Organization of the mitochondrial ribosomal RNA genes of maize

David B. Stern, Tristan A. Dyer and David M. Lonsdale

Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, UK

Received 6 April 1982; Accepted 10 May 1982

ABSTRACT

The organisation of the mitochondrial ribosomal RNA genes in maize is described. Each of the rRNAs is encoded by a single gene. The 5S and 18S rRNA genes are close together, and separated from the 26S rRNA gene by 16 kb of DNA. There is no evidence of heterogeneity in this gene arrangement.

INTRODUCTION

Mitochondria are semi-autonomous organelles, having a unique translation system that uses specific mitochondrial encoded transfer, messenger and ribosomal RNAs (rRNA). All mitochondria contain two high-molecular-weight (HMW) rRNAs, the larger of which is approximately twice the size of the smaller. Only in higher plant mitochondria, however, has a small (5S) rRNA component been detected (1), a feature unique to plant mitochondrial ribosomes, though common to all other non-mitochondrial types of ribosome.

The mitochondrial DNA (mtDNA) sequences coding for rRNAs have been mapped in a number of organisms. In some, among them Drosophila melanogaster and Neurospora crassa, the genes for the rRNAs are close together and probably co-transcribed (2-6). In the human and mouse mtDNA, all the bases between the two rRNA genes form a tRNA molecule which is excised during a maturation process (7,8). However, these genes in Aspergillus nidulans are separated by 5 kb, and in yeast by 15 kb (9,10). Thus, there is considerable variation in rRNA gene organisation.

In wheat mitochondria, the genes for the two HMW rRNAs (18S and 26S) are far apart, with the 5S rRNA and 18S rRNA genes close together (11). In maize, we show that the 5S rRNA gene is indeed closely linked to the 18S rRNA gene, and 16 kb away from that of the 26S rRNA. Each gene is present in a single copy. This represents the first physical mapping of the rRNA genes in plant mitochondria.
MATERIALS AND METHODS

a) Extraction of maize total RNA

*Zea mays* WF9-N seeds were surface-sterilized for 30 seconds in 5% sodium hypochlorite, washed 5-10 times in sterile, deionized water and imbibed overnight. Seeds were germinated in the dark at 30°C, in sterile porcelain trays lined with dampened paper towels.

RNA was extracted from 5-7 day old coleoptiles as described {12}, except that instead of CsCl centrifugation, the nucleic acids were suspended in 10 ml 0.15 M sodium acetate and 0.65 M sodium chloride, pH 5.6 and centrifuged for 3 h at 260,000 x g at 2°C (MSE 10 x 10 ml Ti rotor). The pellet, containing the HMW RNA, was resuspended in 1 ml 0.15 M sodium acetate, 0.5% SDS pH 6, and 2 ml ethanol were added. The RNA was stored as a suspension at -20°C, and suitable aliquots were removed for electrophoresis.

b) Preparation of mtRNA

Mitochondria were purified from 5 day old dark-grown coleoptiles on sucrose gradients {13}. The mitochondria were gently resuspended in 3 volumes of a solution containing 0.2 M mannitol, 10 mM Tricine pH 7.2, 1 mM EGTA. Following centrifugation at 10,000 rpm for 20 min at 4°C (MSE High Speed 18), the pellet was resuspended in a buffer containing 50 mM Tris-HCl, 10 mM EDTA pH 8.8. SDS was added to 2% and the aqueous phase extracted twice with equal volumes of phenol before ethanol precipitation of the RNA.

c) Mitochondrial DNA cosmid bank

The cosmid bank established from WF9-N maize mtDNA {14} was screened to identify cosmids containing the rRNA genes. Cosmid DNA was isolated from 100 ml cultures as previously described {14}.

d) Preparation of cosmid DNA fragments

10-20 pg of cosmid DNA was digested with an appropriate endonuclease, and then electrophoresed overnight in a 30 ml 1% agarose tube gel containing ethidium bromide (250 x 13 mm). The fragments were located with long wave ultraviolet light, and the bands excised. DNA was isolated as described {15}.

e) Labelling of nucleic acids and hybridizations

DNA was labelled by nick translation {16,17} using {α-32P}dATP (Amersham International Ltd., 400 Ci/mmol). RNA was 5'-labelled following partial alkaline hydrolysis {18} with γ-32P ATP (Amersham International Ltd., 2,000 Ci/mmol) and polynucleotide kinase. Labelled DNA was separated from unincorporated nucleotides on a 1.2 ml Sephadex G100 column.

RNA was fractionated in agarose/2.2 M formaldehyde denaturing gels {19} and transferred to nitrocellulose (Schleicher-Schüll, BA-85) in 10 x SSC.
5 mm x 10 cm strips of the nitrocellulose were prehybridised for at least one hour in 15 ml screw-top tubes (21) in 1.5 ml of 50% deionized formamide/5 x SSC/10X Denhardt’s containing 15 μg/ml sonicated, denatured salmon sperm DNA. The labelled DNA probe was then added to the tubes which were shaken rapidly at 42°C for 16-48 hr before washing and autoradiography (Kodak XAR-5 film, -80°C).

DNA-DNA hybridizations were carried out as described elsewhere (22,23) following gel electrophoresis and transfer to nitrocellulose (20). The mitochondrial rRNAs were sized by comparison to cytosolic and chloroplast rRNAs (24,25) and the chloroplast ribulose-1,5-bisphosphate carboxylase large subunit mRNA (26).

Enzymes were purchased from Bethesda Research Ltd., and digests were carried out under conditions specified by the manufacturer.

RESULTS

1. Identification and mapping of cosmids containing ribosomal genes

As the rRNA forms by far the largest proportion of the mtRNA, the total RNA without fractionation can be used as a probe for the rDNA sequences. The cosmid bank (14) was therefore probed with 5'-labelled mtRNA to identify the cosmids containing these sequences. Out of a bank of 160 clones, 23 hybridized to 5'-labelled mtRNA. DNA prepared from these cosmids was digested with the restriction endonucleases Sst II and Sma I and a restriction map constructed. Probing of these restriction digests and digests of total mtDNA identified several fragments which hybridized to 5'-labelled mtRNA (Fig. 1). These fragments could be classified into two groups on a contiguous physical map, and the rDNA cosmids containing these fragments were of three types. One type, of which 2c32 is typical, contained both rRNA hybridizing regions, demonstrating their physical linkage (see Fig. 3, track 3). The other two types of cosmids were identified as containing only one of the two rRNA hybridizing regions, e.g. 2c13 and 2c21. The two regions hybridising to 5'-labelled mtRNA were separated by 16.1 kb (Fig. 2).

In addition to the restriction fragments containing the rRNA genes, weaker hybridization (<5%) to 1.58 kb and 1.75 kb Sst II fragments was observed when mtDNA was probed with 5'-labelled mtRNA (see Fig. 1). These two Sst II restriction fragments are known to be contiguous on the physical map and a minimum of 50 kb from the rDNA region. The cosmids which contain these fragments, typified by 2c7, do not hybridise to the 18S mitochondrial
rRNA transcript (see Fig. 3, track 1).

2. Identification of the 26S and 18S rRNA genes

The genes coding for HMW RNAs were localized by probing total RNA with the cosmids containing the mitochondrial rDNA (Fig. 3). Hybridization of 2c21 to HMW RNA identified a transcript of approximately 1925 nucleotides (18S rRNA - Fig. 3, track 2). In contrast, cosmid 2c13 hybridized to a 3580 nucleotide transcript (26S rRNA - Fig. 3, track 4). Therefore, it would appear that the 26S rRNA gene is in 2c13 and the 18S rRNA gene in 2c21. Cosmid 2c32 hybridized to both transcripts (Fig. 3, track 3), con-
firing the linkage of the two genes on the physical map (Fig. 2).

The other minor hybridizations to 2c13 and 2c32 are probably specific breakdown products of 26S rRNA. Additionally, when total RNA is probed with 2c7, 2c21 or 2c32 (Fig. 3), minor hybridization to an RNA of 1541 nucleotides is observed. This RNA has the same electrophoretic mobility as the chloroplast 16S rRNA, the presence of which is demonstrated when total RNA is probed with the cloned chloroplast 16S rDNA sequence P6 [24]. The homology between 2c7 and the chloroplast 16S rRNA will be discussed elsewhere (Stern & Lonsdale, manuscript in preparation).

3. Localization of the 5S rRNA gene

In order to determine the position of the mitochondrial 5S rRNA gene, rDNA cosmids were used to probe total mtRNA which had been fractionated in 2% agarose/6% formaldehyde denaturing gels, and transferred to nitrocellulose. Only cosmids 2c21 and 2c32, and not 2c13, hybridized to a low-molecular-weight (LMW) mtRNA, thus demonstrating that the 5S rRNA gene is close to that of the 18S rRNA, and not to the 26S rRNA gene.

The 1.52 kb, 1.16 kb and 5.4 kb Sst II fragments of cosmid 2c21 all hybridized to the 1925 nucleotide mitochondrial transcript and to chloroplast 16S rRNA (data identical to Fig. 3, track 2). Because of the arrangement of these fragments, it can be assumed that the 1.16 kb Sst II fragment is an internal fragment of the 18S rRNA gene. Therefore, to
identify the 5S rRNA coding sequence mtRNA was probed using the isolated 1.52 kb and 5.4 kb Sst II fragments. The 1.52 kb Sst II fragment hybridized to a LMW RNA species but the 5.4 kb fragment did not (Fig. 4). This RNA comigrates with wheat cytosol 5S rRNA (121 nucleotides) which was identified using the probe pTa704 which contains cloned nuclear 5S rDNA [27].

4. Ribosomal RNA gene structure

The total length of the 5S-18S rDNA region, as estimated from densitometric scans of the autoradiograph shown in Figure 1, is about 2158 base pairs. This agrees well with the estimated nucleotide content of the 5S and 18S rRNAs (2046 nucleotides). As the exact position of the coding sequences for the 18S and 5S rRNAs within the 1.52 kb and 5.4 kb Sst II fragments cannot be exactly determined from our data, the existence of one or more introns in the 18S rRNA gene cannot be excluded. However, as the 26S rRNA gene occupies only the Sma I/Sst II fragment (Fig. 2), it cannot contain any large introns.

DISCUSSION

The plant mitochondrial genome is unique in several respects. In maize mtDNA, for example, there are several repeated sequences [14 and Lonsdale and Hodge, unpublished data] and the genome size of plant mitochondria is much larger than that found in mitochondria of other types of organisms [28,29]. Also, it contains a 5S rRNA gene. In fact, one of the few

Figure 4. Mitochondrial RNA Fractionated in a 2% agarose/ 2.2 M formaldehyde gel and probed with 2c13 (1), 5.4 kb Sst II Fragment from 2c21 (2), pTa704 (3) and 1.52 kb Sst II fragment from 2c21 (4).
features that is shared between most mitochondrial genomes is that they contain single copies of the rRNA genes (2-10). The exception to this is *Tetrahymena* mtDNA, which has two copies of the 21S rRNA gene (30).

In plants, studies on the rRNA gene structure indicate that both chloroplast and mitochondrial rDNAs had a prokaryotic progenitor. This is demonstrated here by the cross-hybridization between the chloroplast 16S rRNA and the cloned mtDNA 2c21, and is substantiated by the prokaryotic nature of both these RNAs as shown by sequence studied (31-35). But whereas the chloroplast rRNA genes are in exactly the same orientation as in prokaryotes (5' 16S+23S+5S 3') (36-44), as shown here, the plant mitochondrial rRNA genes are arranged quite differently.

We have no evidence that the rRNA genes occur in any other configuration in the mitochondrial genome of maize, indicating that the genome is not heterogenous (45,46).

**ACKNOWLEDGEMENTS**

We would like to thank Mr. T.P. Hodge for his excellent technical assistance, the Agricultural Research Council for supporting this work and Pioneer Hibred International for supplying the seed stocks from which the material necessary for this work was grown. D.B.S. is a Winston Churchill Foundation Fellow.

**REFERENCES**