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TRANSCRIPTION OF A CLONED OVALBUMIN ds-cDNA IN XENOPUS LAEVIS OOCYTES

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SUMMARY

A recombinant plasmid containing a double-stranded DNA copy of the ovalbumin messenger RNA (pOV230 DNA) was successfully transcribed by injecting the purified DNA into Xenopus laevis oocyte nuclei. Injection of pOV230 DNA resulted in the synthesis of plasmid sequences and ovalbumin messenger RNA sequences while injection of plasmid DNA resulted in plasmid RNA transcription only. 21-38% of the total [^{3}H]RNA isolated from pOV230 DNA injected oocytes was capable of hybridizing to pOV230 DNA. Strand selectivity experiments revealed that 80-100% of the ovalbumin RNA synthesized was coding strand RNA.

The cloning of eucaryotic genes which has recently been developed opens new approaches to the study of control mechanisms involved in gene expression. The Xenopus oocyte offers a potentially powerful assay system to study the transcriptional and translational capacities of cloned DNAs. Mertz and Gurdon (1) and Brown and Gurdon (2) have shown that purified DNAs can be transcribed in Xenopus oocytes if the DNA is injected into the nucleus. Cloned DNA, including Xenopus 5S DNA inserted into the plasmid pMB9 (3) and Drosophila histone DNA inserted into Col E1 (1), has been shown to be transcribed in Xenopus oocytes.

As a first step in cloning the ovalbumin gene, a double-stranded DNA copy of ovalbumin mRNA has been inserted into the plasmid pMB9 (4). Sequence determination has shown that the ovalbumin DNA in the plasmid (pOV230) contains all sequences but the terminal 12 base pairs present at the 5'-end of $mRNA_{ov}$ (5). We report here that this cloned DNA is capable of being transcribed in Xenopus oocytes, as shown by hydridization analysis using DNA filters.

Abbreviations: pOV230, E. coli plasmid pMB9 containing a full-length double-stranded DNA copy of ovalbumin DNA; $mRNA_{ov}$, messenger RNA for ovalbumin.

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MATERIALS AND METHODS

Xenopus oocytes: Mature female Xenopus laevis were immobilized on ice and portions of the ovary removed through a small abdominal incision. Stage V and VI oocytes (6) were manually isolated and placed in modified Barth's solution (7) until they were injected.

DNA injection: The DNA for injection, previously purified according to Katz et al., (8), was precipitated with 0.25 M NaCl and 2 volumes 100% ethanol, washed twice with 100% ethanol, dried on a flash evaporator and resuspended in 88 mM NaCl/15 mM Tris-HCl, pH 7.8. [^{3}H]UTP alone [Schwartz/Mann (18 Ci/mM)] or [^{3}H]UTP and [^{3}H]CTP together [New England Nuclear [^{3}H]UTP (35.4 Ci/mM), [^{3}H]CTP (22 Ci/mM)] were flash evaporated and dissolved in the DNA solution. The final injection solution contained 200 µg/ml DNA and 10 mCi/ml [^{3}H]UTP (Experiment 1) or 100 µg/ml DNA and 10 mCi/ml each [^{3}H]UTP and [^{3}H]CTP (Experiments 2-5). This solution was taken up into a glass micropipette attached to a multiple injection syringe. Individual oocytes were injected by placing them on a dry microscope slide and either directing the micropipette tip towards the nucleus or the cytoplasm, as described by Gurdon (7). Approximately 50 nl was injected into each oocyte. Injected oocytes were incubated in modified Barth's solution for 48 hours. At the end of the labeling period, the oocytes were washed with distilled H_2O and frozen at -70°C until the RNA was extracted.

RNA extraction: The RNA was extracted from the oocytes as described by Mertz and Gurdon (1) with one additional step. After the DNase treatment, the RNA was applied to a .7x30 cm Sephadex G-50 column (Pharmacia) and the [^{3}H]RNA fractions pooled and precipitated with 0.25 M NaCl and 2.5 volumes of 100% ethanol.

In vitro synthesis of RNA from pMB9 DNA: Unlabeled pMB9 RNA used for the competition experiments described below was synthesized from purified pMB9 DNA according to methods previously published (9), by incubating 50 mM Tris, pH 7.9, 5 mM $MgCl_2$, 100 mM $(NH_4)_2SO_4$, 2 mM β-mercaptoethanol, 1 mM each ATP, CTP, UTP, and GTP, 0.8 mM potassium phosphate, E. coli RNA polymerase (320 µg/ml) and pMB9 DNA (250 µg/ml) for 2 hours at 37° C. At the end of the incubation period, DNase (Worthington, RNase free) was added at 20 µg/ml for 30 minutes at 37° C. The RNA was extracted twice with 1:1 Sevag:phenol and the aqueous phase ethanol precipitated. The precipitated RNA was washed twice with 100% ethanol, dried on a flash evaporator, dissolved in distilled H_2O and passed through a Sephadex G-50 column. The RNA peak was precipitated, dissolved in distilled H_2O and kept frozen until needed.

Purified ovalbumin mRNA ($mRNA_{OV}$): Ovalbumin mRNA was kindly supplied by Dr. Savio Woo. It was purified as previously described (10).

[^{3}H]RNA-DNA filter hybridization: RNA extracted from oocytes was hybridized to pOV230 DNA immobilized on millipore filters according to previously published procedures (9). The filters (0.65 mm dia.) were placed in small vials containing a total of 40 µl hybridization solution. The solution contained 50% formamide, 1.4XSSC[1], 5 mM EDTA and from 6,000 to 88,000 dpm [^{3}H]RNA, depending upon the experiment. A small amount of [^{32}P] $cRNA_{OV}$ was added to most vials to determine the efficiency of the hybridization reaction. The [^{32}P]$cRNA_{OV}$ was transcribed from $cDNA_{OV}$ using E. coli polymerase.

To test for strand specificity, the DNA filters were preincubated with 5 µg $mRNA_{OV}$ overnight and subsequently washed with 2XSSC[2] before the oocyte

[1] 1.4xSSC: 0.21 NaCl, 0.021 M sodium citrate, pH 7.0

[2] 2xSSC: 0.3 M NaCl, 0.03 M sodium citrate, pH 7.0
0.1xSSC: 0.015 M NaCl, 0.0015 M sodium citrate, pH 7.0

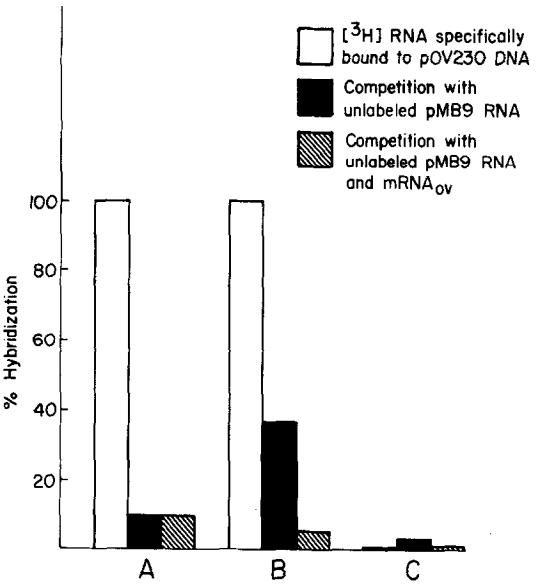

Figure 1: Transcription of pOV230 and pMB9 DNA: [^{3}H]RNA hybridization to pOV230 DNA and competition with pMB9 RNA and $mRNA_{ov}$. Oocyte [^{3}H]RNA was hybridized to pOV230 DNA immobilized on filters (0.79 µg DNA/filter). Approximately 6000-8000 dpm [^{3}H] RNA were added to each hybridization reaction. Each reaction was done in duplicate. 100% hybridization refers to the amount of [^{3}H]RNA specifically bound to pOV230 DNA (820 dpm for A and 732 dpm for B). A. pMB9 DNA injected into oocyte nuclei; B. pOV230 DNA injected into oocyte nuclei; C. pOV230 DNA injected into oocyte cytoplasm. Group C graphed using amount of specifically bound [^{3}H] RNA in group B as 100%.

[^{3}H]RNA was hybridized to the filters. Competition experiments were carried out by the addition of 10 µg pMB9 RNA and 5 µg $mRNA_{ov}$ to the incubation reaction. After incubating overnight at 42° C the filters were washed extensively with 2XSSC and 0.1XSSC and treated with RNase (20 µg/ml) for one hour to remove non-specifically bound RNA. The filters were dried, dissolved in Piersolve (Amersham) and the amount of [^{3}H]RNA bound determined by scintillation spectroscopy.

RESULTS

Microinjection of dye into groups of oocytes was used to determine the number of times the nucleus was successfully hit. After injection the oocytes were briefly fixed in a boiling H_2O bath and opened. Using this technique the success rate was estimated to be 33-50%.

Transcription of pOV230 and pMB9 DNA (Figure 1):

pOV230 DNA and [^{3}H]UTP were injected together into two groups of oocytes. In one group the micropipette tip was aimed at the nucleus and in the other group the

DNA was injected into the cytoplasm. A third group of oocytes was injected with pMB9 DNA, aiming at the nucleus. After 48 hours, the RNA was extracted from each group of oocytes and hybridized to pOV230 DNA immobilized on filters. Figure 1 shows that while no detectable pOV230 hybridizable RNA was produced in the group of oocytes in which the DNA was cytoplasmically injected (C), hybrids were formed in those groups of oocytes in which the DNA was injected into the oocyte nucleus. Competition experiments carried out by the addition of unlabeled pMB9 RNA to the hybridization reaction revealed a difference in the radioactive transcripts produced in the oocytes injected with pMB9 DNA (A) and pOV230 DNA (B). The amount of [^{3}H]RNA bound to the filters was decreased by over 90% in group A (pMB9 DNA injection) while only about 65% of the [^{3}H]RNA was competed by unlabeled pMB9 RNA in group B (pOV230 DNA injection). It is clearly shown by competition with both pMB9 RNA and $mRNA_{ov}$ that only in group B (pOV230 DNA injection into nuclei) can an additional 30% of the [^{3}H]RNA be competed by $mRNA_{ov}$.

Strand Specificity (Table 1):

pOV230 DNA, [^{3}H]UTP and [^{3}H]CTP were injected into oocytes from two females (X and Y) and the [^{3}H]RNA hybridized to pOV230 DNA on filters. In two experiments shown in Table 1, half of the filters were preincubated with 5 μg $mRNA_{ov}$ while the remaining half were preincubated in hybridization solution without $mRNA_{ov}$. During the preincubation in the presence of $mRNA_{ov}$, the $mRNA_{ov}$ hybridized to the coding strand DNA_{ov} sequences on the filter, leaving only the anticoding strand DNA_{ov} sequences open. Therefore, during the subsequent hybridization, only the radioactive anti-RNA_{ov} sequences could hybridize to the DNA_{ov} on the $mRNA_{ov}$ presaturated filters. Competition with $mRNA_{ov}$ during the hydridization reaction caused the cold $mRNA_{ov}$ to hybridize with the DNA_{ov} coding sequences on the filter, and, since the cold $mRNA_{ov}$ was present in vast excess, it also annealed to any [^{3}H]anti-$mRNA_{ov}$ sequences in solution before the radioactive strands could hybridize to the DNA on the filter. This competition, therefore, blocked hybridization of both coding and anticoding strand [^{3}H]$mRNA_{ov}$ to the filter.

TABLE 1

Transcription of pOV230 DNA after injection into Xenopus oocytes.
[^{3}H]RNA hybridization to pOV230 DNA immobilized on filters (0.5 μg DNA/filter).

Donor Animal	Input (DPM)	Hybrid† (DPM	$mRNA_{ov}$ Presaturated Filters	% Competition		% Coding and Anticoding Strand $mRNA_{ov}$	% Anti-$mRNA_{ov}$
				pMB9 RNA	pMB9 RNA + $mRNA_{ov}$		
X	33,000	11,000	-	88.2[a]	97.7[b]	9.5[c]	
	73,400	27,800	-	86.1[a]	95.5[b]	9.4[c]	
	73,400	28,100	+	94.9[d]	97.8[e]		1.9[f]
Y	49,100	16,600	-	82.7[a]	88.6[b]	5.9[c]	
	89,400	19,100	-	89.1[a]	95.9[b]	6.8[c]	
	89,400	21,000	+	96.8[d]	95.6[e]		0[f]

†Total amount [^{3}H]RNA capable of hybridizing to the pOV230 DNA. ([^{32}P] $cRNA_{ov}$ was included as an internal control in each experiment). Each number is the average of duplicate determinations.

NOTE: c = b-a; f = e-d

As shown in Table 1, from 21-38% of the input [^{3}H]RNA hybridized to the pOV230 DNA on control filters. Competition with unlabeled pMB9 RNA decreased the [^{3}H]RNA bound by 82.7-89.1% on control filters (experiment a), while it decreased the [^{3}H]RNA bound on $mRNA_{ov}$ presaturated filters by 95.9-96.8% (experiment d). Competition with both pMB9 RNA and $mRNA_{ov}$ decreased the amount of [^{3}H]RNA bound by 95.5-97.7% (experiment b) on control filters and by 95.6-97.8% (experiment e) on $mRNA_{ov}$ presaturated filters. Thus, coding and anticoding strand [^{3}H]RNA_{ov} sequences accounted for 6.8-9.5% (experiment c) of the total radioactivity bound on control filters, while anticoding strand RNA_{ov} accounted for less than 2% (experiment f) of the radioactivity bound on $mRNA_{ov}$ presaturated filters. These results suggest that the coding strand was preferentially transcribed under the experimental conditions.

DISCUSSION

Purified pOV230 DNA was capable of being transcribed in Xenopus oocytes if the DNA was injected into the nucleus, while cytoplasmically directed injections did not support the synthesis of [^{3}H] pOV230 RNA. (Figure 1). This is in accordance with the finding of Mertz and Gurdon (2) and may reflect the predominant nuclear localization of DNA-dependent RNA polymerase, or the presence of a nuclear factor(s) required for the stability of injected DNA (11).

The amount of pOV230 RNA synthesized was estimated to be from 21-38% of the total amount of radioactive RNA transcribed during the incubation period (Table 1). This amount is in the same range as that of viral-specific RNA synthesized after the injection of SV40 DNA (1).

It was also shown by competition experiments with unlabeled pMB9 RNA and $mRNA_{ov}$ that $mRNA_{ov}$ sequences were synthesized in oocytes injected with pOV230 DNA, but not in oocytes injected with only plasmid DNA (pMB9 DNA) (Figure 1). Two separate strand selectivity experiments indicate that 80-100% of the RNA_{ov} sequences were coding strand RNA (Table 1). It is interesting that strand-specific transcription was also observed in the oocytes following the microinjection of bacteriophage ϕX174-RFI DNA (1) as well as 5S DNA (2).

It is possible that in these experiments the RNA_{ov} was being transcribed as part of a larger RNA molecule containing pMB9 sequences or transcription could have been initiated at the A-T region of the recombinant molecule. At the present time, it is not known whether there is random initiation of transcription around the recombinant pOV230 molecule, in which case, additional factors probably conferred the strand specificity, or whether what we observed was the read-through transcription of the ovalbumin gene initiated on a plasmid promotor which furtuitously resulted in the preferential expression of the coding strand of $mRNA_{ov}$. Preliminary experiments failed to detect immunoprecipitable ovalbumin in oocytes injected with pOV230 DNA.

Experiments are currently in progress to study the transcription of the cloned natural ovalbumin gene after injection into the oocyte. It is anticipated that such studies on the natural gene will provide further information on the regulation of the expression of the ovalbumin gene.

REFERENCES

1. Mertz, J.E. and Gurdon, J.B. (1977) Proc. Natl. Acad. Sci. USA 74, 1502-1506.
2. Brown, D.D. and Gurdon, J.B. (1977) Proc. Natl. Acad. Sci. USA 74, 2064-2068.
3. Brown, D.D. and Gurdon, J.B. (1978) Proc. Natl. Acad. Sci. USA 75, 2849-2853.
4. McReynolds, L.A., Catterall, J.F. and O'Malley, B.W. (1977) Gene 2, 217-231.
5. McReynolds, L.A., O'Malley, B.W., Nisbet, A.D., Fothergill, J.E., Givol, D., Fields, S., Robertson, M. and Brownlee, G.G. (1978) Nature 273, 723-728.
6. Dumont, J.N. (1972) J. Morphol. 136, 153-179.
7. Gurdon, J.B. (1976) J. Embryol. Exp. Morphol. 36, 523-540.
8. Katz, L., Kingsbury, D.T. and Helinski, D.R. (1973) J. Bacteriol. 114, 577-591.
9. Roop, D.R., Nordstrom, J.L., Tsai, S.Y., Tsai, M-J., and O'Malley, B.W. (1978) Cell 15, 671-685.
10. Woo, S.L.C., Rosen, J.M., Liarakos, C.D., Choi, Y.C., Busch, H., Means, A.R., O'Malley, B.W. and Robberson, D.L. (1975) J. Biol. Chem. 250, 7027-7039.
11. Wyllie, A.H., Gurdon, J.B., and Price, J. (1977) Nature 268, 150-152.