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# The Transcription and Translation of DNA Injected into Oocytes

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# The transcription and translation of DNA injected into oocytes

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## Introduction

Previous work from this group has shown that purified DNA injected into frog eggs is replicated semi-conservatively (Laskey & Gurdon 1973; Ford & Woodland 1975; Laskey & Melton 1978), and that purified messenger RNA injected into eggs or oocytes is efficiently translated (Gurdon, Lane, Woodland & Marbaix 1971). For the last few years we have tried to obtain transcription of genes injected into oocytes or eggs, either as whole nuclei or as purified molecules of DNA. Genes contained in whole nuclei are transcribed and translated into recognizable proteins (Gurdon, De Robertis & Partington 1976; De Robertis, Partington, Longthorne & Gurdon 1977; further references in De Robertis, Partington & Gurdon 1978, this volume). We summarize here our evidence that purified DNA is transcribed into RNA and translated into proteins after injection into oocytes and eggs of the frog *Xenopus laevis*.

## Aim of experiments

Ideally we would obtain correct transcription and translation of purified genes injected into eggs or oocytes as purified DNA. This would be useful for two reasons. First, it would be possible to map regulatory sequences in DNA molecules, such as promoters, using restriction enzymes to cut, and possibly cloning procedures to propagate, defined segments of genes and their adjacent DNA. Secondly, we could hope to inject purified genes and reisolate them still attached to the associated molecules of oocytes or eggs. For further discussion of these possibilities and their relevance to development, see Gurdon (1977).

## Transcription of injected DNAs in eggs

The first success in transcribing purified genes in injected frog cells came from the use of fertilized eggs. Purified 5*S* DNA (coding for 5*S* RNA) or ribosomal DNA (which carries the genes which code for 18*S* and 28*S* RNAs) was injected into eggs and transcription was recognized by labelling transcripts which were then hybridized back to DNA of the type injected initially (Gurdon & Brown 1977). Because of the substantial level of endogenous transcription, transcripts from the injected DNAs constituted a small proportion of the total labelled RNA, and were not characterized further. The most interesting conclusions from this extensive series of experiments were (1) that the injected genes were not rapidly broken down, a substantial fraction being present in tadpoles several days after this injection, and (2) that mouse satellite DNA which is not transcribed in mouse tissues was also not transcribed to a detectable extent in injected frog eggs. The first conclusion is consistent with the earlier finding by Colman (1975) that the artificial DNA polymer poly[d(A–T).d(A–T)] results in the formation of poly r(A.U) in injected eggs and oocytes.

### Transcription of injected genes in oocytes

The successful transcription of purified DNA injected into oocytes was worked out by Mertz & Gurdon (1977) using SV40 supercoiled circular DNA. At an early stage of these experiments, it became evident that the injected DNA was transcribed only if it was aimed at the nucleus of the oocyte; DNA injected into oocyte cytoplasm never yielded any complementary transcripts. Subsequent experiments (Wyllie, Gurdon & Price 1977) have shown that oocyte nuclei contain a component or condition which stabilizes circular SV40 DNA in a supercoiled configuration, a state which may well be required for its transcription. DNA injected into oocyte cytoplasm is converted into linear molecules which are gradually degraded.

Table 1. The use of injected *Xenopus* oocytes to transcribe *Drosophila melanogaster* histone genes after integration into, and excision from, bacterial plasmid DNA†

| DNA injected‡ | 1st hybridization§ DNA (percentage of count hybridized) | | 2nd hybridization‖ DNA (percentage of count hybridized) | |
|---|---|---|---|---|
| experiment A¶ | | | | |
| Col E1–histone | Col E1 | (7.9) | Col E1 | (1.2) |
| | | | Col E1–histone | (7.4) |
| Col E1–histone | Col E1–histone | (15.0) | Col E1 | (1.6) |
| | | | Col E1–histone | (2.4) |
| experiment B | | | | |
| Col E1–histone | Col E1 | (1.0) | — | |
| | Col E1–histone | (1.9) | — | |
| Bam H1–histone | Col E1 | (0.01) | — | |
| | Col E1–histone | (0.6) | — | |

† Details of these experiments have been described by Mertz & Gurdon (1977) and Laskey *et al.* (1978).

‡ The types of DNA used for injection and filter hybridization were as follows: Col E1, complete circular DNA from the plasmid Col E1. Col E1–histone, complete circular DNA from the recombinant plasmid cDM500, in which 1.6 repeats of a *Drosophila melanogaster* histone gene cluster have been integrated into Col E1; this plasmid was kindly made available by Dr D. Hogness and Dr R. Karp. Bam H1–histone, a single repeat of the *Drosophila* histone gene cluster excised from cDM500 by the restriction enzyme Bam H1, and used in linear form.

§ Hybridization involves the binding of [$^3$H]RNA extracted from oocytes to DNA immobilized on Millipore filters. In this column DNA refers to the type of DNA injected and bound to filters for hybridization. Percentage of count refers to the proportion of total RNA count which hybridized to DNA on filters.

‖ The unhybridized [$^3$H]RNA from the first reaction (previous column) was used for hybridization to further DNA filters. The results of this second hybridization reaction are presented as for the previous column.

¶ Experiments A and B were conducted on different days with different samples of oocytes. The percentage of total transcripts complementary to the injected DNA was much lower than usual in experiment B. Experiment A gave typical results in which 10–20% of all synthesized RNA was complementary to the injected DNA.

To inject DNA into the nucleus of an oocyte requires practice, because the pigment and yolk which large oocytes contain make it impossible to see the nucleus through the cytoplasm. The procedure which we use (Gurdon 1976) was developed in the course of our nuclear injection experiments (referred to above and in the preceding article). In our experience so far, we usually deposit a substantial proportion of the DNA in the oocyte nucleus in about 50% of all injections. Other technical details affecting the transcription of DNA in oocytes are given by Mertz & Gurdon (1977).

As a result of our early work with SV40, it became evident that many kinds of purified DNA are transcribed when injected into oocyte nuclei. This includes prokaryotic DNAs of bacteria, bacteriophages, and bacterial plasmids as well as various kinds of vertebrate chromosomal DNA and animal virus DNA. Of particular importance is the fact that a single repeat of the *Drosophila*

histone gene cluster is transcribed, after excision from the plasmid (in which it was cloned) by use of a restriction enzyme (table 1). As discussed above, experiments of this type could eventually be used for the application of oocyte injections to the mapping of eukaryotic promoters. Mouse satellite DNA is not transcribed in injected oocytes to a significant extent, thus confirming the results obtained initially with injected eggs.

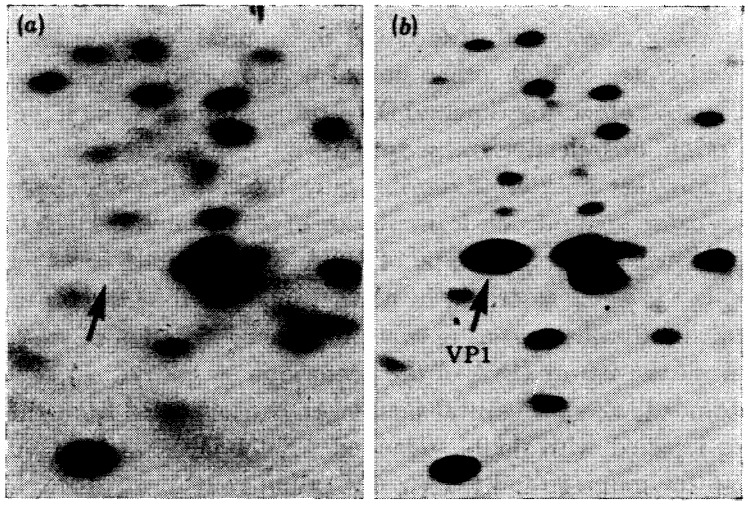


FIGURE 1. The use of *Xenopus* oocytes to translate injected DNA. Twenty oocytes were injected with 5 ng of SV40 form I DNA or mock-injected with saline, aiming for the oocyte nucleus. The oocytes were incubated for 3 days in unlabelled medium, and then for 6 h in medium containing [$^{14}$C]amino acids. The extracted proteins were analysed by two dimensional gel electrophoresis, as described by De Robertis *et al.* 1977. The gels were dried and autoradiographed. Only a small part of the complete gel autoradiograph is shown in each figure. (*a*) Mock-injected oocytes; (*b*) DNA-injected oocytes. Both figures show the same distribution of oocyte-coded proteins. In addition, (*b*) shows the presence of a protein having the electrophoretic properties of virion protein 1 of SV40.

## TRANSLATION OF INJECTED DNAS IN OOCYTES

The recognition of transcripts in these experiments depended on the hybridization of labelled RNA synthesized in oocytes to DNA immobilized on filters. This is a good choice of method for determining the total amount of RNA sequences transcribed from injected DNA, but it does not require that the transcripts are of full length or of normal composition throughout. Proof that at least some of the transcripts contain RNA of normal composition would be provided if proteins coded for by the injected genes could be demonstrated in injected oocytes. This has been achieved for SV40 DNA. The clearest demonstration of the translation of injected DNA is provided by the protein VP1; oocytes injected with wild-type SV40 DNA synthesize substantial amounts of a protein which migrates in two dimensional gels in the expected position, no such protein being present in uninjected oocytes (figure 1). Moreover, when wild-type DNA and DNAs from mutants carrying a partial deletion for genes which code for the virion proteins VP1 and VP3 were injected, proteins were synthesized which were reduced in size by the expected amount (De Robertis & Mertz 1977).

## Selective transcription

To show that injected DNA molecules make transcripts after injection into oocytes and eggs is not in itself of special interest, since transcripts can be obtained more conveniently by incubating DNA *in vitro* with bacterial RNA polymerase. However, transcription of pure DNA *in vitro* is notoriously lacking in fidelity. There is little if any indication that the transcription of DNA *in vitro* makes use of natural promoters, since the wrong strand of DNA is transcribed as often as the naturally transcribed strand, and the natural initiation and termination sites are not used more frequently than other sites. Injected oocytes would have an enormous analytical advantage over other experimental DNA transcription systems if they provided a high fidelity of transcription, or 'selective transcription'.

The first indication that oocytes transcribe pure DNA with a fidelity not provided by transcription *in vitro* came from the non-transcription of mouse satellite DNA in eggs (Gurdon & Brown 1977) as outlined above. This type of DNA is transcribed *in vitro* by *Escherichia coli* RNA polymerase, but is not transcribed in normal mouse cells. Oocytes therefore treat this DNA as it is handled naturally, not as *in vitro*. A further indication of selective transcription in oocytes came from the use of DNA of the bacteriophage $\phi$X174. In replicative form this DNA is double-stranded, and all natural messenger is transcribed from the so-called minus strand. In oocytes injected with double-stranded $\phi$X174 DNA at least 90% of the stable transcripts are from the minus strand (Mertz & Gurdon 1977).

The most clear-cut demonstration of transcriptional fidelity has come from the injection of 5*S* DNA of *Xenopus laevis* and *X. borealis* into oocytes. When this kind of DNA is transcribed *in vitro* with RNA polymerase from *E. coli* or from *Xenopus*, the wrong strand is transcribed extensively, and transcripts of the correct size are not seen more abundantly than transcripts of other sizes. Yet the labelled RNA synthesized by oocytes injected with 5*S* DNA shows a very high selectivity of transcription. At least 90% of the 5*S* DNA transcripts are from the naturally transcribed strand, and, as in normal cells, there is a predominance of stable transcripts from the gene as opposed to spacer regions of the DNA. Most remarkable, however, is the fact that nearly all of the 5*S* DNA transcripts are of the correct size, a result which implies the correct initiation and termination of transcription or post-transcriptional processing. Furthermore, a complete oligonucleotide analysis of the labelled 5*S* RNA shows the presence of all the expected oligonucleotides, and the absence of others, most notably the oligonucleotides which immediately precede and follow the gene sequence. To prove that the 5*S* transcripts made in these oocytes are synthesized from the injected DNA and not by an enhanced transcription of the endogenous oocyte genes, an oligonucleotide analysis was carried out on 5*S* RNA synthesized by *X. laevis* oocytes injected with *X. borealis* 5*S* DNA; the results showed clearly that the transcripts were coded for by the injected *X. borealis*, and not by the endogenous *X. laevis*, genes. Details of these experiments are given by Brown & Gurdon (1977). The main conclusion is that DNA injected into oocytes is transcribed with remarkable fidelity or selectivity, of a kind not so far reported for any experimental system in which purified DNA is used.


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## Future applications of DNA injections into oocytes

The two major directions in which we are currently pursuing this work are the following.

(i) The mapping of the functionally important regions of DNA which are concerned with regulating the activity of coding regions requires a system by which isolated segments of DNA can be faithfully transcribed. We have yet to show that selective transcription can be achieved when single repeats of tandemly arranged genes (such as *5S* and histone genes) are injected into oocytes. The finding (our unpublished results) that linear DNA molecules are eroded from their ends even in nucleus-injected oocytes, whereas circular DNA is not, suggests that linear molecules may need to be circularized for stable and selective transcription. We also have indirect reasons for suspecting that selective transcription may be dependent upon supercoiling of DNA, which probably reflects its assembly in chromatin (Laskey *et al.* 1978). We hope that it will eventually be possible to obtain selective transcription of plasmid-cloned segments of DNA, possibly after circularization using other transcriptionally inert pieces of DNA. If this is achieved it should be possible to prepare segments of DNA containing genes but lacking nearby sequences (e.g. promoters). The transcription of such variant DNA molecules should identify functionally important sequences.

(ii) Attempts are being made to isolate oocyte molecules such as proteins which may regulate gene activity. Unpublished experiments have shown that injected DNA may be recovered as a nucleoprotein complex. It is not yet known whether this complex is transcriptionally active and whether it is of the composition required to give selective transcription. If it is, the identification of its structure and composition could reveal the molecular basis of selective transcription.

Looking further ahead, we cannot necessarily assume that oocytes contain molecules which regulate the activity as well as inactivity of different genes, though the results of nuclear injections suggest that oocytes have both kinds of regulatory components (see De Robertis *et al.* 1978, this volume). We hope to use this experimental system to selectively withdraw gene-specific molecules, including embryologically important determinants, from oocytes and eggs (Gurdon 1977). If *all* genes injected into oocytes as pure DNA are transcribed, we would hope to identify regulatory molecules either by the progressive removal of proteins, etc., from whole nuclei or chromatin, or by the reconstitution of DNA–protein complexes using cell-type specific molecules, such as non-histone chromosomal proteins.

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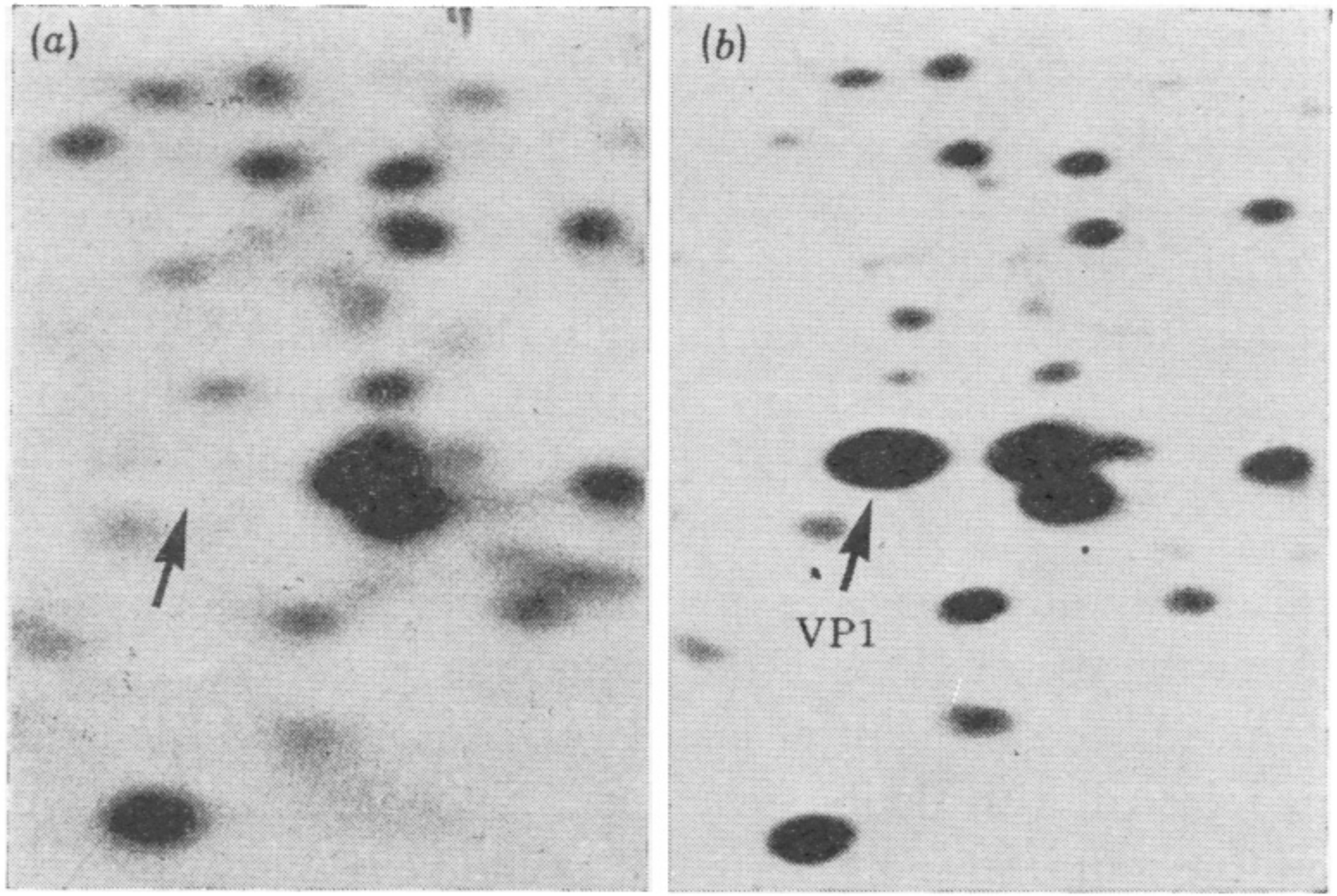


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