Transcription Initiation Sites in Mitochondria of *Oenothera berteriana*

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Toward the identification of promoter structures in dicot plant mitochondria, transcription initiation sites were localized by *in vitro* capping of mitochondrial RNAs from *Oenothera berteriana*. By this approach at least 15 initiation sites are detectable in the genome. Primary 5′-transcript termini were determined for coxII, atpA, and an 18 S rRNA precursor. Alignment of the sequences surrounding the transcript termini defines a conserved sequence block of 28 nucleotides between positions -20 and +9. A core of 9 nucleotides between positions -7 and +2 (5′-CRTAg3′) is conserved with soybean transcription initiation sites. All transcripts analyzed begin with the underlined G–dinhucleotide. Transcription initiation of these protein coding and ribosomal RNA genes at similar sequence elements suggests common binding specificities of the RNA polymerase or its cofactors for both types of genes. The putative promoter consensus motif for dicots is very different from monocot mitochondrial promoter sequences. Only the 5′-CRTA-3′ tetranucleotide is conserved between both plant groups.

Mitochondria have retained a number of characteristics of prokaryotic organisms that are presumably related to the endosymbiotic ancestors of these organelles (Gray, 1989). Such prokaryotic features include primary gene sequences and aspects of gene expression, including transcription and translation processes. Transcription initiation signals, however, appear to be distinct from those in prokaryotes (Gray, 1989; Gray et al., 1992).

Experimental analyses of transcription initiation in mitochondria took advantage of the observation that transcripts in these organelles are as in prokaryotes not capped *in vivo*. This allows specific identification of initiation sites by adding labeled cap structures specifically to primary 5′-transcript termini. Transcription and particularly promoter structures have been extensively investigated in mitochondria of mammal and fungi, but very little is known about plants in this respect (Chang and Clayton, 1984, 1986a, 1986b; Chang et al., 1986, 1987; Clayton, 1991; Hixson and Clayton, 1985), while about 20 promoter sites have been identified on the 75-kb *Saccharomyces cerevisiae* mitochondrial DNA. These initiation sites are characterized by a conserved sequence motif of 9 nucleotides that is sufficient for promoter function *in vitro* (Christianson and Rabinowitz, 1983; Costanzo and Fox, 1990; Edwards et al., 1982; Schinkel and Tabak, 1989). The large size of the plant mitochondrial genomes (200–2400 kb) suggests that similarly several promoters are required to direct transcription of the numerous genes encoded in this organelle.

Plant mitochondrial promoters have been identified in the monocots wheat and maize by *in vitro* capping analyses and *in vitro* transcription experiments (Covello and Gray, 1991; Gray et al., 1992; Mulligan et al., 1988a, 1988b; Rapp and Stern, 1992). A loose consensus sequence has been defined by conserved nucleotide identities at the sites of transcript initiation in these two monocots. Correct initiation at these sites was confirmed by *in vitro* studies (Hanic-Joyce and Gray, 1991; Rapp and Stern, 1992). One of the maize mitochondrial promoters has been further characterized *in vitro* by mutagenesis of the surrounding sequences. These deletion studies showed a core sequence of 11 nucleotides to be essential for initiation in *vitro* (Rapp and Stern, 1992). Virtually nothing is known about mitochondrial promoter sequences in dicot plants, but multiple promoters are expected for transcription of the numerous genes. The genome size of *Oenothera* is currently estimated to be about 400 kb, large enough to encode more than 100 genes. In the 186-kb *Marchantia* mitochondrial genome 34 potential genes have been located (Oda et al., 1992), suggesting the number in higher plant mitochondria to be in this range, with large parts of the remaining DNA taken up by nuclear and plastid sequences (Schuster and Brennicke, 1988).

So far only transcripts of the atp9 gene in soybean have been successfully capped *in vitro* (Brown et al., 1991). Three other small RNA molecules could also be analyzed in this plant, but their function remains unclear. Comparison of the soybean atp9 primary transcript end with steady state 5′-transcript termini of other dicots like *Oenothera berteriana* and *Petunia hybrida* revealed sequence similarities at some of these termini (Brown et al., 1991; Schuster et al., 1987). Their definite identification as primary or processed transcript termini, however, requires experimental verification.

To obtain more information about mitochondrial transcription initiation sites in dicot plants we investigated 5′-transcript termini in *O. berteriana* mitochondria by *in vitro* cap-
pigin. In the experiments reported here primary transcript termini of two mRNAs and one rRNA precursor could be identified. From these data a putative dicot promoter consensus sequence can be defined that shows only a core of four nucleotides conserved with the consensus proposed for monocot mitochondrial promoters (Gray et al., 1992).

**EXPERIMENTAL PROCEDURES**

**Mitochondrial Nucleic Acids—**Mitochondrial DNA was prepared from *O. berteriana* tissue culture cells as described previously (Schuster et al., 1988). For isolation of RNA, mitochondria were purified by differential centrifugation and a discontinuous Percoll gradient (Marchfelder et al., 1990). Mitochondria were lysed in a buffer containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% Sarkosyl, and 0.1 M β-mercaptoethanol. After incubation at room temperature for 5 min 0.1 volume of 2 M NaOAc (pH 4.0), 1 volume of water-saturated phenol, and 0.2 volumes of chloroform:isoamyl alcohol (24:1) were added and mixed extensively. The sample was kept on ice for 15 min and centrifuged in a Beckman SJ 13.1 rotor at 10,000 rpm for 15 min. RNA was precipitated from the aqueous phase by addition of 1 volume of isopropyl alcohol and incubation at -20 °C for at least 2 h prior to centrifugation as above. On average between 200 and 400 μg of mitochondrial RNA were obtained from 1.5 kg of callus culture cells.

**Gene-specific Clones—**Mitochondrial DNA fragments containing the genes coxII, atpA, atpB, and 18 S rRNA and/or the respective upstream sequences were cloned and subcloned in pBR322, pUC19, or pBluescript vectors following standard protocols (Sambrook et al., 1989).

**Blotting and Hybridization Conditions—**For Southern blot and dot blot analysis GeneScreen+ (Du Pont) membranes were used according to manufacturer's instructions. In hybridizations with *in vitro* capped RNA, 100 μg of the labeled mitochondrial RNA were used.

**In Vitro Capping—**The *in vitro* capping reactions were carried out in a volume of 20 μl with 100 μg of mitochondrial RNA, 50 mM Tris-HCl (pH 7.9), 1.25 mM MgCl2, 2.5 mM dithiothreitol, 80 units of RNase inhibitor, 1 μM GTP, 250 μCi of [α-32P]GTP (3,000 Ci/mmol), and 15 units of guanylyltransferase for 30 min at 37 °C. The reaction was terminated by adding 20 μg of proteinase K and digestion for 15 min at 37 °C. RNA was purified by extraction with 0.5 volumes of water-saturated phenol and 0.5 volumes of chloroform:isoamyl alcohol (24:1) and by two extractions with 1 volume of chloroform:isoamyl alcohol (24:1). RNA was precipitated from the aqueous phase by addition of ammonium acetate to a final concentration of 2.5 M and 70% ethanol. Most of the unincorporated label was removed by three washes with 70% ethanol.

**RNase Protection—**About 6 μg of cap-labeled mitochondrial RNA and 1 μg of antisense RNA (or 500 pg to 1 ng of antisense oligonucleotide, respectively) were denatured at 75 °C for 10 min in 30 μl of hybridization buffer containing 40 mM Pipes (pH 6.4), 0.4 mM NaCl, 1 mM EDTA, and 80% formamide. Hybridization between sense and antisense RNAs was allowed to proceed overnight at 30-45 °C with the temperature adjusted accordingly for the oligonucleotides. For the RNase reaction, 300 μl of mitochondrial RNA mixture (0.3 μM Nac, 10 mM Tris-HCl (pH 7.4), 5 mM EDTA, 700 units/ml RNase T1, 40 μg of RNase A) were added and incubated at 30 °C for 1 h. The reaction was terminated by phenol/chloroform extractions and ethanol precipitation.

**Miscellaneous Methods—**Primer extension experiments were done following standard protocols (Sambrook et al., 1989). Primers used in these experiments were, coxII, 5'-GAATTCTAAAAAAGGAAAA-GCGG-3' with the 3' terminal nucleotide located at +49 downstream from the 5' end of the mRNA; atpA, 5'- CGTGGCTCC- AACGAACCTG-3' with the 3' terminal nucleotide located at +51 downstream of the mRNA 5' end; 18 S rRNA, 5'-CTTGGATTCTC- TCTGTAGTC-3' with the 3' terminal nucleotide located at +49 downstream of the 5' end of the 18 S rRNA precursor. DNA sequencing was performed with a T7 sequencing kit (Pharmacia).

**RESULTS**

**Capping of Mitochondrial RNA—**Cap structures are added *in vivo* to most of the nuclear primary transcripts that specifically contain di- or trinucleotides at their 5' termini. Since mitochondrial transcripts are generally not capped *in vivo* this reaction can be used *in vitro* to directly identify sites of transcription initiation in these genomes.

Experiments with different mitochondrial RNA preparation and isolation procedures showed the efficiency of the *in vitro* capping reaction to be very sensitive to contaminants. Purification of mitochondria through Percoll gradients, for example, proved superior to sucrose gradients in removing plant compounds inhibitory to the labeling reaction. Mitochondrial RNA thus isolated reproducibly resulted in a number of RNA species with an *in vitro* added cap structure. RNA molecules up to sizes of 2 kb could be identified in autoradiographs. Most of these RNAs, however, do not incorporate enough radioactivity to allow further analysis or even direct sequencing. One of the smaller RNA species consistently copurified with the mitochondrial RNA and capped efficiently is probably the nuclear encoded 5 S rRNA (data not shown).

**Minimal Number of O. berteriana Mitochondrial Promoters—**The number of transcription initiation sites in the mitochondrial genome can be estimated from the number of genomic regions from which primary transcripts originate. To determine this number, total mitochondrial RNAs were labeled by *in vitro* capping and hybridized to Southern blots of restricted total mitochondrial DNA. Between 10 and 15 prominent and several less intensively labeled fragments, together between 12 and 18 fragments, are detected in each of the different digests (Fig. 1). RNase digestion of the hybridized blots did not alter the number of hybridizing fragments, probably due to the rather small size of the capped transcripts.

**Fig. 1.** Hybridization of capped mitochondrial RNA reveals a minimum of 12-18 sites of transcription initiation in *O. berteriana* mitochondria. Restriction digests of total mitochondrial DNA (3 μg each) with EcoRI (lane 1), BamHI (lane 2), and HindIII (lane 3) were size fractionated in an agarose gel, blotted, and hybridized with cap-labeled RNA. RNase A and T1, digestion of the blot did not alter the number of hybridization signals, most likely due to the small size of the capped RNA species. The sizes of DNA length standards are given in kb.
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Fig. 2. Localization of the site of transcription initiation for the coxII gene. Restriction digests of clone pC1 containing the coxII coding region and adjacent sequences were blotted and hybridized with capped mitochondrial RNA. The autoradiograph of the blot on the left hand side shows digests of EcoRI/BstEII in lane 1, BstEII/HpaI in lane 2, EcoRI/HpaI in lane 3, and EcoRI/PstI in lane 4. Sizes of marker DNA fragments are given in kb on the left margin. The fragments hybridizing with the capped RNA are indicated as black bars under the restriction site map on the right. Restriction sites are given for EcoRI (E), BstEII (Bs), HpaI (Hp), and PstI (P). The dotted vertical lines border the BstEII/HpaI fragment as the smallest hybridizing fragment containing the transcription initiation site.

This observation suggests each hybridization signal to be derived from transcription initiation sites within the DNA fragments (not shown). Assuming that each transcription initiation site is connected with a bona fide promoter, their number in the *O. berteriana* mitochondrial genome can be estimated to be around 15. Some of the primary transcripts may be processed too rapidly to be detectable in this assay, while other hybridization signals may be derived from duplications of the promoter sequences. The comparatively few large RNA species labeled in the in vitro capping reaction, however, suggest processing or degradation to be the major sources of error in this estimation. Multiple promoters may be present on large restriction fragments that are not resolved in such experiments. Therefore the estimated 15 promoters have to be considered a minimal number in this genome.

Correlation of in Vitro Capped Transcripts with Specific Genes—Primary transcripts of individual genes were identified by hybridizing the in vitro cap-labeled RNA to dot blots with clones containing specific mitochondrial genes (not shown). The strongest signal was obtained for the coxII gene coding for subunit II of the cytochrome oxidase. Comparatively high concentrations of cappable transcripts were found for several of the ATP genes encoding subunits of the mitochondrial ATPase. In descending order of signal strength primary transcripts for atpA, atp9, 18S rRNA, and atp6 were identified.

The genes with the most abundant detectable primary transcripts were investigated further to define their initiation sites on the genomic DNA.

Transcription Initiation of coxII—A single 1250-nucleotide transcript of the coxII gene is found in the steady state mRNA in *O. berteriana* mitochondria (Hiesel and Brennicke, 1983, 1985). To identify the genomic region where primary transcript(s) of coxII originate, restriction digests of the cloned coxII coding and upstream sequences were hybridized with capped mitochondrial RNA (Fig. 2). The most distal upstream hybridization signal was located on a 0.5-kb BstEII/HpaI restriction fragment.

The primary transcript ends were determined by RNase protection of the capped mitochondrial RNA with in vitro synthesized coxII antisense RNAs covering the BstEII/HpaI fragment (Fig. 3). A synthetic oligonucleotide covering the region of the 5' end in antisense orientation was used for further fine mapping and confirms the 5' end of the primary transcript to be about 210 nucleotides upstream of the open reading frame (not shown). The in vitro capped RNA terminus coincides with the 5'
Terminus of the steady state RNA. Previous S1 protection experiments had identified the steady state transcript terminus in this region (Hiesel and Brennicke, 1985). Examination of the precise 5' ends by primer extension locates the transcript ends at the guanosine, 207 nucleotides upstream of the open reading frame, 4 nucleotides shorter than previously estimated from nuclease protection (Fig. 3).

The data obtained for the primary RNA end by S1 protection, primer extension, and RNase protection are confirmed by direct sequence analysis of a small capped RNA species (data not shown). This RNA was isolated from a preparative gel separation of in vitro capped total mitochondrial RNA as one of the best labeled small RNAs and thus amenable to sequencing. Sequence analysis revealed this RNA to be derived from the coxII locus, presumably a distinct degradation product of the 5' end of the coxII mRNA, with the 5'-nucleotide located at the position identified by the other methods.

Transcription Initiation of atpA—The steady state transcript of the atpA gene in O. berteriana mitochondria is identified as a single 2100-nucleotide species (Schuster and Brennicke, 1986). The 5' end of this RNA has been mapped precisely by primer extension experiments to a guanosine residue 209 nucleotides upstream of the open reading frame (Fig. 4). In the same region hybridization of capped mitochondrial RNA identified the site of transcription initiation within a 200-base pair SpeI/BglII restriction fragment upstream of the atpA open reading frame (Fig. 4). RNase protection of the capped RNA with a synthetic antisense oligonucleotide covering the region of the 5' end of the steady state transcript located the exact 5'-primary transcript end to the 5' -nucleotide of the steady state mRNA (data not shown). The single atpA transcript of 2.1 kb identified in Northern blot experiments thus appears to be unprocessed at the 5' end in O. berteriana mitochondria.

Transcription Initiation of atp9—As in mitochondria of most other higher plants, a number of steady state transcripts are observed for the atp9 gene in O. berteriana. In maize mitochondria several promoters are responsible for some of the different 5' ends (Mulligan et al., 1988b). In O. berteriana, two different 5'-transcript termini have been mapped upstream of atp9, the proximal of which was proposed to originate from a processing event (Schuster and Brennicke, 1989; Schuster et al., 1987).

Hybridization with capped RNA to restriction digests indicates several primary RNA termini upstream of the open reading frame (not shown). Multiple RNA termini are also detected by primer extension and as yet did not allow unambiguous identification of transcription initiation sites for this gene. Transcription of the atp9 gene in O. berteriana thus appears to be rather complex with at least two transcription initiation sites and additional processing events (Schuster and Brennicke, 1989) that require more detailed analysis for definition.

Transcription Initiation of 18 S rRNA—The O. berteriana gene for the 18 S rRNA is transcribed into a 5'-extended precursor molecule. The 5' end of this precursor had been

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FIG. 5. Identification of the transcription initiation site for the 18 S rRNA precursor. Hybridization of capped RNA to restriction fragments of the 18 S rRNA gene identifies fragments on both sides of the PstI restriction site immediately upstream of the mature 5' terminus (lane 1 in the blot on the left hand side and bar 1 under the map of clone H5/2 in the center part). Hybridization downstream of the PstI site is most likely due to unprocessed precursor transcripts. Further analysis of subclone 5/2-1 from the upstream region revealed that the smallest region hybridizing with capped RNA was a 120-base pair SfuI/PstI fragment (indicated by the dotted vertical lines), which is not visible in this reproduction. Fragments identified in the different digests are shown as black bars under the map and were determined in digests with HindIII/PstI (lane and fragment 1) of clone H5/2 and BamHI/SfuI (lane and bar labeled 2), PstI/SfuI (lane and bar labeled 3), and BamHI/SpeI (lane and bar 4) of subclone 5/2-1. Sizes of the DNA length standard are given in kb. Restriction sites shown in the map are BamHI (B), HindIII (H), PstI (P), SfuI (Sf), and SpeI (Sp). The right hand side shows a primer extension gel of the rRNA precursor, determining the exact first nucleotide as the G, 125 nucleotides upstream of the mature 18 S rRNA marked with an arrow above the sequence (bottom part).

mapped previously by S1 protection and has now been confirmed by primer extension experiments (Fig. 5). Hybridization of capped mitochondrial RNA to cloned restriction fragments of this region show this terminus to be derived from de novo initiation of transcription. The smallest hybridizing restriction fragment of 120 nucleotides is located between the first transcribed nucleotide of the precursor (the G residue at position -125) and the 5' terminus of the mature 18 S rRNA (Fig. 5). The amount of capillary precursor transcripts was not sufficient for RNase protection experiments and more precise mapping. The absence of any hybridization signals with capped RNA further upstream correlates with the absence of any other, especially larger, primer extension signals. This observation together with the small size of the identified fragment are considered strong evidence for this precursor to be the 18 S rRNA primary transcript.

Hybridization of capped RNA with the restriction fragment downstream of the mature end of the 18 S rRNA is most likely derived from unprocessed precursor RNA (Fig. 5, lane 1). A second promoter at the mature 5' end cannot be excluded, but this region shows no similarity with any other identified transcript initiation sites. The mature 18 S rRNA must be excised from this precursor by both 5' and 3' endonucleolytic cuts since the 18 S rRNA is cotranscribed with the 5 S rRNA encoded further downstream (Wissinger et al., 1988).

DISCUSSION

Number of Transcription Initiation Sites in O. berteriana Mitochondria—The number of promoters in the large plant mitochondrial genomes is unclear to date. The multiple primary RNA ends detectable in O. berteriana mitochondria allow an estimation of promoter frequency in this genome. The 15 identified sites of transcription initiation must be considered a minimal number since many primary transcripts may not be detectable by capping like in soybean, where relatively few sites of transcription initiation are detected by this approach (Auchincloss and Brown, 1989; Brown et al., 1991). A likewise relatively small number of mitochondrial RNAs can be capped in the monocots maize and wheat, where 11 and 4 respective transcription initiation sites could be assigned to specific genes (Covello and Gray, 1991; Kennell and Pring, 1989; Mulligan et al., 1988a, 1988b).

Compared with the 20 identified promoters on the yeast mitochondrial genome of about 75 kb, the 15 transcription initiation sites for the approximate 400-kb mitochondrial DNA of O. berteriana, with many more genes, appears to be rather small. A limited number of plant mitochondrial promoters requires large polycistronic transcription units as those observed in mammalian mitochondria (e.g. Chang and Clayton, 1986a, 1986b). Plant mitochondrial multicistronic mRNAs have indeed been observed, as for example, the O. berteriana 18 S rRNA-5 S RNA-nad5 exons a and b cistron (Wissinger et al., 1988), but many genes like the open reading frames for coxII and atpA are transcribed into monocistronic mRNAs (Hiesel and Brennicke, 1983, 1985; Schuster and Brennicke, 1986). Closely spaced multiple promoters for some genes as detected, e.g. for atp9 in maize mitochondria, further increase the possible number of promoters (Mulligan et al., 1988a, 1988b).
sequences derived from two RNA termini (Brown et al., 1991). Comparison with the soybean sequence shows considerable from the dicot sequence (Covello and Gray, 1991; Gray et al., 1992). Conserved sequence blocks at the termini of primary transcripts are indicative of some function in promoting transcription initiation. The conserved sequence motif derived in this investigation of *O. berteriana* mitochondria is therefore here referred to as "promoter" although definitive identification is required by *in vitro* transcription systems. Since the corresponding elements in wheat and maize mitochondria have been shown to promote correct transcription initiation *in vitro*, we consider the analogy strong enough to allow tentative classification of these related elements in *O. berteriana* as promoters.

Conserved Dicot Mitochondrial Promoter(s)—Comparison of the conserved sequence of the *O. berteriana* mitochondrial promoters with the consensus sequence of the two transcription initiation sites of soybean mitochondria shows a high degree of sequence similarity between the two dicot plant species (Fig. 6).

The 29-nucleotide long *O. berteriana* consensus can be narrowed to 9 nucleotides in the comparison with the two soybean sequences (Brown et al., 1991). The refinement seems plausible since within this sequence both soybean elements match the *O. berteriana* consensus in most nucleotide positions. Within this region the nonanucleotide (5'-CRTAAGAGAAGAAAGN) is best conserved between nucleotides (−7 and +2) is best conserved (Fig. 6). The first two nucleotides of the soybean mitochondrial primary transcripts are as in *O. berteriana* GA. The high degree of sequence conservation suggests that for at least these two dicots the nonanucleotide constitutes a conserved sequence element that is likely to be related or even identical with the dicot mitochondrial promoter. The common A-rich region upstream of this element may contribute to the promoter.

Most of the investigated transcription initiation sites in dicots begin in these conserved sequences while one of the soybean termini cannot be aligned with the proposed consensus motif (Brown et al., 1991). Whether a second type of promoter element is active in dicot plant mitochondria requires further investigation.

Differences between Dicot and Monocot Promoters—Comparison of the soybean transcription initiation sites with the monocot promoter sequences showed very little sequence conservation (Brown et al., 1991). The high conservation of the sequences around transcription initiation sites between soybean and *O. berteriana* now suggests a similar type of initiation sequence in dicot plant mitochondria. Transcription initiation signals appear to be even better conserved in dicots, since the proposed promoter sequences vary considerably more between the two monocots wheat and maize than between the two dicots (Covello and Gray, 1991; Gray et al., 1992; Mulligan et al., 1988a, 1988b). Only the 5'-CRTA-3' central motif is conserved between the two monocots and in the dicot promoter region. The low similarity between monocot and dicot promoters suggests rather divergent RNA polymerases and/or binding factors.

Mitochondrial Promoters in Animals, Fungi, and Plants—Promoters in prokaryotes, eukaryotes, and plant chloroplasts, unlike those in mitochondria, show distinct spacing between conserved sequence element, recognition site, and the start site of transcription. A common characteristic of all mitochondrial promoters analyzed to date, including animals, fungi, and plants, is a conserved sequence element that covers the transcription initiation sites (Chang and Clayton, 1984, 1988).
1986a, 1986b; Chang et al., 1986, 1987; Christianson and Rabinowitz, 1983; Clayton, 1991; Covello and Gray, 1991; Hixson and Clayton, 1985; Kennell and Lambowitz, 1989; Mulligan et al., 1988a, 1988b; Rapp and Stern, 1992). This position of the promoter elements results in highly conserved 5'-transcript termini, initiated from different promoters of a mitochondrial genome. In dicot plants, particularly at the three transcription initiation sites defined here in O. berteriana and the two soybean sites, all primary transcripts start with the 5'-GA-3' dinucleotide. Four nucleotides (5'-TAAG-3') are conserved between the dicot consensus sequence and the yeast mitochondrial promoter (Fig. 6).

In fungal mitochondria one type of promoter has been found to transcribe protein coding and tRNA and rRNA genes (Schink et al., 1989). In O. berteriana the presence of the conserved promoter element in front of mono- and polycistronic transcripts as well as protein- and ribosomal RNA-coding regions suggests that these two types of genes are transcribed from this one class of promoter elements. This broad competence of the conserved element and plant mitochondrial transcription in general is further supported by the previous observation of cotranscription of the 18 S and 5 S rRNAs together with the first two exons of the nad5 gene in O. berteriana.

The putative promoter elements identified in the dicot O. berteriana now allow the development of in vitro transcription systems for the further dissection of these elements and the requirements of transcription initiation in dicot plant mitochondria.

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