Proc. Natl. Acad. Sci. USA
Vol. 75, No. 10, pp. 4819–4823, October 1978
Biochemistry

Specific transcription of eukaryotic tRNA genes in *Xenopus* germinal vesicle extracts

(eukaryotic precursor tRNAs/promoter function/cloned DNA/eukaryotic transcription system)

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Communicated by Frank H. Ruddle, July 26, 1978

ABSTRACT **Cloned tRNA genes from *Drosophila* and from yeast have been transcribed faithfully in extracts prepared from *Xenopus* germinal vesicles. The newly formed RNA is composed of precursor tRNAs (of 5S RNA size) and of tRNAs. The plasmid pCIT12 carries genes for *Drosophila* $tRNA^{Lys}$, $tRNA^{Arg}$, and $tRNA^{Asn}$. Nucleotide analysis of one RNA species transcribed from pCIT12 DNA showed it to be identical to *Drosophila* $tRNA^{Lys}$; it even contained some of the modified nucleotides expected for this tRNA. This RNA species is formed in the germinal vesicle extract via a larger precursor tRNA molecule that does not contain nucleotide modifications. This simple transcription system should aid studies aimed at defining the regulatory DNA regions responsible for eukaryotic gene transcription. In addition, it may provide tRNA precursors that are needed for detailed investigations of eukaryotic tRNA biosynthesis.**

Our understanding of the process of RNA biosynthesis in eukaryotic cells and of its regulation is limited (1), due in part to the lack of recognized mutants affecting tRNA biosynthesis, even in genetically well-studied eukaryotic organisms such as yeast and *Drosophila*. The development of techniques for molecular cloning of DNA (reviewed in ref. 2) and for rapid determination of nucleotide sequences (3–5) has given some insight into the organization of tRNA genes in the genome (6) and allowed elucidation of the actual DNA sequence of some tRNA genes (7, 8). However, these findings have not provided an experimental strategy for defining the detailed sequence of enzymatic steps leading to mature tRNA, nor have they shed light on the importance of the intervening sequences found in some tRNA genes (7, 8). But it is clear that novel enzymes must be involved to exclude such nucleotide sequences from the mature tRNA (9).

The availability of tRNA genes emphasized the need for routine tests of their biological function. In particular, an *in vitro* transcription system is desirable in which cloned eukaryotic tRNA genes can be easily transcribed. This should help to define the presumptive sites of initiation and termination of transcription and also to make available precursor tRNAs for studies of their enzymatic conversion to mature tRNA. The *Xenopus* oocyte is a promising system for such studies. DNA injected into the oocyte nucleus can be transcribed *in vivo* (10, 11). Cloned *Xenopus* genes for 5S RNA (12) and for the initiator tRNA (13) have been faithfully transcribed into their mature products in this fashion. Brown and his colleagues (14) have greatly simplified this procedure by their demonstration that cloned *Xenopus* 5S DNA can be efficiently transcribed *in vitro* in an extract prepared from germinal vesicles.

In this paper we show that cloned tRNA genes from *Drosophila* and from yeast can be faithfully transcribed in extracts from *Xenopus* germinal vesicles. The major products of transcription are tRNA precursors which can be processed to mature tRNA in the germinal vesicle extract.

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MATERIALS AND METHODS

DNA. Covalently closed circular DNA of plasmids pCIT12 (6), ColE1 (see ref. 2), pBR313 (see ref. 2), and pYM7 was prepared. pYM7 is pBR313 carrying a 2.5-kilobase (kb) *Hin*dIII fragment of *Schizosaccharomyces pombe* DNA which specifies an unknown *S. pombe* tRNA (unpublished results). Relaxed DNA was prepared from covalently closed supercoiled DNA by treatment with S1 nuclease (15) and phenol extraction. The product was analyzed by electrophoresis in agarose gels. Growth of recombinant DNA plasmids and handling of recombinant DNA was carried out under P2 EK1 conditions.

tRNAs. Individual tRNA species were purified from *Drosophila melanogaster* larvae by standard procedures involving benzoylated DEAE-cellulose, Sepharose 4B, or reversed-phase (RPC-5) chromatography (ref. 16; U. Pedersen and D. DeFranco, unpublished results). Their specific activities (pmol/A_{260} unit) in homologous charging reactions were $tRNA^{Arg}$ (940), $tRNA^{Asn}$ (1500), and $tRNA_2^{Lys}$(1900). For hybridization ^{32}P-5′-end-labeled tRNAs were used. They were obtained by reaction with polynucleotide kinase (16) and purified by two-dimensional polyacrylamide gel electrophoresis (17).

Preparation of Germinal Vesicle Extracts from *Xenopus laevis* Oocytes. Extracts were prepared as described (14). Ovarian lobes were extracted from *X. laevis* females anaesthesized with ethyl-*m*-aminobenzoate methylsulfonate (1%). Stage V and VI oocytes (18) were stripped with forceps and transferred onto petri dishes containing oocyte priming medium (10 mM $MgCl_2$/5 mM Tris·HCl, pH 8.0). After 1 hr at 0° they were transferred into J buffer [70 mM NH_4Cl/70 mM $MgCl_2$/10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), pH 7.4/0.1 mM EDTA/2.5 mM dithiothreitol), and the oocyte envelope was disrupted with forceps. The germinal vesicles were separated from the yolk, washed, and taken up in J buffer (1 μl of buffer per germinal vesicle). Then the germinal vesicles were ruptured by gently sucking the suspension up and down in an Eppendorf pipette (about three to five strokes). After centrifugation for 5 min at 5000 × *g*, the supernate was promptly used for incubation.

Transcription of DNA in Germinal Vesicle Extract. This was slightly modified from the procedure of Birkenmeier *et al.* (14). The incubation mixture contained DNA (normally 0.03 μg/μl of germinal vesicle supernate), three unlabeled nucleoside triphosphates (0.2 mM), one nucleoside [α-^{32}P]triphosphate (0.01–0.03 mM, 10–40 Ci/mmol), and germinal vesicle supernate (75% by volume of the total incubation mixture). When more than one radioactive nucleoside triphosphate was used in the same reaction, they were present at 0.01 mM. In later experiments α-amanitin (5 μg/ml) was present routinely. Incubation was at 23°–24° for up to 180 min. The reaction was stopped by addition of sodium dodecyl sulfate to a final concentration of 0.5% and incubation with proteinase K (0.1 mg/ml) for 30 min at room temperature. After addition of 15

Abbreviation: kb, kilobases.

mM NaCl/10 mM Tris·HCl, pH 7.8/5 mM EDTA (to a final volume of 0.2 ml) and 50 μg of *Escherichia coli* carrier tRNA, the solution was extracted with phenol and the RNA was recovered by repeated ethanol precipitation.

RESULTS

tRNA Genes on pCIT12 and pYM7. Since most of our work was performed with the DNA of plasmid pCIT12, we needed to know which specific tRNA genes were contained on it. This ColE1 plasmid, carrying a 9.3-kb piece of *Drosophila* DNA, was constructed by Davidson and his colleagues (6). Their initial characterization showed it to contain at least four tRNA genes: *Drosophila* tRNA hybridized to fragments A, B, D, and G of plasmid DNA digested with *Hpa* I/*Eco*RI (see also Fig. 1). They also showed that the tRNA genes on DNA fragments B, D, and G are identical and differ from the one located on fragment A.

Since we had purified a dozen different *Drosophila* tRNA species by conventional chromatographic techniques, we attempted to identify the nature of the tRNA genes coded on pCIT12 by hybridization with these tRNA preparations. Plasmid DNA was digested with *Hpa* I and *Eco*RI and the DNA fragments were separated by gel electrophoresis (6). After denaturation and transfer onto nitrocellulose (19), they were hybridized to ^{32}P-5′-end-labeled tRNA. The results are shown in Fig. 1. $tRNA^{Lys}$ hybridized to bands B, D, and G, while $tRNA^{Arg}$ bound to fragment A. Thus, $tRNA^{Lys}$ probably corresponds to Davidson's tRNA-2, 3, and 4, and $tRNA^{Arg}$ to tRNA-1 (6). The assignments agree with results of *in situ* hybridization to *Drosophila* salivary gland chromosomes (S. Hayashi, I. C. Gillam, A. Delaney, R. Dunn, G. M. Tener, T. Kaufman, T. Grigliatti, and D. T. Suzuki, unpublished results) which show that $tRNA_2^{Lys}$ and $tRNA_2^{Arg}$ hybridize to 42A, the region of the *Drosophila* chromosome 2R to which pCIT12 DNA also hybridizes (6). $tRNA^{Asn}$ also hybridizes to the plasmid DNA fragments B, G, and I and possibly A (Fig. 1). We have no explanation for the different efficiency of hybridization of our pure $tRNA^{Asn}$ preparation (as judged by acceptor activity), which might be a mixture of two isoacceptors with different nucleotide sequence. In this case the $tRNA^{Asn}$ genes on fragment B and fragments G and I might be different, as indicated by the hybridization properties of RNAs 1, 2, and 4. On the other hand, contamination of the $tRNA^{Asn}$ preparation by a minor unrelated tRNA species may also explain the data. Only further studies will indicate the total number of kinds of tRNA genes on pCIT12.

Plasmid pYM7 contains gene(s) for an unknown *S. pombe* tRNA, as shown by hybridization of unfractionated *S. pombe* [^{32}P]tRNA to restriction endonuclease fragments of the plasmid DNA (data not shown).

Use of *Xenopus* Germinal Vesicle Extract for Transcription of tRNA Genes. *Xenopus* 5S DNA can be transcribed into mature 5S RNA in extracts prepared from *Xenopus* germinal vesicles (14). Therefore we reasoned that this system should also be good for transcribing tRNA genes, since these two classes of low molecular weight RNA are formed by the same enzyme, RNA polymerase III (20). We decided to use cloned *Drosophila* and yeast tRNA genes to test this idea.

Plasmid DNA was transcribed and the RNA was analyzed by polyacrylamide gel electrophoresis (Fig. 2). Several bands were observed in the region of 4–5S RNA (near XCFF). These products appeared to arise from insertion of *Drosophila* DNA into the plasmid, since virtually no products were formed when the DNA of the parent plasmid ColE1 was used as template. DNA of other control plasmids (pBR313 or pMB9) had only little template activity; the transcription products were mostly longer than tRNA (data not shown). A similar experiment with DNA of the yeast plasmid pYM7 gave a strong 4S RNA band (Fig. 2). However, there was a low level of unspecific transcription products, probably derived from the bacterial plasmid DNA. In an attempt to identify which RNA polymerase was responsible for RNA formation, we included various concentrations of α-amanitin in reactions with pYM7 DNA as template. RNA polymerase II is inhibited by low concentrations of α-amanitin, while high concentrations of this compound are needed to inhibit the action of RNA polymerase III (20). Low levels of α-amanitin significantly reduced the unspecific transcription, while the synthesis of the 4S RNA product was not diminished (Fig. 2). This may be taken as an indication that RNA polymerase II transcribed bacterial plasmid DNA. However, at higher α-amanitin concentrations the tRNA gene transcription is virtually abolished (Fig. 2). Thus it appears that

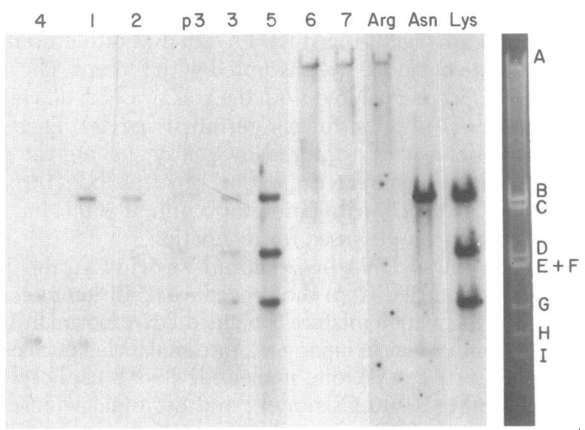

FIG. 1. DNA fragments obtained from digestion of pCIT12 DNA with *Hpa* I and *Eco*RI and hybridization (18) of the fragments with radioactive RNA species. The DNA fragments were electrophoresed in 1% agarose. *Right* column shows the ethidium bromide staining pattern. Length of DNA fragments (kb) (ref. 6): A (8.9), B (1.47), C (1.35), D (0.85), E (0.77), F (0.75), G (0.50), H (0.36), and I (0.27). Radioactive RNA species: Lys, Arg, and Asn denote purified *Drosophila* tRNAs, numbers denote RNA species obtained by transcription of pCIT12 DNA and separated by two-dimensional polyacrylamide gel electrophoresis (see Fig. 3). Careful inspection of hybridization filters shows that DNA fragments C, E, and F do not hybridize to these DNAs.

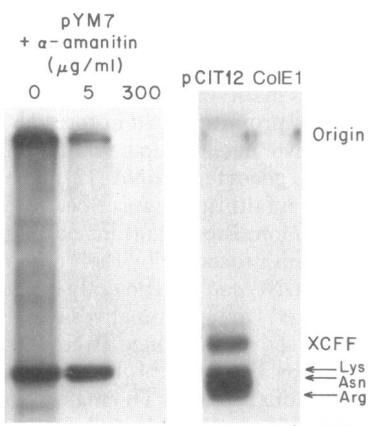

FIG. 2. Polyacrylamide gel electrophoresis of RNA transcription products from pYM7, pCIT12, and ColE1 DNA. Electrophoresis was on a 10% polyacrylamide gel, pH 8.3. XCFF, position of the xylene cyanol FF marker (5.5 cm from origin). Arrows, positions of *Drosophila* $tRNA^{Lys}$, $tRNA^{Arg}$, and $tRNA^{Asn}$. Transcription reaction of pYM7 DNA was 150 min; that of pCIT12 and ColE1 DNA, 90 min. α-Amanitin concentrations in the pYM7 reactions are indicated. RNA was labeled with [α-^{32}P]CTP and detected by autoradiography.

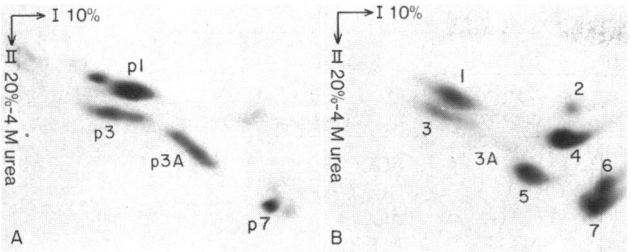

FIG. 3. Separation of [α-^{32}P]CTP-labeled transcription products of pCIT12 DNA by two-dimensional polyacrylamide gel electrophoresis (16). RNA was extracted from a 20-min, 80-μl reaction (*A*) or 130-min, 20-μl reaction (*B*).

RNA polymerase III is responsible for formation of the 4S RNA species in these reactions.

Characteristics of Transcription Reactions. The nucleoside triphosphate concentration stated in *Materials and Methods* was experimentally determined to work well. Since the amount of endogenous nucleoside triphosphates present in the germinal vesicle extract is unknown, the absolute concentration of these RNA precursors in the incubation mixture cannot be stated. However, the concentration of the radioactive triphosphate added (0.01 mM) is rate limiting, but there is sufficient product formed for subsequent biochemical studies. For instance, in a 120-min transcription of pCIT12 DNA the sum of all RNAs recovered after two-dimensional electrophoresis (Fig. 3*B*) gave 7000 dpm/μl of reaction mixture. This represents 0.7% of the input radioactivity. With pYM7 or pCIT12 DNA as template, the incorporation is linear for at least 3 hr.

The optimal DNA concentration is 0.03–0.05 μg/μl of germinal vesicle extract; higher or lower concentrations lead to decreased RNA formation. Since we obtained excellent incorporation with the total plasmid DNA, we did not test the template activity of linear purified *Drosophila* DNA fragments. However, covalently closed supercoiled DNA has a severalfold higher template activity than relaxed DNA prepared by S1 nuclease treatment (data not shown).

Analysis of Transcription Products. The nucleotide sequence of only one *Drosophila* tRNA species, $tRNA_2^{Lys}$, is known (16). Therefore, standard RNA sequence analysis could not be used for identification of all transcription products of pCIT12 DNA as tRNA gene products. Instead, we decided to test the specificity of the transcription reaction by hybridizing the RNA formed *in vitro* to fragments of pCIT12 DNA produced by restriction endonuclease. Separation of the transcription products by two-dimensional gel electrophoresis gave at least seven distinct RNA species (Fig. 3*B*). Species 2, 4, 5, 6, and 7 have tRNA length, while RNA-1 and -3 migrate like 5S RNA. This length estimate is substantiated by the number of T1 oligonucleotides found upon fingerprint analysis of these RNA species. Hybridization of the RNA species to separated pCIT12 DNA fragments is shown in Fig. 1. Of the tRNA-sized transcription products, RNA-5 hybridized to the same DNA fragments as did $tRNA^{Lys}$. RNA-6 and RNA-7 showed the same characteristics as $tRNA^{Arg}$. RNA-2 hybridized to fragment B, while RNA-4 bound to fragments G and I. Bands B, G, and I are the DNA regions to which $tRNA^{Asn}$ hybridizes. Whether this behavior is caused by a sequence heterogeneity between the different $tRNA^{Asn}$ genes needs to be clarified by sequence analysis of the RNA transcripts and of the DNA. The larger RNA species 1 and 3 are very likely precursors of the shorter RNAs (see below) and hybridize like $tRNA^{Asn}$ and $tRNA^{Lys}$, respectively. All the major RNA products of pCIT12 DNA transcription hybridize to the same DNA fragments that the purified tRNAs recognize. While this does not constitute proof, it is strong evidence that the RNA species formed are transcripts of the tRNA genes on pCIT12. Similarly, the RNA formed after transcription of pYM7 DNA hybridizes to the same DNA fragment as does unfractionated yeast tRNA (data not shown).

Detailed Analysis of RNA-5. Since $tRNA_2^{Lys}$ is the only *Drosophila* tRNA species of known sequence (16) and since it hybridizes to the same DNA fragments as did RNA-5, we conducted a detailed analysis (21) of this RNA molecule. We thus prepared separate RNA transcripts labeled with different nucleoside [α-^{32}P]triphosphates. Fig. 4 shows the RNase A and T1 RNase fingerprints of the U- and C-labeled and G-labeled RNA, respectively. Analyses of the eluted oligonucleotide fragments by RNase A, T1 RNase, and T2 RNase are presented in Tables 1 and 2. When compared to the known tRNA sequence, the results indicate that RNA-5 is mature $tRNA_2^{Lys}$. Like the mature tRNA, its 5′-terminus is pG-C and its 3′-terminal sequence is C-C-A_{OH}. This sequence is probably added by tRNA nucleotidyltransferase (22), since the first (located on

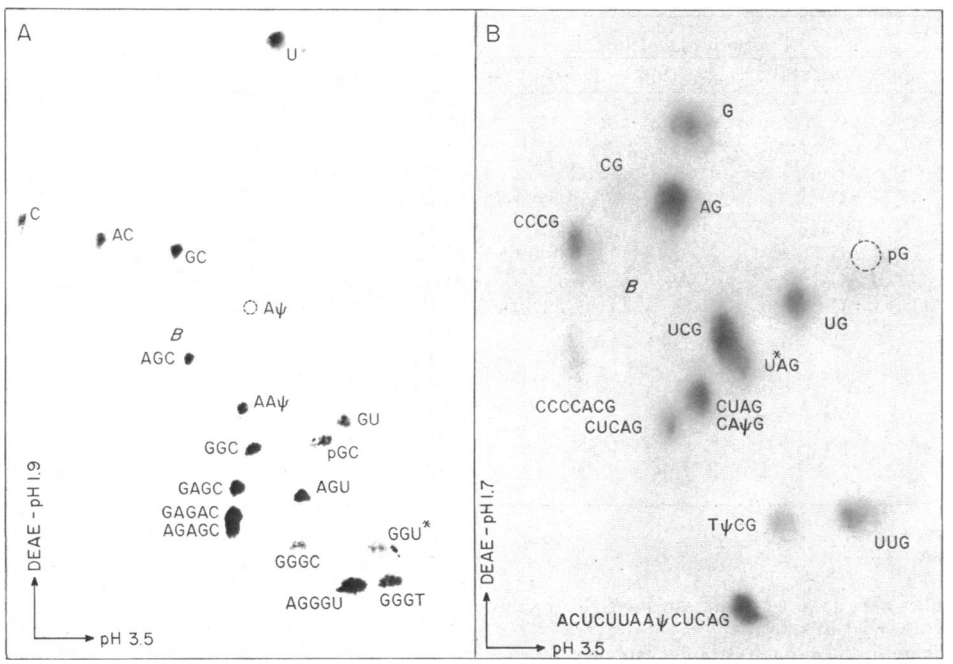

FIG. 4. Fingerprint analysis of RNA-5 (Fig. 3*B*). (*A*) RNase A digest of RNA labeled with [α-^{32}P]UTP + [α-^{32}P]CTP. (*B*) T1 RNase digest of RNA labeled with [α-^{32}P]GTP. The 3′-terminal fragment C-C-A_{OH} is not found in this fingerprint, since it is not labeled by radioactive GTP. It was isolated from an [α-^{32}P]CTP-labeled RNA digest and analyzed as described in Table 2. Asterisk, state of modification of U_{20} not known.

Table 1. Pancreatic RNase end products of RNA-5

Fragment*	Transcript labeled with [α-^{32}P]UTP†	[α-^{32}P]CTP†	[α-^{32}P]UTP + [α-^{32}P]CTP†	Deduced‡
pGC	—	pG,C	pG,C	pGC*C*
GGC	C	G	C,G	GGC*U*
Am^{2}GC	C	AG	C,AG	AGC*U*
AGD	AG	U	AG,U	AGU*C*
GGD	G	—	G	GGU§
AGAGC	—	AG	AG	AGAGC
AΨ	AU	AΨ	AΨ	AΨ
GAGAC	AC	AC	AC	GAGAC*U*
t^{6}AAΨ	AAU	AAU	AAU	AAU*C*
AGGm^{7}GD	G	U	G,U	AGGGU*C*
GU	2G,1U	—	2G,1U	GU + GU*U*
GGGTmΨ	G,T,U,	—	G,T,U	GGGT*U* + GGGU*U*
Gm^{1}AGC	—	AG,C	AG,C	GAGC*C*
AC	—	AC	AC	AC
GGGC	—	G	G	GGGC
GC	—	G,C	G,C	GC*C*
C	C	C	C	C*C*,C*U*
U	U	U	U	U*C*,U*U*

* From known sequence.
† Digested with T1 RNase.
‡ Bases indicated by italics were deduced from nearest-neighbor analysis.
§ State of modification of U_{20} is not known.

fragment B in Fig. 1) of the tRNA^{Lys} genes of pCIT12 whose sequence was determined does not contain these nucleotides in its DNA sequence (unpublished results). We have ascertained the state of modification of all nucleotides in RNA-5, which, from knowledge of the tRNA sequence, were expected to be modified.* As can be seen from Tables 1 and 2, ribothymidine and all three pseudouridines were formed in the germinal vesicle extract, while m^{1}A was found only in fractional amounts. However, the other modified nucleosides (m^{2}G, D, t^{6}A, m^{5}C, Tm, and m^{7}G) present in mature tRNA were absent in the transcript. In their oocyte injection experiments of *Xenopus* tRNA$_i^{Met}$ genes, Kressmann *et al.* (13) also observed significant undermodification of m^{7}G in the tRNA formed.

4S RNA Transcripts Are Formed from Larger Precursor RNAs. When the products of a short-term reaction (20 min) were analyzed by two-dimensional gel electrophoresis (Fig. 3A), very few RNA molecules of 4S size were observed. Hybridization data indicate that RNA-p3 binds to tRNA^{Lys} genes (Fig. 1), while RNA-p1 appears to be a precursor of tRNA^{Asn} (data not shown). The time course of formation of RNA-p7 suggests it to be a precursor to RNA-6 and RNA-7. Fingerprint analysis of RNA-p3 has confirmed its assignment as a tRNA$_2^{Lys}$ precursor; with exception of the 3′- and 5′-terminal fragments, all oligonucleotides of the mature tRNA are present in addition to some fragments not found in tRNA$_2^{Lys}$. RNA-p3 contains no modified

* This was omitted only in U_{20} (position in the mature tRNA sequence), for which analysis of A-labeled RNA would have been necessary.

Table 2. T1 RNase end products of RNA-5

Fragment*	Transcript labeled with [α-^{32}P]GTP†	[α-^{32}P]GTP‡	[α-^{32}P]CTP‡	Deduced§
pG	pG			pG
CCCG	C,G			CCCG*G*
CUAm^{2}G + CAΨG	AG,AU	Ψ	G	CUAG*C* + CAΨG
CUCAG	AG			CUCAG
DCG + m^{7}GDm^{5}CG	2C,1G		U	UCG + CUG*G*
DAG	AG			U¶AG
AG	AG	A	G	AG + AG*C*
ACUCUUt^{6}AAΨCUCAG	AG	G	1A,2U,1Ψ	ACUCUUAAΨCUCAG*G*
UG	U,G			UG*G*
TmΨCG	C		Ψ	TΨCG
CCCCACG	AC			CCCCACG
UUG	U,G			UUG*G*
CG	C		G	CG*C*
CCA$_{OH}$	—		C	CCA$_{OH}$‖
G	G			G*G*

* From known sequence.
† Digested with RNase A.
‡ Digested with RNase T2.
§ Bases indicated by italics were deduced from nearest-neighbor analysis.
¶ State of modification of U_{20} is not known.
‖ Identified by electrophoretic and chromatographic mobility.

nucleosides (data not shown). Similar analyses of RNA-1 and RNA-3 (Fig. 3*B*) have indicated that they are precursors to RNA-4 and RNA-5.

DISCUSSION

We have demonstrated that eukaryotic tRNA genes cloned in plasmid vectors can be transcribed specifically in extracts from germinal vesicles. This is shown by the fact that the majority of the RNA transcripts synthesized appear to be tRNA precursors or mature tRNA. Inhibition studies with α-amanitin have implicated RNA polymerase III in this process. The enzyme appears to recognize selectively transcription initiation sites of tRNA genes; only on a low level of transcription of DNA regions devoid of tRNA genes was observed. As in any *in vitro* study, we cannot assess whether accurate transcription initiation has taken place. This will need a careful sequence comparison of initial transcription products formed *in vitro* and *in vivo*. However, the observed size of the precursor tRNA-like transcripts suggests that initiation took place at or close to the true site.

How general is this finding? We have evidence (unpublished results in collaboration with J. Abelson) that pBR313 plasmids containing *Saccharomyces cerevisiae* tRNA genes can be transcribed selectively to yield precursor tRNAs that still contain intervening RNA sequences (9) and also to form dimeric precursor tRNAs. Likewise, tRNA gene transcription was observed in ColE1, pMB9, or pBR322 plasmids containing *Drosophila* or *S. pombe* tRNA genes. Therefore it is unlikely that only certain bacterial plasmid DNAs allow the specific transcription of cloned tRNA genes. It is expected that tRNA genes from other organisms, especially of mammalian origin, can also be transcribed by *Xenopus* germinal vesicle extracts, since very great sequence homology between tRNAs from a large variety of mammalian sources is found (23).

No definitive conclusions about the presence of processing nucleases or tRNA-modifying enzymes in the germinal vesicle can be drawn from the results that mature $tRNA_2^{Lys}$ was formed in our transcription experiments, albeit without its full complement of modified nucleosides. Contaminating ooplasm adhering to the exterior of the germinal vesicles could have contributed some of these enzymes since extensive washing was not done. On the other hand, we may not have detected all tRNA-modifying enzymes present since we did not use optimal reaction conditions. It may be interesting to see whether, upon supplementation of the germinal vesicle extract with the proper cofactors (e.g., *S*-adenosylmethionine), additional modified nucleosides are formed or whether these enzymes are missing in the extract.

The ability of the germinal vesicle extracts to transcribe eukaryotic tRNA genes specifically has two obvious implications. It provides a system in which the DNA regions responsible for the initiation and termination of transcription of these genes can be studied. Nucleotide sequence analysis of a specific tRNA gene (isolated by molecular cloning) and of its initial precursor RNA product (produced in short-time incubations in the germinal vesicle extract) should indicate the presumptive promoter and terminator sites for such genes. *In vitro* chemical modification of these regions in isolated DNA fragments (24) and subsequent tests of such "mutant" DNAs for their transcription efficiency should permit the definitive localization of these sites.

A second area to which these studies will contribute is the detailed investigation of tRNA biosynthesis in eukaryotes. The germinal vesicle transcription system may be used as a tool to produce precursor tRNAs from any cloned eukaryotic tRNA gene; these can be used as substrates in the homologous system to identify and characterize the tRNA processing or modification enzymes. Since in yeast, for instance, many temperature-sensitive mutants are known, some of which may have defects in tRNA processing (25), the availability of yeast precursor tRNAs will be a valuable tool for characterizing these mutants.

The faithful transcription of tRNA genes altered genetically or chemically in regions corresponding to the mature tRNA sequence could provide mutant precursor tRNAs or tRNA whose effect on the processing and modification enzymes could be examined and whose biological activity (e.g., suppressor tRNAs) could be tested in protein-synthesizing systems *in vitro*.

We thank Dr. D. D. Brown for acquainting us with the techniques of germinal vesicle preparation and for his counsel and optimism during this project. We thank Drs. N. Davidson and P. Yen for their gift of the plasmid pCIT12 and advice on characterizing its DNA. This work was supported by grants from the National Institutes of Health (HD 09167 and GM 22854). O.S. and B.H. are recipients of postdoctoral fellowships from the Deutsche Forschungsgemeinschaft.

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