Volume 5 Number 12 December 1978 Nucleic Acids Research

Cloning of nematode tRNA genes and their expression in the frog oocyte

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Received 9 October 1978

ABSTRACT

Transfer RNA genes of the nematode Caenorhabditis elegans have been cloned in E. coli using the plasmid Col E1 as vector. The tRNAs coded by 3 hybrid plasmids were purified by hybridisation of labelled nematode tRNA with the plasmid DNAs. Each plasmid appears to code for a single distinct tRNA species. The expression of the cloned DNAs was analysed in vivo by injection into nuclei of Xenopus laevis oocytes. Evidence is presented which suggests that these nematode tRNA genes are accurately transcribed and processed in frog oocytes. Analysis of one hybrid plasmid shows that a 300 base pair DNA fragment contains both the structural gene and those regions required for its transcription in vivo. The results show that cloned eukaryotic DNAs from a heterologous source can be tested for functional gene activity in X. laevis oocytes.

INTRODUCTION

Transfer RNA genes are well suited for investigating the control of gene expression in that their gene products are precisely defined and easily characterized. This set of functionally related genes is known to show two forms of regulation. In eukaryotes, cells usually express the entire gene family uniformly (1), however, there are instances where cells differentially enhance the transcription of specific tRNA genes (2,3). Thus, a comparative and functional study of this gene family may help clarify the mechanisms by which individual genes are controlled and consequently, the molecular basis for their coordinate expression.

The potential for combining a genetic and biochemical approach to the study of eukaryotic gene regulation is available in the free-living nematode Caenorhabditis elegans (4). This report describes the identification of cloned nematode tRNA genes and their in vivo expression on injection into Xenopus laevis oocytes.

Injected frog oocytes constitute a living cell system wherein the biological function of nuclei and/or purified macromolecules can be tested

(5). The injection of purified DNAs into X. laevis oocytes initiates the synthesis of RNA transcripts and, in some instances, the proteins encoded by the injected genes (6-9). In particular, Gurdon and Brown have demonstrated that Xenopus 5S ribosomal RNA genes support the sustained synthesis of an authentic 5S ribosomal RNA when injected into the nucleus of Xenopus laevis oocytes (10,11). Similarly, the experiments reported here show that cloned C. elegans tRNA genes direct the synthesis of bona fide tRNAs when injected into Xenopus oocytes. In addition, we have used this injection system to demonstrate that a relatively small DNA segment, 300 base pairs, contains all the information required for the in vivo expression of a tRNA gene. While this work was in progress Kressmann et al. (9) have shown that in a homologous system cloned Xenopus tRNA genes can be expressed on injection into Xenopus oocytes.

MATERIALS AND METHODS

DNA and tRNA from C. elegans. Sulston and Brenner (12) have described the growth of C. elegans and isolation of its DNA. Transfer RNA was isolated after disruption of the nematodes in a French pressure cell at 4^{o} (12) followed by phenol extraction (13). After precipitation with ethanol, the crude RNA was dissolved in 0.01 M tris-chloride pH 7.4, applied to a DEAE cellulose column and successively eluted with tris buffer containing 0.1 M and 1 M NaCl. The 1 M NaCl eluent containing the tRNA was precipitated with ethanol, dissolved in 0.01 M tris-chloride pH 7.4 and dialysed against the same buffer. Polyacrylamide gel electrophoresis showed that this fraction also contained 5S RNA and small amounts of degraded ribosomal RNA. The tRNA can be aminoacylated using aminoacyl tRNA synthetases from C. elegans or wheat germ (T. Tranquilla, unpublished).

Cloning of tRNA genes. C. elegans tRNA genes were cloned in E. coli $Kr^{-}m^{+}$ via the plasmid Col E1 using the single Eco R1 restriction site on this plasmid DNA (14). These experiments were done in Category 2 containment conditions with the approval of GMAG. Hybrid plasmids were contructed by ligating Eco R1 restriction fragemnts of nematode DNA to Eco R1 digested plasmid DNA. 1 µg restricted Col E1 DNA and 5 µg restricted nematode DNA were incubated with T_4 DNA ligase (1 unit) in 100 µl of 30 m M tris-chloride pH 7.6, 10 m M $MgCl_2$, 1 m M dithiothreitol, 1 m M ATP, for 4 days at 4^{o}. E. coli was transformed with the ligated plasmids (15) and plated on tryptone-yeast extract plates containing colicin E1. About 1500 colonies were picked on to gridded plates and these screened for nematode tRNA genes by the colony hybridisation technique of Grunstein

and Hogness (16). Plasmid characterisation on agarose gel electrophoresis using the toothpick assay of Barnes (17) showed that 40% of the colonies contained plasmids with inserted DNA. The hybridisation probe was nematode tRNA labelled at the 5' terminus with ^{32}P (1-3 x 10^6 cpm/µg tRNA). This was prepared by removing the tRNA 5' terminal phosphate with bacterial alkaline phosphatase and re-introducing a ^{32}P labelled terminus with T_4 polynucleotide kinase and ^{32}P-γ-ATP (18). Positively scoring colonies were re-purified and plasmid DNA prepared according to Clewell (19).

Since the nematode tRNA preparation contained rRNA fragments the screening procedure also selected clones containing nematode ribosomal DNA sequences. Plasmids carrying nematode tRNA gene sequences were distinguished by hybridisation to tRNA specifically labelled at the 3' terminal adenylic acid residue with ^{32}P-α-ATP by yeast tRNA nucleotidyl transferase (0.5-1 x 10^6 cpm/µg tRNA). Purification of the yeast enzyme and labelling of tRNA was as described by Sternbach et al. (20). Plasmid DNAs were separated by electrophoresis on 1% agarose gels and DNA bands were detected by fluorescence after ethidium bromide staining. They were transferred to cellulose nitrate strips using the Southern procedure (21) and the strips hybridised separately with 5' and 3' terminally labelled nematode tRNA in 5 x SSC containing 50% v/v formamide at 37° for 16 h (SSC is 0.15 M NaCl, 0.015 M Na citrate pH 7). After washing in 5 x SSC in 50% formamide at 37° and 20° and then in 2 x SSC, the strips were autoradiographed by the method of Laskey (22).

Purification of plasmid specified tRNAs. Transfer RNAs coded by plasmids were purified by hybridisation of labelled nematode tRNA to the plasmid DNAs. Heat denatured plasmid DNA was bound to cellulose nitrate filters (23). Filters with 20 µg DNA were hybridised to 3' terminally labelled nematode tRNA (10^6 cpm/µg tRNA; 1 µg/filter) in 2 x SSC at 65° for 16 h. The filters were extensively washed with 2 x SSC at 20°, hybridised tRNA eluted at 100° for 15 min in 0.01 x SSC, and the eluted tRNA precipitated with ethanol from 0.2 M sodium acetate.

Preparation of DNAs for injection. Whole plasmid DNA was banded in CsCl with ethidium bromide, extracted with isobutanol and dialysed against water. DNA restriction fragments separated by agarose or acrylamide gel electrophoresis, were extracted with isobutanol to remove the ethidium bromide stain and chromatographed on G25 Sephadex.

Injection and Labelling of Oocytes. Stage V and VI oocytes (24) were removed from anaesthetized Xenopus laevis females and cultured at 20-22° in

Hepes-buffered Barth solution (MBS-H) until injected (25). 50 nanolitres of DNA (100 µg/ml) mixed with ^{32}P-α-GTP (10 mC/ml; 350 Ci/mmole) were injected into the oocytes' germinal vesicle with approximately 50% success. Unless otherwise stated, about 20 oocytes were injected for each DNA sample, incubated in MBS-H at 20° for five hours and frozen at -70° until the RNA was extracted.

RNA extraction and analysis. RNA was extracted from frozen oocytes by homogenization and digestion in proteinase K (100 µg/ml), 50 mM Tris HCl, pH 7.5, 5 mM EDTA and 0.5% SDS. The homogenate was extracted twice with phenol : chloroform (1:1) and precipitated with ethanol. The ethanol precipitate was dissolved in 8 M urea and resolved by electrophoresis in a high resolution acrylamide gel. The gel is composed of a 4% acrylamide stacking top equilibrated in 40 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA, 7 M urea pH 7.7 and a 10% acrylamide resolving bottom equilibrated in 90 mM Tris, 90 mM H_3BO_3, 3 mM EDTA, 7 M urea pH 8.2. RNA bands were detected by autoradiography using Kodak X-omatic film.

RNA species were eluted from gels by smashing the acrylamide with a glass rod and incubating the gel fragments in 10 volumes of 0.5 M ammonium acetate, 0.01 magnesium acetate, 0.1% SDS, and 0.1 mM EDTA at 37°C for 18 hrs. The mixture was diluted with two volumes of water and applied to a DEAE cellulose column. After washing with 0.2 M ammonium acetate the RNA was eluted with 1.2 M ammonium acetate and precipitated with ethanol.

For fingerprint analyses, RNAs were digested with ribonuclease T_1 and subjected to electrophoresis on cellulose acetate strips, followed by electrophoresis on DEAE cellulose paper in 7% formic acid according to Barrell (26). The modified base composition of an RNA species was determined by digestion with snake venom phosphodiesterase to give 5' labelled ribonucleotides which were resolved by two dimensional thin layer chromatography (27).

Enzymes. Restriction endonuclease Eco R1 was from Miles Research Products, Hind III and Sma I from Boehringer, and Taq 1 a gift from Dr. J.I. Harris. T_4 DNA ligase and T_4 polynucleotide phosphokinase were from Worthington or P-L biochemicals.

RESULTS

Arrangement and cloning of nematode tRNA genes. The haploid DNA content of C. elegans is 8 x 10^7 bp (about 20 times that of E. coli) and contains approximately 300 tRNA genes and 55 genes for (18 + 28) S rRNA (12). Hybridisation of tRNA to separated nematode RNA restriction fragments showed that the tRNA genes are not highly clustered. Nematode

tRNA labelled with (^{32}P) ATP at the 3' terminal A residue by yeast tRNA nucleotidyl transferase was separated by polyacrylamide gel electrophoresis into six bands. Using the Southern transfer method (21) each of these was hybridised to the set of Eco R1 restriction endonuclease digestion products of nematode DNA separated by size on electrophoresis in 1% agarose gels. Between 30-50 separable DNA fragments hybridise to tRNA (Figure 1).

Eco R1 fragments of nematode DNA containing tRNA genes were cloned in E. coli via the plasmid vector Col E1 which has a single Eco R1 restriction enzyme site. The clones were screened for hybridisation with ^{32}P labelled nematode tRNA by the Grunstein and Hogness colony hybridisation technique (16). The tRNA was labelled in the 5' terminal phosphate with ^{32}P-γ-ATP and T_4 polynucleotide kinase. The tRNA preparation contained significant amounts of degraded ribosomal RNA so that this initial screening also selected clones with inserted nematode 18 and 28 S ribosomal DNA sequences. Clones carrying tRNA genes were specifically distinguished by hybridisation of their plasmid DNA with tRNA labelled at the 3' terminal residue with ^{32}P-α-ATP using yeast tRNA nucleotidyl transferase. This enzyme has as substrate tRNA and the tRNA-like structure at the 3' terminus of some plant viral RNAs. No other naturally occurring RNAs are known to be substrates for the enzyme, so that this hybridisation can be used to distinguish tRNA sequences coded in the DNA. 20 clones scoring as positive in the colony hybridisation screening were purified and plasmid DNAs isolated. These were separated by agarose gel electrophoresis, transferred to cellulose nitrate sheets (16) and the transfers hybridised separately to 5' and 3' end labelled nematode tRNA (Figure 2).

Seven plasmids hybridised only to the 5' end labelled probe; they also hybridised to (^{32}P) labelled (18 + 28)S nematode rRNA and so contained ribosomal but not transfer RNA sequences. When initially cloned the DNA of these plasmids comprised 12000 bp corresponding to insets of 6500 bp of nematode DNA which is the size of the single Eco R1 fragment of nematode DNA which hybridises to 18 and 28S rRNA. All seven plasmids were unstable, and during the growth of bacterial cultures for plasmid DNA preparations most of the inserted DNA was lost leaving a fragment of about 300 bp which when isolated from Eco R1 digests still hybridised to (18 + 28)S rRNA.

12 plasmids hybridised to both 5' and 3' end labelled tRNA and so contained tRNA sequences. The three used in the experiments described here are designated Cet 1, 7 and 18 and contain inserted nematode DNA segments of 5500, 5000 and 2500 bp respectively. Unlike the plasmids containing rDNA

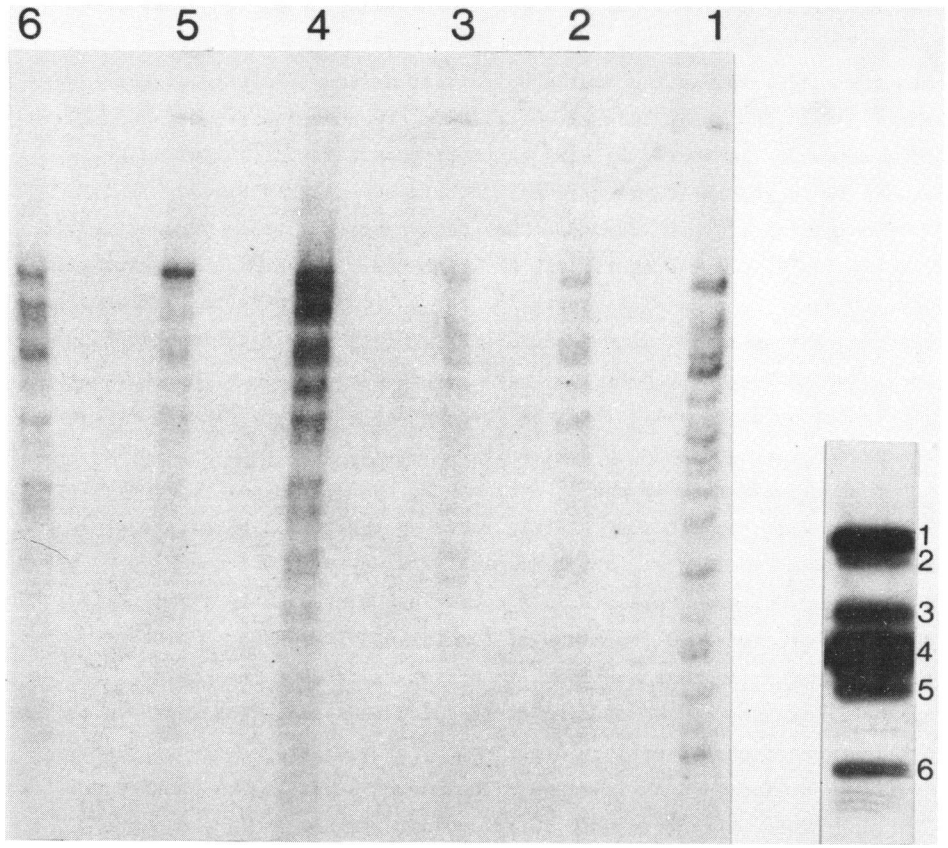

Figure 1: Hybridisation of ^{32}P 3' end labelled nematode tRNA to Eco R1 restriction digestion fragments of nematode DNA. The labelled tRNA was separated by electrophoresis on a 10% acrylamide, 7 M urea, tris-borate gel (see Methods) into the 6 bands shown on the right. Eco R1 digests of nematode DNA were separated by electrophoresis on 1% agarose gels, transferred to cellulose nitrate strips (21) hybridised separately with each of the 6 tRNA fractions and the strips autoradiographed. (Migration is from top to bottom).

sequences, these were stable and in Cet 1 and 7 the inserted sequences had not undergone detectable rearrangement after cloning. The experiment described in Figure 3 shows that the inserted nematode DNA and its restriction enzyme digest fragments correspond in size to those equivalent fragments of genomic nematode DNA which hybridise to the ^{32}P labelled plasmid DNA. This result also shows that these DNA inserts do not contain detectable sequences repeated elsewhere in the genome.

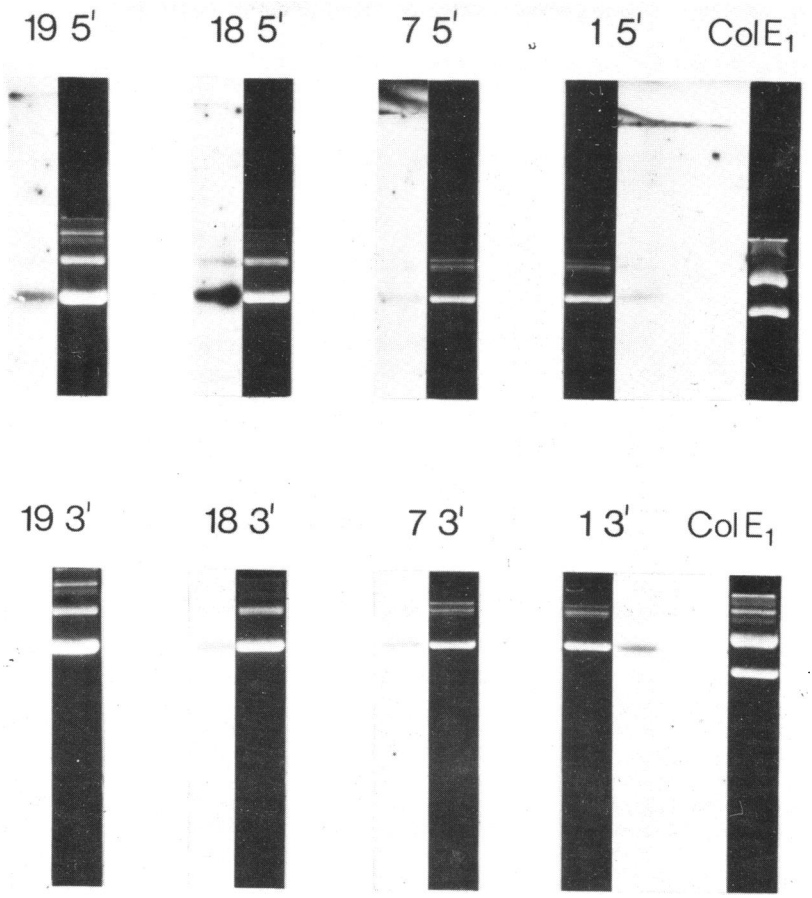

Figure 2: Identification of hybrid plasmids containing nematode tRNA and rRNA sequences. Plasmid DNAs were separated by electrophoresis on 1% agarose gels and stained with ethidium bromide (white bands). The DNAs were transferred to cellulose nitrate strips and hybridised with 5' end labelled tRNA, containing labelled rRNA fragments (upper row) or tRNA specifically labelled at the 3' terminus (lower row). Plasmids 1, 7 and 18 contain tRNA sequences; plasmid 19 contains rRNA sequences.

Plasmid coded tRNAs. The tRNAs coded by plasmids Cet 1, 7 and 18 were isolated by hybridisation of ^{32}P 3' end labelled nematode tRNA to each plasmid DNA immobilised on cellulose nitrate filters. The hybridised RNAs were eluted from the filters and separated on single dimensional polyacrylamide gel electrophoresis (Figure 4) and also on two dimensional gels. The fact that we were unable to resolve more than one band from each of the 3

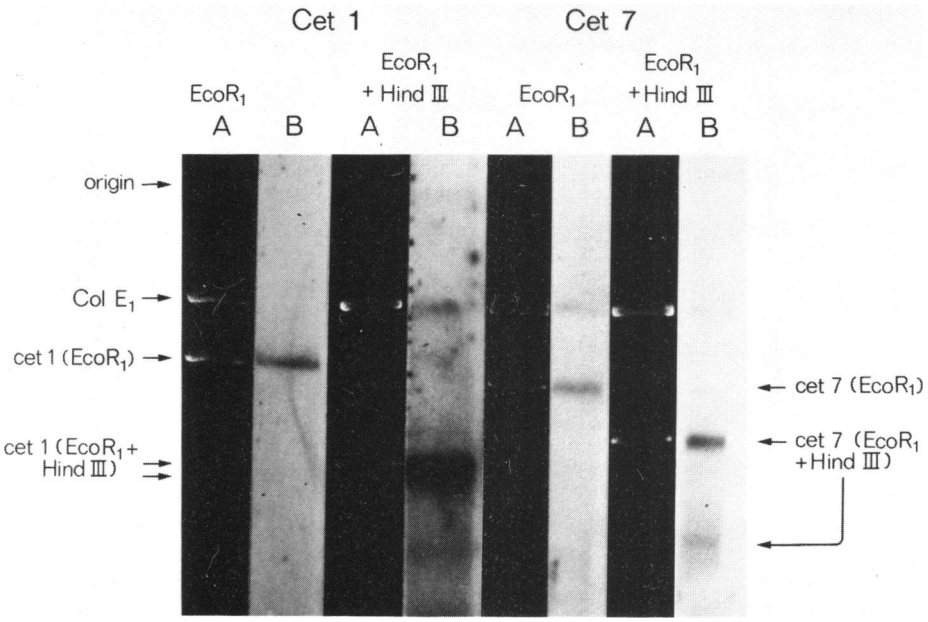

Figure 3: Comparison by restriction enzyme digestion of the inserted nematode DNA in plasmids 1 and 7 with the corresponding sequences in genomic nematode DNA. Lanes A show agarose gel electrophoresis (after ethidium bromide staining) of Cet 1 and 7 DNA digested either with Eco R1 or Eco R1 + Hind III. In lanes B the corresponding enzymic digests of genomic nematode DNA were run and, after transfer to cellulose nitrate, hybridised with Cet or Cet 7 DNA labelled with ^{32}P-α-dATP by nick translation using E. coli DNA polymerase I (28). These strips were autoradiographed. Fragment positions are indicated at the sides of the figure.

tRNAs suggests that each plasmid may only code for a single tRNA species. This can only be established when the nucleotide sequences of the tRNAs or the plasmid DNAs have been determined. However the fingerprint of the T_1 ribonuclease products from the 4S RNA synthesised in the oocyte in response to injection of Cet 7 DNA strongly suggests that this product is a single RNA species (see below and Figure 6).

Cloned nematode DNA directs 4S RNA synthesis in oocytes. Each of the 12 plasmids identified as containing tRNA sequences was tested for functional tRNA genes by microinjection into oocytes. Ten of these direct the synthesis of a 4S RNA species when injected into the nucleus of an oocyte. Two Cet 1 and 7, were chosen for further analysis. Figure 5 shows that Cet 1 and Cet 7 DNA each direct the synthesis in oocytes of a 4S RNA species migrating on gel

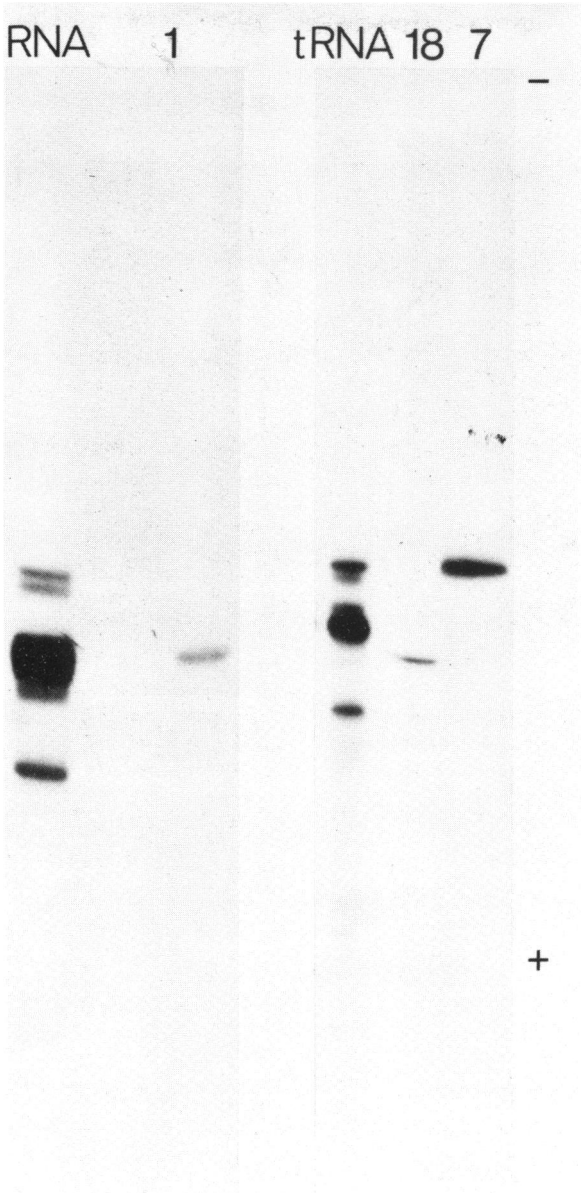

Figure 4: Transfer RNAs coded by plasmids 1, 7 and 18 purified by hybridisation of plasmid DNA to ^{32}P 3'-terminally labelled nematode tRNA. RNA is total labelled nematode tRNA; 1, 7 and 18 are after hybridisation of this to the appropriate plasmid DNA. For 7 and 18 electrophoresis is in the 12½% acrylamide, 7 M urea tris-borate system; for plasmid 1 in 10% acrylamide, 7 M urea, 0.036 M tris, 0.03 M $N_aH_2PO_4$, 0.001 M EDTA, pH 7.7.

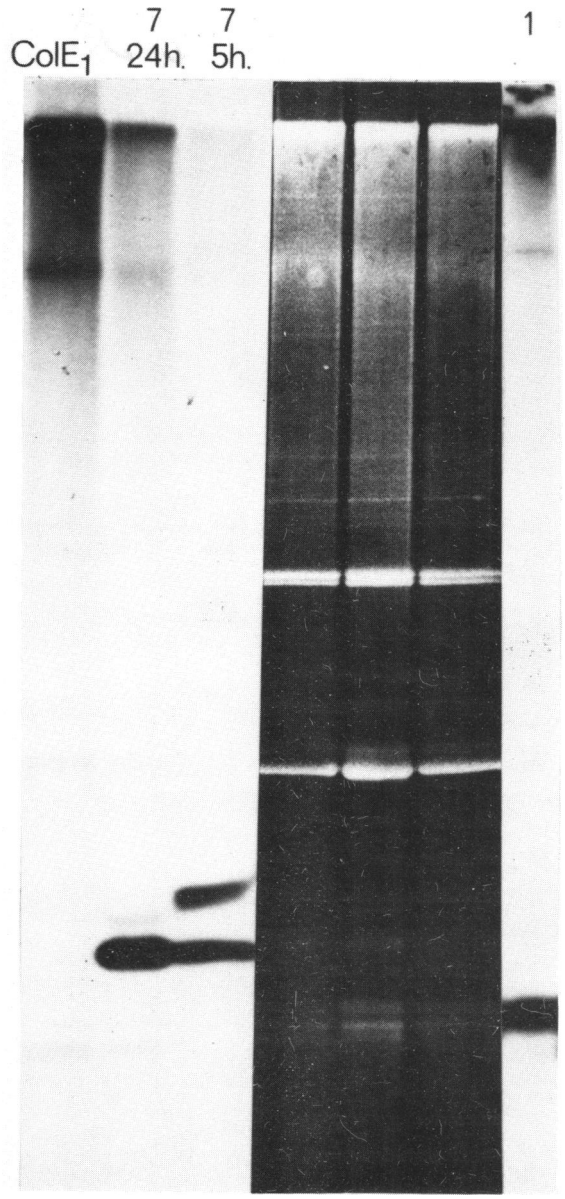

Figure 5: Separation by gel electrophoresis of the RNAs synthesised following injection of plasmids Col E1, Cet 7 and Cet 1 into frog oocytes. ^{32}P-α-GTP was injected simultaneously and the RNAs isolated after 5h (Col E1, Cet 1 and Cet 7) and after 24h (Cet 7). Separation was on the 10% acrylamide, 7 M urea, tris-borate gel system (see Methods) and the gels were autoradiographed. Lanes 4, 5, and 6 were stained with ethidium bromide to show the positions of unlabelled oocyte RNAs.

electrophoresis in the size range of unlabelled Xenopus tRNAs. The two 4S RNAs are distinctly different in size, indicating that they are encoded by different genes. Control oocytes injected with Col E1 DNA and radioactive nucleoside triphosphates or the radioactive nucleoside triphosphates alone do not synthesise any such major or distinct 4S RNA. (The endogenous tRNA synthesis at this stage of Xenopus oogenesis is relatively low, the bulk of the maternal tRNAs being synthesised earlier on in pre-vitellogenic oocytes.)

Cet 7 DNA directs the synthesis in oocytes of an RNA larger than 4S in addition to the predominant 4S RNA product (Figure 5). In contrast, Cet 1 DNA directs the synthesis of just one 4S RNA species. One distinct 4S RNA is invariably synthesised when Cet 7 DNA is injected into oocytes, but the relative amount of the larger RNA species varies between experiments. Fingerprint analyses (Figure 6) show that these two RNA species are very closely related. It is likely that the larger RNA is a maturation precursor of the smaller rather than a transcript of a different gene.

Characterisation of the 4S RNA synthesised in oocytes. The 4S RNA synthesised in oocytes injected with either Cet plasmid 1 or 7 was compared on polyacrylamide gel electrophoresis with the nematode tRNAs specified by plasmids 1 and 7 isolated by hybridisation to plasmid DNA. Figure 7 shows that the 4S synthesised in the oocyte after injection of Cet 1 DNA co-migrates with Cet 1 nematode tRNA. The nematode tRNA which hybridises to Cet 7 DNA appears to migrate very slightly faster than the 4S RNA synthesised in oocytes injected with Cet 7 DNA. Using this gel system the difference in migration observed is less than that expected for two RNAs differing by a single base and could be attributed to a difference in base modification.

Overall, one can conclude that the tRNA synthesised in oocytes injected with a Cet plasmid and the nematode tRNA which hybridises to the same plasmid are remarkably similar in their electrophoretic properties. This conclusion is strengthened by the fact that the tRNAs of plasmids 1 and 7 are quite different in size.

In addition to their characteristic size (70-90 nucleotides in length) all transfer RNAs contain modified bases (27). The 4S RNA synthesised when oocytes are injected with Cet 1 DNA has been assayed for modified bases. Oocytes were injected with Cet 1 DNA together with either ^{32}P-α-ATP, CTP, GTP or UTP. (Preliminary experiments have demonstrated that radioactivity in one ribonucleotide is not transferred to another base during incubation in the oocyte.) The tRNA products were digested and resolved by thin layer chromatography. Figure 8 shows that most of the radioactivity is found in the

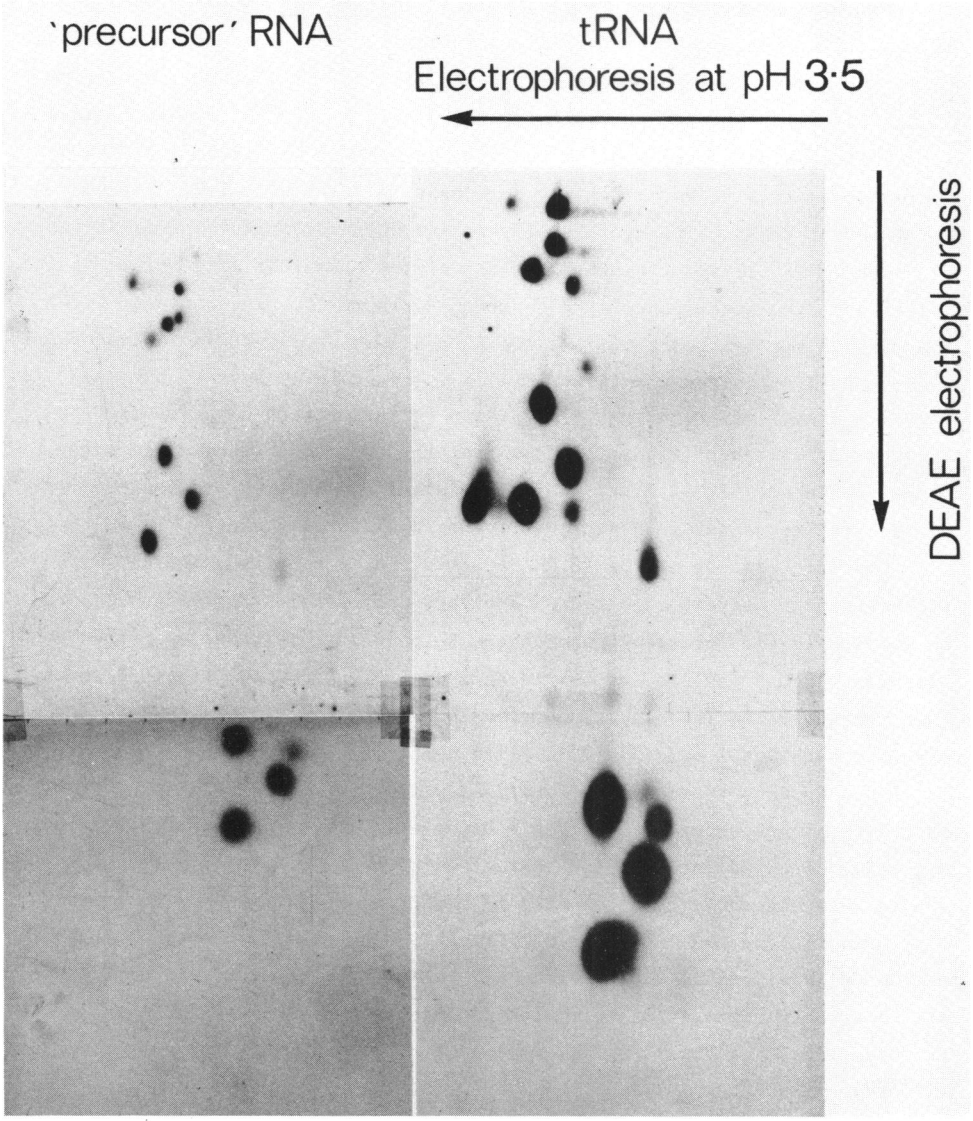

Figure 6: Comparison of T_1 ribonuclease digestion products from the two RNAs synthesised during 5h after injection of Cet 7 DNA into oocytes. After in vivo labelling with ^{32}P-α-GTP the RNAs were separated as shown in Figure 6 where the lower band corresponds in size to Cet 7 specified nematode tRNA and the upper band is the 'precursor' RNA. The T_1 digestion products were separated by electrophoresis on cellulose acetate at pH 3.5 followed by electrophoresis on DEAE cellulose paper in 7% formic acid.

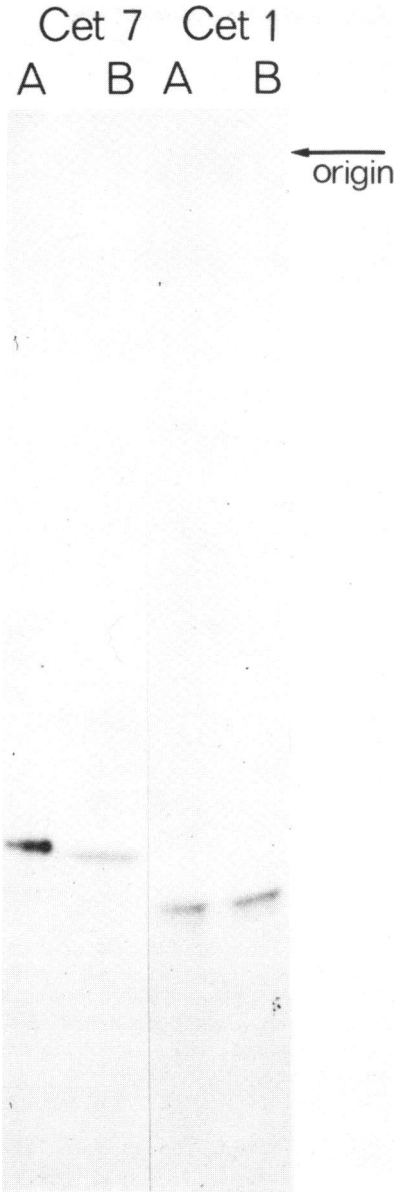

Figure 7: Comparison of the tRNAs specific by Cet 1 and 7 with the 4S RNAs synthesised on injection of these plasmids into oocytes nuclei. Electrophoresis was in a 10% acrylamide, 7 M urea tris borate gel (see Methods). A is the ^{32}P labelled RNA from oocytes; B is the ^{32}P 3' end labelled nematode tRNA hybridisable to the plasmid DNA.

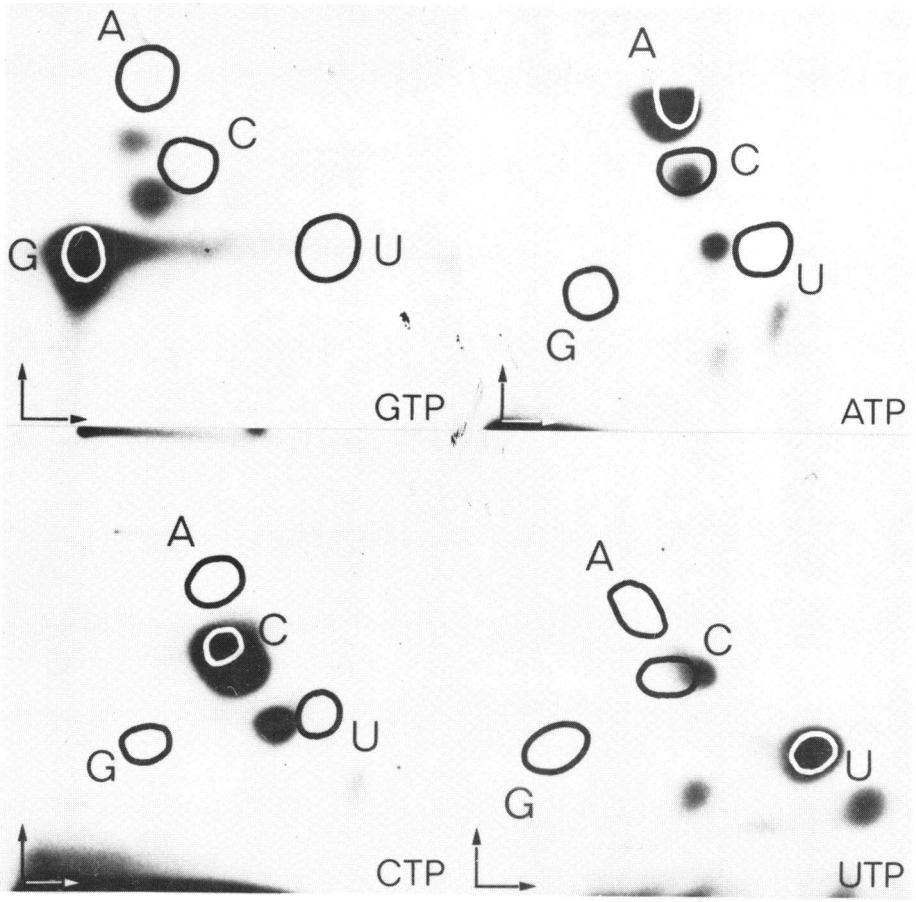

Figure 8: Minor base analysis of the 4S RNA synthesised in oocytes following injection of Cet 1 DNA. The oocyte germinal vesicles were injected with Cet 1 DNA together with ^{32}P-αGTP, ATP, CTP or UTP. The 4S RNA was purified by gel electrophoresis, hydrolysed with venom phosphodiesterase and the 5'-nucleotides separated by 2-dimensional thin layer chromatography (27). A, G, C and U mark the positions of the major nucleotides.

ribonucleotide which had been injected, though some label is found in other positions. These minor spots signify modified bases some of which can be tentatively identified (see legend to Figure 8). Thus, Cet 1 DNA directs the synthesis of a 4S RNA which contains several modified bases. These results show that some of the frog's RNA modifying enzymes recognise nematode tRNAs.

Cet DNA restriction fragments support tRNA synthesis in oocytes. The experiments presented above indicate that Cet plasmids contain both the

structural tRNA gene and the DNA region(s) responsible for controlling its transcription. An operational test for functional genes, genes containing both structural and regulatory regions, is provided by injection into oocytes. With this in view, linear DNA-restriction fragments of Cet 1 DNA have been injected. Figure 9 summarises the results obtained when various portions of Cet 1 DNA are injected into oocytes. In each case the tRNA synthesised is indistinguishable, as judged by migration in acrylamide, from that produced by injection of the whole Cet 1 plasmid. The 5500 nucleotide base pair fragment is the entire nematode DNA insert of Cet 1. The 750 base pair Taq fragment contains both Col E1 and nematode DNA segments (see Figures 9 and 10), and was identified as the only DNA fragment resulting from a Taq digest of Cet 1 which hybridised to ^{32}P-labelled nematode tRNA. Other Taq fragments, injected into oocytes as controls, do not support the synthesis of a tRNA. Using this method of injecting DNA restriction fragments purified from acrylamide gels, we have narrowed down the DNA segment containing functional tRNA gene to 300 base pairs (Figures 9 and 11). Neither an Hae III nor a Hinf II restriction digest of this DNA fragment supports tRNA synthesis when injected into oocytes. The relative position of the structural and regulatory segments with respect to these restriction enzyme sites will be established from the sequence of this DNA fragment.

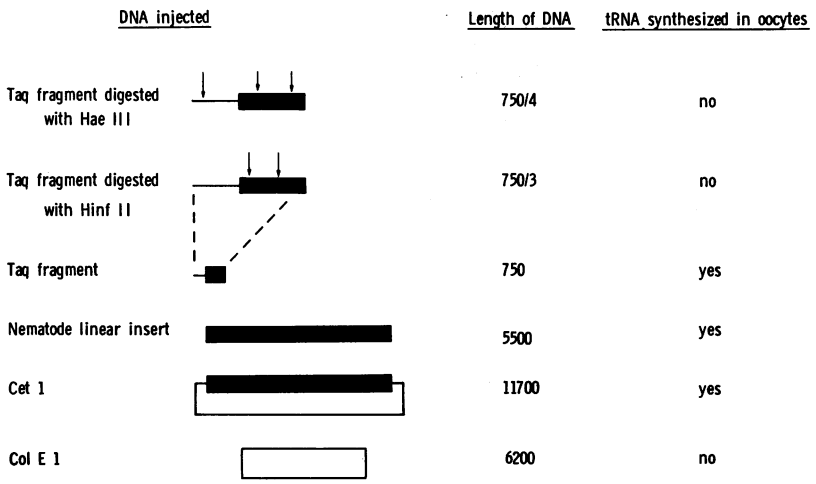

Figure 9: The restriction fragments of plasmid Cet 1 DNA which were injected into oocytes.

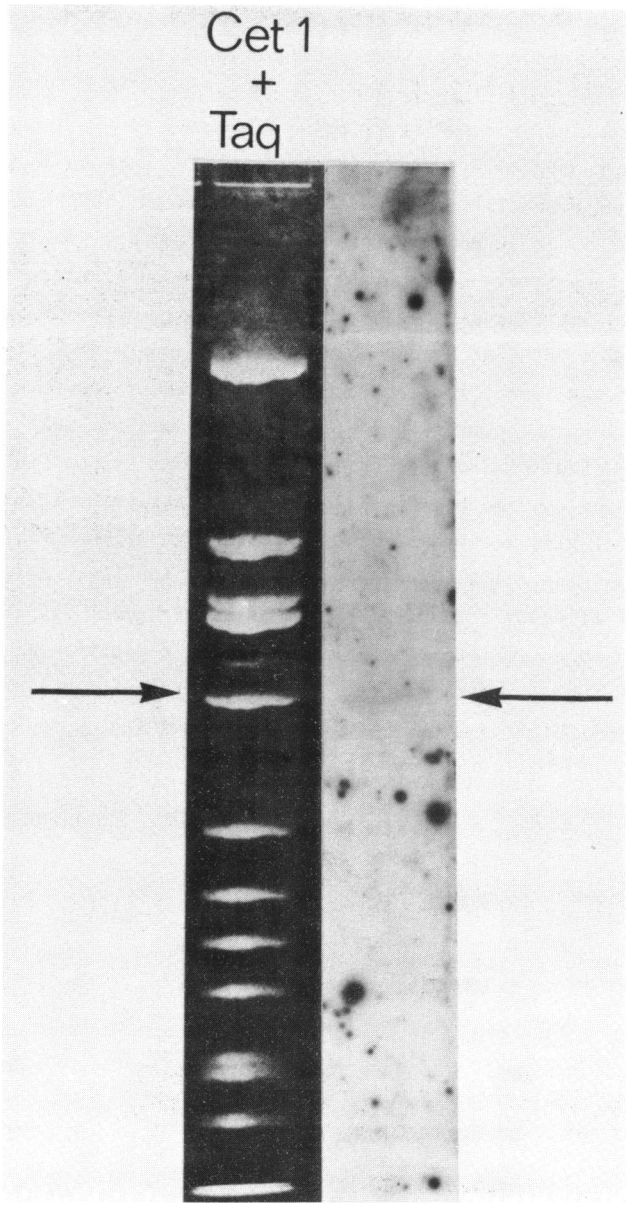

Figure 10: Hybridisation of ^{32}P 3'-terminally labelled nematode tRNA to Taq restriction fragments of plasmid Cet 1 DNA. One the left is shown the separation of the DNA fragments by electrophoresis on a 5% acrylamide gel after staining with ethidium bromide. These were transferred to a cellulose nitrate strip and hybridised with the labelled tRNA. The autoradiograph is shown in the right.

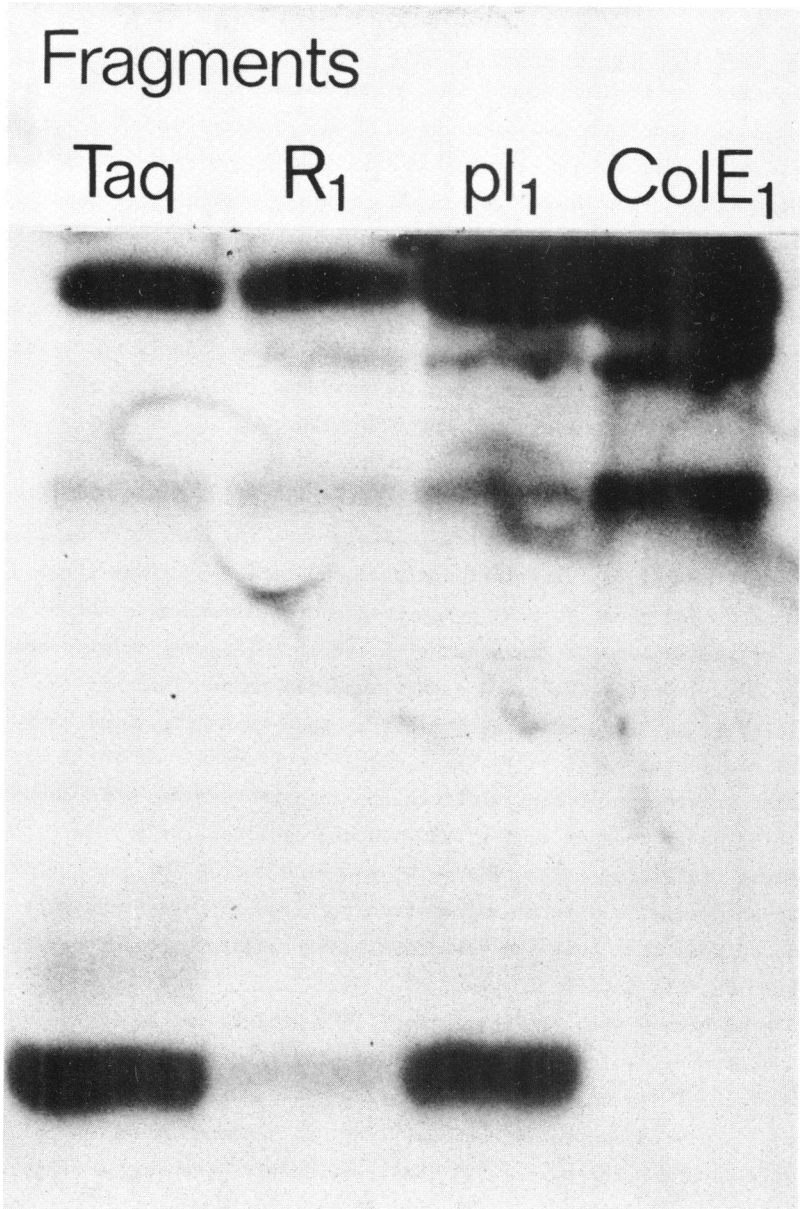

Figure 11: RNAs synthesised in oocytes after injection of plasmid Cet 1 DNA and isolated fragments derived by restriction enzyme digestion. Separation was by electrophoresis on a 12½% tris-borate gel (see Methods). The DNAs injected were (from left to right): the Cet 1 Taq digest fragment hybridisable to tRNA; the Eco R1 fragment comprising the nematode DNA insert in Cet 1; Cet 1; Col E1. The lower faster migrating band corresponds to tRNA.

DISCUSSION

Our data show that Xenopus oocyte injections can be used as an operational assay for functional tRNA genes cloned from a heterologous source. The hybrid plasmids isolated were unequivocally identified as containing tRNA sequences by hybridisation to nematode tRNA labelled with (^{32}P) ATP using tRNA nucleotidyl transferase; an enzyme acting specifically on tRNAs (with the exception of some plant virus RNAs). We have not yet identified these tRNAs by their sequence or aminoacylation specificity. Nevertheless the fact that the 4S RNAs synthesised in the oocyte in response to plasmid DNA injection co-migrate on gel electrophoresis with the plasmid specified nematode tRNAs, and contain modified nucleotides, implies that the final 4S RNA product is indeed the plasmid specific tRNA.

Unpublished results of Melton and Cortese showing that the E. coli $tRNA_I^{Tyr}$ gene when integrated into ϕ80 or E. coli pacl plasmid DNA is not expressed on injection into oocytes indicate that the oocyte recognises eukaryotic transcription initiation signals specifically. Our results suggest that injection of nematode tRNA genes into oocytes results in normal transcriptional and subsequent RNA processing events. The possibility remains that the DNAs injected into oocytes are randomly transcribed and that the specificity of the product is determined by post-transcriptional processing. However, the injection of restriction digestion fragments of Cet 1 not only shows that transcription begins within the inserted nematode DNA sequence but has enabled us to dissect this hybrid plasmid and identify a relatively small DNA segment containing a tRNA gene. We conclude that a 300 nucleotide base pair segment contains all the information required for its transcription in vivo and suggest that both the structural and regulatory regions are closely associated in this eukaryotic gene.

In conjunction with DNA sequencing, this project can be extended to further dissect these tRNA genes. For example, it may be possible to alter the structure of the DNA in vitro and assay the putative mutants for functional genes by oocyte injections. The DNA sequence of mutant plasmids which still contain the structural tRNA gene should help define promoter or regulatory regions. Finally, with similar information on other Cet plasmids, a comparative anatomy of the nematode tRNA genes may help clarify the molecular basis by which this gene family is regulated.

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