Stability and Movement of mRNAs and Their Encoded Proteins in *Xenopus* Oocytes

D. R. DRUMMOND, M. A. McCRAE, and A. COLMAN
Medical Research Council Developmental Biology Group, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL United Kingdom

ABSTRACT The stability and movement of several polyadenylated (poly A^+) and nonpolyadenylated (poly A^-) mRNAs in *Xenopus* oocytes have been examined. At least 50% of the poly A^+ mRNA molecules (9S rabbit globin mRNA, chicken ovalbumin, and lysozyme) were stable in oocytes over a 48-h period, irrespective of the amount injected. About 50% of injected poly A^- reovirus mRNAs was degraded within the first 24 h of injection, irrespective of the amount injected, although no further degradation was observed over an additional 24 h.

The movement of all poly A^+ mRNAs injected at either the animal or vegetal pole of the oocyte was very slow. Little movement of RNA from the animal half to the vegetal half was observed even 48 h after injection. In contrast, similar amounts of mRNA were present in both halves 48 h after vegetal pole injection. Similar results were obtained after injection of poly A^- reovirus mRNAs.

The movement of the proteins encoded by the poly A^+ mRNAs was studied in the 6-h period after injection when little mRNA movement had occurred. 85% of the globin synthesized accumulated in the animal half irrespective of injection site. The movement of the sequestered secretory proteins ovalbumin and lysozyme in the same oocytes as globin was much slower; very little lysozyme appeared in the half of the oocyte opposite the site of injection.

The large size and ample store of molecular components have made the amphibian oocyte a popular choice for the assay of biological materials after microinjection. The oocyte has proved an excellent vehicle for investigation of mRNA translation (1–3), DNA transcription (4, 5), RNA splicing (6), chromatin assembly (7, 8), protein segregation and secretion (9, 10), and nucleocytoplasmic movements of RNA (11). When you consider the large size of the oocyte (>1.2 mm), it is surprising that there has been no systematic study of the rate of movement of injected macromolecules (except polysaccharides [12]) from the site of injection, although this could be because considerations of macromolecular diffusion rates are of limited significance for many of the uses cited above. However, in several studies the extent of competition of injected mRNAs with endogenous mRNAs has been used to investigate spare translational capacity (13) or relative mRNA translational efficiencies (14, 15), or to assess the translational status of maternal mRNA pools (16). Richter and Smith (16) have also used co-injection or sequential injections of different RNAs to demonstrate the existence of distinct translational pools in oocytes for secretory and nonsecretory mRNAs. In the quantitative interpretation of all the competition data cited above, it is implicitly assumed that injected mRNAs diffuse rapidly within the oocyte to achieve a homogenous equilibrium distribution. However, various studies attest to the complex spatial arrangement of the endogenous macromolecules in the oocyte. For example, several groups demonstrated distinct gradients of specific classes of RNA within oocyte and fertilized egg cytoplasm (17–19). Also, yolk and other proteins show a pronounced concentration gradient along the animal–vegetal axis of these cells (20, 21). Given this complexity, the behavior of injected RNAs and proteins cannot be assumed. Studies of mRNA movement have been made in fertilized *Xenopus* embryos (22, 23); however, it is inappropriate to extrapolate the findings to oocytes since the cytoplasmic consistency and organization of developing embryos are very different from those in oocytes.

In this paper we examine the movement of a variety of mRNAs and the proteins they encode after microinjection into various regions of the *Xenopus* oocyte.

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MATERIALS AND METHODS

Chemicals

All chemicals were of analytical quality and were obtained from British Drug Houses (Poole, U.K.). Cycloheximide, progesterone, deoxynucleotide triphosphates, dextran sulfate, polyvinyl pyrrolidone, S1 nuclease, salmon sperm DNA, and phenylmethylsulfonyl fluoride were purchased from Sigma Chemical Co. (Poole, U.K.). Rabbit anti-chicken ovalbumin antibodies were obtained from Miles Laboratories Inc. (Elkhart, IN). Rabbit anti-chicken lysozyme antibodies were a kind gift from D. Cutler (EMBL, Heidelberg).

Radiochemicals

[^{32}P]dGTP (3000 Ci/mmol), [^{32}P]dCTP (3,000 Ci/mmol), [^{35}S]methionine (>800 Ci/mol), [^{32}P]UTP (3,000 Ci/mmol), and [^{3}H]fucose (40–70 Ci/mmol) were obtained from the Radiochemical Centre (Amersham, U.K.).

Preparation of mRNAs and DNAs for Microinjection

Total polyadenylated (poly A$^+$)[1] oviduct RNA was purified from the oviducts of laying Rhode Island Red hens as described by Cutler et al. (24). Rabbit reticulocyte poly A$^+$ RNA was fractionated on sucrose gradients as described by Berridge and Lane (25) and a 9S fraction that contained predominantly globin mRNA was isolated. Reovirus mRNA was synthesized in vitro as follows: viral cores were prepared from purified reovirus type 3 (Dearing) virions as described by McCrae and Joklik (26). These cores were used to synthesize capped and methylated viral mRNA basically according to the method of Skehel and Joklik (27). The only modification during synthesis was a reduction in the level of nonradioactive uridine-triphosphate to one-tenth of that for the other nucleoside triphosphates and the inclusion of [α^{32}P]uridine 5′-triphosphate at 100 μCi/ml in the reaction mixture. All mRNAs were resuspended in sterile water for microinjection.

A recombinant plasmid DNA containing a complete coding region of cloned ovalbumin cDNA (28) was purified by standard cesium chloride centrifugation (29). For injection it was dissolved at 150 μg/ml in sterile water.

Microinjection of Oocytes

50-nl aliquots of mRNAs were injected into oocytes at random sites or just beneath the animal or vegetal poles as described earlier (30). *Xenopus* oocytes were microinjected with mRNA or DNA as described by Colman (3, 5). Injected oocytes were cultured for various times in modified Barths saline (3). For protein labeling studies, this saline was supplemented with [^{35}S]methionine, 1 mCi/ml.

Dissection of Oocytes

At the end of a culture period oocytes were either frozen at −70°C; enucleated (3) before storage at −70°C of pooled nuclei and enucleate cytoplasms; or sectioned into animal and vegetal halves before storage at −70°C of pooled halves. To section the oocytes we positioned them on masking tape mounted on strips of aluminum. Excess liquid was removed and the metal strips were transferred to a bed of dry ice. We bisected frozen oocytes along the animal–vegetal equator using freehand a scalpel blade (<0°C), and we monitored the process with a stereomicroscope.

Maturation of Oocytes

Oocytes were matured by incubation overnight in Barths saline containing 5 μg/ml progesterone (31).

RNA Extraction of Oocytes or Oocyte Fragments

Groups of 10–20 oocytes or oocyte halves were extracted for RNA as described by Kressmann et al. (32). Isolated nuclei were extracted similarly except that unlabeled oocytes were added to provide carrier RNA in the homogenization buffer. Extracted RNA was resuspended in distilled water.

RNA Gel Electrophoresis

Unless otherwise mentioned, we denatured samples of RNA equivalent to 2.5 complete or 5 half oocytes (~10 μg RNA) by heating them for 5 min at 60°C in MOPS buffer containing 50% (vol/vol) deionized formamide, 15% (vol/vol) formaldehyde. (MOPS buffer contains 20 mM 3-(*N*-morpholino)propanesulfonic acid, 5 mM sodium acetate, 1 mM EDTA, pH 7.0.) After samples were quenched in ice, 0.1 vol of 50% (vol/vol) glycerol, 0.01% (wt/vol) bromophenol blue was added and the samples were immediately loaded onto a 1.5% agarose gel containing MOPS buffer with 15% (vol/vol) formaldehyde. After electrophoresis at 100 V for 5 h gels were soaked in 10% glycine for 30 min before being stained with ethidium bromide (10 μg/ml) and destained in distilled water.

Northern Blotting

The contents of stained gels were usually transferred to 0.45-μm-pore nitrocellulose (Schleicher & Schuell, Keene, NH) and hybridized to nick-translated DNA probes as described by Thomas (33) before autoradiography with Fuji RX film with an intensifying screen.

Alternatively, when radioactive RNA was used, gels were dried down on to Whatman 3 MM paper (Whatman Inc., Clifton, NJ) and autoradiographed directly.

S1 Nuclease Mapping

HYBRIDIZATION: In each hybridization assay, 4–8 μg of extracted RNA was mixed with ~200 ng of double-stranded DNA probe, in a total volume of 7 μl of S1 hybridization buffer (80% vol/vol) deionized formamide, 0.4 M NaCl, 40 mM PIPES, 1 mM EDTA, pH 6.4). Each mixture was sealed in a glass capillary tube and the contents were denatured by a 10-min incubation at 80°C, followed by immediate transfer to 52°C. After hybridization for 15 h at 52°C, the contents of each capillary were diluted into 150 μl of S1 assay buffer (0.28 M NaCl, 4.5 mM zinc acetate, 20 μg/ml sonicated, denatured salmon sperm DNA, 0.05 M sodium acetate, pH 4.6) containing 150 U S1 nuclease. The reactions were incubated at 37°C for 30 min before being stopped by addition of 6 μl 0.2 M EDTA. Samples were then extracted with 150 μl of phenol/chloroform (1:1) followed by ethanol precipitation. Precipitates were dissolved in 80% deionized formamide, 0.3% xylene cyanol ff, 0.3% bromophenol blue, 20 mM EDTA, pH 7.6, and electrophoresed on 6 or 8% acrylamide/urea sequencing gels as described by Sanger et al. (34).

PREPARATION OF LABELED DNA PROBE: The two 5′ termini of a Hind III restricted pTK$_2$ vector (Fig. 6 *b*) were phosphatased with calf intestinal phosphatase and then kinased by the use of [γ-^{32}P]ATP and T4 polynucleotide kinase. The probe shown in Figure 6 *b* was prepared via a gel purification step after restriction of the kinased fragment with Bam HI.

Preparation of Nick-translated DNA

Hind III digestion of the constructs pTK$_2$OV+ or pTK$_2$Lys+ (28) released fragments of 1,318 or 485 base pairs, which contained the complete coding sequences of chicken ovalbumin or lysozyme, respectively. Hae III digestion of pβG1 (35) released a 333-base pair fragment which contained part of the rabbit β-globin coding sequence. Fragments were purified from individual bands after electrophoresis on agarose gels. We nick-translated the purified fragments (36) using [α^{32}P]dGTP or [α^{32}P]dCTP to an estimated specific activity of 10^7–10^8 dpm/μg.

Quantitation of Injected Reovirus RNA

The stability of radioactive reovirus RNA in injected oocytes was quantitated in two ways. First, aliquots of oocyte homogenates were spotted onto Whatman No. 1 filter squares and counted directly or precipitated by two washes with ice-cold 10% (wt/vol) trichloroacetic acid, followed by several washes in ethanol and finally acetone. Second, labeled bands on the dried-down RNA gels (see above) were excised and counted. In addition, in order to assess the comparative stabilities of the different reo mRNAs, we subjected autoradiographs of electrophoresed RNA to quantitative densitometry using a Joyce-Loebl densitometer (Joyce, Loebl and Co. Ltd, Gateshead, U.K.).

RESULTS

Stability of Injected mRNA

The stability of an mRNA is an important factor in the design and interpretation of experiments to examine its movement. Therefore we have attempted to check the stability in oocytes of the various mRNA preparations employed in this study. Fig. 1, *a* and *b*, shows the results of two experiments

[1] *Abbreviations used in this paper:* poly A$^+$, polyadenylated; poly A$^-$, nonpolyadenylated.

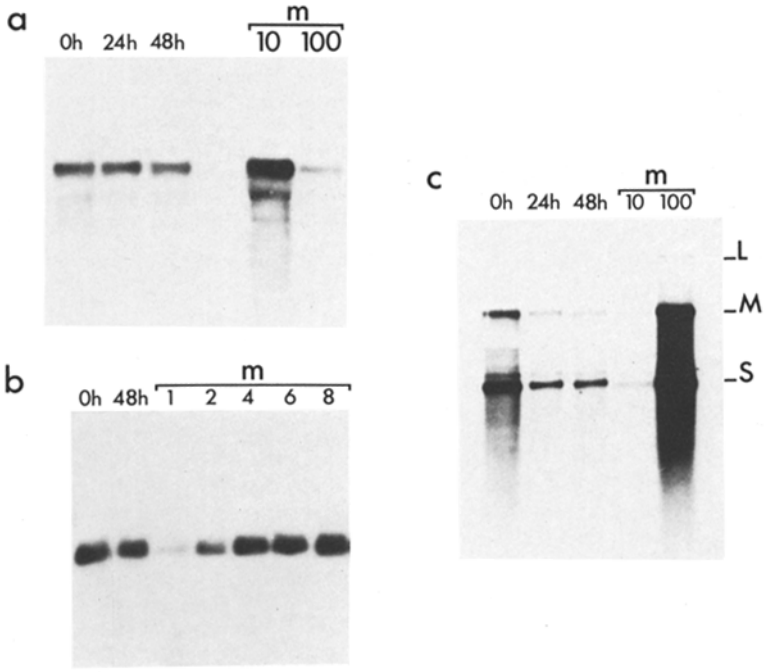

FIGURE 1 Stability of RNAs in oocytes. At the times indicated RNA was extracted from whole oocytes injected with 50 ng/oocyte oviduct mRNA (*a*), 50 ng/oocyte globin mRNA (*b*), or 60 ng/oocyte ^{32}P-labeled reovirus RNA, and run on denaturing agarose/formaldehyde gels. Gels were blotted onto nitrocellulose (*a* and *b*) before probing with nick-translated ^{32}P-labeled ovalbumin (*a*) or globin (*b*) DNAs, or dried down and autoradiographed directly (*c*). Marker lanes (*m*) contain uninjected RNA (*1*, 0.001 μg; *2*, 0.002 μg; *4*, 0.004 μg; *6*, 0.006 μg; *8*, 0.008 μg; *10*, 0.01 μg; *100*, 0.1 μg). *L*, *M*, and *S* are the large (25S), medium (18S), and small (12S) size classes of reovirus mRNA.

in which ~50 ng of chick oviduct poly A$^+$ RNA, or rabbit reticulocyte 9S globin mRNA, was injected into oocytes at random sites, and the RNA was extracted at times up to 48 h after injection. The RNA was then electrophoresed on a formaldehyde-agarose gel before transfer to a nitrocellulose filter and hybridization to ^{32}P-labeled nick-translated ovalbumin DNA or globin DNA. Visual comparison of the hybridization signal from the ovalbumin mRNA recovered at the various times indicates only a small amount of degradation. The same result was found with lysozyme mRNA which is also present in the oviduct poly A$^+$ RNA preparation (data not shown, but see Fig. 2). Comparison of the ^{32}P content of the oocyte and calibration lanes in Fig. 1*b* by scintillation counting indicates that only 30% of the globin mRNA present at 0 h has been degraded within 48 h. Using similar analysis (data not shown) we have also established that this relative stability is found whether the site of injection is the animal, vegetal, or equatorial region of the oocyte.

To monitor mRNA stability by an alternative and more quantitative method, we prepared and injected radioactive reovirus mRNA. This mRNA was made by in vitro transcription, and the products (Fig. 3) contain the three main size classes of reovirus RNA, large (*L*), medium (*M*), and small (*S*), and a background smear of incomplete transcripts. Although this RNA is not polyadenylated, experiments involving injections of 8.5–13 ng/oocyte have shown it to be translationally active and quite stable ($t_{½}$ = 72 h) in oocytes (37; see also reference 38). The fate of larger (~60 ng/oocyte) amounts of injected reovirus mRNA over a 48-h period is shown in Fig. 1*c* where an agarose gel was directly autoradiographed without a transfer step. Table I displays a quantitative analysis of the reovirus RNA recovered at the different time points. Total recoverable acid insoluble radioactivity decreased by 50% over the first 24 h postinjection, but very little further decrease occurred between 24 and 48 h postinjection. This decrease may be an overestimate of RNA degradation, since some RNA usually leaks out of injected oocytes in the hours after injection; however the bracketed figures (Table I) indicate the minimal amount of degradation that may have occurred and demonstrate the conversion of some injected RNA to an acid soluble form. This RNA degradation is not selective, as judged by the similar reduction seen in the three major RNA classes over the 48-h period. We have obtained results very similar to the above when less (~12.5 ng/oocyte) RNA was injected (results not shown), and we conclude that a significant amount (~50%) of this nonpolyadenylated mRNA (poly A$^-$) is stable in oocytes irrespective of the amounts injected.

This demonstrated stability of the injected poly A$^+$ and poly A$^-$ mRNAs allows experimental investigation of the movement of injected mRNAs between different regions of the oocyte.

Movement of Injected poly A$^+$ mRNA in Oocytes

We adopted the following strategy in studying the movement of mRNA after injection. RNA was deposited at either the animal or vegetal pole of the oocyte as described in Materials and Methods. Oocytes were then either frozen immediately or after incubation for various periods. Frozen oocytes were bisected along the animal–vegetal equator (defined here as the border between the pigmented and nonpigmented hemispheres) and RNA was extracted from pooled "halves" (actually the animal half comprises only 45% of the total volume); ~60% of the oocyte's ribosomal RNA content was found in these animal halves. Extracted RNA was subjected to electrophoresis and hybridization as described in Fig. 1. Fig. 2 shows the results from an experiment where the movement of chick ovalbumin and lysozyme mRNAs and rabbit globin mRNA were studied in this way. The results displayed in Fig. 2 indicate that for all three types of mRNA, very little movement occurred out of the animal half even 48 h postinjection. In contrast, significant movement of mRNA out of the vegetal region occurred, and mRNA levels in both halves were similar. Unfortunately, the inaccuracies inherent in this type of analysis prevent any assessment of the relative rates of movement of the three mRNA types. However, we conclude that for all the mRNAs, the rate of movement away

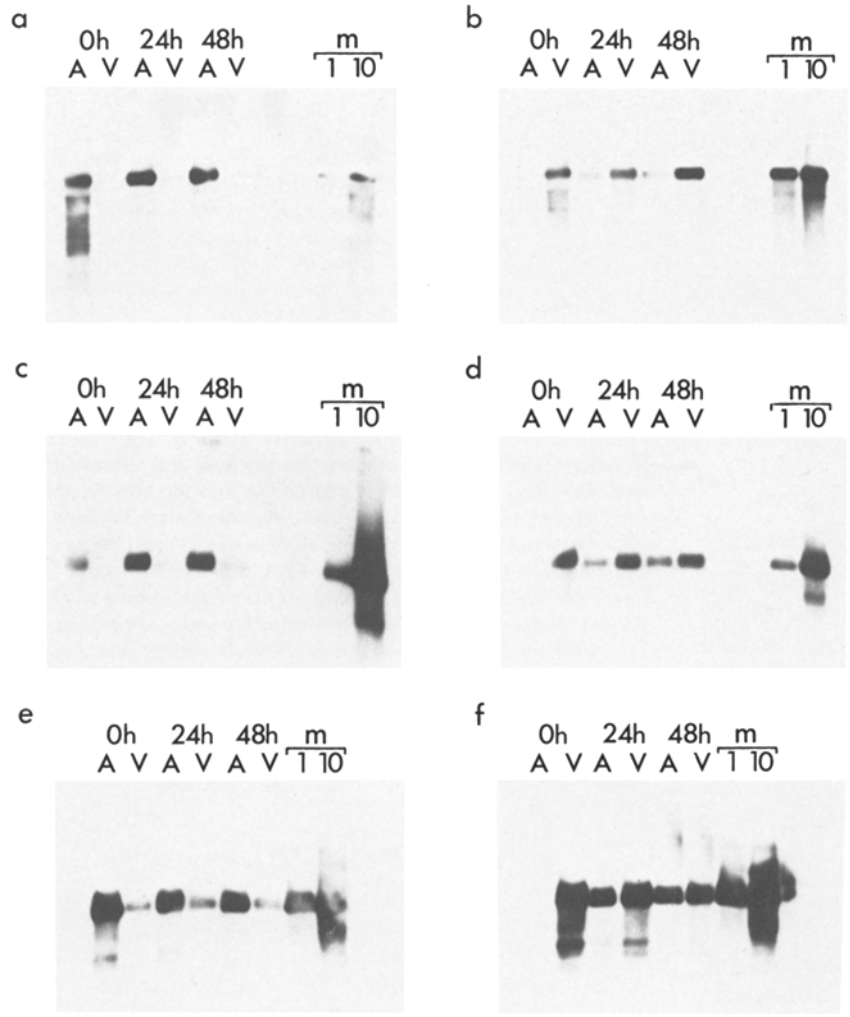

FIGURE 2 Movement of ovalbumin, lysozyme, and globin mRNA. Oviduct poly A^+ RNA (0.5 mg/ml) and globin mRNA (0.5 mg/ml) were co-injected into either the animal or vegetal poles of oocytes. RNA was extracted from the pooled animal and vegetal halves at 0, 24, and 48 h. Aliquots from each sample were run on three gels. The gels were blotted and probed with labeled nick-translated ovalbumin (*a* and *b*), lysozyme (*c* and *d*), and globin (*e* and *f*) DNAs. *a*, *c*, and *e* are from animal pole–, and *b*, *d*, and *f* are from vegetal pole–injected oocytes. *A*, animal half; *V*, vegetal half. All marker lanes (*m*) contain aliquots of the appropriate uninjected RNA (*10*, 0.1 μg; *1*, 0.01 μg).

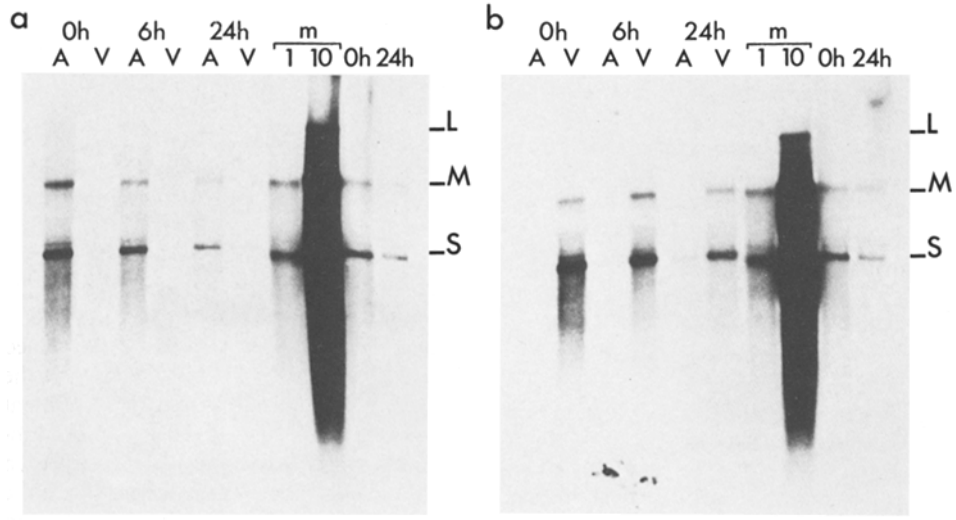

FIGURE 3 Movement of reovirus mRNA. ^{32}P-labeled reovirus mRNA at 0.25 mg/ml was injected into either the animal (*a*) or vegetal (*b*) poles of oocytes. RNA extracted from the pooled animal (*A*) or vegetal (*V*) halves (0, 24, and 48 h later) was run on gels, which were dried down and autoradiographed. Complete oocytes were also extracted at 0 and 24 h (shown in last two lanes of *a* and *b*). Marker lanes (*m*) contain uninjected RNA (*10*, 0.1 μg; *1*, 0.01 μg RNA). The major classes of RNA are indicated; *L*, large; *M*, medium; *S*, small.

from the injection site depends on the position of the site in the oocyte.

Movement of poly A⁻ RNA in Oocytes

The above data indicate that injected poly A^+ mRNA movement within oocytes is slow. There are several possible explanations for this slow rate (see Discussion), one of which is that presence of the poly A tail results in the mRNA molecules becoming adsorbed to the cytoskeleton via poly A-binding proteins. However, as seen in Fig. 3, a similar slow rate of movement was found with injected poly A^- reovirus RNAs. In this experiment, densitometer tracings of the autoradiographs revealed no differential rates of movement between the major RNA classes (small and medium) present even though the medium species have approximately double the molecular weights of the small class.

TABLE I

Stability of Reovirus mRNA

RNA	Amount of RNA		
	0 h	24 h	48 h
Total	100 (100)	55 (74)	48 (68)
25S	100	31	51
18S	100	37	39
12S	100	47	51

The experiment shown in Fig. 1 *c* was further quantitated in two ways. (*a*) Total RNA: Aliquots from each time point were precipitated with trichloroacetic acid. Unbracketed figures show the acid-insoluble counts for each time point as a percentage of the 0-h value. Bracketed figures show the same counts expressed as a percentage of the total counts present in the aliquot and corrected to the 0-h value (arbitrarily set at 100). (*b*) 25S, 18S, and 12S: The appropriate bands were cut from the gel shown in Fig. 1 *c* and counted in a scintillation counter. The count for each time point is expressed as a percentage of the 0-h value.

Influence of the Oocyte Nucleus on Movement

The oocyte nucleus is present only in the animal half. It is therefore possible that the lack of movement of the poly A^+ RNA deposited at the animal pole could result from irreversible sequestration into the nucleus as a result of misdirected microinjection, or after movement of the mRNA into the nucleus. These possibilities are excluded by the results displayed in Fig. 4. Fig. 4, *a* and *b* shows that no lysozyme or ovalbumin mRNA could be found in the GV within 3 h of an animal pole injection and, in Fig. 4 *c*, that although globin mRNA is present in the animal half 48 h after a vegetal pole injection, none was detectable within the oocyte nucleus. We conclude that injected mRNA has an exclusively cytoplasmic localization.

However, although the large nucleus does not sequester mRNA it could act as a significant obstacle to the movement of mRNA from the animal pole. This could in principle be tested using enucleated oocytes. However, we were concerned that the damage inevitably inflicted by enucleation procedures could affect the subsequent experiment. Instead we removed the nucleus "physiologically" by using progesterone treatment to induce the oocytes to undergo maturation. The results in Fig. 5 indicate that the rate of movement of injected lysozyme mRNA from the animal to vegetal half is similar in matured and control oocytes. The rate of movement after injection in the vegetal pole of the matured oocytes is less clear since although the amounts of mRNA in both halves are similar after the 6-h incubation there is obviously a substantial proportion of the injected mRNA already in the animal (uninjected) pole at 0 h. We found this consistently after injecting into the vegetal pole of both matured oocytes and unfertilized eggs; it may reflect the increased fluidity of the cytoplasm after maturation, which may lower the resistance of the vegetal cytoplasm to the surge of injected material.

Movement of Endogenous mRNA in Oocytes

During the animal pole injections shown in Figs. 2 and 3, mRNA was deposited just above the oocyte nucleus. In the experiments described in Fig. 6, the ovalbumin RNA is transcribed in the oocyte nucleus after injection of an expression vector containing cloned ovalbumin cDNA (28). The ovalbumin RNA transcripts present in both halves of the oocyte 48 h after injection were analyzed by quantitative S1 nuclease analysis. We found similar amounts of transcript in each half of the oocyte (Fig. 6). Given the position of the nucleus and the behavior of ovalbumin mRNA injected at the animal pole (Fig. 2, *a*), this distribution of the endogenous ovalbumin mRNA was unexpected.

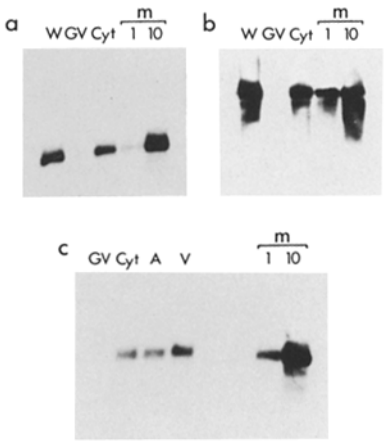

FIGURE 4 Partition of RNA between nucleus and cytoplasm. (*a* and *b*) Oviduct poly A^+ RNA (1 mg/ml) was injected into the animal pole of oocytes. Oocytes were manually enucleated 3 h later and RNA extracted from oocyte germinal vesicles (*GV*) and the respective enucleated cytoplasms (*Cyt*). RNA was also extracted from whole oocytes (*W*). Equivalent aliquots from each sample were run on gels, blotted, and hybridized with nick-translated lysozyme (*a*) or ovalbumin (*b*) DNAs. (*c*) Globin mRNA (1 mg/ml) was injected into the vegetal pole. RNA was extracted 48 h later from pooled animal (*A*) or vegetal (*V*) halves or germinal vesicles (*GV*) and their enucleated cytoplasm (*Cyt*), electrophoresed, blotted, and hybridized to nick-translated globin DNA. Marker lanes (*m*) contain the uninjected appropriate RNA (*10*, 0.1 μg; *1*, 0.01 μg).

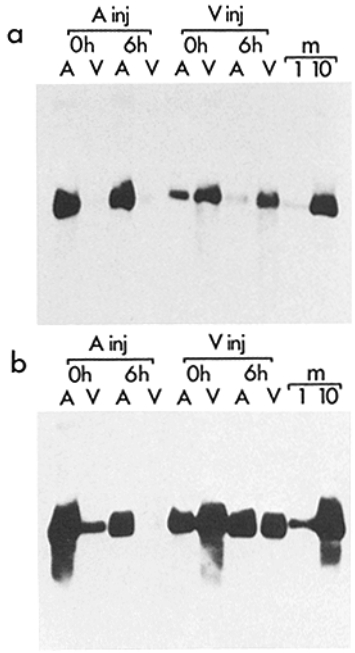

FIGURE 5 Movement of lysozyme mRNA in matured oocytes. Oviduct mRNA (1 mg/ml) was injected at the animal pole (*A inj*) or vegetal pole (*V inj*) of control (*a*) or progesterone-matured (*b*) oocytes. RNA extracted from pooled animal (*A*) or vegetal (*V*) halves 0 and 6 h later was run on a gel, blotted, and hybridized to nick-translated lysozyme DNA. Marker lanes (*m*) contain uninjected RNA (*10*, 0.1 μg; *1*, 0.01 μg).

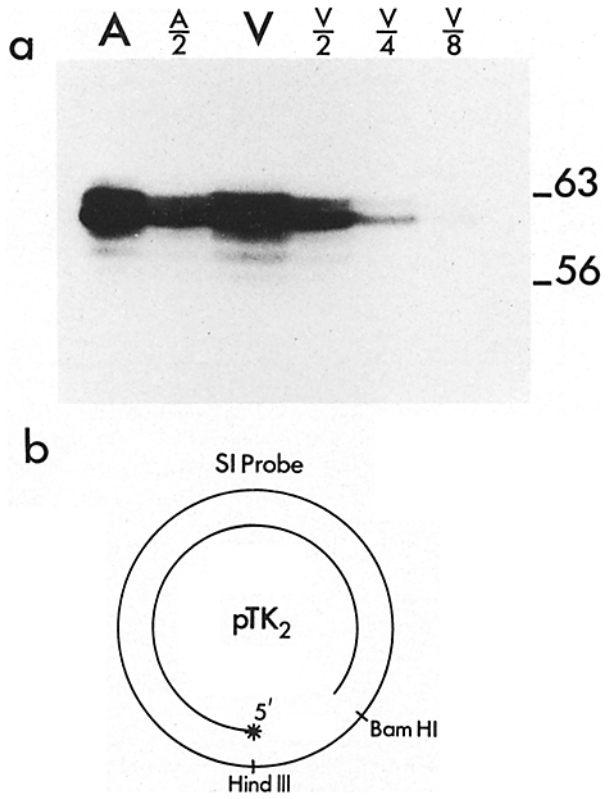

FIGURE 6 Distribution of ovalbumin transcripts transcribed from injected DNA. pTK_2OV+ DNA (0.15 mg/ml), a construct containing full length ovalbumin cDNA downstream from the herpes simplex thymidine kinase promoter (28), was injected into oocyte germinal vesicles and the oocytes were cultured for 48 h. RNA was then extracted from animal (*A*) or vegetal (*V*) halves and analyzed by the use of S1 nuclease protection of a region of a ^{32}P-5′-labeled DNA probe (see *b*). Protected DNA was electrophoresed on a 6% polyacrylamide, 8 M urea sequencing type gel. *a* shows the protected fragment(s), sized with the aid of a calibrated sequence track (not shown) provided by P. Turner (University of Liverpool, U. K.). Diluted RNAs (*A/2; V/2, V/4*, and *V/8*) were also used to establish the quantitative nature of the hybridization.

The Synthesis and Movement of Foreign Proteins in Oocytes

A proportion of mRNAs is gradually converted into polysomes after injection into oocytes (16, 25). It seemed possible that preferential polysome formation in the animal half might contribute to the low rate of movement of many mRNAs injected into this region, especially mRNAs that encode secretory proteins (e.g., ovalbumin and lysozyme) where the emerging nascent polypeptide chains could anchor the polysome to local endoplasmic reticulum membranes (for review of the secretory process, see reference 39). However, the results shown in Fig. 7 (lanes *6* and *7*) indicate that similar amounts of ovalbumin were made in oocytes within the 24-h period after oviduct mRNA injection at the animal or vegetal pole of oocytes. Virtually no movement of mRNA into the noninjected half was detectable in this experiment (data not shown; see the legend to Fig. 7). This result suggests that translation is not a factor in the differential movement of mRNAs. This conclusion is strengthened by the observation that injection of mRNA into oocytes treated with cycloheximide to inhibit completely translation (Fig. 7, lane *1*) had no effect on mRNA movement (data not shown).

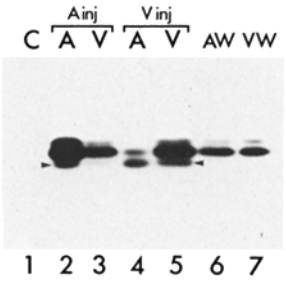

FIGURE 7 Ovalbumin synthesis and movement in oocytes. Oviduct poly A^+ RNA was injected at the animal (*AW*) or vegetal (*VW*) pole of oocytes, and the oocytes were cultured for 24 h in media containing 1 mCi/ml [^{35}S]methionine (for protein analysis) or unlabeled media (for RNA analysis). Whole [^{35}S]methionine-labeled oocytes were then homogenized and immunoprecipitated with anti-ovalbumin antibody before electrophoresis on 12.5% polyacrylamide gels. Unlabeled oocytes were extracted for RNA and analyzed as in Fig. 1 (data not shown). Lane *1* shows an immunoprecipitated sample from oocytes that were incubated for 2 h in media containing 100 μg/ml cycloheximide (*C*) before being injected with mRNA (resuspended in water containing 100 μg/ml cycloheximide) at the animal pole. The oocytes were then cultured in radioactive media (as above) for 24 h. A different batch of oocytes was also injected at the animal (*A inj*) or vegetal (*V inj*) pole and processed as for lanes 6 and *7* except that protein (and RNA) was analyzed from pooled animal (*A*) or vegetal (*V*) halves 6 h after injection. Note the presence of the band migrating at the position of nonglycosylated, miscompartmentalized ovalbumin (arrowheads; 40) only in this batch of oocytes.

Fig. 7 also shows that although the ovalbumin mRNA is confined to the injected half of the oocyte during the 6-h period of the experiment, a substantial proportion of the encoded protein enters the uninjected half. Three ovalbumin polypeptides are resolved in Fig. 7; the two slower forms are both glycosylated species (40) and segregate within the oocyte's secretory apparatus. The fastest species are unglycosylated ovalbumin molecules, which are synthesized in some batches of oocytes and which remain miscompartmented within the cytosol of the oocyte (40). It is interesting that the miscompartmented form appears to move more rapidly than the major glycosylated form from the vegetal to the animal half, but not in the reverse direction.

Fig. 8 shows an extension of this type of analysis, in which we monitored the distribution of ovalbumin, lysozyme, and globin proteins 6 h after the co-injection into oocytes of all three encoding mRNAs. The specific gel bands were excised from the fluorographs shown in Fig. 8 and the ^{35}S content of each protein was quantitated and displayed in Fig. 9. Within the same oocytes each protein demonstrated a different distribution: both ovalbumin and lysozyme moved more rapidly into the animal half from the vegetal half than vice versa, although the degree of movement was much greater for ovalbumin, whereas rabbit globin markedly accumulated in the animal half irrespective of the site of mRNA injection.

We conclude that as with the encoding mRNAs, there is a tendency for all of these proteins to move more rapidly from the vegetal to animal half than in the reverse direction. Also, like mRNA, this movement is not due to the proteins entering the nucleus as either globin (A. Wilson, personal communication) or ovalbumin and lysozyme (Colman, A., unpublished observation), translated in the oocyte, are detected in the oocyte nucleus.

To investigate whether the protein distribution was simply due to the accessible cytoplasm (see Discussion) present in the two halves we injected [^3H]fucose, a sugar apparently not metabolized in oocytes. This formed a 65:35 equilibrium distribution in favor of the animal half (Fig. 9). Such a distribution does not explain the different rates of movement

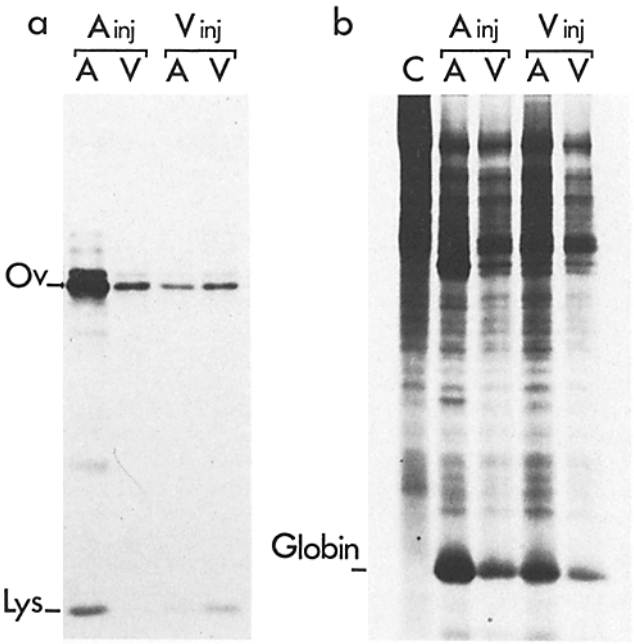

FIGURE 8 Distribution of ovalbumin, lysozyme, and globin in oocytes. Oocytes were coinjected with oviduct poly A^+ RNA and globin mRNA (1 and 0.5 mg/ml final concentration, respectively) at the animal (*A inj*) or vegetal (*V inj*) pole. After 6 h culture in media containing 0.5 mCi/ml [^{35}S]methionine, pooled animal (*A*) and vegetal (*V*) halves were prepared and homogenized as in Materials and Methods. Clarified homogenates were either first immunoprecipitated with antiovalbumin and antilysozyme (a) or directly electrophoresed on 12.5% polyacrylamide gels (*b*). The positions of ovalbumin (*Ov*), lysozyme (*Lys*), and globin are shown. Lane *C* in *b* is a noninjected oocyte sample.

displayed by the three proteins nor the 85:15 equilibrium distribution displayed by globin.

DISCUSSION

The large size and resilience of *Xenopus* oocytes has resulted in numerous studies that involved their microinjection with various macromolecules (1–7). In particular the ability of oocytes to translate exogenous mRNAs has been widely exploited. In the interpretation of most of these mRNA injection experiments the topological fate of the injected mRNA is of only minor consequence. However, for some experiments, particularly those that involve multiple injections of mRNA, the possibility of a nonequilibrium or localized distribution of the injected mRNA within the oocyte, which would complicate the interpretation of the experiment, has been either overlooked or ignored. So whereas it has been established that microinjected proteins (41) and small RNAs (11) can diffuse rapidly in the oocyte, no systematic investigation of the movement of microinjected mRNA has been reported. In this paper we have examined the stability and distribution of foreign mRNAs, and the proteins they encode, after injection into specific regions of the oocyte. Our general strategy was to introduce mRNA at the animal or vegetal pole and, at various times after injection, to bisect the oocyte along the border between the pigmented animal and unpigmented vegetal hemispheres. RNA extracted from these regions was analyzed in two different ways: poly A^+ RNA distribution was analyzed by use of filter hybridization of electrophoretically separated RNA molecules, and ^{32}P-reovirus RNA was analyzed by direct

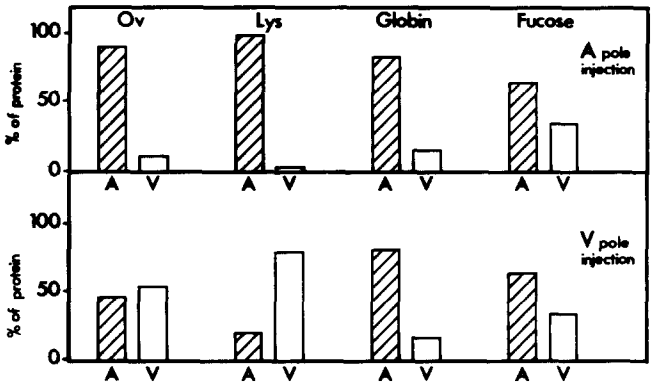

FIGURE 9 Movement of proteins and fucose in oocytes. The appropriate bands corresponding to ovalbumin, lysozyme, and globin in Fig. 8 were excised along with similar regions from control tracks, and their radioactive contents were assessed by scintillation counting. In the figure the amount of each protein in either the animal (*A*) or vegetal (*V*) half of the oocyte is expressed as a percentage of the total amount of the protein present. Also shown is the distribution of [^{3}H]fucose after injection into either pole. 50 nl of [^{3}H]-fucose (1 mCi/ml) was injected, and oocytes were frozen and sectioned 3 h later. Oocyte halves were homogenized in 100 mM NaCl, 10 mM Tris/Cl pH 7.6, 5 mM $MgCl_2$ before aliquots were counted.

autoradiography followed by band excision and scintillation counting or quantitative densitometry.

Stability of Injected RNA

Using the above methods we have established that all of the poly$^+$ mRNAs tested appear to be at least 50% stable over a 48-h period, even at the highest injection levels. The semiquantitative nature of the hybridization analysis precludes more accurate measurement, although by using several marker lanes we have established that after injection of up to 50 ng of globin mRNA per oocyte, 70% remains stable. This value is certainly different from the >90% degradation seen by Richter and Smith (16) after the injection of similar amounts of rabbit globin mRNA. With poly A^- reovirus mRNA we found that 50% was degraded within 24 h but that no further degradation occurred over the next 24 h. Similar biphasic behavior has been demonstrated for both vesicular stomatitis mRNA and slime mold mRNA (42), although previous studies on reovirus mRNA stability indicated simple first-order kinetics of degradation with half-life values of 8 h (38) or 72 h (37). Evidently with both poly A^+ and poly A^- mRNAs different workers obtain results that differ greatly. A similar discrepancy, which arose over the stability of human polyadenylated interferon mRNA (cf 43 and 44), was later attributed to differences that exist between the oocytes obtained from wild or laboratory bred female frogs (P. Seghal, personal communication). Such differences may account for the more recent discrepancies cited above.

Having established that mRNA injected into our oocytes is stable within acceptable limits we were able to investigate the movement of injected mRNA along the animal–vegetal axis of the oocyte after injection into either the animal or vegetal pole. We found that the movement of the poly A^+ mRNAs (ovalbumin, lysozyme, and globin) from either injection site was slow, and within 6 h of microinjection very little movement into the opposite half could be detected. Even 48 h after injection there was only limited movement from the animal

to the vegetal half, whereas movement in the opposite direction resulted in similar amounts of mRNA in both halves. This very slow movement of exogenous mRNA in oocytes contrasts with the rapid movement of endogenous poly A^+ mRNA microinjected back into fertilized *Xenopus* eggs; Capco and Jeffery (22) found that specific gradients of the RNA are established within 6 h of microinjection and that similar gradients form irrespective of the site of injection. However, we recently injected ovalbumin mRNA into fertilized eggs at the one-cell stage and found that ovalbumin can be detected only at the gastrula stage in those regions corresponding in origin to the sites of injection (L. Jones, H. Woodland, and A. Colman, unpublished observations). It is therefore possible that endogenous mRNA has specific sorting signals absent from foreign mRNAs.

As outlined above, two separate phenomena may be distinguished in our results on mRNA movement: mRNA movement is slow and shows a directional bias in its rate. With hindsight, the slow movement is not particularly surprising when you consider the relatively slow movements of molecules in amphibian and other cellular cytoplasm. Paine et al. (12) determined the diffusion coefficients for a range of small molecules in the cytoplasm of *Rana* oocytes and found that diffusion coefficients over a 70-fold molecular weight range are from 0.4 to 0.1 of their values in water. With larger macromolecules, for example bovine serum albumin in fibroblast cells (45), values 0.015 of the equivalent values in water have been found. This decreased rate of diffusion mentioned above in oocytes can be largely explained by an increased diffusional path length in the crowded cytoplasm (46). In addition to considerations of increased diffusion paths, interactions of mRNAs with components of the cytosol might contribute to slower movement; such interactions could include the involvement of mRNAs with poly A (47) or "cap"-binding (48) proteins, or their assembly into ribonucleoprotein particles (49, 50).

Each RNA injected into oocytes appeared to move more rapidly from the vegetal to the animal pole than in the opposite direction. The result of these differential movements, if sustained over periods longer than those studied, would be a net accumulation of injected mRNA in the animal half. A higher concentration of both ribosomal and poly A^+ RNA is found in the animal pole of the early embryo (18). In addition, injected globin mRNA also concentrates in the animal pole of the developing *Xenopus* embryo (23). Gradients of other oocyte components have been reported before. For example, a concentration of ribosomes has been described in the animal half of *Xenopus* oocytes (20); in agreement with this finding, we observed that the animal half contains 60% of the ribosomal RNA. There is also a gradient of yolk platelets along the opposite axis (20). The yolk gradient results in an imbalance in the volumes of solute-accessible cytoplasm between the animal and vegetal halves. Using fucose (a sugar not readily metabolized in oocytes) we found that an equilibrium distribution of 65:35 (animal/vegetal) is established within 3 h of injection of either pole. However, like other sugars (46), fucose penetrates into the nucleus in contrast to the injected mRNAs. When the contribution of the nucleus to the total accessible volume of the oocytes is taken into account (12% [41]), we calculate that an equilibrium distribution for a molecule that does not enter the nucleus should be 60:40 (animal/vegetal). Thus, although we expected to see a slightly skewed distribution in mRNA at equilibrium, toward the animal half, clearly this distribution is not achieved even 48 h after injection into the animal pole. This contrasts with the more rapid equilibration shown by other macromolecules (e.g. transfer RNAs, 5S RNA, 7S RNA, and small nuclear RNAs (11) or large proteins [41]). It is curious that the distribution of ovalbumin mRNA made from injected DNA differs from that after injection of mRNA into the animal pole. It is tempting to suggest that the endogenous mRNA is translocated differently, but the less extreme position of the oocyte nucleus makes this interpretation premature.

Although we cannot explain the molecular basis of the slow mRNA movement, we can exclude a role for translation in this process. First, translation of ovalbumin mRNA is quantitatively similar in both animal and vegetal halves, yet mRNA movement between the two halves is very different. Second, inhibition of translation with cycloheximide before and after mRNA injection did not affect rates of movement. Finally, the slow movement of even the globin mRNA argues against the membrane association of secretory mRNAs (e.g., ovalbumin and lysozyme) during translation (see 16, 39) as contributing to that slow movement.

Our results have implications for the interpretation of competition experiments involving the injection of various mRNAs into oocytes. The injection of increasing amounts of mRNA into oocytes causes the incorporation of amino acids into endogenous protein to fall by 50% at the highest levels of injected mRNA (14, 16). In discussing the observed competition both Laskey et al. (14) and Richter and Smith (16) assumed that the injected mRNA equilibrates, at least spatially, with the endogenous mRNA pool. However, since the oocytes were incubated for only 5 h (14) or 10 h (16) after injection, we feel these assumptions were unjustified. These reservations could apply even more strongly to experiments in which mRNAs were injected into the same oocytes at different times (17), unless similar injection sites were used.

The slow movement of injected mRNAs allowed us to examine the movement of the proteins translated from them. In all cases tested the protein movement is considerably more rapid than that of the encoding mRNA. Globin is particularly fast and achieves an equilibrium distribution within 6 h of mRNA injection, with an 85:15 distribution between the animal and vegetal halves irrespective of the site of mRNA injection. This result is surprising in that Bonner (41) found that no regional accumulation occurred after microinjection of a large range of proteins. Behavior similar to that of globin was observed with the nonglycosylated and miscompartmented ovalbumin polypeptide. This species moved rapidly from the vegetal to the animal half but not in the reverse direction. Glycosylated ovalbumin moved less rapidly, however, although the occurrence of movement between the two regions indicates the continuity of the cisternae of the endoplasmic reticulum in which this species of ovalbumin is sequestered. That lysozyme, the smallest of the proteins examined (which is also sequestered in the endoplasmic reticulum), moves least of all is surprising. It is interesting that lysozyme is translocated through the secretory apparatus 12 times faster than ovalbumin (25). We speculate that although physical continuity exists throughout the oocyte at the level of the endoplasmic reticulum, this is absent in the secretory apparatus involved in the later stages of translocation, which, we suggest, channel secretory proteins to nearby regions of the plasma membrane.

This report demonstrates our interest (30) in exploiting the

large size of the oocyte to examine spatial constraints that operate in a single living cell. However, the movements of mRNA and protein described here are limited in that they refer only to the bulk transfer of the molecules across the equatorial plane. We are now repeating some of the RNA-injection experiments using in situ hybridization (17). This technique should be especially useful for resolving whether mRNA transcribed within the oocyte (Fig. 6) is transported differently from injected mRNA.

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REFERENCES

1. Asselbergs, F. M. 1979. Postsynthetic fate of the translation product of messenger RNA microinjected into *Xenopus* oocyte. *Mol. Biol. Rep.* 5:199–208.
2. Lane, C. D. 1983. The fate of genes, messengers and proteins introduced into Xenopus oocytes. *Curr. Top. Dev. Biol.* 18:89–116.
3. Colman, A. 1984. Translation of eukaryotic mRNA in *Xenopus* oocytes. *In* Transcription and Translation—A Practical Approach. D. Hames and S. Higgins, editors. IRL Press, Oxford. 271–301.
4. Gurdon, J. B., and D. Melton. 1981. Gene transfer in amphibian eggs and oocytes. *Ann. Rev. Genet.* 15:189–218.
5. Colman, A. 1984. Transcription of exogenous DNA in *Xenopus* oocytes. *In* Transcription and Translation—A Practical Approach. D. Hames and S. Higgins, editors. IRL Press, Oxford. 49–69.
6. Green, M., T. Maniatis, and D. Melton. 1983. Human α-globin pre-mRNA synthesized in vitro is accurately spliced in *Xenopus* oocyte nuclei. *Cell.* 32:681–694.
7. Laskey, R. A., B. M. Honda, A. D. Mills, N. R. Morris, A. H. Wyllie, J. E. Mertz, E. M. De Robertis, and J. B. Gurdon. 1978. Chromatin assembly and transcription in eggs and oocytes of *Xenopus laevis*. *Cold Spring Harbor Symp. Quant. Biol.* 42:171–178.
8. Wyllie, A. H., R. Laskey, J. Finch, and J. B. Gurdon. 1978. Selective DNA conservation and chromatin assembly after injection of SV40 DNA into oocytes. *Dev. Biol.* 64:178–188.
9. Colman, A., S. Bhamra, and G. Valle. 1984. Posttranslational modification of exogenous proteins in *Xenopus laevis* oocytes. *Biochem. Soc. Trans.* 12:932–937.
10. Lane, C. D. 1981. The fate of foreign proteins introduced into *Xenopus* oocytes. *Cell.* 24:281–282.
11. De Robertis, E. M., S. Lienhard, and R. F. Parisot. 1982. Intracellular transport of microinjected 5S and small nuclear RNAs. *Nature (Lond.).* 295:572–577.
12. Paine, P. L., L. C. Moore, and S. B. Horowitz. 1975. Nuclear envelope permeability. *Nature (Lond.).* 254:109–114.
13. Moar, V. A., J. B. Gurdon, C. D. Lane, and G. Marbaix. 1971. Translational capacity of living frog eggs and oocytes, as judged by messenger RNA injection. *J. Mol. Biol.* 61:93–104.
14. Laskey, R. A., A. D. Mills, J. B. Gurdon, and G. A. Partington. 1977. Protein synthesis in oocytes of *Xenopus laevis* is not regulated by the supply of messenger RNA. *Cell.* 11:345–351.
15. Asselbergs, F. A. M., W. J. Van Venrooij, and H. Bloemendal. 1980. Preferential translation of mRNAs in an mRNA-dependent reticulocyte lysate. *Eur. J. Biochem.* 109:159–165.
16. Richter, J. D., and L. D. Smith. 1981. Differential capacity for translation and lack of competition between mRNAs that segregate to free and membrane-bound polysomes. *Cell.* 27:183–191.
17. Capco, D. G., and W. R. Jeffery. 1982. Transient localizations of messenger RNA in *Xenopus laevis* oocytes. *Dev. Biol.* 89:1–12.
18. Phillips, C. R. 1982. The regional distribution of poly(A) and total RNA concentrations during early Xenopus development. *J. Exp. Zool.* 223:265–275.
19. Carpenter, C. D., and W. H. Klein. 1982. A gradient of poly(A)$^+$ RNA sequences in *Xenopus laevis* eggs and embryos. *Dev. Biol.* 91:43–49.
20. Niewkoop, P. D., and J. Faber, editors. 1967. Normal Table of *Xenopus laevis*. Second ed. North Holland, Amsterdam.
21. Moen, T. L., and M. Namenwirth. 1977. The distribution of soluble proteins along the animal-vegetal axis of frog eggs. *Dev. Biol.* 58:1–10.
22. Capco, D. G., and W. R. Jeffery. 1981. Regional accumulation of vegetal pole poly(A)$^+$ RNA injected into fertilized Xenopus eggs. *Nature (Lond.).* 294:255–257.
23. Froehlich, J. P., L. W. Browder, and G. A. Schuttz. 1977. Translation and distribution of rabbit globin mRNA in separated cell type of *Xenopus laevis* gastrulae. *Dev. Biol.* 56:356–371.
24. Cutler, D., C. D. Lane, and A. Colman. 1981. Non-parallel kinetics and the role of tissue-specific factors in the secretion of chicken ovalbumin and lysozyme from *Xenopus* oocytes. *J. Mol. Biol.* 153:917–931.
25. Berridge, M., and C. D. Lane. 1976. Translation of *Xenopus* liver messenger RNA in *Xenopus* oocytes: vitelloginin synthesis and conversion to yolk platelet proteins. *Cell.* 8:283–297.
26. McCrae, M. A., and W. K. Joklik. 1978. The nature of the polypeptide encoded by each of the 10 double-stranded RNA segments of reovirus type 3. *Virology.* 89:578–593.
27. Skehel, J. J., and W. K. Joklik. 1969. Studies on the *in vitro* transcription of reovirus RNA catalyzed by reovirus cores. *Virology.* 39:822–831.
28. Krieg, P., R. Strachan, E. Wallis, L. Tabe, and A. Colman. 1984. Efficient expression of cloned complementary DNAs for secretory proteins after injection into *Xenopus* oocytes. *J. Mol. Biol.* 180:615–643.
29. Clewell, D. B., and D. R. Helinski. 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. *Proc. Natl. Acad. Sci. USA.* 62:1159–1166.
30. Colman, A., J. Besley, and G. Valle. 1982. Interactions of mouse immunoglobulin chains within Xenopus oocytes. *J. Mol. Biol.* 160:459–474.
31. Smith, L. D., and R. E. Ecker. 1969. Role of the oocyte nucleus in physiological maturation in *Rana pipiens*. *Dev. Biol.* 19:281–309.
32. Kressmann, A., S. G. Clarkson, V. Pirotta, and M. Birnsteil. 1978. Transcription of cloned tRNA gene fragments and subfragments injected into the oocyte nucleus of *Xenopus laevis*. *Proc. Natl. Acad. Sci. USA.* 75:1176–1180.
33. Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* 77:5201–5205.
34. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.
35. Jeffreys, A. J., and R. A. Flavell. 1977. The rabbit β-globin gene contains a large insert in the coding sequence. *Cell.* 12:1097–1108.
36. Rigby, P. W. J., M. Dieckmann, C. Rhoder, and P. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* 113:237–251.
37. McCrae, M. A., and H. R. Woodland. 1981. Stability of non-polyadenylated viral mRNAs injected into frog oocytes. *Eur. J. Biochem.* 116:467–470.
38. Furuichi, Y., A. La Fiandra, and A. J. Shatkin. 1977. 5′-terminal structure and mRNA stability. *Nature (Lond.).* 266:235–239.
39. Sabatini, D. D., G. Kreibach, T. Morimoto, and M. Adesnik. 1982. Mechanisms for the incorporation of proteins in membranes and organelles. *J. Cell Biol.* 92:1–22.
40. Colman, A., C. D. Lane, R. Craig, A. Boulton, T. Mohun, and J. Morser. 1981. The influence of topology and glycosylation on the fate of heterologous secretory proteins made in Xenopus oocytes. *Eur. J. Biochem.* 113:339–348.
41. Bonner, W. M. 1975. Protein migration into nuclei. *J. Cell Biol.* 64:421–430.
42. Allende, C. C., J. E. Allende, and R. A. Firtel. 1974. The degradation of ribonucleic acids injected into *Xenopus laevis* oocytes. *Cell.* 2:189–196.
43. Sehgal, P. B., H. Soreq, and H. Tamm. 1978. Does 3′-terminal poly(A) stabilize human fibroblast interferon mRNA in oocytes of *Xenopus laevis*? *Proc. Natl. Acad. Sci. USA.* 75:5030–5033.
44. Colman, A., and J. Morser. 1979. Export of foreign proteins from Xenopus oocytes. *Cell.* 17:517–526.
45. Wojcieszyn, J. W., R. A. Schlegel, and K. A. Jacobson. 1981. Measurements of the diffusion of macromolecules injected into the cytoplasm of living cells. *Cold Spring Harbor Symp. Quant. Biol.* 46:39–44.
46. Horowitz, S. B. 1972. The permeability of the amphibian oocyte nucleus in situ. *J. Cell Biol.* 54:609–625.
47. Richter, J. D., and D. C. Evers. 1984. A monoclonal antibody to an oocyte-specific poly(A) RNA-binding protein. *J. Biol. Chem.* 259:2190–2194.
48. Zumbé, A., C. Stahli, and H. Trachsel. 1982. Association of a M_r 50,000 cap-binding protein with the cytoskeleton in baby hamster kidney cells. *Proc. Natl. Acad. Sci. USA.* 79:2927–2931.
49. Richter, J. D., and L. D. Smith. 1983. Developmentally regulated RNA binding proteins during oogenesis in *Xenopus laevis*. *J. Biol. Chem.* 258:4864–4869.
50. Darnbrough, C. H., and P. J. Ford. 1981. Identification in *X. laevis* of a class of oocyte-specific proteins bound to messenger RNA. *Eur. J. Biochem.* 113:415–424.