A transfer RNA^{Arg} gene of *Pelargonium* chloroplasts, but not a 5S RNA gene, is efficiently transcribed after injection into *Xenopus* oocyte nuclei

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Received 10 August 1984; Revised 2 October 1984; Accepted 15 October 1984

ABSTRACT

We present the primary structure of a chloroplast tRNA^{Arg}_{ACC} gene of the plant, *Pelargonium zonale*, and its faithful expression in *Xenopus* oocyte nuclei. This tRNA^{Arg} gene is located 250 bp downstream of a 5S RNA gene within a cloned 5kb long ribosomal DNA segment (Fig.1). The *Pelargonium* tRNA^{Arg} gene shares 97% and 86% sequence homology with tRNA^{Arg}_{ACC} genes of *Spirodela oligorhiza* and *Euglena gracilis* chloroplasts, respectively, and also extensive homology (70%) with the corresponding gene of *E.coli*. It lacks an intervening sequence and, like eukaryotic tRNA genes, does not code for the 3' terminal CCA nucleotides. Moreover, the chloroplast tRNA^{Arg} gene carries all the sequence elements essential for transcription by vertebrate RNA polymerase III since it is efficiently expressed in *Xenopus* oocyte nuclei, even in the presence of 1µg/ml α-amanitin. In *Xenopus* oocyte nuclei, no transcripts of the chloroplast 5S RNA gene were detected.

INTRODUCTION

The ribosomal RNA genes of chloroplasts in higher plants are usually part of an inverted repeat unit which consists of, in the order of transcription, the 16S rDNA, two transfer RNA genes (for isoleucine and alanine), the 23S rDNA, 4.5S rDNA and 5S DNA (for reviews see 1 and 2). Apart from the occurrence of 4.5S rDNA that apparently arose from a 3' segment of 23S rDNA (3) and the existence of 700-950bp long intervening sequences within each of the two tRNA genes (4,5), this gene arrangement parallels in its overall structure the organization of *E.coli* rDNA operons. In contrast to the polycistronic transcription of *E.coli* rDNA operons, the 5S DNA seems to be independently transcribed in chloroplasts. Evidence in favor of this conclusion is the existence of promoter-like DNA sequences in front of the 5S DNA (6), and the labelling kinetics of 5S RNA (7). To elucidate the pattern of chloroplast rDNA transcription

in more detail we injected a cloned rDNA segment of Pelargonium zonale into Xenopus oocyte nuclei. This 5kb long DNA segment contains, in 5' to 3' orientation, a large part of 23S rDNA, the 4.5S rDNA, 5S DNA, and about 1.5kb of 3' flanking DNA sequences (see Fig.1). Upon injection into Xenopus oocytes we observed the efficient transcription of a transfer RNA^{Arg} gene. Like a tRNA^{Arg} gene in Spirodela chloroplasts (8), this tRNA gene resides 250bp downstream from the 5S DNA. The 5S DNA is not expressed in oocytes. The Pelargonium tRNA^{Arg} gene shares sequence homologies with the corresponding genes of Spirodela and Euglena chloroplasts and E.coli of 97%, 86% and 70%, respectively. It lacks an intervening sequence and is also efficiently expressed in Pelargonium chloroplasts.

MATERIALS AND METHODS

The clone pRB40 (Fig.1) was constructed by cleavage of Pelargonium zonale chloroplast DNA with BamHI and insertion of the BamHI fragment into the BamHI site of pBR322. Ribosomal DNA containing clones were isolated by colony hybridization of recombinant, tetracycline-sensitive colonies with ³²P-labelled cloned rDNA from spinach chloroplast. pRB40 DNA was mapped by digestion with the restriction enzymes BamHI, EcoRI and HindIII and its 1.8kb EcoRI fragment by partial restriction enzyme mapping of 5' end-labelled DNA according to (9). After labelling of the 1.8kb fragment and subsequent digestion by PvuII or SacI (which cut the 1.8kb fragment once, as did BglII), the two resulting DNA segments were partially digested by TaqI and the digestion products fractionated on polyacrylamide sequencing gels along with end-labelled HpaII-pBR322 marker DNA fragments. Apart from the tDNA^{Arg} and its flanking sequences (Fig.2), large portions of pRB40, including the major part of 23S rDNA and the 5S DNA, have been sequenced (Lazarus and Metzlauff, unpublished results).

For sequencing the tRNA^{Arg} gene, the 0.33kb TaqI and 0.27kb TaqI-EcoRI fragments (Fig.1) were isolated after TaqI digestion of the 1.8kb fragment from low melting agarose and either 5' end-labelled with γ -³²P-ATP by polynucleotide kinase or 3' end-labelled with α -³²P-CTP by the Klenow fragment of DNA polymerase I. By the use of α -CTP, the TaqI site of the 0.27kb TaqI-EcoRI

fragment could be selectively labelled. The sequence shown in Fig.2 was obtained by sequencing both strands of the 0.33kb and 0.27kb fragments from the *TaqI* site within the tRNA^{Arg} gene in opposite directions (Fig.1) according to Maxam and Gilbert (10). Although we did not sequence a fragment overlapping the *TaqI* site both the results of restriction mapping and sequence analyses (see Fig.2 and 3) render unlikely the formal possibility that a short *TaqI* fragment escaped our attention.

Chloroplast RNA was isolated according to (11) and separated on 2mm thick 10% polyacrylamide gels prepared with 40mM Tris, pH 7.6, containing 20mM Na-acetate, 2mM EDTA. The tRNA was isolated from ethidium bromide stained gels as described for the isolation of short DNA fragments from polyacrylamide gels (10). For *S1* nuclease mapping, 0.4-1.6µg of tRNA were hybridized overnight at 30°C with $1.5-2 \times 10^4$ cpm each of single stranded 5' end-labelled probes of the 0.33kb *TaqI* fragment in 20µl of 80% formamide containing 40mM MOPS buffer, pH 6.4, 400mM NaCl, 1mM EDTA. The nucleic acids were then digested by the addition of 40(-200) units *S1* nuclease in 200µl of 30mM Na-acetate, pH 4.6, 250mM NaCl, 1mM ZnSO₄ for 90 min at room temperature. After phenol/chloroform treatment the nucleic acids were precipitated, dissolved in 5µl loading buffer and fractionated on 8% polyacrylamide sequencing gels (see Fig.5).

For oocyte nuclei injection, pRB40 DNA was purified by two subsequent ethidium bromide gradient centrifugations followed by one centrifugation in a normal CsCl gradient and extensive dialysis. The 5S DNA clone *Xbs* 1 containing one repeat unit of *Xenopus borealis* somatic 5S DNA cloned in the *HindIII* site of pBR322 was a gift of D. Brown (Carnegie Institution, Baltimore). The *X. laevis* tDNA^{Met} and tDNA^{Leu} clones were gifts of H. Hofstetter (Ciba Geigy, Basel). Each clone consists of one tDNA segment of 72bp (tDNA^{Met}) or 83bp DNA (tDNA^{Leu}), flanked by 50-120bp DNA at the 5' and 3' sides, and cloned in pBR322. The sequences as well as the transcription characteristics of the three clones in *Xenopus* oocytes have been published in detail (12-14). Usually, about 10ng DNA dissolved in injection solution (15) were injected per nucleus after centrifugation of oocytes according to (16). About 0.2µCi of α-³²P-GTP (410 Ci/mMol,

Fig. 1. Restriction map and gene arrangement of clone pRB40 from the chloroplast genome of *Pelargonium zonale*.

A) Map of the 5kb long chloroplast DNA insert of pRB40. Gene segments are shown by open boxes, spacer DNA by a thick line. The transcription direction of all gene sequences is from left to right.

B) Enlargement of the 1.8kb EcoRI fragment. The position of the TaqI site was determined by partial restriction enzyme analyses of the 5' end-labelled EcoRI fragment. The position of the single PvuII and SacI sites used in these analyses are denoted by thick arrows. The right-most TaqI site within the tRNA^{Arg} gene was used for sequencing both strands of 0.33kb and 0.27kb DNA in opposite directions (see Materials and Methods).

Amersham, U.K.) were injected together with pRB40 DNA, and the oocytes were incubated in modified Barth's medium at room temperature for 16 h. Oocyte RNA was isolated by proteinase K digestion and phenol treatment as described (17), and the RNA was fractionated on 12% polyacrylamide sequencing gels (10).

RESULTS

Identification of a tRNA^{Arg} gene downstream of a 5S RNA gene in the *Pelargonium* chloroplast genome

The rDNA containing clone pRB40 of the *Pelargonium* chloroplast genome comprises the 3' half of one rDNA repeat unit including the major part of 23S rDNA, the 4.5S rDNA, 5S DNA, and about 1.5kb of 3' flanking DNA sequences (see Fig.1). Injection of pRB40 DNA into *Xenopus* oocyte nuclei resulted in the efficient transcription of a low molecular weight RNA. We determined a size of about 77 nucleotides for this RNA by co-electrophoresis of pRB40-directed transcription products along

Fig. 2. Nucleotide sequence of tRNA^{Arg}_{ACC} gene from *Pelargonium* chloroplasts and its 5' and 3' flanking sequences. The corresponding sequences of tRNA^{Arg} genes from *Spirodela oligorhiza* (8), *Euglena gracilis* (21) and *E. coli* (22) are given below the *Pelargonium* tRNA gene sequence. Identical positions are denoted by dashes, deletions by points. +1 denotes the first nucleotide of mature tRNA (see also Fig.5). The upstream sequence of the tRNA^{Arg} gene is also compared with a sequence of tobacco chloroplast DNA located at a similar position in the tobacco chloroplast genome (6).

with 5S RNA, tRNA^{Met} and tRNA^{Leu}, synthesized from the corresponding *Xenopus* DNA clones injected into oocyte nuclei, in 12% polyacrylamide sequencing gels (Fig.4). This size agrees well with a transfer RNA but not with the expected 5S RNA of about 120 nucleotides, or the chloroplast-specific 4.5S rRNA for which a size of about 100 nucleotides has been determined (6,18).

In order to localize and to identify the putative tRNA gene, pRB40 DNA was cleaved by EcoRI, BamHI, and HindIII (singularly or in double digests), blotted onto nitrocellulose filters, and hybridized with labelled oocyte tRNA synthesized after injection of pRB40. The shortest specifically labelled DNA fragment of these digests was the 1.8kb EcoRI fragment of the 5kb chloroplast DNA (results not shown). The 1.8kb EcoRI fragment was then isolated from preparative gels and subsequently cleaved by TaqI. Since all known tRNAs carry the consensus sequence UCA^G within their pseudouridine loop (19; see also Fig.3) and most tRNA genes contain the corresponding TaqI site TCGA (20; see Fig.2), we expected to obtain a suitable start point for sequencing the tRNA gene from a TaqI digestion.

Fig. 3. Putative cloverleaf structure of tRNA^{Arg}_{ACG} from *Pelargonium* chloroplasts.

The secondary structure of tRNA was derived from the tRNA^{Arg} gene primary structure shown in Fig.2. The numbering is according to the "standard" tRNA sequence (see 19). Open circles denote differences to the tRNA^{Arg} of *Eugenia gracilis*, stippled (gray) circles differences to the tRNA^{Arg} of *E.coli*, and filled (black) circles differences to both tRNAs^{Arg} (see 21,22). Note the chloroplast tRNA^{Arg}_{ACG} specific deletion of nucleotide 17 (denoted by α) and the unpaired nucleotides 5-68 and 6-67 within the aminoacyl stem. According to the anticodon structure of tRNA^{Arg} from *E.coli* (22) and of many eukaryotic tRNAs, the anticodon ACG (underlined) is probably converted to ICG during maturation of pre-tRNA^{Arg} (see 41).

By hybridizing with labelled chloroplast tRNA, a 0.33kb TaqI and a 0.27kb TaqI-EcoRI fragment were found to contain the predicted tRNA gene (see Fig.1). Sequencing of these DNA fragments according to Maxam and Gilbert (10) resulted in the identification of a tRNA^{Arg}_{ACG} gene.

The structure of the tRNA^{Arg}_{ACG} gene

The sequence of the tRNA^{Arg} gene and of additional 5' and 3' flanking nucleotides is presented in Fig.2. The primary structure of the tRNA^{Arg}_{ACG} gene shows 97% and 86% homology with the chloroplast tRNA^{Arg}_{ACG} genes of *Spirodela oligorhiza* (8) and *Euglena gracilis* (21) and 70% homology to the same gene of

E.coli (22). The sequence of all genes coincides completely in their anticodon and T Ψ loop segments (see Figs.2 and 3). The chloroplast tRNA^{Arg} genes lack a nucleotide at position 17 of the "standard" tRNA sequence and carry a section of two unpaired "base pairs" within their aminoacyl stem (Fig.3). These mismatches seem to be specific for the secondary structure of chloroplast tRNA^{Arg} since the E.coli tRNA^{Arg}, like the overwhelming majority of tRNAs, possesses a perfectly paired aminoacyl stem (22). Almost all nucleotide differences between the chloroplast and E.coli tRNAs^{Arg} affect stem regions. In each case, the changes resulted in the retention of base pairs. Notable base pair alterations occur at position 50/64 within the T Ψ stem and 10/25 of the D stem. At position 50/64 of chloroplast tRNA^{Arg} there is a weak GoU pair instead of a G $\bullet$ C pair in E.coli tRNA^{Arg}, and vice versa, a strong G $\bullet$ C pair is found at position 10/25 instead of a weak GoU pair in E.coli tRNA^{Arg} (see Fig.3).

The first 75 nucleotides upstream of the chloroplast tRNA^{Arg} gene of Pelargonium contain neither a Pribnow box-like sequence nor the -35 sequence TTG which is involved in the binding of prokaryotic RNA polymerase to the DNA template (23). They show, however, conspicuous homologies with the upstream sequence of the Spirodela tRNA^{Arg} gene and a stretch of nucleotides at a similar position in the tobacco chloroplast genome (see Fig.2; 23). It remains to be established whether in the tobacco chloroplast genome a tRNA gene follows the homologous DNA segment as well. A notable peculiarity of this sequence is a run of pyrimidines (13 Ts in Pelargonium and 9Ts in tobacco, interrupted by one or two Cs, respectively) mapping to approximately position -45 of the Pelargonium tRNA^{Arg} gene.

On the other hand, no oligo(dT)-stretch was observed within the first 40 nucleotides downstream of the chloroplast tRNA gene. This is in striking contrast to the structure of terminator sequences of many prokaryotic genes (see 24) and of all eukaryotic genes transcribed by the RNA polymerase III (see Discussion).

Transcription of the tRNA^{Arg} gene in Xenopus oocyte nuclei

As mentioned above and shown in Fig.4, the injection of pRB40 into Xenopus oocyte nuclei gave rise to the synthesis of

Fig. 4. Electrophoretic analysis of transcription of clone pRB40 in *Xenopus* oocyte nuclei.

In (A) 10ng of pRB40 DNA was injected per oocyte and separated on a 12% sequencing gel (lane 4). For comparison, the transcripts of 5S DNA clone Xbs1 (lane 5) and of tDNA^{Met} (lanes 1 and 2) and tDNA^{Leu} (lane 3) from *Xenopus* were co-electrophoresed in the same gel. In lanes 1 and 2, the RNA of 2 and 6 oocytes, respectively, in all the other lanes, the RNA of 3 oocytes was separated. In (B) pRB40 DNA was injected together with 0 (lane 2), 1µg/ml (lane 3) or 100µg/ml α-amanitin (lane 4). Note the inhibition of tRNA^{Arg} synthesis by 100µg/ml α-amanitin and of the 74nt long, minor transcript by 1µg/ml α-amanitin. In (C) *Xenopus* tDNA^{Met} was injected with 0 (lane 1), 1µg/ml (lane 2) and 100µg/ml α-amanitin (lane 3). Nt = nucleotides.

tRNA^{Arg} of about 77 nucleotides. This size agrees well with the sequence of this tRNA^{Arg} gene for which a length of 74bp was determined, and the finding that, as for all eukaryotic tRNAs, the 3' terminal CCA residues of chloroplast tRNAs are post-transcriptionally added (see Discussion). Since the chloroplast tRNA^{Arg} migrates electrophoretically like the two tRNAs of Xenopus (see Fig.4), it seems likely that the tRNA^{Arg} is additionally modified like eukaryotic tRNAs in the Xenopus oocytes (see Fig.3).

Besides the tRNA^{Arg}, a minor RNA fraction of about 74 nucleotides was synthesized after injection of clone pRB40 into oocyte nuclei (see Fig.4 A,B). This RNA comprises about one tenth of α -³²P-GTP incorporated into the tRNA^{Arg} main fraction. Two-dimensional separations of pRB40-directed transcription products on a 12% polyacrylamide gel in the first dimension and on a 20% gel in the second gave the same radioactive pattern, i.e. one main and one minor spot corresponding to the tRNA and the minor 74 nucleotide transcription product (results not shown).

The synthesis of tRNA^{Arg} is specifically inhibited by 100 μ g/ml α -amanitin and is, therefore, catalyzed by RNA polymerase III of oocyte nuclei. As shown in Fig.4, the co-injection of 1 μ g/ml α -amanitin (referred to the nuclear volume) along with pRB40 or Xenopus tDNA^{Met} did not affect the transcription of tRNA^{Arg} and tRNA^{Met} species, whereas injections of 100 μ g/ml α -amanitin completely inhibited the transcription of both RNAs (Figs.4B, lane 4, and 4C, lane 3). In striking contrast to the amanitin-sensitivity of tRNA fractions, the synthesis of the 74 nucleotide long minor fraction is already inhibited by 1 μ g/ml α -amanitin and should, therefore, be catalyzed by RNA polymerase II (Fig.4B, lane 3). No further analyses were done to characterize the minor 74 nucleotide RNA in more detail.

Transfer RNAs are usually synthesized as precursor molecules with additional 5' and 3' nucleotides. In the course of a multistep process these nucleotides are removed, many nucleotides of pre-tRNAs become modified, and the CCA sequence is added to the 3' end (25). As described in detail for yeast

Fig. 5. Identification of expression of tRNA^{Arg}_{ACG} gene in *Pelargonium* chloroplasts by S1 nuclease mapping of chloroplast tRNA.

Chloroplast tRNA was hybridized with single stranded DNA of 0.33kb TaqI fragment (see Fig.1), digested by S1 nuclease, and the DNA was fractionated on an 8% sequencing gel (see Materials and Methods for details). Lanes 1-4: Hybridization of tRNA-complementary DNA strand of 0.33kb TaqI fragment with 0 (lane 1), 0.4µg (lanes 2 and 3) and 1.6µg tRNA (lane 4). The samples were digested with 40 units (lanes 1,2 and 4) or 200 units of S1 nuclease (lane 3). Lanes 5+6: Hybridization of non-coding DNA strand of 0.33kb TaqI segment with 0 (lane 5) or 0.4µg tRNA (lane 6) followed by digestion with 40 units of S1 nuclease. For comparison, the sequence ladder of the non-coding strand of 0.33kb TaqI fragment is shown at the left hand. The oligo(dT) segment in the upstream region, the promoter box A for vertebrate polymerase III and the anticodon are denoted by brackets, the 5' end of mature tRNA sequence is denoted by an arrow.

pre-tRNAs, almost all of these reactions take place in the Xenopus oocyte nucleus (26,27). To see if processing also occurs with our pre-tRNA^{Arg} we did some time course experiments by injecting oocytes with pRB40 DNA and incubating for 1,3 and 16 hrs. The transcription of pRB40 in oocytes incubated for 1 or 3 hrs unexpectedly did not give rise to a pronounced amount of larger precursor tRNA. Like the transcripts of tRNA^{Met} and tRNA^{Leu} genes of Xenopus, the pre-tRNA^{Arg} of Pelargonium chloroplasts seems to be very rapidly processed to mature tRNA. Only by overexposing our gels, an RNA band of 88-90 nucleotides was observed which is probably the primary transcription product of the chloroplast tRNA^{Arg} gene in oocyte nuclei (data not shown).

The tRNA^{Arg} gene is expressed in chloroplasts

In order to determine whether the tRNA^{Arg} gene transcribed in Xenopus oocytes is also naturally expressed in Pelargonium chloroplasts, we fractionated whole chloroplast RNA on a 10% polyacrylamide gel, isolated and subsequently analyzed the tRNA by S1 nuclease mapping. As the radioactive probe, the 0.33kb TaqI fragment (see Fig.1) was 5' end-labelled and the tRNA-complementary DNA strand isolated from a strand separation gel. The results of the S1 digestion are shown in Fig.5 and demonstrate the pronounced hybridization of chloroplast tRNA with the tRNA^{Arg} gene. As predicted the tRNA fraction protected a DNA segment of about 56 nucleotides which corresponds to the tRNA^{Arg} gene segment from the TaqI site at position +56 to the first nucleotide of mature tRNA^{Arg} (compare Figs.1 and 5).

DISCUSSION

Structure and expression of the chloroplast tRNA^{Arg} gene in Xenopus oocyte nuclei

It is generally accepted that the chloroplast genomes in higher plants code for the complete set of transfer RNAs which are required for the synthesis of chloroplast-specific proteins (see 1). The primary structure of tRNA^{Arg} gene of Pelargonium chloroplasts resembles a typical chloroplast tRNA gene. Like those, it lacks both the 3' terminal CCA sequence and shares extensive sequence homology (70%) with the corresponding E.coli

gene and significantly less (about 50%) with the corresponding eukaryotic tRNA genes. This conclusion is corroborated by the very high sequence homology of 97% and 86% with the tRNA^{Arg}_{ACC} genes of Spirodela and Euglena chloroplasts, respectively. Moreover, all three chloroplast tRNAs^{Arg} contain two mismatches in their aminoacyl stem (see Fig.3). Similar mismatches seem to be common also among mitochondrial tRNAs (see 20 and 21).

We demonstrate the faithful expression of a chloroplast tRNA gene from higher plants by RNA polymerase III following injection into Xenopus oocyte nuclei. Similar results were reported for the expression of two injected tRNA genes from E.coli (28) and of cloned mitochondrial tRNA genes from fungi and plants in Xenopus oocytes (29). Moreover, transcription of a tRNA^{Val}-tRNA^{Asn}-tRNA^{Arg} gene cluster from Euglena in a HeLa cell in vitro system demonstrates that tRNA genes from algal chloroplasts are transcribed by the mammalian RNA polymerase III (30). All these results support the view that many (but not all: see, e.g., 31) prokaryotic mitochondrial and chloroplast tRNA genes contain the internal promoter sequences necessary for the faithful transcription by vertebrate RNA polymerase III (see below). It remains, however, to be established whether or not the efficient expression of the tRNA^{Arg} gene in Xenopus oocyte nuclei reflects its natural mode of transcription and expression in Pelargonium chloroplasts.

The expression of tRNA genes in plant chloroplasts

In chloroplasts of higher plants there are at least two different RNA polymerases. One of these seems to be specific for the transcription of tRNA genes (32). Moreover, transcription analyses of cloned spinach tRNA genes in a homologous chloroplast in vitro system led to the conclusion that the promoter for the transcription of tRNA genes is located within 150bp upstream of the first nucleotide of mature tRNA sequence. The functional relevance of specific upstream sequence motifs has, however, not been determined. This is also the case for the consensus sequence GT^A_TNTA^{AA}_{TT} which was found upstream of all Euglena and some plant tRNA genes (32) but is absent in the upstream sequence of the Pelargonium tRNA^{Arg} gene (see Fig.2).

The 3' boundary of all RNA polymerase III genes is delimited by a run of at least 4 T residues which represents the

Fig. 6. Sequence comparison amongst the consensus sequences of promoter boxes of transfer RNA genes and the *Xenopus borealis* somatic 5S DNA with the corresponding sequences of the *Pelargonium* chloroplast tRNA^{Arg} and 5S RNA genes. Common nucleotides are in boxes. The consensus sequence of tRNA genes is from (39), the *X.borealis* 5S DNA sequence is from (12), the plant 5S DNA sequences are from Lazarides (unpubl.results; *Pelargonium*), (6; tobacco) and (42; *Spinacia*).

signal for transcription termination by RNA polymerase III (33). Surprisingly, no oligo(dT) segment was found 3' to the tRNA gene (Fig.2). Moreover, no distinct stem-loop structures which are supposed to be involved in the transcription termination of the *Spirodela* tRNA^{Arg} gene (8) are present in the downstream sequences of the *Pelargonium* tRNA^{Arg} gene (see Fig.2). The tRNA^{Arg} gene is a typical RNA polymerase III template

The initiation of transcription of eukaryotic tRNA and 5S RNA genes by RNA polymerase III takes place by the interaction of the enzyme with intragenic nucleotides, which is mediated by protein factors. Functional analyses have demonstrated that two sequence boxes, A and B, are essential for the promotion of transcription of tRNA genes. These promoter boxes are separated by 33-43bp and were located within the D- and TΨ-stem and -loop segments around the positions +9 to +19 and +55 to +64 (13,14,35-37). The sequences of both boxes are highly conserved in all tRNA genes. From a comparison of sequences of all eukaryotic tRNAs, a consensus sequence (+9)RRYNNARY-GG(+19) for box A and of (+53)G^TTCRANNC(+61) for box B was derived (R represents a purine, Y a pyrimidine and N any nucleotide; see Fig.6 and 38). Except for one nucleotide (A instead of Y at position +16; see Figs.2 and 6) the structures of boxes A and B of the tRNA^{Arg} gene of *Pelargonium* chloroplasts coincide with the consensus sequences for both promoter boxes. Position +16 is anyhow not ubiquitously conserved because other RNA polymerase

III-transcribed genes, such as the adenoviral VAI genes and Alu-type sequences of the hamster genome, have a G or A, respectively, at this position (see 39).

Why is the chloroplast 5S RNA gene not transcribed in *Xenopus* oocyte nuclei?

In striking contrast to the tRNA^{Arg} gene, the 5S RNA gene of *Pelargonium* chloroplasts is not expressed in *Xenopus* oocyte nuclei. The DNA segment of *Pelargonium* 5S DNA corresponding in its position to the internal promoter of vertebrate 5S RNA genes is presented in Fig.6 and shows only a few nucleotides in common with the *X.borealis* somatic 5S DNA. Unlike the *Xenopus* 5S DNA, there is no sequence homology between the nucleotides of about +50 to +60 and the promoter box A of tRNA genes. Since this sequence motif is one of the essential promoter elements for RNA polymerase III (see above), the chloroplast 5S DNA therefore seems not to be recognized and transcribed by the RNA polymerase III of *Xenopus*.

Based on the primary structure of all eukaryotic genes transcribed by RNA polymerase III Ciliberto et al. (39) have subdivided them into two classes. Class 1 includes all tRNA genes and a number of short viral and chromosomal genes whereas class 2 genes are represented by the 5S RNA genes. In class 1 genes the promoter box A is located 10-20bp from the start of transcription and about 30-60bp from promoter box B. In class 2 genes promoter box A is located about 50bp away from the transcription start, and the additional promoter elements are near the box A motif. All class 1 genes appear to be related to a common (tRNA-like) ancestral gene. This seems not to be the case for 5S RNA genes. Moreover, the emergence of the promoter box A-equivalent in 5S RNA genes of higher eukaryotes seems to be a recent event (39), and evolutionary ancient 5S RNA genes, such as those of *Schizosaccharomyces pombe* (40) and of *Pelargonium* chloroplasts (this paper), are not transcribed by RNA polymerase III of *Xenopus* oocytes.

ACKNOWLEDGEMENTS

We are very much indebted to Sabine Skiebe for expert technical assistance and to Donald Brown and Hans Hofstetter for the gifts of DNA clones. E.S. is very grateful to Walter

Schaffner for providing the opportunity to finish this work in his laboratory. Moreover, he wishes to thank Sandro Rusconi and Oleg Georgiev for helpful discussions and advice. For critical reading the manuscript, we are indebted to Max Birnstiel, Greg Gilmartin, Walter Schaffner and Fred Schaufele. Finally, we thank Fritz Ochsenbein for the excellent preparation of figures and Silvia Oberholzer for typing the manuscript.

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