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Transcription of cloned tRNA gene fragments and subfragments injected into the oocyte nucleus of *Xenopus laevis*

(oocyte injection/cloned DNA/$tRNA_I^{Met}$ biosynthesis/transcriptional units/surrogate genetics)

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ABSTRACT **Cloned 3.18 kilobase fragments of *Xenopus laevis* DNA containing genes for $tRNA_I^{Met}$ and for at least one other 4S RNA species are transcribed rapidly after their injection into the nucleus of *X. laevis* oocytes. The newly synthesized RNA can be resolved by gel electrophoresis into a few predominant 4S RNA species and a series of slower migrating components. One of the 4S RNA species appears to be identical, by fingerprint analysis, to the $tRNA_I^{Met}$ isolated by hybridization of somatic cell RNA to this cloned tRNA gene fragment (tDNA). Thus, the $tRNA_I^{Met}$ produced after injection can be both fully processed and modified. Its rate of synthesis is estimated to be about 6×10^9 molecules/hr in each oocyte injected with 2 ng of tDNA. When the tDNA fragment is cleaved into two halves with the restriction endonuclease *Sst* I, each injected half gives rise to a subset of the RNAs produced after injection of the intact fragment. This experiment thus suggests the presence of at least two transcriptional units on this cloned tDNA. This simple way of biologically testing defined restriction fragments may be of value for analyzing the functional organization of other cloned eukaryotic DNA units.**

Although certain eukaryotic genes have been available in highly enriched form for some time, such as those coding for *Xenopus* ribosomal RNA (1), 5S RNA (2), and $tRNA_I^{Met}$ (3) and for sea urchin histone mRNAs (4), it is with the development of techniques for site-specific cleavage and molecular cloning of DNA (reviewed in ref. 5) and for rapid DNA sequencing (6, 7) that huge strides have been made in the analysis of these repetitive genes. These structural studies have revealed some interesting sequence homologies which may be interpreted in a tentative way on the basis of their locations relative to structural genes or to the presumptive sites of initiation and termination of transcription or translation (8). It has become evident, however, that a sequence alone is not enough. To make even just a start toward understanding the strategy behind the organization of eukaryotic gene units requires a biological test of their function. In some cases this can be provided by the techniques of classical genetics. An alternative "surrogate genetics" (8) approach is to study the expression of cloned DNA fragments, both in unmodified form and after suitable sequence alteration, transported into a living cell by a viral vector or by mechanical injection (9).

A promising system for such an approach is the *Xenopus* oocyte which is capable of transcribing injected eukaryotic and prokaryotic DNA (9–12). A difficulty with this system, however, is that the DNA must be injected into the oocyte nucleus for it to be transcribed (11). Because the oocyte is opaque and the nucleus is hidden from view, this is not technically straightforward. The problem can be obviated by a brief centrifugation of the oocytes onto a nylon grid prior to injection (9).

Here we first discuss a few quantitative aspects of transcription by oocytes injected in this manner. Second, we report on the quality of the RNA produced after injection of a cloned fragment of *X. laevis* DNA known to contain genes for $tRNA_I^{Met}$ and for at least one other 4S RNA species. Third, we present evidence to suggest the existence of at least two transcriptional units in this cloned tDNA fragment.

MATERIALS AND METHODS

DNA. Fragments of *X. laevis* 3.18-kilobase (kb) tDNA from clone λt210 were recovered from the recombinant phage DNA by *Hin*dIII digestion, preparative agarose gel electrophoresis, and DEAE-cellulose chromatography.‡ The same methods were used to recover the 1.76- and 1.42-kb fragments from *Sst* I digests of the purified tDNA.

Ooctye Isolation and Centrifugation. Ovarian lobes were removed fully from *X. laevis* females anesthetized with MS-222 (Sandoz). Individual stage V and VI oocytes (13) were stripped free with a platinum loop and were transferred to 5-cm-diameter plastic petri dishes containing 6–8 ml of modified Barth's solution (14). A nylon net (0.8-mm mesh) had been fixed previously to the bottom of each dish with chloroform and sterilized with 0.1% diethylpyrocarbonate and UV irradiation. The oocytes were oriented with their animal (brown) pole upward. The petri dishes and contents were placed with a minimum of shaking onto the flat bottom of swing-out buckets of an MSE Cool-Spin 6 × 1 liter rotor and centrifuged at 1000 × *g* for 10 min at 18°. During centrifugation, the oocytes are squeezed into the mesh of the nylon net and the pigment granules are displaced to form a dark brown ring. As ascertained by serial sectioning, the nucleus is located just below this pigment ring. The oocytes, 50–150 at a time, were routinely injected within 30 min of centrifugation but they maintained their typical pigment ring pattern for at least an additional 1 hr. Centrifugation at 450 × *g* may be better for the long-term survival of the oocytes, although the location of the nucleus is less obvious (15).

Oocyte Injection. DNA for injection was ethanol-precipitated, washed twice with 80% ethanol, dried under reduced pressure, and redissolved in 88 mM NaCl/10 mM Tris·HCl, pH 7.5. Radioactive GTP (New England Nuclear or the Radiochemical Centre, Amersham) was lyophylized and dissolved directly in the DNA solution. The final injection solution contained in 10 μl: 1–2 μg of DNA and 50 μCi of [^{3}H]GTP (10 Ci/mmol) or [α-^{32}P]GTP (100–200 Ci/mmol). This was taken

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Abbreviation: kb, kilobase.

‡ S. G. Clarkson, V. Kurer, and H. O. Smith, unpublished.

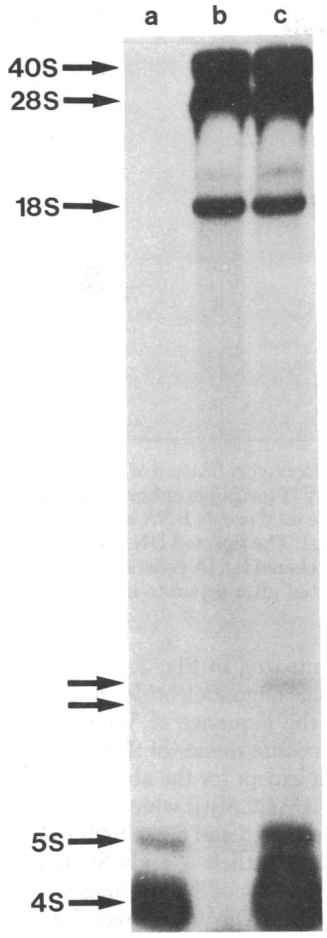

FIG. 1. Gel electrophoresis (3.5% polyacrylamide/0.5% agarose). Lanes: a, 5S and 4S RNA from *X. laevis* tissue culture kidney cells labeled for 3 days with [^{32}P]orthophosphoric acid; b, total RNA from *X. laevis* oocytes incubated for 5 hr after injection of [α-^{32}P]GTP; c, total RNA from oocytes incubated for 5 hr after simultaneous injection of [α-^{32}P]GTP and 3.18-kb tDNA fragments from clone λt210. Unlabeled arrows indicate the migration positions of RNAs found after tDNA injection and having apparent lengths of 200–250 nucleotides.

up in a glass injection needle with a tip diameter of 20–30 μm. The needle was fixed to a micromanipulator via a needle holder and was connected, by plastic tubing filled with paraffin oil, to a 5-μl syringe (Hamilton 85NE). The syringe was driven by a foot-operated motor via a micrometer drive to give a flow rate of 0.2 μl/min. A constant injection volume was maintained by keeping the needle in each oocyte for a fixed time during continuous liquid flow. Injections were done by first moving the petri dish to bring an oocyte close to the needle. The micromanipulator was then adjusted so that the needle pierced the oocyte in the center of the pigment ring, with the needle tip just below the surface. Approximately 20 nl containing 2–4 ng of DNA and 0.1–0.2 μCi of labeled GTP was injected into each oocyte. Oocytes surviving this treatment (generally about 90%) were detached from the nylon net by gently blowing medium over them with a pasteur pipette. They were then transferred into fresh modified Barth's medium and incubated at 18° for 4–7 hr. Injections of the purified tDNA fragment, or its subfragments, were carried out under P2 containment conditions.

RNA Extraction and Analysis. At the end of the labeling period the oocytes were washed twice in modified Barth's solution and then were transferred to 10 mM Tris·HCl, pH 7.5/1.5 mM $MgCl_2$/10 mM NaCl containing proteinase K at 1 mg/ml. Approximately 50 oocytes were resuspended per ml of this medium. Sodium dodecyl sulfate was added to 2% and the mixture was intermittently vortexed during 15 min at room temperature. NaCl was added to 0.3 M and the turbid brown solution was extracted three times with phenol/$CHCl_3$, 1:1 (vol/vol), and three times with $CHCl_3$ alone. RNA was recovered by ethanol precipitation, washed twice with 80% ethanol, and dissolved at about 1 mg/ml in 0.2% sodium dodecyl sulfate. Incorporated radioactivity was assayed by trichloroacetic acid precipitation in the presence of 1% sodium pyrophosphate (16).

Total oocyte RNA was fractionated on composite slab gels (0.2 × 25 × 25 cm) of 3.5% polyacrylamide/0.5% agarose in 90 mM Tris·borate, pH 8.3/2.5 mM EDTA (17). Electrophoresis was at 8 V/cm for 3 hr at room temperature. For more detailed analysis of low molecular weight RNA, the total RNA was first centrifuged through 5–20% sucrose gradients in 10 mM Tris·HCl, pH 7.5/0.1 M NaCl/0.5% sodium dodecyl sulfate in a Beckman SW 40 rotor at 38,000 rpm for 5 hr at 23°. RNA in the 3S–10S region of the gradients was ethanol precipitated and fractionated by two-dimensional polyacrylamide gel electrophoresis in the above Tris·borate/EDTA buffer at 4° (18). Electrophoresis in the first dimension (10% gel) was at 10 V/cm for 10 hr and in the second (20% gel) it was at 12 V/cm for 30 hr.

Total RNA from *X. laevis* tissue culture kidney cells labeled for 3 days with [^{32}P]orthophosphoric acid was hybridized under RNA excess conditions with the 3.18-kb tDNA fragments of clone λt210.‡ The hybridized RNA was eluted and directly analyzed on two-dimensional gels.

RNA recovered from gels was digested with RNase T_1 and the oligonucleotides were fingerprinted (19) by ionophoresis on cellulose acetate strips at pH 3.5 followed by homochromatography on DEAE-cellulose plates with homomix VI of Jay *et al.* (20). The oligonucleotides were eluted and digested with RNase A, and the products were analyzed by one-dimensional ionophoresis on DEAE-81 paper in pyridine acetate at pH 3.5 (19).

RESULTS

Quantitative Aspects of GTP Incorporation by Centrifuged Oocytes. Centrifuged stage V and VI oocytes of *X. laevis* incorporated radioactive GTP into RNA rapidly within 1 hr of injection and then at a slower rate for at least the next 3 hr. After this time, 2–3% of the injected label was found in RNA. The kinetics of incorporation were similarly biphasic for uncentrifuged oocytes, as has been reported (21). The same level of incorporation occurred irrespective of whether the GTP was injected into the nucleus or cytoplasm. From this we infer that the injected GTP equilibrates rapidly with the GTP pool which, for these stage oocytes, is at least 150 pmol (22, 23). Our standard injection of 0.1 μCi/oocyte has a negligible effect on the size of this pool and thus gives it a specific activity of about 0.67 Ci/mmol. Under these conditions, centrifuged oocytes incorporated an average of 2.3×10^3 dpm of labeled GTP within 1 hr. This represents an incorporation rate of 1.55 pmol of GTP per oocyte per hr which is 70% of the initial rate of RNA synthesis in uncentrifuged stage VI oocytes (21). Hence, the synthetic capacity of oocytes was little impaired by centrifugation prior to injection. Moreover, centrifuged oocytes have recently been shown to be active in coupled transcription–translation experiments (15).

Transcription of tDNA in Injected Oocytes. After the first few hours of incubation, most of the GTP incorporated by

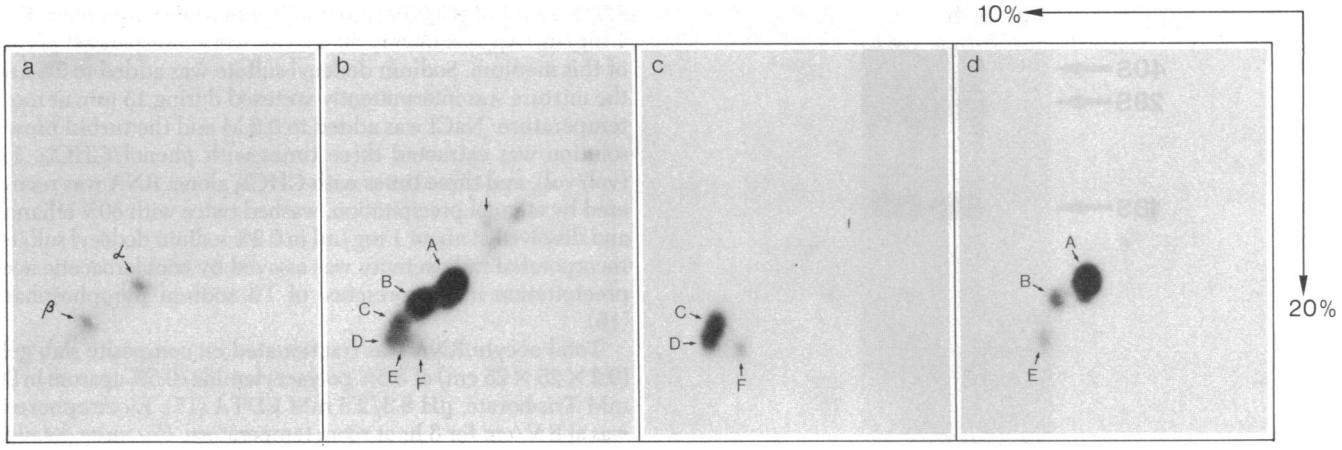

FIG. 2. Two-dimensional polyacrylamide gel electrophoresis. (*a*) The RNA recovered after hybridization of 3.18-kb tDNA fragments from clone λt210 with total RNA from *X. laevis* tissue culture kidney cells labeled for 3 days with [^{32}P]orthophosphoric acid; (*b*, *c*, and *d*) RNA from *X. laevis* oocytes incubated for 4 hr after simultaneous injection of [α-^{32}P]GTP and DNA. The total oocyte RNA was first fractionated by sucrose gradient centrifugation; the RNA in the 3S–10S region was applied to the first-dimension gel. The injected DNA was the intact 3.18-kb tDNA fragment in *b*, the left arm 1.76-kb fragment in *c*, and the right arm 1.42-kb fragment of the cloned tDNA generated by *Sst* I digestion in *d* (see Fig. 4). The unlabeled arrow in *b* indicates the 4S–5S RNA component that is not detected after separate injection of the two *Sst* I fragments.

centrifuged oocytes was found in the 40S ribosomal RNA precursor and its processing products whereas endogenous synthesis of 5S RNA and 4S RNA was hardly detectable (see lanes a and b, Fig. 1). In contrast, a greatly enhanced synthesis of 4S RNA occurred after injection of cloned 3.18-kb fragments of *X. laevis* DNA known to contain genes for $tRNA_1^{Met}$ and for at least one other 4S RNA species (lane c, Fig. 1). The same extent of 4S RNA synthesis occurred after injection of either 2 or 4 ng of linear tDNA fragments per oocyte, which suggests that both doses are saturating. The transcription required the injection of the tDNA into the oocyte nucleus, as has been observed with other templates (9, 11, 12). In our hands, this was achieved in about 50% of the injected oocytes. Synthesis and processing of the ribosomal RNA precursor were not impaired by the 4S RNA synthesis, which can account for 30–70% of the incorporated GTP in 4–7 hr of incubation. Additional minor bands of radioactivity were found in the 4S–5S and 5S RNA regions of composite polyacrylamide/agarose gels and in some larger RNA species which, from their mobilities in such gels, had lengths of 200–250 nucleotides (lane c, Fig. 1).

The 4S RNA synthesized after injection of linear 3.18-kb tDNA fragments could be resolved into a limited number of spots by two-dimensional polyacrylamide gel electrophoresis of the 3S–10S region of sucrose gradients (9). With more extensive electrophoresis in each dimension, four major and two minor spots were found in the 4S region of the gels and at least three minor spots in the 4S–5S region (Fig. 2*b*). Only two stable RNA species from *X. laevis* tissue culture cells are capable of hybridizing with this cloned tDNA.‡ These two species (α and β in Fig. 2*a*) are in the 4S range and they coelectrophorese with two of the major spots produced after tDNA injection (A and C of Fig. 2*b*).

From its electrophoretic mobility, we suspected that the prehybridized spot β was $tRNA_1^{Met}$. Therefore, β and C were recovered from the gels and digested with RNase T_1, and the ensuing oligonucleotides were fractionated by ionophoresis on cellulose acetate followed by homochromatography on DEAE-cellulose. Each oligonucleotide from spot β was then further analyzed by RNase A digestion; similar analyses were performed on the RNase T_1 products of spot C but yielded less-complete information because they had been labeled only with [α-^{32}P]GTP. The two fingerprints and their assigned sequences are compared in Fig. 3. The prehybridized RNA β contained all the oligonucleotides anticipated for *X. laevis* $tRNA_1^{Met}$ from the sequence of Wegnez *et al.* (24). Spot C, synthesized as a consequence of tDNA injection, had an identical fingerprint except for the absence of the 3′-terminal oligonucleotide ($CUACCA_{OH}$) which should not be labeled with [α-^{32}P]GTP (Fig. 3*b*). β and C are therefore identical, with the possible exception of their 3′ ends. Some molecules of spot C may be slightly undermodified as suggested by the apparently lower content of m^7GDm^5CG. Nevertheless, this oligonucleotide is clearly present and we conclude, therefore, that oocytes are capable of producing $tRNA_1^{Met}$ faithfully and in fully modified form after injection of the appropriate tDNA.

A further conclusion concerns the rate of synthesis of this tRNA species. From the radioactivity recovered from spot C for fingerprint analysis, we estimate that each successfully injected oocyte incorporated about 3.5×10^2 dpm of labeled GTP per hr into this RNA. At a specific activity of 0.67 Ci/mmol (see above), this corresponds to a rate of synthesis of about 6×10^9 $tRNA_1^{Met}$ molecules per hr.

Transcription of Subrepeat Fragments of tDNA. The distribution of known genes within the 3.18-kb tDNA fragment of clone λt210 is shown in Fig. 4. It is based on restriction enzyme analysis coupled with RNA·DNA hybridization‡ and on sequence analysis of selected regions of the DNA (F. Müller and S. G. Clarkson, unpublished data). Two $tRNA_1^{Met}$ genes are present on the same DNA strand—about 0.35 kb apart in the left half of the repeat—and are transcribed in the direction shown in Fig. 4. A further two 4S RNA genes (and perhaps more) are located somewhere to the right of the single *Sst* I cleavage site.

Two contrary modes of transcription of these genes can be envisaged: (*i*) coordinate transcription in the form of a polycistronic precursor, in which case *Sst* I digestion should prevent transcription of those genes located downstream of the cleavage site; and (*ii*) independent transcription of each gene, in which case *Sst* I digestion, if it has any inhibitory effect at all, would be expected to impair the transcription of only one gene. We have attempted to distinguish between these possibilities by studying the effects of separate injection of the two fragments of tDNA generated by *Sst* I digestion.

Each of these fragments was transcribed rapidly in the oocyte

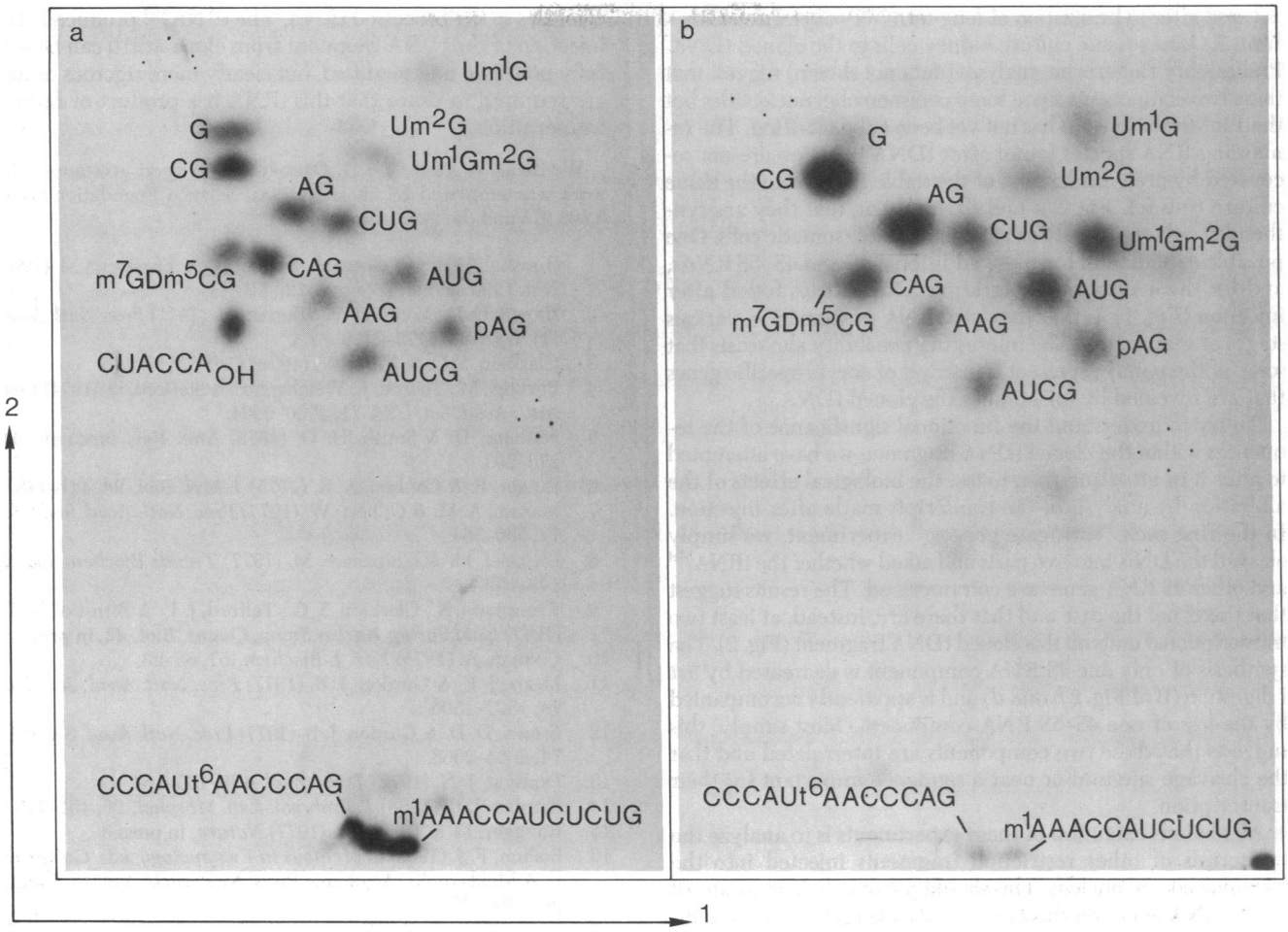

FIG. 3. Comparison of RNase T_1 fingerprints. (*a*) The uniformly ^{32}P-labeled spot β (Fig. 2*a*) recovered from hybrids between 3.18-kb tDNA fragments from clone λt210 and total RNA from *X. laevis* tissue culture cells. (*b*) Spot C (Fig. 2*b*) synthesized by *X. laevis* oocytes after injection of the 3.18-kb tDNA fragments and [α-^{32}P]GTP. The oligonucleotide sequences were assigned by analyses of the mobilities of the RNase A digestion products. Faint spots in the middle of *b* are contaminants from spot B (Fig. 2*b*). First dimension: ionophoresis on cellulose acetate at pH 3.5. Second dimension: homochromatography on DEAE-cellulose.

nucleus although at a somewhat decreased rate compared to that of the intact tDNA. When their low molecular weight transcripts were analyzed by two-dimensional gel electrophoresis, newly synthesized RNA components were found and were unique to each *Sst* I fragment (Fig. 2 *c* and *d*). Moreover, these components together accounted for all of the labeled spots found after injection of the intact tDNA, with the exception of one of the 4S–5S RNA components (marked with an arrow in Fig. 2*b*). Only one 4S RNA component (spot B) is underrepresented (cf. Fig. 2 *b*, *c*, and *d*). We conclude that 4S RNA synthesis from either half of this tDNA fragment does not require the presence of the 4S RNA genes in the other half. Hence, the low molecular weight RNA species synthesized in oocytes after tDNA injection are unlikely to be derived from a common polycistronic precursor.

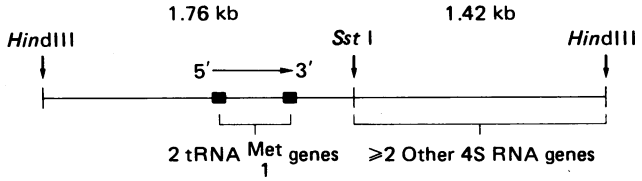

FIG. 4. Simplified restriction endonuclease map of the 3.18-kb tDNA fragment from clone λt210. The direction of transcription of the two $tRNA_1^{met}$ genes is indicated.‡ The polarity and exact locations of the other 4S RNA genes of this fragment have not yet been determined.

DISCUSSION

This paper demonstrates that $tRNA_1^{Met}$ is faithfully produced as a consequence of the injection of a cloned tDNA fragment into the oocyte nucleus of *X. laevis* (Figs. 1, 2, and 3). Endogenous 4S RNA synthesis is barely detectable whereas in each successfully injected oocyte up to 70% of the incorporated GTP is found in 4S RNA species which are synthesized at a rate of about 3×10^{10} molecules per hr. Of these, $tRNA_1^{Met}$ identified by fingerprinting (Fig. 3) comprises about 6×10^9 molecules. This is 10% of the $tRNA_1^{Met}$ content of a mature oocyte (24). Hence, injected oocytes are capable of producing, in a few hours, as much $tRNA_1^{Met}$ as they synthesized over a period of at least 100 days prior to injection. This extensive transcription is not unusual, however, because one-quarter to one-half of all newly synthesized RNA can be derived from other injected templates, such as simian virus 40 (11) or sea urchin histone DNA (9), although there is no evidence that all these molecules represent *bona fide* mRNA.

In addition to $tRNA_1^{Met}$, several other low molecular weight RNA species are synthesized after injection of the cloned tDNA fragment. One of these (A of Fig. 2*b*) has the same electrophoretic mobility as a 4S RNA (α of Fig. 2*a*) that can be re-

covered after hybridization of long-term ^{32}P-labeled total RNA from *X. laevis* tissue culture kidney cells to the cloned tDNA. Preliminary fingerprint analyses (data not shown) suggest that these two components share some common oligonucleotides but their interrelationship has not yet been fully clarified. The remaining RNA species found after tDNA injection are not recovered by prehybridization of the stable RNA from the tissue culture cells (cf. Fig. 2 *a* and *b*), implying that they are synthesized only transiently or not at all in these somatic cells. One possible explanation for these additional 4S and 4S–5S RNAs, and for the even slower migrating RNA species found after injection (Fig. 1), is that they are tRNA precursors in various stages of maturation. The interesting possibility also exists that some of them may represent transcripts of oocyte-specific genes that are revealed by injection of the cloned tDNA.

To try to understand the functional significance of the sequences within this cloned tDNA fragment, we have attempted to alter it *in vitro* and then to test the biological effects of the alteration by analysis of the transcripts made after injection. In the first such "surrogate genetic" experiment, we simply cleaved the DNA into two parts and asked whether the $tRNA_1^{Met}$ and other 4S RNA genes are cotranscribed. The results suggest that this is not the case and that there are, instead, at least two transcriptional units on this cloned tDNA fragment (Fig. 2). The synthesis of only one 4S RNA component is decreased by *Sst* I digestion (B of Fig. 2 *b* and *d*) and is apparently accompanied by the loss of one 4S–5S RNA component. Most simply, this suggests that these two components are interrelated and that the cleavage site is in or near a sequence important for their transcription.

An obvious extension of these experiments is to analyze the transcripts of other restriction fragments injected into the *Xenopus* oocyte nucleus. This should not only help to locate all the 4S RNA genes on this cloned tDNA fragment but also indicate whether each gene can be transcribed independently. The value of this system for understanding gene expression, however, will only be realized by the demonstration of faithful transcription of the injected DNA rather than of post-transcriptional processing of random transcripts. Some caution is also required, therefore, for those experiments in which expression of the injected template is assayed only by hybridization of the transcripts to the injected DNA or, in the case of DNA transcribed by RNA polymerase II, by the ability of some of the transcripts to be translated. In the case of injected tDNA, we expect three essential steps leading to the appearance of mature tRNA molecules: the synthesis of the tRNA precursor, the removal of extra non-tRNA sequences from the precursor by processing enzymes, and the modification of certain nucleotides in the processed tRNA. The $tRNA_1^{Met}$ produced after injection of the tDNA fragment from clone λt210 can be both fully processed and modified, but clearly more rigorous criteria are required to prove that this tRNA is a product of faithful transcription.

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1. Birnstiel, M. L., Wallace, H., Sirlin, J. L. & Fischberg, M. (1966) *Nat. Cancer Inst. Monogr.* **23,** 431–447.
2. Brown, D. D., Wensink, P. & Jordan, E. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 3175–3179.
3. Clarkson, S. G. & Kurer, V. (1976) *Cell* **8,** 183–195.
4. Birnstiel, M., Telford, J., Weinberg, E. & Stafford, D. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 2900–2904.
5. Nathans, D. & Smith, H. O. (1975) *Ann. Rev. Biochem.* **44,** 273–293.
6. Sanger, F. & Coulson, A. R. (1975) *J. Mol. Biol.* **94,** 441–448.
7. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
8. Birnstiel, M. & Chipchase, M. (1977) *Trends Biochem. Sci.* **2,** 149–152.
9. Kressmann, A., Clarkson, S. G., Telford, J. L. & Birnstiel, M. L. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** in press.
10. Colman, A. (1975) *Eur. J. Biochem.* **57,** 85–96.
11. Mertz, J. E. & Gurdon, J. B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1502–1506.
12. Brown, D. D. & Gurdon, J. B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2064–2068.
13. Dumont, J. N. (1972) *J. Morphol.* **136,** 153–164.
14. Gurdon, J. B. (1968) *J. Embryol. Exp. Morphol.* **20,** 401–415.
15. Rungger, D. & Türler, H. (1977) *Nature,* in press.
16. Bollum, F. J. (1968) in *Methods in Enzymology,* eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 12, Part B, pp. 169–173.
17. Peacock, A. C. & Dingman, C. W. (1968) *Biochemistry* **7,** 668–674.
18. Ikemura, T. & Dahlberg, J. E. (1973) *J. Biol. Chem.* **248,** 5024–5032.
19. Barrell, B. G. (1971) in *Procedures in Nucleic Acid Research,* eds. Cantoni, G. L. & Davies, D. R. (Harper & Row, New York), Vol. 2, pp. 751–779.
20. Jay, E., Bambara, R., Padmanabhan, R. & Wu, R. (1974) *Nucleic Acids Res.* **3,** 331–353.
21. Anderson, D. M. & Smith, L. D. (1977) *Cell* **11,** 663–671.
22. Woodland, H. R. & Pestell, R. Q. W. (1972) *Biochem. J.* **127,** 597–605.
23. LaMarca, M. J., Smith, L. D. & Strobel, M. C. (1973) *Dev. Biol.* **34,** 106–118.
24. Wegnez, M., Mazabraud, A., Denis, H., Petrissant, G. & Boisnard, M. (1975) *Eur. J. Biochem.* **60,** 295–302.