[25] The Use of *Xenopus* Oocytes for the Expression of Cloned Genes

By J. B. GURDON and M. P. WICKENS

General Properties of Oocytes Compared to Other Recipient Cells

The injection of amphibian oocytes was one of the first systems in which purified DNA was correctly transcribed and expressed as protein.[1] Since then, cell-free systems have been developed which initiate transcription accurately, and the expression of eukaryotic genes has been obtained by infecting cells with genetically manipulated viruses, by transfecting Ca-precipitated DNA into cultured cells, and by the direct injection of DNA into cultured cells or mouse eggs. Compared to these other systems, amphibian oocytes have three characteristics. First, a very small amount of DNA needs to be injected into a single oocyte to obtain recognizable transcription and expression (see sections Transcription and Expression as Protein). Second, the expression of DNA can be monitored within a few hours of injection, during which time it is not replicated or integrated into host cell chromosomes, but is assembled with nucleosomes into an apparently normal chromatin structure. In most other expression systems, DNA is integrated and replicated, as a result of which it may undergo genetic changes and its expression may be influenced by the properties of adjacent host DNA. Third, oocyte injection makes it possible to introduce any cell components, such as RNA, chromatin, or nuclear proteins; this is likely to be particularly valuable for analyzing the regulation of gene expression.

An amphibian oocyte is a single large cell, surrounded by several thousand small follicle cells. It is in meiotic prophase, and active in RNA and protein synthesis, but totally inactive in DNA synthesis. Its composition and general properties are summarized in Table I.

[1] Many of the conclusions stated in this chapter have been documented in recent reviews which should be consulted for all unreferenced statements [see J. B. Gurdon and D. A. Melton, *Annu. Rev. Genet.* **15**, 189 (1981); A. Kressman and M. L. Birnstiel, *in* "Transfer of Cell Constituents into Eukaryotic Cells" (J. Celis, ed.) pp. 383–407 Plenum, New York, 1980; M. P. Wickens and R. A. Laskey, *in* "Genetic Engineering" (R. Williamson, ed.), Vol. 1, pp. 103–167. Academic Press, New York, 1981]. We supply original references in this article only when they provide a source of technical information.

ISBN 0-12-182001-7

Methods

Oocytes

The collection and culture of oocytes, as well as the instruments and techniques needed for injection, have all been described.[2] This source supplements the information given below.

In a typical experiment, 25 nl of DNA at 200 μg/ml (i.e., 5 ng) is injected into an oocyte. The best medium for culturing oocytes is modified Barth solution (MBS) (see Table II).

Labeling of RNA and Protein

For labeling RNA it is generally best to inject 1 μCi of [^{32}P]GTP or [^{32}P]CTP per oocyte (mixed with DNA if desired). ATP or UTP may be used, but the pool sizes are larger (see Table I) and UTP is efficiently converted in oocytes into non-RNA molecules. Nucleosides and amino acids, but not nucleotides, are taken up from the medium. Thus RNA may also be labeled by adding [^{3}H]guanosine to the medium. In this case, transcripts synthesized in the large number of follicle cells account for more than 90% of the total ^{3}H-labeled RNA (see below for follicle cell removal).

The most generally useful procedure for labeling proteins is to incubate an oocyte in 10 μl MBS containing 5 μCi of [^{35}S]methionine for 18–24 hr. The amount of incorporation is not greatly affected by the volume of medium in which the oocytes are incubated, at least between 3 and 15 μl per oocyte.

With isotopes of high specific activity, the amount of incorporation is directly proportional to the amount of radioactive precursor used, at least up to 10 μCi per oocyte.

Injection Technique

The transcription of injected DNA takes place only in the nucleus or germinal vesicle of an oocyte, which is not normally visible. DNA can be deposited in the germinal vesicle with about an 80% success rate by penetrating the oocyte in the center of the black pigmented hemisphere, until the pipette tip is judged to be one-third of the way from this point to the opposite pole of the oocyte.[3] This technique may be readily learned by practising the injection of a concentrated trypan blue solution. Opening the oocyte with forceps just after injection will show whether the dye was

[2] J. B. Gurdon, *Methods Cell Biol.* **16**, 125, 139 (1977).
[3] J. B. Gurdon, *J. Embryol. Exp. Morphol.* **36**, 523, 540 (1976).

TABLE I

COMPOSITION AND GENERAL PROPERTIES OF A FULL SIZED (1250 μm DIAMETER) OOCYTE OF *Xenopus laevis*[a]

	Cytoplasm	Nucleus	Follicle cells
Volume (% of total)[b]	0.5 μl (90%)	40 nl (~10%)	—
DNA content			
Chromosomal[c]	None	12 pg	30,000 pg
Nucleolar[c]	None	25 pg	30 pg
Mitochondrial[c]	4000 pg	None	150 pg
DNA synthesis per day[d]	None	None	Significant
RNA content			
Ribosomal (28 + 18 S)[e]	5 μg (10^{12} ribosomes)	—	
5 S[f]	60 ng	—	
4 S[f]	60 ng	—	
Poly(A)-containing[g]	70[h] ng (10% polysomal)	10 ng	
RNA accumulation[i] (total per day)	Whole oocyte: 20 ng (1 ng mitochondrial)		
Protein content			
Yolk	250 μg	None	
Nonyolk	25 μg	2.5 μg	
Histones[j]	70 ng	70 ng	
Nucleoplasmin[k]	5 ng	250 ng	
RNA polymerase[l]	—	10^5 × somatic cell	
Protein synthesis (total accumulated/day)[m]	400 ng	None	

	Content per oocyte, ignoring follicle cells (pmol)
rATP[n]	1700
rUTP	1200
rCTP	500

rGTP	250
dTTP	7
Methionine[o]	44
Glutamic, aspartic acids	2900, 1600
Other amino acids	30–300

[a] Much of the information in this table on synthesis is discussed in detail in Ref. 28. —, not known.
[b] Total volume is 1 μl, of which half is yolk and is therefore metabolically unavailable space.
[c] Tetraploid nucleus with 1000-fold amplified ribosomal genes. The haploid genome consists of about 3×10^9 base pairs or 3 pg DNA. See Refs. 34–37 for genomic and mitochondrial DNA values. About 5000 follicle cells surround each oocyte.
[d] No replication of double-stranded DNA; single-stranded DNA becomes double-stranded (see Ref. 16). About 5% of follicle cells take up [^{3}H]thymidine.
[e] See Refs. 38 and 39.
[f] See Ref. 40.
[g] See Refs. 41, 44, 49, and 51; 90% is untranslated, not polysome-associated.
[h] Partly mitochondrial, see Refs. 36, 37, and 46.
[i] The typical transcription rate for an oocyte is 15 nucleotides per second, with RNA polymerases spaced 100–200 bp apart (Ref. 28). See also Refs. 11, 36, 42, and 45. At this rate of RNA accumulation (<20 ng/day), it would take 250 days for a full-sized oocyte (5 μg RNA) to be formed.
[j] See Ref. 43.
[k] See Ref. 32.
[l] An oocyte has equal activities of polymerases I, II, and III. Its activity exceeds that of a cultured cell by 60,000 for polymerases I and II, and by 500,000 for polymerase III. Almost all oocyte activity except for IIA is in its nucleus. See Ref. 33.
[m] See Ref. 47, p. 136 and Ref. 48. The rate given may increase by a few fold under some physiological conditions. The typical translation rate for an oocyte is one codon (3 nucleotides) per second, with ribosomes spaced 100 nucleotides apart. Protein synthesis does not include yolk, which is synthesized in the liver and transported to growing oocytes.
[n] For measurement of nucleotide pools, see Ref. 50.
[o] For measurement of amino acid pools, see Ref. 29.

TABLE II
MODIFIED BARTH SOLUTION (MBS) AND ITS PREPARATION

	Concentration in medium (m*M*)	10 × stock[a] (g/liter)
NaCl	88	51.3
KCl	1.0	0.75
$NaHCO_3$	2.4	2.0
Hepes, pH 7.5	10.0	23.8
$MgSO_4 \cdot 7H_2O$	0.82	2.0
$Ca(NO_3)_2 \cdot 4H_2O$	0.33	0.78
$CaCl_2 \cdot 6H_2O$	0.41	0.90

[a] The 10 × stock solution should be prepared by adding reagents in the above order to 900 ml H_2O, finally making up to 1 liter. After filter sterilization, this stock may be stored for months at +4°. After dilution for use, the pH should not need adjustment, but penicillin and streptomycin may be added to give a final concentration of 10 mg/liter.

deposited in the oocyte's nucleus. Some workers prefer to centrifuge oocytes lightly so as to bring the germinal vesicle to the surface where it can be seen,[4] but this can reduce viability. Usually a manually controlled syringe is used to control the volume of fluid injected,[2] but a more sophisticated apparatus has been described[5] which avoids problems of the pipette becoming blocked by backflow.

A single injected oocyte is often adequate for the detection of RNA or protein. However, individual oocytes vary somewhat in the amount of RNA or protein which they synthesize from an injected template. Such individual differences are easily averaged out by injecting 10 or more oocytes from the same ovary with each sample.

Follicle Cell Removal

Each oocyte is closely surrounded by about 5000 follicle cells, which greatly affect the composition and synthesis of ovarian material (Table I) unless removed. This can be done by gently swirling small clusters of oocytes (20 per cluster) overnight in MBS containing 2 mg/ml collagenase.[6] This procedure removes all follicle cells as judged by scanning electron

[4] A. Kressman, S. G. Clarkson, V. Pirrotta, and M. L. Birnstiel, *Proc. Natl. Acad. Sci. U.S.A.* **75,** 1176 (1978).
[5] D. L. Stephens *et al.*, *Anal. Biochem.* **114,** 299 (1981).
[6] T. J. Mohun, C. D. Lane, A. Colman, and C. C. Wylie, *J. Embryol. Exp. Morphol.* **61,** 367 (1981).

microscopy, and leaves oocytes with unimpaired viability.[6] It seems preferable to the use of pronase.[7] In many cases, the presence of follicle cells can be ignored and not removed, e.g., after labeling RNA by the injection of ^{32}P-labeled nucleotides which do not penetrate the follicle cells from oocyte cytoplasm.

RNA Extraction

For the analysis of transcripts, RNA may be extracted from oocytes with good recovery and minimal risk of degradation by the following procedure (other procedures have also been reported[8-11] and may be used successfully). Homogenize 5–15 oocytes in 0.5 ml of 0.3 *M* NaCl, 2% SDS, 50 m*M* Tris, pH 7.5, 1 m*M* EDTA (room temperature). This is conveniently done in a 1.0-ml glass homogenizer. Quickly transfer the homogenate to a 1.5-ml microfuge tube containing 0.5 ml of phenol:chloroform (1:1), and vortex immediately. Centrifuge in a microfuge for 5–10 min. Remove the aqueous phase. Add 0.5 ml of the same homogenization buffer to the phenol: chloroform phase, vortex, and centrifuge again. Remove the aqueous phase, combine with the first, and add 2 volumes of ethanol. A large flocculent white precipitate of carbohydrate (see below for removal) will form immediately upon addition of the alcohol. Ethanol precipitate as desired (−20° for 15 min usually is adequate); then recover the precipitate by centrifugation and wash it with 70% ethanol. Again drain off the alcohol, and dry briefly. If the precipitate is contaminated with brown material, as is often the case when extracting 15 or more oocytes per 0.5 ml, redissolve the precipitate in 50 m*M* Tris, pH 7.5 and extract once more with an equal volume of phenol:chloroform (1:1). Remove the aqueous phase, adjusting to 0.3 *M* NaCl, and precipitate with 3 volumes of ethanol.

Proteinase K may also be included in the homogenization buffer (at 1 mg/ml) when isolated germinal vesicles and cytoplasms are being analyzed.[12] It is simplest to transfer the nucleus or cytoplasm directly into homogenization buffer. Using proteinase K-supplemented buffer, cytoplasm and nuclei may be accumulated for at least 15 min without the RNA suffering any degradation.

When preparing RNA from single oocytes, homogenize each oocyte in 0.2–0.4 ml as above. It is not necessary to add carrier, since each oocyte

[7] L. D. Smith and R. E. Ecker, *Dev. Biol.* **19,** 281 (1969).
[8] J. E. Mertz and J. B. Gurdon, *Proc. Natl. Acad. Sci. U.S.A.* **74,** 1502 (1977).
[9] D. D. Brown and E. Littna, *J. Mol. Biol.* **8,** 669 (1964).
[10] A. Kressmann *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **42,** 1077 (1978).
[11] D. M. Anderson and L. D. Smith, *Cell* **11,** 663 (1977).
[12] E. Probst *et al.*, *J. Mol. Biol.* **135,** 709 (1979).

contains 5 μg of rRNA and a considerable amount of carbohydrate. However, in preparing RNA from single germinal vesicles which contain little nucleic acid or carbohydrate, it is prudent to add exogenous RNA or DNA (e.g., tRNA, to a final concentration of 50 μg/ml) to the above homogenization buffer, so as to minimize losses during extraction and precipitation.

Analysis of Labeled RNA

Any standard method used to analyze labeled or unlabeled RNA can, in principle, be applied to transcripts from oocytes. Each of the techniques which follow is discussed in detail elsewhere in this series. Here we discuss only those points which are particularly important for work with oocytes.

1. Direct gel electrophoresis. For injected genes transcribed by RNA polymerase III (tRNA and 5 S RNA), this clearly is the method of choice. Enough radioactive RNA is synthesized in a few hours to be detected by gel electrophoresis of total RNA from less than one oocyte. Although oocytes synthesize tRNA and 5 S RNA from their own genes, this endogenous background is inconsequential in most experiments, since the transcripts of injected genes are usually at least 20 times more abundant. Genes encoding mRNA, transcribed by polymerase II, cannot always be assayed by direct gel electrophoresis of total labeled RNA. This is because (a) such genes initiate 100 to 1000 times less frequently than those transcribed by polymerase III,[1,12] (b) precise initiation and termination are required for the production of a detectable RNA band, and neither process may be efficient on a particular DNA template, and (c) polyadenylation and multiple splicing events may complicate the pattern observed. In addition, several endogenous transcripts are prominent in those regions of an agarose gel in which mRNAs generally lie, and so contribute to the background (see Ref. 46 for a detailed description). In spite of these difficulties, mRNA transcripts from some genes, notably the sea urchin histone genes and the SV40 late genes, are of discrete size, and can be readily detected in total RNA preparations from less than one oocyte.

2. Purification of specific transcripts by hybridization to DNA bound to paper. DNA immobilized on DBM-paper filters may be used to purify template-specific transcripts which are only a small fraction of total oocyte RNA. This reduces the background by two to three orders of magnitude relative to direct gel analysis and provides a reasonable recovery of specific transcripts (10–50% of template-specific transcripts in a 4-hr hybridization). In such experiments as much as 30 oocytes' worth of RNA is redissolved in 100 μl of 50% formamide (deionized), 0.4 *M* NaCl, 0.2% SDS, 20 m*M* Pipes, pH 6.4, 5 m*M* EDTA. Redissolving the RNA at this concentration may require repeated pipetting and some patience. This so-

lution is then hybridized to paper-bound DNA which is then washed and eluted. The large amount of carbohydrate does not increase the background or interfere with the hybridization.

3. Southern filter hybridization. To determine which regions of injected DNA direct the synthesis of labeled transcripts, 50,000–500,000 cpm of ^{32}P-labeled RNA from injected oocytes can be hybridized to a filter bearing DNA restriction fragments transferred after gel electrophoresis.

Analysis of Unlabeled RNA

1. Northern analysis. Sufficient material is generally synthesized from injected genes (Table III) to permit rapid detection. However, it is essential to remove the DNA which was injected from the RNA sample prior to electrophoresis, since it can otherwise contribute a very high background. This may be achieved by standard biochemical techniques, e.g., digestion with RNase-free DNase. It must also be noted that each oocyte contains 5 μg of ribosomal RNA which may prevent the detection of transcripts of similar size.

2. S1 nuclease digestion of RNA:DNA hybrids. The versatility of this method and the large amount of RNA synthesized in oocytes make this technique very useful for analyzing transcripts synthesized by polymerase II. Again, standard techniques may be used without modification. Less than one oocyte's worth of RNA is often adequate for an overnight exposure using an end-labeled DNA fragment probe of only 10^6 cpm/μg specific activity.

A contaminant of oocyte RNA (possibly the carbohydrate) can distort the migration of protected DNA fragments on thin sequencing gels, such that lanes narrow toward the bottom. Results are interpretable, though it may be difficult to deduce precise lengths by comparison with undistorted marker lanes. This problem can be circumvented either by using only a little oocyte RNA in each lane (less than one-tenth oocyte per 5-mm-wide slot), by mixing markers with the protected fragments, or by purifying the RNA free of carbohydrate prior to hybridization [see DBM-paper technique (2) above].

Methods of Protein Analysis

The methods used to prepare homogenates for protein analysis must, of course, vary with the conditions required for the stability of the particular protein examined. A generalized technique useful for direct gel analysis of radioactive proteins is as follows. It includes extraction of the homogenate with freon (see below); this selectively removes yolk proteins, which distort the high-molecular-weight region of SDS polyacrylamide gels, but does not appear to selectively remove any other proteins from an

oocyte homogenate (as judged by SDS–polyacrylamide gel electrophoresis). In contrast, the removal of yolk by direct centrifugation results in highly specific losses of basic proteins (R.A. Laskey, personal communication).

Homogenize 10–30 oocytes in 1 ml of ice-cold 15 m*M* Tris, pH 6.8, and 150 μg/ml PMSF. Add an equal or greater volume of freon (1,1,2-trichlorotrifluoroethane) and vortex. Separate the upper, aqueous phase from the lower, freon phase by centrifugation for 10 min in a microfuge. A large dark interphase containing yolk protein and pigment granules will be obvious. Remove the aqueous phase and, if necessary, centrifuge for 10 min to clarify. This homogenate may then be analyzed directly by SDS–polyacrylamide gel electrophoresis.

Immunological techniques may also be used to recognize proteins synthesized in the oocyte from injected DNA templates. It is advantageous that the oocyte extensively and accurately modifies protein posttranslationally (see section Posttranslational Events). Furthermore the selective secretion by oocytes of only those proteins which are normally secreted results in a considerable purification, since all the endogenous nonsecreted proteins are effectively removed.

To detect materials secreted into the medium,[6,13] oocytes should be isolated individually from the loosely attached follicular tissue. It is essential to remove any dead oocytes, which may release proteolytic enzymes. Incubations should be carried out with one or two oocytes in a 5 or 10 μl drop of MBS (Table II) in a water-saturated atmosphere. Increasing the volume in which the oocyte is incubated does not much decrease the amount of [^{35}S]methionine which it takes up from the medium. The medium may be collected after 1–2 days.

Isolation of Germinal Vesicles and Cytoplasms

For some experiments, it is useful to separate the germinal vesicle and cytoplasm of injected oocytes. When this is anticipated, it is desirable to inject only about 15 nl into the germinal vesicle, so that it is not damaged by inflation. The germinal vesicle and cytoplasm can be separated by opening the oocyte with forceps in MBS (Table II), and removing adhering yolk by passing the germinal vesicle into and out of a pipette. Alternatively, an incision can be made with a syringe needle (26 G) in the oocyte's animal pole (center of pigmented region) and the germinal vesicle gently squeezed out with forceps.[2] Since the germinal vesicles are small, they are best collected in homogenization medium containing proteinase K and carrier DNA, RNA, or protein (see above). Isolated germinal

[13] A. Colman and J. Morser, *Cell* **17**, 517 (1979).

vesicles of DNA-injected oocytes may be used for the electron microscope examination of active transcription complexes. To avoid spending time on the analysis of germinal vesicles which happened to miss an injection of DNA, a trace of any iodinated large protein such as serum albumin, may be added to the DNA which is injected.[14] Individual germinal vesicles are then counted in a drop in a gamma counter before making nuclear spreads.

Transcription

The configuration of DNA injected into oocytes has a substantial effect on the efficiency of its transcription. A small linear molecule (5000 base pairs long) yields 10–20 times less RNA in oocytes than the same kind of DNA in circular form.[1,12] On the other hand all forms of circular molecule are equally well transcribed. Single-stranded molecules, such as M13 phage DNA, are copied into a double-stranded form,[15] and nicked circles are ligated. These are then converted into double-stranded supercoiled molecules and assembled with nucleosomes.[16]

A generally appropriate amount of DNA to inject is 5 ng (25 nl at 200 μg/ml). This amount seems to saturate the transcriptional capacity of an oocyte, at least with genes transcribed by polymerase II and III,[8,17] though an oocyte has sufficient histones to assemble much more than 5 ng DNA (Table I). The efficiency with which DNA is transcribed (transcripts/gene/hour) increases when less DNA is injected, but the absolute amount of gene product is less.

The amount of RNA typically obtained from injected DNA, and its specific activity, can be calculated as follows, assuming circular molecules containing one copy of a gene are injected: 1 μCi (2×10^6 dpm) of [^{32}P]nucleoside triphosphate (either GTP or CTP) is injected. Since the oocyte contains about 250 pmol of GTP (Table I), this corresponds to 8000 dpm/pmol GTP, or 5 dpm/pg RNA, which therefore has a specific activity of about 5×10^6 dpm/μg.

Oocytes not injected with DNA generally incorporate about 5% of the GTP pool in 24 hr, corresponding to 12.5 pmol GTP, or 17 ng RNA. Most of the stable RNA seen after a 24-hr incorporation period is rRNA; in shorter labeling periods, pre-rRNAs predominate. In addition to their endogenous transcription, oocytes injected with DNA transcribed by polymerase II generally incorporate 1% of the GTP pool into RNA com-

[14] M. F. Trendelenburg and J. B. Gurdon, *Nature* (London) **276**, 292 (1978).
[15] R. Cortese, R. Harland, and D. A. Melton, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4147 (1980).
[16] A. H. Wyllie, *et al.*, *Dev. Biol.* **64**, 178 (1978).
[17] J. B. Gurdon and D. D. Brown, *Dev. Biol.* **67**, 346 (1978).

plementary to injected DNA (or about 5% with DNA transcribed by polymerase III). These values correspond to 3 ng (polymerase II) and 8 ng (polymerase III) of transcript accumulated per day in each oocyte (Table III).

The amounts of accumulated transcript per day given above relate to *stable* RNAs and are presented as an aid in experimental design. They are not equivalent to *rates* of synthesis, since as much as 90% of the newly synthesized RNA from some templates—SV40, for instance—may be rapidly degraded.[17a]

α-Amanitin can be used to determine the type of oocyte polymerase which transcribes injected genes (Fig. 1). The injection of α-amanitin (mixed with DNA) at 5 pg per oocyte eliminates polymerase II transcription without decreasing the activity of polymerases III or I; 500 pg α-amanitin per oocyte greatly reduces polymerase III transcription, but even 5 ng per oocyte has no substantial effect on transcription by polymerase I (Fig. 1). It has been found so far that eukaryotic genes are transcribed in injected oocytes by the same type of polymerase as is used in the cells where these genes are normally expressed.

TABLE III
RNA SYNTHESIS IN DNA-INJECTED *Xenopus* OOCYTES[a]

DNA injected (No. of genes)[b]	Synthesis of complementary (5 S or SV40) RNA[b]		
	Total RNA dpm (% of [^{32}P]GTP injected)	dpm in RNA (% of total labeled RNA)	Amount of 5 S or SV40 RNA[c]
None	100,000 (5%)	[5 S: 0.25% (endogenous); SV40: none]	(5 S: 40 pg)
Xenopus 5 S in plasmid[b] (5 ng; 5×10^8 mol)	200,000 (10%)	50,000[d] (25%)	8 ng; 10^{11} mol
SV40 DNA[b] (5 ng; 5×10^8 mol)	120,000 (6%)	20,000 (16%)	3 ng; 10^9 mol[e]

[a] Labeled for 24 hr with 1 μCi [^{32}P]GTP per oocyte. These results apply only to newly synthesized RNA which is stable for several hours.

[b] See Ref. 17 for quantitation of results with 5 S genes in a plasmid, and Refs. 8 and 30 for SV40 transcription.

[c] Values calculated assuming that an uninjected oocyte synthesizes 20 ng RNA per day (Table I).

[d] The remaining 50,000 dpm RNA synthesized from injected DNA is complementary to the plasmid region of the injected DNA.

[e] Assuming an average transcript length of 3000 nucleotides.

[17a] A. A. Miller *et al. Molec. Cell Biol.* (1982) (in press).

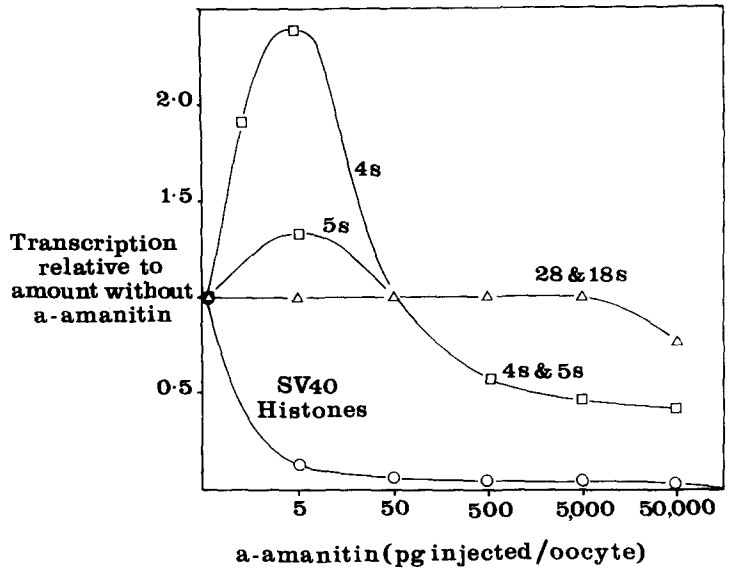

FIG. 1. α-Amanitin sensitivity of transcription in DNA-injected oocytes.

Injected prokaryotic plasmids (pBR322, ColE1, etc.) are transcribed by polymerase II, and produce approximately the same amount of transcript per day per oocyte as do eukaryotic genes which direct accurate initiation and termination, like SV40. Not surprisingly then, read-through transcription from vector DNA into eukaryotic genes inserted into recombinant clones can complicate studies of transcription initiation. Consequently, eukaryotic DNAs are often excised from the vector using a restriction enzyme and are then religated into a circle free of vector DNA prior to injection.[12]

The fidelity with which injected genes are transcribed by oocytes varies considerably according to the type of gene used.[1] Genes transcribed by polymerase III (e.g., 5 S genes and tRNA genes) generally show good strand selectivity, accurate initiation, and good but not perfect termination. Some genes transcribed by polymerase II, such as herpes virus thymidine kinase, certain sea urchin histone genes, and the SV40 late genes also show accurate initiation and termination, while others, such as ovalbumin (in a plasmid or on its own), do not. The cause of this difference in the transcription of these genes is not yet clear.

In those cases in which the oocyte has been used to map promoter regions, it appears that oocytes provide more *in vivo*-like conditions than cell-free systems, in that regions of DNA other than the TATA box are required. In this context the fact that injected DNA is assembled into nucleosomes may be relevant.

Posttranscriptional Processing

RNA polymerase III transcribes tRNA genes injected into oocytes into a primary transcript which is then matured in the nucleus into tRNA by a series of modifications. These include the removal of 5′ and 3′ sequences, base modification, and the precise excision of an intervening sequence. Fully matured tRNA is produced with high efficiency and is transported to the cytoplasm.

Oocytes also carry out several posttranscriptional modifications of mRNA precursors synthesized by polymerase II from injected genes. For example, the proper 3′ end of SV40 late transcripts is synthesized efficiently in oocytes; in infected cells, it has been shown that this requires posttranscriptional cleavage of an RNA precursor.[18] Furthermore, roughly 80% of those transcripts which terminate at the proper position are polyadenylated, as judged by their binding to poly(U)-Sepharose and their electrophoretic mobility.[18] Other genes tested in these respects include histones and herpes virus thymidine kinase. Some of the histone genes direct efficient and accurate termination, while other histone genes[19] and the thymidine kinase gene do not.[20] Neither histone nor thymidine kinase transcripts are efficiently polyadenylated. The presence of a 5′ cap has not been investigated.

The splicing of mRNA precursors has been inferred when proteins are synthesized from genes in which intervening sequences interrupt the protein-coding region. This is true for T antigen of SV40 and for ovalbumin (see the following section). The direct demonstration of RNA splicing has so far been presented only for tRNA genes transcribed by RNA polymerase III and for SV40 late genes transcribed by polymerase II.[18] At present it is difficult to predict whether a particular type of injected gene will produce abundant correctly spliced transcripts.

The passage of transcripts from an occyte nucleus to the cytoplasm has been followed in some detail because it is easy to separate manually the nucleus from the cytoplasm. The most significant result of such investigations is that incorrect or incomplete transcripts generally fail to reach the cytoplasm whereas mature correctly processed transcripts clearly do so. This has been documented for tRNA genes,[1] histone genes,[12] and SV40[18]

[18] M. P. Wickens and J. B. Gurdon, *J. Mol. Biol.* **163,** 1 (1983); J. P. Ford and M.-T. Hsu, *J. Virol.* **28,** 795 (1978).

[19] C. C. Hentschel and M. L. Birnstiel, *Cell* **25,** 301 (1981).

[20] S. L. McKnight and E. R. Gavis, 1980. *Nucleic Acids Res.* **8,** 5931 (1980), and personal communication.

TABLE IV

PROTEIN SYNTHESIS IN INJECTED OOCYTES[a]

Material injected[b]	Complementary RNA synthesized[c]	Functional mRNA	Protein synthesis due to injection: dpm in specific protein (% of total)[d]	Protein synthesis due to injection: Amount[e]
SV40 DNA[b] (1 ng; 10^8 mol)	3 ng; 10^9 mol	10^6 mol[f]	5000 dpm VP1 (0.5%)	1.25 ng; 3×10^{10} mol
Chick ovalbumin[b] DNA in plasmid (5 ng; 3×10^8 mol)	3 ng; 3×10^8 mol	10^4 mol[f]	100 dpm (0.01%)	25 pg; 5×10^8 mol
Rabbit β-globin[b] mRNA (1 ng)	—	3×10^9 mol	500,000 dpm (50%)	120 ng; 5×10^{12} mol

[a] Labeled for 24 hr, with 5 μCi [^{35}S]methionine per oocyte.

[b] Results based on Ref. 31 (for SV40 DNA), Ref. 30 (for chick ovalbumin DNA), and Ref. 21 (for rabbit globin mRNA).

[c] Based on values in Table III for SV40, and in Ref. 30 for ovalbumin.

[d] One oocyte incubated in 5 μCi [^{35}S]methionine in 10 μl MBS for 24 hr synthesizes a total of 10^6 dpm protein.

[e] Calculated from percentage values in last column assuming that an oocyte synthesizes 250 ng protein per day (Table I).

[f] Estimated assuming that each molecule of mRNA makes 30 proteins per minute (Ref. 21).

Expression as Protein

DNA injected into oocytes has been shown to be expressed as protein for SV40 and polyoma virus (T antigens and virion proteins), *Drosophila* and sea urchin histones, chick ovalbumin, and thymidine kinase.[1] Protein will possibly be found whenever sufficiently sensitive methods of detection are used. The amount of protein synthesized can be estimated as follows. Under standard conditions (see Methods section) an oocyte incorporates about 10^6 dpm of [^{35}S]methionine into protein per day, during which time it synthesizes 250 ng of total protein. Virion protein 1 of SV40 is synthesized in DNA-injected oocytes in greater amounts than most other proteins coded for by injected genes (Table IV). It constitutes 0.5% of total synthesis, corresponding to 5000 cpm (1 ng) after standard labeling conditions for 1 day. A similar value is obtained if a calculation is based on the specific activity of an oocyte's methionine pool (44 pmol) and the methionine content of the protein.

There is considerable variation in the yield of protein synthesized by different types of injected DNA. For example 50 times less ovalbumin is synthesized than SV40 VP1 from similar amounts of DNA (Table IV). The efficiency with which oocytes translate mRNA is known from the injection of many different kinds of purified mRNA. A pure preparation of rabbit β-globin mRNA is translated 30 times per minute.[21] If we assume that fully processed cytoplasmic mRNA for SV40 proteins and ovalbumin are all translated at the same rate, we can deduce the amounts of these mRNAs in DNA-injected oocytes. It is clear from Table IV that only a small fraction of the total RNA transcribed from injected DNA becomes translatable mRNA.

Posttranslational Events

Many proteins are accurately modified in oocytes after translation. These posttranslational steps include the modification of amino acids, proteolytic cleavage of a primary protein chain, and the transport and secretion of selected proteins within and from a cell.[22] The ability of oocytes to carry out these activities has been tested in mRNA rather than DNA-injection experiments. In general oocytes perform correctly most posttranslational events characteristic of wholly unrelated cells of different types and different species. For example,[23] the N-terminal methionine is

[21] J. B. Gurdon, "The Control of Gene Expression," p. 59. Harvard Univ. Press, Cambridge, 1974.

[22] C. D. Lane, *Cell* **24**, 281 (1981).

[23] C. D. Lane and J. S. Knowland, *in* "Biochemistry of Development" (R. Weber, ed.), Vol. 3. Academic Press, New York, 1974.

acetylated on calf lens crystalline, a terminal 15 amino acids are removed from mouse light chain immunoglobulin, ovalbumin is glycosylated, and the primary polypeptide of mouse encephalomyocarditis virus is cleaved into virion proteins.

The selective secretion of proteins also takes place in injected oocytes.[13] For example, mammalian interferon and the milk protein casein are secreted from mRNA-injected oocytes, as they are from cells in which they are normally synthesized. In contrast, globin and other proteins not normally secreted are also not secreted from oocytes. The recovery of materials secreted by oocytes is described in the Methods Section.

It seems, from these examples, that the cellular mechanisms responsible for posttranslational events are fairly universal in their occurrence in cells, and particularly in oocytes. Proteins synthesized from injected eukaryotic genes of nonamphibian species are therefore very likely to undergo their normal modifications even though synthesized in oocytes. The selective secretion of proteins can greatly help their identification. The recovery of proteins from the oocyte culture medium rather than from a crude oocyte homogenate already eliminates most of the high background of normal oocyte proteins, and antibody precipitations can be further used to recognize minute amounts of material.

Other Uses of Oocytes

Assay for mRNA Purification. Gene isolation usually requires at least partially pure mRNA for the preparation of cDNA or for screening a genomic DNA library directly. Since oocytes were first used for translating mRNA, cell-free systems have been greatly improved and generally have a lower background than oocytes. However, the efficiency of mRNA translation in oocytes is much greater than cell-free systems. Thus if only small amounts of mRNA are available, oocytes provide an especially sensitive assay.[24] As far as is known, all types of eukaryotic mRNA [including some that do not normally carry a poly(A) tail] are translated in oocytes, whether they come from mammals, nonamphibian vertebrates, or invertebrates. Large amounts of contaminating rRNA or tRNA can be tolerated (up to 1 mg/ml or 50 ng per oocyte) but, since all mRNAs are in competition for a limited translational capacity, contaminating mRNAs reduce the efficiency of translation of the one being assayed.

DNA Expression in Somatic Cells. DNA can be conveniently injected into the cytoplasm of fertilized eggs. More than 250 pg per egg usually causes abnormal development,[25] and least damage is sustained if about

[24] S. Nagata *et al. Nature (London)* **284,** 316 (1980).
[25] J. B. Gurdon, *Nature (London)* **248,** 772 (1974).

30 nl containing 200 pg (or 2×10^7 molecules of 5 kb DNA) of DNA is injected into the vegetal pole of eggs undergoing cleavage into the two-cell stage. The injected DNA replicates, and by the late blastula stage has increased the injected amount by 10 times or more.[26,27] Some of the injected DNA is expressed and is probably integrated into the host chromosomes. The large amount of yolk in amphibian eggs makes it impossible to see the egg pronuclei (as can be done in mouse eggs), but DNA deposited in the cytoplasm has a good chance of becoming included in nuclei as they undergo over 10 rounds of rapid division during the 12 hr that follow fertilization.

Acknowledgments

We gratefully acknowledge the comments of L. Dennis Smith, E. J. Ackerman and P. Farrell.

[26] M. M. Bendig, *Nature (London)* **292,** 65 (1981).
[27] S. Rusconi and W. Schaffner, *Proc. Natl. Acad. Sci. U.S.A.* **78,** 5051 (1981).
[28] E. H. Davidson, "Gene Activity in Early Development." Academic Press, New York, 1976 (contains original references).
[29] J. J. Eppig, Jr., and J. N. Dumont, *Dev. Biol.* **28,** 531 (1972).
[30] M. P. Wickens *et al., Nature (London)* **285,** 628 (1980).
[31] D. Rungger and H. Turler, *Proc. Natl. Acad. Sci. U.S.A.* **75,** 6073 (1978).
[32] A. D. Mills *et al., J. Mol. Biol.* **139,** 561 (1980).
[33] R. G. Roeder, *J. Biol. Chem.* **249,** 249 (1974).
[34] I. B. Dawid, *J. Mol. Biol.* **12,** 581 (1965).
[35] D. D. Brown and I. B. Dawid, *Science* **160,** 272 (1968).
[36] J. W. Chase and I. B. Dawid, *Dev. Biol.* **27,** 504 (1972).
[37] A. Webb and L. D. Smith *Dev. Biol.* **56,** 219 (1977).
[38] D. D. Brown and E. Littna, *J. Mol. Biol.* **20,** 95 (1966).
[39] L. Golden, U. Schafer, and M. Rosbash, *Cell* **22,** 835 (1980).
[40] M. Mairy and H. Denis, *Dev. Biol.* **24,** 143 (1971).
[41] M. Rosbash and P. J. Ford, *J. Mol. Biol.* **85,** 87 (1974).
[42] I. B. Dawid, *J. Mol. Biol.* **63,** 201 (1972).
[43] H. R. Woodland and E. D. Adamson, *Dev. Biol.* **57,** 118 (1977).
[44] M. O. Cabada *et al., Dev. Biol.* **57,** 427 (1977).
[45] A. C. Webb, M. J. La Marca, and L. D. Smith, *Dev. Biol.,* **45,** 44 (1975).
[46] E. Rastl and I. B. Dawid, *Cell* **18,** 501 (1979).
[47] E. D. Adamson and H. R. Woodland, *Dev. Biol.* **57,** 136 (1977).
[48] W. J. Wasserman, J. D. Richter, and L. D. Smith, *Dev. Biol.* **89,** 152 (1982).
[49] G. J. Dolecki and L. D. Smith, *Dev. Biol.* **69,** 217 (1979).
[50] J. Maller, M. Wu, and J. C. Gerhart, *Dev. Biol.* **58,** 295 (1977).
[51] D. G. Capco and W. R. Jeffrey, *Dev. Biol.* **89,** 1 (1982).