High-fidelity transcription of 5S DNA injected into *Xenopus* oocytes

(purified genes/RNA synthesis/5S RNA/living oocytes/nuclear injection)

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ABSTRACT   Purified DNA, from *Xenopus* erythrocytes, containing genes for 5S ribosomal RNA (5S DNA) is transcribed faithfully when it is injected into the nucleus of a *Xenopus* oocyte. Full length 5S RNA of the correct sequence is transcribed from the injected DNA, and the newly synthesized transcripts hybridize predominantly with the coding strand of 5S DNA. The majority of transcripts hybridize with the gene region as opposed to the spacer region of the coding strand. Accurate transcription starts within the first 3 hr and continues for up to 4 days after injection of 5S DNA. Radioactive 5S RNA is detected in as short a labeling time as 1 hr.

A "dual" 5S RNA gene system exists in *Xenopus* and other amphibians (1). Growing oocytes synthesize a mixture of 5S RNAs, the principal component of which differs in several nucleotides from the 5S RNA made by somatic cells. The DNAs that contain the structural genes for the oocyte-type 5S RNA have been purified from *Xenopus laevis* (Xlo) (2) and *Xenopus borealis* (Xbo) (3) and their structures have been partly characterized. Because of their simple well-defined transcripts, these 5S DNAs are particularly attractive for studies aimed at reconstructing faithful RNA transcription *in vitro*. Attempts to produce faithful transcripts of purified 5S DNA by the DNA-dependent RNA polymerase of *Escherichia coli* (4) or by the various forms of *Xenopus* polymerases (5) have been unsuccessful. These enzymes synthesize RNA abundantly from the noncoding strand as well as from the spacer sequences of naked 5S DNA. The size of these transcripts is heterogeneous, being usually much greater than 5S RNA and even longer than a full repeat of 5S DNA (6).

An alternate approach toward an experimental transcription system has been to inject DNA into *Xenopus* eggs and oocytes. A low level of transcripts was synthesized from 5S DNA and ribosomal DNA that had been injected into *Xenopus* eggs (7). Recently it has been found that simian virus 40 and several other kinds of DNA are transcribed after their injection into the oocyte nucleus (germinal vesicle, GV) (8). In this paper we show that 5S DNA injected into oocytes is transcribed with a high degree of fidelity into a 5S RNA that cannot be distinguished from the 5S RNA naturally transcribed from these genes in unmanipulated oocytes.

MATERIALS AND METHODS

Purification of 5S DNA. The 5S DNAs used for these experiments were the principal oocyte types purified from erythrocyte DNA of *Xenopus laevis* (Xlo) (2) and *Xenopus borealis* (Xbo) (3). The strands of these two kinds of 5S DNA were separated by alkaline CsCl density gradient centrifuga-

Abbreviations: The four different kinds of 5S RNA and their respective genes (5S DNA) are Xbo, Xbs, Xlo, and Xls—*Xenopus borealis* oocyte-type and somatic-type, *Xenopus laevis* oocyte-type and somatic-type. *Xenopus borealis* is the correct name for the species referred to in our previous publications as *X. mulleri*. GV, germinal vesicle (nucleus) of an oocyte.
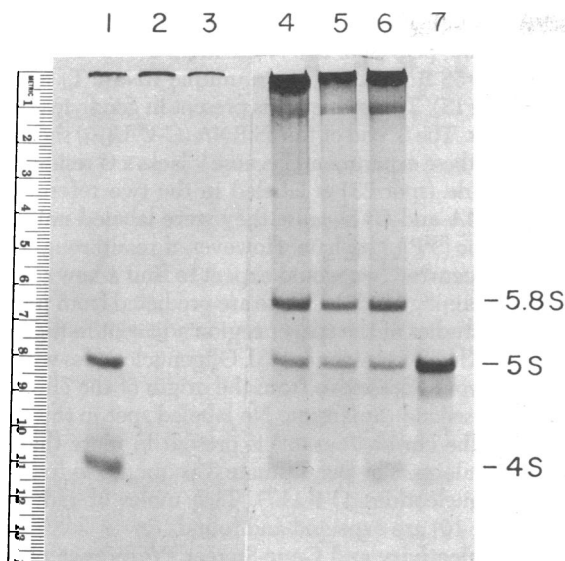
tion. The coding strand of both 5S DNAs is the light (L) strand.

Preparation, Injection, Culturing, and Labeling of Oocytes. Stage V and VI oocytes (9) were extracted from Nembutal-anaesthetized frogs (10), cultured at 19° in MBS-H medium (10), and injected with DNA and radioactive precursors for RNA synthesis. DNA was injected into the GV, which is not visible in living full-sized oocytes (10). DNA is deposited successfully in the GV in about 50% of all attempted GV injections. However, DNA is never found in the GV when it is introduced into the cytoplasm at the vegetal pole (A. Wylie and J. B. Gurdon, unpublished).

Except where otherwise stated, oocytes were incubated for 24 hr after injection of 50 nl of DNA (at 200 μg/ml), and then given a second injection of about 50 nl of the radioactive precursor at 10 mCi/ml into the cytoplasm. Oocytes were labeled with [³H]GTP (10 Ci/mmol) or with [α-³²P]GTP (11 Ci/mmol) (both obtained from the Radiochemical Centre, Amersham). At the end of the desired labeling period oocytes were treated with Pronase (11), defolliculated by hand, and frozen at −70°.

RNA Extraction and Analysis. RNA was extracted from frozen oocytes as described (8). In brief, the method involves digestion with proteinase K, two phenol extractions at pH 7.5, two more at pH 8.9, and ethanol precipitation. The ethanol precipitate was dissolved in buffer containing sodium dodecyl sulfate until it was analyzed.

The radioactive RNA prepared from injected oocytes was electrophoresed into 8% nondenaturing acrylamide slab gels (12). In some cases the gels were sliced and each piece was incubated overnight at 37° with 1 ml of 0.1 M NaOH; 10 ml of Biofluor (New England Nuclear) was added and the radioactivity of the samples was measured. Alternatively, radioautography of ³²P-labeled samples or fluorography (13) of ³H-labeled samples was carried out on the gels. Gels were stained for 30 min with 2 μg/ml of ethidium bromide and photographed (14).

Radioactive RNA samples were hybridized with 5S DNA or its separated strands that had been immobilized on nitrocellulose (HA Millipore) filters. The 5S DNA to which the radioactive RNA was hybridized was the same as that used for the injection. About 1 μg of DNA was adsorbed to each filter. The hybridization medium was 50% (vol/vol) formamide, 4 × SET (1 × SET is 0.15 M NaCl/5 mM EDTA/50 mM Tris at pH 7.8). About 5000 cpm of ³²P-labeled complementary RNA (cRNA) was introduced into each hybridization reaction. This RNA was transcribed from purified Xbo or Xlo 5S DNA by *E. coli* RNA polymerase using [α-³²P]CTP as a precursor. The extent to which cRNA hybridized to homologous 5S DNA in the presence of different ³H-labeled RNA samples normalized possible artifacts in separate hybridization reactions. It also provided a standard for the strand specificity of the [³H]RNA that was synthesized in injected oocytes (see Table 2).

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FIG. 1.   Polyacrylamide gel electrophoresis of [³H]RNA synthesized by *X. laevis* oocytes after injection of Xbo 5S DNA (0 hr) followed by [³H]GTP at 24 hr; the oocytes were extracted at 48 hr. The RNA applied to each slot was extracted from about seven oocytes. Endogenous low-molecular-weight RNAs are visible in the stained gel (slots 4–7). The radiofluorogram is on the left (slots 1–3). Slots 1 and 4, DNA injected into the germinal vesicle; slots 2 and 5, DNA injected into the oocyte cytoplasm; slots 3 and 6, saline injected into the germinal vesicle; slot 7 is known 5S RNA purified from oocytes. (The faster moving bands in slot 7 are degradation products.)

RNA labeled with [α-³²P]GTP was purified from gels and treated with RNase T₁ and the oligonucleotides were fingerprinted by two-dimensional electrophoresis (15). Fractionation in the first dimension was by ionophoresis on cellulose acetate strips at pH 3.5; in the second dimension it was by ionophoresis on DEAE-paper with 7% (vol/vol) formic acid. Individual oligonucleotides were quantitated by cutting them out of the paper and measuring radioactivity directly. Their identity was inferred from their position in the fingerprint. The fingerprints of known Xlo and Xbo 5S RNAs shown in Fig. 4 as references were prepared by R. D. Brown. They were produced from gel-purified 5S [³²P]RNA synthesized by whole ovaries that were incubated with ³²P-labeled inorganic phosphate (16).

The following types of RNA were prepared from unmanipulated materials. Immature ovaries were removed from animals about 4 weeks after metamorphosis and incubated for 2 hr in culture medium containing 1 mCi/ml of [³H]guanosine (17). Total radioactive RNA was extracted from these ovaries by homogenization in 1 × SET, 1% sodium dodecyl sulfate, and an equal volume of water-saturated phenol. The RNA was collected by ethanol precipitation. Nonradioactive 5S RNA was extracted from *X. laevis* ovaries (18) and purified by gel electrophoresis. A sample of this purified 5S RNA was labeled *in vitro* with [¹²⁵I]iodine (19).

RESULTS

Oocytes Injected with 5S DNA Synthesize 5S RNA. A major component of the labeled RNA prepared from oocytes injected with Xbo 5S DNA has the same size as 5S RNA (Figs. 1 and 3). Endogenous 5S RNA in the oocyte extracts is visualized by staining with ethidium bromide; this permits the exact alignment of the radioactive band with the stained 5S RNA band. The 5S DNA must be injected into the GV to obtain this 5S RNA synthesis. Two negative controls are provided by the injection of 5S DNA into the cytoplasm of oocytes and of saline
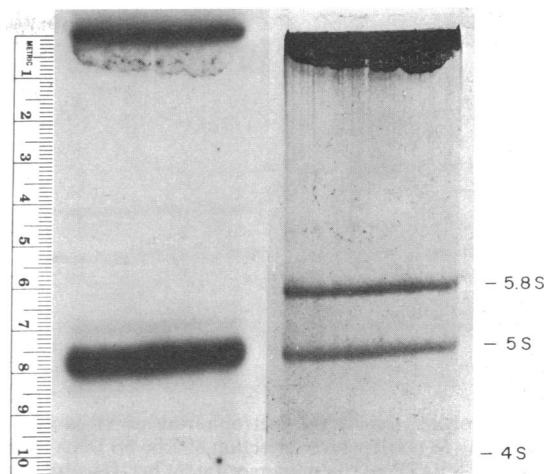

FIG. 2.   Polyacrylamide gel electrophoresis of radioactive RNA synthesized by about 25 oocytes that had been injected with Xlo 5S DNA and [α-³²P]GTP at 0 hr. The oocytes were extracted at 26 hr. The stained gel is on the right. The radioautogram of the same gel is on the left. The labeled 5S RNA was used for the fingerprint in Fig. 4B.

solution into the GV (Fig. 1). In neither case is labeled 5S RNA seen. The endogenous synthesis of 5S RNA is too low to be detected by radioactivity in these radioautograms (see Table 2). The RNA synthesized in oocytes as a consequence of injecting Xbo 5S DNA into the GV includes, in addition to the 5S RNA, two other products (Figs. 1 and 3). These two additional bands of radioactivity migrate coincidentally with stained bands of 4S RNA and an RNA species that is slightly larger than 4S RNA—possibly 4S RNA precursor (see *Discussion*). This latter band is lighter than the 4S or 5S RNA bands and is only visualized when an extract of more than 30 oocytes is electrophoresed and when the gel is stained for more than 1 hr with ethidium bromide (see Fig. 3).

Injected Xlo 5S DNA (Fig. 2) results in a major band of radioactive RNA that comigrates with full-length endogenous 5S RNA. About 60% of all acid-precipitable radioactivity in these oocytes comigrates with 5S RNA of the correct size. Trace bands of radioactivity, excluding that at the gel origin, together comprise less than 5% of the total radioactivity in the sample.

The 5S-sized RNA products were identified as transcripts of the injected 5S DNAs from their oligonucleotide fingerprints. The labeled 5S RNAs synthesized by *X. laevis* oocytes that had been injected with Xbo and Xlo 5S DNA were fractionated on polyacrylamide gels (Figs. 2 and 3). The 5S [³²P]RNAs were digested with RNase T₁ and the oligonucleotides were fingerprinted. The two oligonucleotide patterns are compared in Fig. 4B and D and Table 1 with those expected from the known 5S RNA sequences (Fig. 4A and C) (16). The fingerprints are unequivocally identifiable as those of the two kinds of 5S RNA encoded for by the two 5S DNAs used for injection. All expected oligonucleotides are present in each fingerprint. Endogenous Xlo 5S RNA synthesis is too low to contribute significantly in these experiments. It usually comprises about 0.3% of the total stable RNA synthesized in these oocytes (see Table 2), while the 5S RNA synthesized from the injected Xbo and Xlo 5S DNA was about 20% and 60%, respectively, of all labeled RNA in these experiments. The known oligonucleotide composition of Xlo and Xbo 5S RNAs is listed in Table 1. Note especially that the presence or absence of nucleotides 2, 3, 20, B, and C distinguishes the oocyte-type 5S RNAs from the two species of *Xenopus*. The oligonucleotides 7 and 19 are characteristic of
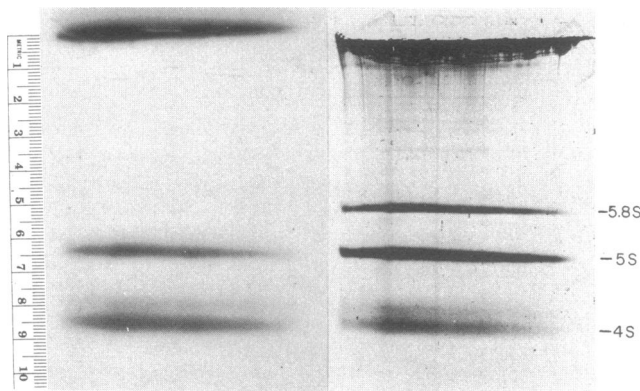
FIG. 3. Polyacrylamide gel electrophoresis of ^{32}P-labeled RNA synthesized by 56 oocytes after injection of Xbo 5S DNA (0 hr) followed by [α-^{32}P]GTP as the precursor (at 24 hr). Oocytes were extracted at 48 hr. The stained gel is on the right. The radioautogram of the same gel is on the left. The labeled 5S RNA was used for the fingerprint in Fig. 4D.

the somatic-type 5S RNAs, and they are present in either low or undetectable amounts in these fingerprints. Oocytes synthesize small amounts of somatic-type 5S RNA (see ref. 16) so that oligonucleotides specific for Xbs and Xls 5S RNAs are detected in the two reference 5S RNAs (Fig. 4A and C). The trace spots seen on the chromatogram probably reflect heterogeneity in the thousands of genes for 5S RNA (16). However, no single trace oligonucleotide is present in more than 0.2 molar equivalents. One spot is present in lower molar equivalents than predicted from the known sequence of these 5S RNAs. Spot 15 is the largest T$_1$ oligonucleotide and, in our experience, is easily overdigested by RNase T$_1$.

The 5'-terminal nucleotide of all four types of *Xenopus* 5S RNA is G (16). In the living cell, even within mature ribosomes, 5S RNA can be mono-, di-, or tri-phosphorylated at its 5' end

(20). This fingerprint detects unequivocally only one of these possible termini, pGp (spot 22). Nucleotides 2–7, counting from the 5' end of 5S RNA, comprise a unique RNase T$_1$ hexanucleotide (spot 13). This sequence is present in good yield in the labeled RNAs. The 3' end of the 5S RNA (C-U-U$_{OH}$) should not be labeled in these experiments because it lacks a G residue. This oligonucleotide (spot 23) is labeled in the two reference 5S RNAs (Fig. 4A and C) because they were labeled uniformly with inorganic [^{32}P]phosphate. However, if readthrough at the 3' end had occurred, we would expect to find a new oligonucleotide containing four Us. These are predicted from previous sequencing studies of the spacer region adjacent to the 3' end of Xbo and Xlo 5S RNA genes (18). Oligonucleotides with four U residues would not move from the origin of the chromatogram in the second dimension. No labeled spot in this region (the top of the chromatogram) is present in more than 0.04 molar equivalents. The penultimate 3' sequence in 5S RNA is U-A-G-G (nucleotides 114–117). Two moles of radioactive U-A-G (spot 10) are expected and found.

Strand Selectivity and Gene-Spacer Preference of Transcription. One experiment was carried out with each of a range of DNA injection and RNA labeling times (Table 2). Synthesis time varied from 1 to 24 hr. The DNA and labeled precursor were injected together or the DNA was injected for 3 hr to several days before the isotope. In all cases, radioactive 5S RNA of the correct size and in excess of the endogenous level was detected by polyacrylamide gel electrophoresis. Transcripts of the coding strand (L) were much more abundant than those of the noncoding strand (H). The amount of spacer transcripts varied but was usually less than gene transcripts as determined by competition with excess unlabeled 5S RNA (data not shown). When Xbo 5S DNA was injected, more than 50% of the RNA that hybridized with Xbo 5S DNA was competed out by excess nonradioactive 5S RNA in most cases. This can be compared with a value of about 25% when the same experiment was carried out with [^{3}H]cRNA synthesized by *E. coli* RNA poly-
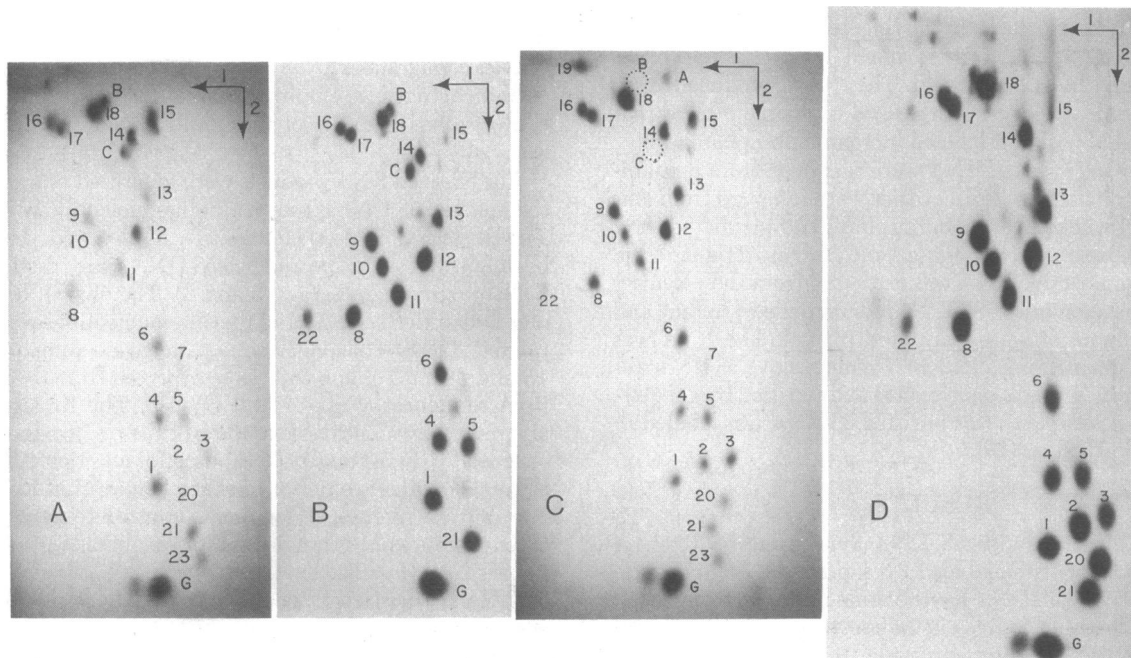

FIG. 4. A comparison of RNase T$_1$ oligonucleotide profiles of uniformly labeled Xlo and Xbo oocyte-type 5S RNAs from uninjected oocytes (A and C, respectively) with the 5S RNAs synthesized by *X. laevis* oocytes following injection with Xlo and Xbo 5S DNAs and [α-^{32}P]GTP (B and D, respectively). Table 1 summarizes the molar yield of each oligonucleotide and the identification by base sequence. The oligonucleotides in B and D are identified only by their position. They have not been sequenced further in these experiments.

Table 1. Yields of oligonucleotides from RNase T$_1$ digestion of ^{32}P-labeled 5S RNA synthesized by X. laevis oocytes injected with X. borealis and X. laevis oocyte-type 5S DNA

Spot	Oligonucleotide	Yield from Xbo 5S RNA		Yield from Xlo 5S RNA		Found in 5S RNA‡
		Theoretical*	Actual†	Theoretical*	Actual†	
G	G	14 (4)	3.4	14 (4)	4.7	C
1	A-G	1	1.7	1	1.7	C
2	C-A-G	1 (2)	2.1	0	0.1	Xls, Xbs, Xbo
3	C-C-C-G	1	0.9	0	0	Xls, Xbs, Xbo
4	A-A-G	1	1.1	1	1.2	C
5	A-C-C-G	1	0.9	1	1.1	C
6	A-A-A-G	1	0.9	1	0.8	C
7	C-C-A-A-G	0	0.02	0	0.07	Xls and Xbs
8	U-G	2	2.6	2	2.8	C
9	A-U-G	2 (3)	3.3	1 (2)	2.6	C
10	U-A-G	1 (2)	1.9	1 (2)	2.1	C
11	U-C-G	1	1.3	2 (3)	3.2	C
12	C-C-U-G	2 (4)	4.2	3 (5)	5.5	C
13	C-C-U-A-C-G	1 (2)	1.5	1 (2)	1.3	C
14	A-A-U-A-C-C-A-G	1 (2)	1.5	1 (2)	1.1	C
15	C-C-A-C-A-C-C-A-C-C-C-U-G	1	0.6	1	0.3	Xlo, Xls, Xbo
16	U-U-A-G	1	1.0	1	1.0	C
17	U-C-U-G	1	1.1	1	1.4	C
18	A-U-C-U-C-G	2 (5)	4.1	1 (3)	2.5	C
	U-A-C-C-U-G	1		1		Xbo and Xlo
19	U-A-C-U-U-G	0	0.1	0	0	Xls and Xbs
20	C-C-G	1 (2)	2.0	0	0.03	Xbo
21	C-G	1	1.5	1	1.6	C
22	pG		0.4		0.7	C
A	C-C-A-U-A-C-C-A-C-C-C-U-G	0	<0.08	0	0	Xbs
B	A-U-C-U-C-A-G	0	<0.03	1	0.9	Xlo
C	A-U-A-C-A-G	0	<0.03	1 (2)	1.1	Xlo

* The sequences of the four kinds of 5S RNA are found in ref. 16. The numbers refer to the moles of nucleotide in each kind of 5S RNA. The numbers in parentheses are the moles of [^{32}P]nucleotides where they differ from the actual moles of nucleotides. The difference is due to the nearest neighbor exchange of [α-^{32}P]GTP-labeled RNA as a result of RNase T$_1$ digestion. Thus, a molecule of [$3'$-^{32}P]GMP is only produced where there are three adjacent G residues. Wherever there are two adjacent G residues an oligonucleotide with two labeled phosphate molecules is produced along with one nonradioactive molecule of 3'-GMP. Xlo 5S RNA is said to be heterogeneous at position 90 (16), yielding 0.5 molar equivalents of A-C-C-G (spot 5) and C-C-G (spot 20). Our data support the sequence of Wegnez et al. (21), who found mainly A-C-C-G at this position.

† The radioactive 5S RNA isolated from the gels is shown in Figs. 2 and 3 and fingerprinted (Fig. 4).

‡ C indicates common to all four 5S RNAs.

merase from Xbo 5S DNA. When the same experiment was carried out with Xlo 5S DNA, about 75% of the hybridized material was competed out by unlabeled 5S RNA.

DISCUSSION

The transcription of 5S DNA that has been injected into the GV of oocytes is faithful by the following criteria: Both X. laevis and X. borealis "oocyte-type" 5S DNA support the synthesis of labeled 5S RNA of the correct length as judged by comigration with known 5S RNA. Fingerprints of the labeled 5S RNAs synthesized following injection of Xbo 5S DNA and Xlo 5S DNA are identical to those of authentic Xbo and Xlo 5S RNA, respectively (Fig. 4 and Table 1). Every oligonucleotide is present and close to the expected amount (Table 1). The labeled transcripts hybridize predominantly with the coding strand (L) of 5S DNA (Table 2). Some spacer sequences are detected but a majority of transcripts hybridized with the gene region only. Selectivity of transcription or of posttranscriptional processing is also observed in oocytes injected with simian virus 40 DNA (J. E. Mertz and J. B. Gurdon, unpublished). The possibility that posttranscriptional processing accounts for the faithful transcripts of 5S DNA was not examined in these experiments. However, there is considerable evidence that mature 5S RNA

is the true transcription unit of 5S DNA in eukaryotes (see ref. 1).

In addition to 5S RNA synthesis, injected Xbo 5S DNA promotes the synthesis of an RNA that comigrates with 4S RNA and an RNA that migrates slower than 4S RNA (see Figs. 1 and 3). The "4S" RNA hybridizes with the injected Xbo 5S DNA. When X. borealis total DNA is banded to equilibrium in either neutral CsCl or actinomycin/CsCl gradients, the DNA sequences homologous to this "4S" RNA band in exactly the same position as Xbo 5S DNA. Hybridization of "4S" RNA to Xbo 5S DNA occurs with about 10% the efficiency of that of 5S RNA. We do not know whether the "4S" RNA is one or more transfer RNAs and if it is, whether the "4S" RNA genes are linked with Xbo 5S DNA or just isolated with them. Injected Xlo 5S DNA does not support the synthesis of 4S RNA (Fig. 2).

In order for it to be transcribed, 5S DNA must be injected into the GV, as has also been observed for simian virus 40 DNA (8). Cytoplasmic injection yields no more transcripts than does the injection of saline solution into the GV, as measured either by gel electrophoresis and determining the radioactivity of the 5S RNA band or by hybridization of the labeled transcripts with 5S DNA (Fig. 1 and Table 2). Endogenous 5S RNA synthesis

Table 2. Fidelity of transcription of
5S DNA injected into oocyte germinal vesicles*

Source of DNA	Time of injection of DNA, hr[†]	Duration of labeling, hr	Percent radioactivity[‡] migrating with		Strand selection of transcription, coding/ noncoding
			5S	"4S"	
Xbo	0	0–3	13	27	10
Xbo	0	3–6	24	29	22
Xbo	0	6–9	23	51	14
Xbo	0	24–27	—	—	26
Xbo	0	24–25	13	40	8
Xbo	0	120–144	4	47	8
Xbo	96	120–140	15	42	9
Xbo	0	24–48	17	38	16
Xlo	0	24–48	60	<0.5	80
Saline	0	24–48	0.3	0.2	—
In vivo synthesis, 2 hr pulse[§]					43
[125]I-labeled 5S RNA					45
Xbo cRNA[¶]					1.3
Xlo cRNA[¶]					0.45

* Each line represents a single experiment.
† Experiment was begun at time 0 by placing the ovarian tissue in culture medium.
‡ Gels were sliced and radioactivity migrating coincidentally with 5 S and with "4S" and the slower moving "precursor 4S" RNA was expressed as a percentage of total radioactivity in gel. Variable degradation of samples gives variation due to increased background. In one sample, degraded high-molecular-weight RNA made it impossible to calculate gel radioactivity.
§ Total [3H]RNA isolated from immature *X. borealis* ovaries labeled for 2 hr with [3H]guanosine (see *Materials and Methods*).
¶ Complementary RNA transcribed from the pure 5S DNAs with *E. coli* RNA polymerase.

in these experiments ranged between 0.1 and 0.5% of all RNA synthesized by control uninjected oocytes. Injected Xlo 5S DNA supported transcription of 60% of all RNA synthesized, or about 200 times more 5S RNA synthesis than the endogenous level. Transcripts from Xbo 5S DNA comprised about the same fraction of newly synthesized RNA except that they include both "4S" and 5S RNAs. These values are underestimates, because we know that the DNA is injected successfully into the germinal vesicles of only 50% of the oocytes. Although this number represents a large increase in 5S RNA synthesis, the number of injected genes represents an even larger increase over the normal content. Each tetraploid *X. laevis* GV contains about 10^5 oocyte-type 5S RNA genes (2). An injection of 10 ng of 5S DNA provides about 10^{10} *X. laevis* 5S RNA genes, or about 10^5 times the endogenous level. Thus, 100,000 times the number of endogenous genes is supporting about 200 times the level of endogenous synthesis in the case of injected Xlo 5S DNA. However, preliminary experiments in which less 5S DNA is injected show that our usual dose of 10 ng is in excess of the oocyte's transcriptional capacity by at least an order of magnitude. It is interesting to note that the excess of injected 5S DNA does not act as template for unfaithful transcription but appears not to be transcribed at all.

A wide variety of injection times and labeling times resulted in high-fidelity transcription including the synthesis of full-length 5S RNA. We emphasize that the results in Table 2 are only qualitative. The numbers cannot be compared with each other because each is a single determination. There are several sources of variation that have not been quantitated. First, the

response of individual oocytes to injected DNA varies depending upon the frequency with which the DNA is introduced successfully into the GV. This variation was minimized by using at least 20 oocytes for each sample. Second, some degradation of high-molecular-weight RNA raised the background of radioactivity in gel regions where 4S and 5S RNA migrated. Third, it was difficult to determine the extent to which endogenous synthesis was reduced in oocytes that had been injected with massive doses of 5S genes. Variability of synthesis for the reasons given above and some variation in the total incorporation of isotope by each oocyte reduce the accuracy of our results.

The oocyte injection system brings full circle the approach to animal genetics that we call "genetics by gene isolation." In the past we had to be content with structural and evolutionary studies of purified genes. However, we can now ask whether a particular DNA sequence is capable of functioning in a living cell. In these experiments genes were isolated from a cell type in which they do not function *in vivo* (the nucleated erythrocyte), and at least some of these genes have been shown to be still capable of being faithfully transcribed. Differentiation could not have caused these unexpressed genes to be altered irreversibly.

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