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Purified DNAs are transcribed after microinjection into *Xenopus* oocytes

(simian virus 40/cloned DNA of *Drosophila melanogaster* histone genes/RNA·DNA filter hybridization/eukaryotic transcription system)

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ABSTRACT **The possibility of using DNA-injected *Xenopus laevis* oocytes and eggs for studying the control of transcription in eukaryotes has been investigated. When purified DNA of simian virus 40 (SV40) is injected into *Xenopus laevis* oocytes, tritiated RNA precursors are incorporated into DNase-I-resistant, RNase-A- and alkali-sensitive material that hybridizes specifically to SV40 DNA. This viral transcription continues for at least 5 days and occurs only when the injected DNA is directed to the nucleus of the oocyte. The quantity of SV40-specific RNA produced is roughly proportional to the amount of DNA injected; above 1 ng per oocyte, most of the nonribosomal RNA made in successfully injected oocytes is virus-specific. Transcription also occurs, although at a lower efficiency, after injection of the DNA into unfertilized eggs. The DNAs of adenovirus 5, cloned *Drosophila melanogaster* histone genes, and even bacteriophage φX174 replicative form, bacteriophage φ80p*lac*, and the ColE1 plasmid are also transcribed after injection into oocytes or eggs.**

Due to their large size (1 mm diameter) and ready availability, oocytes and eggs of the African clawed frog, *Xenopus laevis*, have been widely used in a variety of experiments involving microinjection. Previous work has shown that injected DNA serves as a template for DNA synthesis in eggs (1, 2) and that purified mRNAs are translated efficiently after injection into oocytes (3). In both types of processes, injected living cells function more efficiently than present-day cell-free systems. Similarly, eukaryotic *in vitro* systems that use isolated nuclei or partially purified RNA polymerases for the study of transcription are not efficient and exhibit poor fidelity (4, 5). Living cells might therefore offer some advantages over cell-free systems for investigating transcription in eukaryotes. When the synthetic DNA polymer poly[d(A-T)·d(A-T)] (6) or *Xenopus* ribosomal DNA (7) was injected into oocytes and eggs, some template-specific RNA synthesis was detected. In this paper we present a detailed analysis of the use of *Xenopus* oocytes for studying the transcription of various kinds of injected DNAs.

MATERIALS AND METHODS

DNAs. Covalently closed circular DNA (Form I) of simian virus 40 (SV40) strain *WT830* (8) was prepared as previously described (9). ColE1 DNA was grown from *Escherichia coli* C600 strain EQ39 (obtained from S. Brenner) using the chloramphenicol amplification procedure of Clewell (10). *cDm500* DNA (11) was similarly obtained from *E. coli* strain HB101 carrying this plasmid (generously supplied by R. Karp), with the growth medium supplemented with colicin E1 to kill bacterial segregants lacking the plasmid. The covalently closed circular forms of these DNAs were isolated by the "cleared lysate" method of Katz *et al.* (12), concentrated by precipitation with 10% polyethylene glycol 6000/0.5 M NaCl (13), and purified by centrifugation to equilibrium in ethidium bromide/CsCl gradients. Human adenovirus 5, bacteriophage φ80p*lac*$_1$, and bacteriophage φX174 RFI (double-stranded replicative form) and viral ("+" strand) DNAs were the gifts of W. Russell, A. Travers, and C. Hutchison, respectively. The DNA samples were diluted into "injection medium" [10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (pH 7.4)/0.5 mM EDTA/88 mM NaCl] prior to injection.

Injection of DNAs into Oocytes and Eggs. Stage V and VI oocytes (14), obtained from female frogs that had not ovulated for at least 2 months, were prepared as previously described (15) and stored in Hepes-buffered modified Barth's solution (MBS-H) (15) until injected. Unfertilized eggs were obtained by standard procedures (16). Oocytes and eggs were injected with 60 ± 10 nl of sample, using microforge-sharpened pipettes, as described elsewhere (17). Ref. 15 describes how to inject DNA into the nucleus (germinal vesicle, GV) or cytoplasm of an oocyte; because the GV is invisible in the living oocytes used for injection, successful application of this technique requires practice. After injection, oocytes and eggs were incubated in MBS-H, usually at 19°; 25° incubations gave similar results. In some experiments, oocytes were labeled by incubation in [5,6-^{3}H]uridine (43 Ci/mmol) and [8-^{3}H]guanosine (16 Ci/mmol), each at 10 mCi/ml in MBS-H. This procedure leads to the incorporation into RNA of 1 to 4 × 10^3 cpm per oocyte in 1 day. However, labeling by injection of 50 nl per oocyte of [8-^{3}H]GTP (10 Ci/mmol) at 10 mCi/ml in injection medium was found to enhance incorporation by about 3-fold. This latter procedure was thereafter used routinely. Eggs similarly labeled by injection of [^{3}H]GTP were found to incorporate about 0.5 to 5 × 10^3 cpm into RNA in 1 hr. After incubation with radioactive RNA precursors for the indicated period of time, eggs were dejellied using cysteine (17) and oocytes were defolliculated following treatment with Pronase (500 μg/ml for 3 min at 19°) (18). Both cell types were then frozen dry at −70° until processed for extraction of RNA as described in the legend to Fig. 1. In some of the early experiments, oocytes were not defolliculated. However, because 90–97% of the total [^{3}H]RNA in these samples came from the follicle cells surrounding the oocytes (data not shown), the results are presented as the number, rather than %, of hybridizable ^{3}H cpm.

Biohazard Considerations. Work with *cDm500* was performed in accordance with the British guidelines for experiments involving recombinant DNAs; these conditions are analogous to a P2 level of physical containment. All materials

Abbreviations: SV40, simian virus 40; *cDm500*, a recombinant plasmid consisting of 1.8 repeat units of the segment of *Drosophila melanogaster* DNA that codes for the histone proteins and ColE1 DNA joined by poly(dA)·poly(dT) linkers (25); 1 × SSC, 0.15 M NaCl/0.015 M disodium citrate; GV, germinal vesicle, the nucleus of an oocyte; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MBS-H, Hepes-buffered modified Barth's solution.

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Table 1. Transcription of SV40 after injection into oocytes: ^{3}H cpm hybridizing to SV40 DNA on filters

Form of SV40 injected into "GV"	Labeling period, days post injection 0–1	1–2	3–4
SV40-infected cell nuclei*			
Mock-infected	5	16	13
12 hr post infection	14	16	17
43 hr post infection	124	146 (545)†	33
SV40 (I) DNA (0.93 mg/ml)	304	413 (1299)†	ND‡
No material injected	7	11	ND‡

Injected oocytes were frozen immediately after incubation in tritiated nucleosides at 25° for 24 ± 3 hr. Incorporation of radioactivity into virus-specific RNA was determined as described in the legend to Fig. 1. Each number is the cpm in five oocytes' worth of [^{3}H]RNA that hybridized to a filter containing 0.5 μg of SV40 DNA. Blank filters and filters containing bacteriophage λ DNA had an average of 6 and 11 cpm, respectively, when incubated in the same hybridization reactions.

* CV-1 cells, an established line of African green monkey kidney cells (9), were infected with SV40 virions at a multiplicity of infection of 40 plaque-forming units per cell. After incubation at 37° for the indicated times, the infected cells were removed from the flasks by mild treatment with trypsin and EDTA and their nuclei were isolated and injected into the "GV" of oocytes as previously described (26).

† The numbers in parentheses are the total hybridizable ^{3}H cpm per five oocytes as determined by the extrapolation method illustrated in Fig. 1. When these RNA samples were treated with RNase A prior to incubation with the DNA filters, less than 15 cpm were bound.

‡ Not determined.

coming in contact with plasmid-containing bacteria, SV40, adenovirus 5, or *cDm500* DNA were decontaminated before disposal by autoclaving or exposure to Clorox.

RESULTS

SV40 DNA Is Transcribed in Oocytes. Simian virus 40 is an easily obtainable eukaryotic virus with a small (5.2×10^3 base pairs), well characterized, double-stranded circular DNA genome (see ref. 19 for review). Previous work (20) has shown that HeLa cell nuclei injected into *Xenopus* oocytes synthesize RNA for up to several weeks. We first looked at transcription of SV40 in oocytes injected with the natural, transcriptionally active nucleoprotein complexes that are contained within the nuclei of SV40-infected monkey cells. Table 1 shows that, whereas no detectable SV40-specific RNA is produced when mock-infected monkey cell nuclei are injected into oocytes, viral transcripts can be detected in oocytes injected with late-infection monkey cell nuclei. However, control of transcription of DNA in injected whole nuclei would be difficult to analyze due to the numerous components present in a nucleus. Fortunately, purified SV40 (I) DNA injected by itself is also transcribed, suggesting that the DNA itself is the only exogenous component needed for template-specific transcription.

Characteristics of the Transcription System. Fig. 1 illustrates the filter binding assay used for the detection of the radiolabeled virus-specific RNA made in oocytes that have been injected with SV40 DNA and incubated with tritiated RNA precursors; the radioactivity is incorporated into DNase-I-resistant, RNase-A- and alkali-sensitive material that hybridizes specifically to SV40 DNA. The bound RNA elutes from the DNA filters in 1 × SSC (SSC is 0.15 M NaCl/0.015 M disodium citrate) at a melting temperature of approximately 85–90° (data not shown). Furthermore, because micrograms of SV40 DNA are needed to hybridize the virus-specific RNA completely, we conclude that large (i.e., ng) amounts of SV40-specific RNA are synthesized in SV40-DNA-injected oocytes.

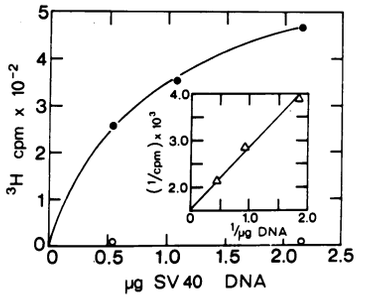

FIG. 1. Assay for synthesis in oocytes of RNA specific for injected DNA: hybridization of ^{3}H-labeled RNA to DNA on filters. RNA was purified from oocytes as performed by E. M. De Robertis, G. A. Partington, and J. B. Gurdon (unpublished). In brief, DNA-injected oocytes, stored frozen at −70° after labeling with tritiated RNA precursors, were homogenized in 50 mM Tris·HCl (pH 7.5)/10 mM EDTA/0.5% sodium dodecyl sulfate containing proteinase K at 500 μg/ml, incubated at 37° for 2 hr, and extracted twice with phenol/chloroform (1:1 wt/vol). The nonaqueous phases were re-extracted with pH 9.0 buffer and nucleic acids were recovered from the pooled aqueous phases by precipitation with ethanol. DNA was eliminated by incubation with DNase I, re-extraction with phenol/chloroform as above, and reprecipitation with ethanol. One to five oocytes' worth of purified [^{3}H]RNA was incubated in 150 μl of 4 × SSC/0.2% sodium dodecyl sulfate at 67–68° for 16–24 hr in the presence of a 6.5 mm diameter nitrocellulose filter (Millipore) containing 0.1–2 μg of denatured DNA (homologous to that used for injection) bound to the filter essentially as described by Raskas and Green (21). (The exact amount of DNA present on each filter was determined in some instances by using ^{32}P-labeled SV40 DNA of a known, low specific activity.) After extensive washing with 2 × SSC and incubation with RNase A (20 μg/ml in 2 × SSC at 30° for 1–2 hr) to remove non-specifically bound RNA, the DNA filters were dried and the amount of ^{3}H cpm hybridized was determined by scintillation spectroscopy with corrections made for machine background (<12 cpm). The *total* amount of [^{3}H]RNA per oocyte capable of hybridizing to an infinite amount of the specific DNA on filters was estimated by extrapolation from a best-fit line using three or more data points on a double-reciprocal plot (see inserted figure for example). In all cases, this calculated value was less than 50% greater than at least one of the data points; for most, it was within 10% of the highest experimental value. To prove that the hybridizable radioactivity was in RNA, samples were treated with RNase A (80 μg/ml in 2 × SSC at 37° for 2 hr) or alkali (0.2 M NaOH at 37° for 16 hr) either prior to incubation with the DNA filters or after elution of the bound radioactive material from the filters by boiling them in 1 mM EDTA (pH 7.5) for 5 min; in all cases tested, less than 5% of the hybridizable material treated in either of these ways was still capable of binding to the filters or of being precipitated with cold 1 M HCl. The actual data shown were obtained from the RNA sample in Table 1 that was prepared following labeling from 1 to 2 days after injection with SV40 (I) DNA. ● and Δ, each hybridization reaction contained 2½ oocytes' worth of ^{3}H-labeled RNA; ○, each hybridization reaction contained 2½ oocytes' worth of RNA that had been treated with RNase A prior to incubation with the DNA filters.

One problem with this transcription system is the severalfold variation in the amount of virus-specific RNA synthesized in comparable groups of oocytes, even when they are treated in as similar a manner as possible (see data in Fig. 3 for examples). The reason for these fluctuations was revealed by assaying the amount of SV40 [^{3}H]RNA produced in *individual* SV40-DNA-injected oocytes. Table 2 shows that, whereas some

Table 2. Transcription of SV40 DNA in single oocytes

Site of injection	^{3}H cpm hybridizing to SV40 DNA on filters
Nucleus	29, 34, 72, 86, 231, 913, 1017, 1063
Cytoplasm	0.4, 1.6, 1.8, 2.0, 2.0, 2.9, 2.9, 3.5
Cytoplasm of enucleates*	1.1, 1.3, 2.5, 2.8, 3.3†, 4.0‡, 4.6

SV40 DNA (16 μg/ml) was injected into oocytes, with the experimenter aiming for the nucleus ("GV"), the nonnuclear animal half ("cytoplasm"), or the center of manually enucleated oocytes. The oocytes were incubated at 20° from ½ to 2½ days after injection in medium containing [^{3}H]uridine. Each value shown is the ^{3}H cpm of RNA extracted from a single oocyte (with 50 μg of yeast RNA added as carrier) that hybridized to a filter containing 0.5 μg of SV40 DNA. The numbers have been arranged in ascending order. Blank filters included in the hybridization reactions had 1–4 cpm above background.

* Oocytes were enucleated as previously described (27) prior to injection.

†, ‡ The total cpm hybridizing from groups of five and ten oocytes, respectively.

"GV"-injected oocytes make large quantities of SV40 RNA, others produce very small or negligible amounts. In addition, when the DNA is injected into the cytoplasm of oocytes or into enucleated oocytes, virus-specific RNA is not detected. Therefore, the region of the oocyte into which the DNA is injected is a crucial factor in obtaining transcription.

To confirm this finding, individual "GV"-injected oocytes were analyzed *both* for retention of the injected DNA by agarose gel electrophoresis and for SV40 RNA synthesis. Five out of nine oocytes injected with 5 ng of SV40 (I) DNA still contained most of the viral DNA as Form I 32 hr after injection, while 22, 19, 18, 16, and 13% of the [^{3}H]RNA synthesized in these oocytes was SV40-specific. However, neither SV40-specific RNA nor SV40 (I) DNA could be detected in the other four oocytes. Because SV40 DNA injected into the cytoplasm of oocytes is not conserved as Form I (A. Wyllie, J. B. Gurdon, and R. A. Laskey, unpublished), these data suggest a correlation between the injection of DNA into the GV and its transcription. Whether the DNA is transcriptionally active only within the nucleus, or whether "GV" injections enable transcription to occur by causing some of the contents of the nucleus to become mixed with cytoplasm, is not yet known. In summary, the variation in the amount of SV40 [^{3}H]RNA synthesized in different oocytes is probably due to our inability to inject the DNA reproducibly into the most effective region of the oocyte. Consequently, oocytes have been processed in groups of 20 or more whenever possible to try to reduce the variability; even so, the numbers obtained are accurate only to within a factor of three or four.

One additional fact is obtained from the above data. The efficiency of the filter hybridization technique used here is only about 50–80% (21). Therefore, the SV40 RNA represents 25% or more of the [^{3}H]RNA produced in oocytes synthesizing viral

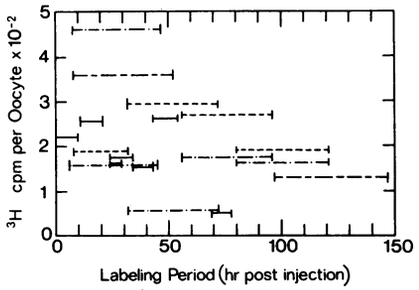

FIG. 2. Incorporation of radioactivity into SV40-specific RNA when the oocytes were labeled at various times after injection. SV40 DNA (100 or 930 μg/ml) was injected into the "GV" of oocytes. After various time intervals, the oocytes were incubated at 19° or 25° in the presence of tritiated RNA precursors until frozen. The amount of radioactivity in each group of oocytes incorporated into SV40-specific RNA was determined as described in the legend to Fig. 1. The positions of each pair of connected vertical bars show the beginning and end of the labeling period for a group of oocytes; the height above the abscissa of the horizontal connecting line indicates the amount of labeled virus-specific RNA present in the sample. The various types of connecting lines denote data compiled from four independent experiments; samples obtained using oocytes from the same female are indicated by a common type of horizontal line.

Table 3. Transcription of various DNA templates in oocytes and unfertilized eggs

DNA injected (100 μg/ml)	DNA used for hybridization	% cpm hybridizable: Oocytes	% cpm hybridizable: Eggs
SV40 (I)	SV40	11.2	0.28
Adenovirus 5	Adenovirus 5	4.7	2.2
ϕ80p*lac*$_1$	ϕ80p*lac*$_1$	2.3	2.5
ϕX174 RFI	ϕX174 RFI	10.9	0.21
	ϕX174 "+" strand	0.60*	0.02
ColE1	ColE1	21.6	0.77
cDm500	ColE1	7.9†	0.40
	cDm500	15.0‡	0.68

25 ± 2 hr after "GV" injection of the indicated DNAs, oocytes were injected with [^{3}H]GTP and incubated at 19° for an additional 27 hr. Unfertilized eggs were labeled for 7 hr at 19° following injection of [^{3}H]GTP mixed with the exogenous DNAs. In each group of defolliculated oocytes or eggs, the percent of the radioactive RNA that could hybridize with the indicated DNAs on filters was determined by the extrapolation procedure described in the legend to Fig. 1. Incubation of the RNA samples with nonhomologous DNAs or with blank filters resulted in less than 0.02% of the input radioactivity binding to the filters, except when RNA from any of the egg samples was hybridized to *cDm500* DNA. In this latter case, a significant amount of cross-hybridization between newly synthesized endogenous *Xenopus* RNA and the *Drosophila melanogaster* histone genes part of *cDm500* was detected, although not at a level high enough to account for the majority of the radioactive RNA from *cDm500* DNA-injected eggs that hybridized to *cDm500* DNA filters. Furthermore, most of the [^{3}H]RNA from *cDm500*-DNA-injected eggs that hybridized to *cDm500* DNA filters eluted from them at the melting temperature of the homologous, rather than heterologous, RNA·DNA hybrids (data not shown).

* As a control, this experiment was also performed by competition hybridization using 0.25 μg of RFI DNA on a filter and 0.5 μg of "+" strand DNA in solution with the RNA; 0.82% of the ^{3}H cpm bound to the filter.

† The RNA that failed to anneal to ColE1 DNA filters in this particular experiment was incubated in a second hybridization reaction with new ColE1 or *cDm500* DNA filters; 1.2 and 7.4% cpm annealed, respectively, to these latter filters. This result directly demonstrates and, therefore, confirms the finding that almost half (i.e., 7%) of the plasmid-specific RNA made in oocytes injected with *cDm500* DNA is specific for the part of the plasmid containing the DNA sequences of the *D. melanogaster* histone genes. Analogous results were also obtained with the RNA from *cDm500*-DNA-injected unfertilized eggs.

‡ As a control for the above experiment, the RNA from *cDm500* DNA-injected oocytes that failed to stick to *cDm500* DNA filters was reincubated with new *cDm500* or ColE1 DNA filters; 1.4 and 1.3% cpm annealed, respectively, in these second hybridization reactions.

Table 4. Transcription of SV40 DNA in single unfertilized eggs

Concentration of injected SV40 DNA (μg/ml)	% ^{3}H cpm hybridizing to SV40 DNA on filters
1000	7.9, 8.7, 9.6, 9.6, 9.6, 10.2
100	1.6, 2.3, 2.5, 2.9, 3.0, 5.0

Unfertilized eggs were injected with SV40 DNA and [^{3}H]GTP, incubated at 19° for 7 hr, and thereafter processed individually as in Table 2. Each value shown is the percent of the total ^{3}H cpm of RNA from a single egg that hybridized to a filter containing 0.5 μg of SV40 DNA.

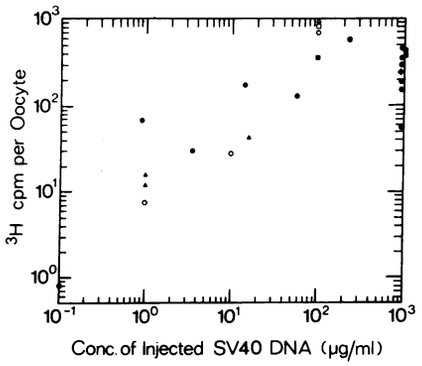

FIG. 3. Relationship between the quantity of SV40 DNA injected and the amount of virus-specific RNA synthesized. Each (50–70 μl) of the indicated concentrations of SV40 DNA was injected into the "GV" of oocytes. The oocytes were subsequently incubated for 1–2 days in the presence of tritiated RNA precursors and processed in groups of 20 or more, as described in the legend to Fig. 1, to determine the average amount of radioactivity incorporated per oocyte into virus-specific RNA. The data shown were compiled from seven independent experiments; points denoted by identical symbols were obtained using oocytes from the same female. Filled-in symbols, oocytes incubated in tritiated nucleosides; open symbols, oocytes injected with [^{3}H]GTP.

RNA. Consequently, most of the nonribosomal RNA made in oocytes successfully injected with SV40 DNA is virus-specific.

Fig. 2 shows the production of SV40-specific RNA at various times after injection. Most of the samples had 100–300 ^{3}H cpm per oocyte of hybridizable RNA regardless of when they were labeled. These data demonstrate that, within the error of our assay, transcription of injected SV40 DNA in oocytes continues at a similar rate for at least 5–6 days.

Fig. 3 shows the quantity of virus-specific RNA made when various amounts of SV40 DNA are injected. Although there is considerable scatter in the points, the general trend indicates that there is a linear relationship between the quantity of DNA injected and the amount of virus-specific RNA synthesized up to a DNA concentration of about 100 μg/ml. Above that concentration, no further increase is seen in the amount of virus-specific RNA produced. This latter phenomenon may be due to a limited supply of some essential oocyte component such as histone proteins or RNA polymerase II.

Various DNAs Are Transcribed. The ability of oocytes to transcribe a variety of other injected DNAs is summarized in Table 3. All of the DNAs tested are transcribed, even the prokaryotic ones. Consequently, transcription of DNA injected into oocytes appears to be a general phenomenon, not confined to special templates such as SV40.

Two points are worth noting here. First, DNA containing the histone genes is transcribed not only in oocytes injected with the ColE1–*D. melanogaster* hybrid DNA *cDm500*, but also in oocytes injected with the purified DNA repeat unit of the histone genes that can be isolated after cleavage of *cDm500* DNA with *Bam*HI restriction endonuclease (data not shown). This finding implies that this segment of the *Drosophila* genome probably contains its own promoter(s) for initiation of transcription. Second, 90–95% of the virus-specific RNA produced in φX174-RFI-injected oocytes is made from the "−" strand of the viral genome. Because the φX174 mRNAs synthesized in *E. coli* are also from the "−" strand (22), this result suggests that correct strand selection for transcription and/or RNA processing can occur with injected DNA and that *Xenopus* RNA polymerases may recognize some bacterial promoters as transcription start signals.

DNAs Are Also Transcribed after Injection into Eggs. The value of unfertilized eggs as a transcription system has also been examined. In summary, we found that transcription occurs; the main advantage over oocytes is the much greater reproducibility in the amount of template-specific RNA made when comparing one injected egg with the next (see Table 4). (This difference is most likely a consequence of nuclear membrane breakdown during the formation of the unfertilized egg from an oocyte with the resulting dispersal of the nuclear contents throughout the cytoplasm.) On the other hand, the disadvantages are: (*i*) less time is available for studying transcription before unfertilized eggs start to deteriorate; and (*ii*) the quantity of template-specific RNA made from a given amount of injected DNA is considerably less for most DNAs tested than that obtained using oocytes (Table 3 and unpublished results).

In conclusion, both oocytes and eggs can be used for studying transcription of injected DNAs; which system is best depends upon the specific subject under investigation.

DISCUSSION

This paper demonstrates that RNA transcripts are synthesized for days from purified DNAs microinjected into eggs or the "GV" of oocytes. Although the detailed steps in this process are as yet undetermined, it is reasonable to expect that the first one would be association of the injected DNA with histone proteins to form a transcriptionally active nucleoprotein complex. Indeed, Laskey *et al.* (23) have recently shown that purified SV40 DNA can be reconstituted into "chromatin-like" structures by incubation with extracts of *Xenopus* eggs; preliminary results suggest that this also occurs after injection of DNA into eggs or oocytes. Such reconstituted structures would then be transcribed using the appropriate cellular RNA polymerase, presumably by initiation at proper promoter sites and with correct processing of the product. On the basis of α-amanitin inhibition experiments (data not shown), we have concluded that injected SV40 DNA is transcribed in oocytes by the same RNA polymerase (i.e., polymerase II) that is used for viral RNA synthesis in its natural host (24). In addition, preliminary RNA sizing and mapping experiments suggest that the majority of the SV40 [^{3}H]RNA obtained from SV40-DNA-injected oocytes is the same size and comes from the same region of the viral genome as the predominant stable viral RNAs produced in monkey cells—i.e., the region coding for the major capsid proteins. Although it is not yet known where transcription starts, this result suggests a high degree of fidelity in selection of the regions being transcribed and/or in processing of the RNA being made. Similar conclusions have also been reached by D. D. Brown and J. B. Gurdon (28) using *Xenopus borealis* 5S ribosomal DNA. Finally, recent experiments by E. M. De Robertis

and J. E. Mertz (unpublished) have indicated that the SV40 RNA produced is translated, because polypeptides coded by the virus can be detected in oocytes injected with SV40 DNA.

The data presented here have shown that transcription of injected DNA is not limited to SV40, thereby eliminating the trivial conclusion that microinjection simply accomplishes artificially the infection of a frog cell by a monkey virus. We have demonstrated transcription in oocytes of many types of injected DNAs and have found that even with prokaryotic genomes, such as that of phage ϕX174, correct strand RNA appears to be synthesized. This experimental system may provide a powerful method for studying control of eukaryotic gene expression.

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