) 8.0 μm; (E) 9.3 μm; (F) 18.3 μm. Arrowheads indicate the forks. Bars are 1.0 μm for A,B and 0.5 μm for C–F.

phenol–chloroform phase separation.

Linear mtDNA molecules were not of a unit size, but were broadly heterogeneous in size, ranging from as small as 1.6 μm to as large as 42 μm. One cannot rule out the possibility that the molecules beyond the lower and upper limits have been overlooked. It is concluded that direct measurement of the contour lengths of linear and 'lasso' molecules may not represent the true values, because close examination of the electron micrographs revealed that varying proportions of the DNA strand are entrapped by protein complexes as condensed knobs (see also [15]) and the twists in linear molecules cause regional protrusions from the supporting plane of nitrocellulose membrane (Koller, Th., personal communication). Because of the broad heterogeneity in size and conformation, small-size linear and 'lasso' structure DNAs did not resolve as discrete bands in agarose gel electrophoresis.

The circles of the 'lasso' structures varied in size from very small up to almost full circle, as shown in fig.1 and fig.2. The swollen tip of the linear DNA molecule in fig.1B is indicative of the initiation of the growing circle. Measurement of the circle and the tail in relation to the unit length would normally assist in understanding the origin of the 'lasso' structure [16,17]. However, no significant relationships between circle and tail were detected because of the broad heterogeneity of the DNA molecules. The fork regions of most of the molecules were double-stranded and completely closed. However, in a few small-size molecules the forks were loose as if the tight supercoil had been locally unwound. Also, note in fig.1E a local unwinding below the circle. The 'lasso' structure has been described only in a limited number of systems [16–18]. Even though direct experimental evidence is lacking, several proposals have been made that the 'lasso' structures might be involved in the replication fork of the mtDNA of *Paramecium* [16], circularization of the linear MVM DNA [17], and transposition of bacteriophage Mu [18]. We obtained electron microscopic evidence suggesting the direct involvement of a 'lasso' structure in DNA replication (to be reported elsewhere). If the reported linear-'lasso' structures represent replicative forms, the genesis of the circular mitochon-

drial genome of pearl millet becomes apparent. Our data with pearl millet mtDNA are consistent with the heterogeneity of size and conformation in higher plant mtDNA. Concatemerization, recombinatorial and transpositional rearrangements, as well as amplification, may all be major contributors to plant mtDNA heterogeneity [4,19]. Findings of intact linear molecules and 'lasso' structures in pearl millet mtDNA in this study, however, provide fresh insight into the formation of various mtDNA species. We are now in a position to study systematically the molecular mode(s) of replication in higher plant mtDNA. In-depth study of the DNA structure at the fork of the 'lasso' structure in relation to its formation and progression remains an intriguing and challenging problem. Isolation of the DNA–protein complex emphasizes the dynamic functional features of mtDNA molecules.

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