The stability and movement of mRNA in *Xenopus* oocytes and embryos

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**INTRODUCTION**

The *Xenopus* oocyte has a distinguished history as an *in vivo* host for the deposition by microinjection of a variety of macromolecules including DNA (Colman, 1984), RNA (Soreq, 1985), proteins (Dingwall, Sharnick & Laskey, 1982) and polysaccharides (Paine, Moore & Horowitz, 1975). In these cases, the oocyte has simply either provided an efficient production system for a product encoded by an injected nucleic acid (Soreq, 1985; Krieg *et al.* 1984) or has been used to investigate mechanisms regulating RNA (De Robertis, Lienhard & Parison, 1982) and protein (Gurdon, 1970; Davey, Dimmock & Colman, 1985) targeting within a living cell. However, microinjection also provides a method for perturbing or simulating processes that occur normally within the oocyte, in order to understand their molecular nature and regulation. For example, Bienz & Gurdon (1982) during their investigations of heat-shock translational control in *Xenopus* oocytes, argued that the heat-shock genes encoding the 70×10^3 M, heat-shock protein (hsp 70) must be constitutively active in oocytes; this was in contrast to the behaviour of injected *Drosophila* hsp 70 genes in unstressed oocytes. Bienz (1984) was subsequently able to simulate the natural situation and confirm her prediction by cloning and then injecting the *Xenopus* hsp 70 gene into oocytes.

A good example of the perturbation of the system comes from studies involving competition between injected and endogenous mRNAs for the available translation machinery (Laskey, Mills, Gurdon & Partington, 1977; Asselbergs, Van Venrooij & Bloemendal, 1979; Richter & Smith, 1981). Most of the studies have used large stage VI oocytes (Dumont, 1972) and the current conclusions are that the oocyte has no spare translational capacity and therefore the low percentage (<2%) of ribosomes present in polysomes (Woodland, 1974) is not simply due to a shortage of available mRNA. Recently similar studies on stage IV oocytes have shown that considerable spare translational capacity exists in these cells (Taylor, Johnson & Smith, 1985).

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Finally, in a different design of experiment, Richter & Smith (1981) investigated the relative translational efficiencies of different exogenous mRNAs which were either coinjected or sequentially injected. They observed no competition between certain mRNA classes and concluded that the mRNAs for secretory and non-secretory proteins entered different translational pools.

In the quantitative interpretation of all the competition data cited above, it is implicitly assumed that injected mRNAs diffuse rapidly and at similar rates 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 have demonstrated distinct gradients of specific classes of RNA within oocyte and fertilized egg cytoplasm (Capco & Jeffery, 1982; Carpenter & Klein, 1982). Also yolk and other proteins show a pronounced concentration gradient along the animal–vegetal axis of these cells (Nieuwkoop & Faber, 1967). Given this complexity, the behaviour of injected RNAs and proteins cannot be assumed. Studies of mRNA movement have been made in fertilized *Xenopus* embryos, however, it is inappropriate to extrapolate the findings to oocytes since the cytoplasmic consistency and organization of developing embryos is very different from that existing in oocytes.

If the above assumptions regarding RNA diffusion in oocytes are incorrect, the results from the competition studies may need reinterpretation and in any event clarification of the issue is required. Resolution of this diffusion problem also now seems more urgent due to the emergence of the technique of anti-sense RNA injection (Melton, 1984) and the discovery of localized RNAs in *Xenopus* oocytes (Rebagliati, Weeks, Harvey & Melton, 1985). The ability of injected anti-sense RNA to hybridize with endogenous RNA will obviously be affected by the ability of the injected RNA to diffuse within the oocyte or embryo. Likewise studies whose objective is to determine mechanisms of RNA localization and whose experimental strategy involves relocalization of injected RNAs, will need to consider the rate of diffusion of injected RNA. In this paper we discuss results that directly address the movement of injected RNAs within oocytes and embryos.

**RESULTS AND DISCUSSION**

*Stability of injected mRNA in oocytes*

The stability of an mRNA in oocytes is an important factor in the design and interpretation of experiments to measure the movement. Stability has also proved an issue of some contention over the years with grossly different values being assigned to the same mRNAs by different workers (e.g. Furiuchi, La Fiandra & Shatkin, 1977; cf. McCrea & Woodland, 1981). We have conjectured that variation between oocyte batches might be responsible for reported differences in mRNA stability (Drummond, McCrea & Colman, 1985) and such batch-dependent variation has recently been observed by Taylor *et al.* (1985). However, of particular concern to our studies was the report of Richter & Smith (1981), which indicated that stability was also related to the amount of mRNA injected (globin
Stability and movement of mRNA in Xenopus or zein mRNAs) even when the same batch of oocytes was used. Such dose-dependent stability could complicate diffusion studies since the diluting effect of diffusion might make RNA molecules in the diffusion 'front' less susceptible to degradation than those remaining at the injection site.

We have used two different methods to examine mRNA stability in oocytes. In the first, poly (A)+ mRNAs extracted from a variety of tissues were injected into oocytes and total RNA recovered from the oocytes at various times after injection. This RNA was then electrophoresed on denaturing agarose gels, transferred to nitrocellulose and then hybridized to 32P-radiolabelled complementary DNA (cDNA) probes. After exposure to X-ray film, the RNA stability was quantified either by microdensitometry or by band excision and scintillation counting. A typical autoradiograph obtained after rabbit globin mRNA injection is shown in Fig. 1A whilst the derived quantification is indicated in Table 1. Alternatively, the final hybridization step can be omitted if the injected RNA is radiolabelled. Under these circumstances the autoradiograph is obtained by exposure of the dried-down gel. Measurements made using this method are more accurate.

Two methods for producing radiolabelled RNA were used. First 32P-reovirus RNAs were synthesized in vitro by adding radioactive nucleotide triphosphates to disrupted reovirus virions (Skehel & Joklik, 1969). The resultant transcripts, which are poly (A)+, were identical to those formed during normal viral infection and we will refer to these RNAs as natural poly (A)+ mRNAs. The second method involves the in vitro transcription of any cloned gene using the Salmonella typhimurium phage SP6 polymerase/vector system (Melton et al. 1984). This method allows the transcription of either sense (i.e. coding) or anti-sense RNA. However, it should be emphasized that even the 'sense' RNAs so obtained will always differ slightly from their natural equivalents at the 5' end and probably differ at the 3' end. We will refer to all these RNAs as synthetic RNAs. When the stability experiment was repeated with radioactive RNAs the results shown in Fig. 1B,C and Table 1 were obtained.

The general conclusion from the results in Table 1 is that all the RNAs (natural or synthetic) are similarly stable over the first 24 h with approximately 50% of injected RNA surviving intact. Further experiments (not shown but see Drummond et al. 1985a; Drummond, Armstrong & Colman, 1985b) indicated that this stability is not affected by the exact site of injection, nor by the amount of RNA injected (ranges of 5–100 ng oocyte−1 tested) nor finally by the participation of the transcript in translation. Indeed similar stabilities were found for anti-sense (i.e. noncoding) RNAs (Drummond et al. 1985b). We have not observed the striking batch-to-batch variations observed by others (e.g. Taylor et al. 1985).

Effect of post-transcriptional modifications on mRNA stability in oocytes

(1) Capping

All eukaryotic and many viral mRNAs have a cap structure consisting of a 7-methylguanosine base joined by a triphosphate bridge to their 5' end. In addition
the first (and sometimes the second) nucleoside of the RNA is methylated (Banerjee, 1980). The cap structure increases the stability of natural mRNAs (McCrea & Woodland, 1981) and SP6 transcripts (Green, Maniatis & Melton, 1983; Krieg & Melton, 1984) injected into Xenopus oocytes. In the experiments

![Image](image-url)

**Fig. 1.** Stability of RNAs in oocytes and embryos. Various RNAs were injected into oocytes (A–C) or fertilized eggs (D) and RNA extracted and analysed at the times or developmental stages indicated. (A) Globin mRNA; (B) reovirus RNAs, 12S (S), 18S (M); (C) SP6-lysozyme RNAs, with (A⁺) or without (A⁻) added poly (A); (D) SP6 lysozyme RNA (LYS), Xenopus histone H4 RNA (H4), Xenopus heat shock, hsp 70 RNA (HS). Marker (m) tracks contain 1, 10 or 100 ng per track. Further details can be found in Drummond et al. (1985a,b).
Stability and movement of mRNA in Xenopus

Table 1. Stability of RNAs in oocytes and embryos

<table>
<thead>
<tr>
<th>Cell type</th>
<th>RNA type</th>
<th>Amount of RNA after specified time</th>
<th>Stage 1 (0h)</th>
<th>Stage 7 (4h)</th>
<th>Stage 8 (5h)</th>
<th>Stage 9 (7h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocytes</td>
<td>rabbit globin</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>chick ovalbumin</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>37</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>reovirus 12S</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>47</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>reovirus 18S</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>SP6 lys, no cap</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>... 1me, cap</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>... 2me, cap</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>SP6 lys, lme cap</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>SP6 lys, lme cap, poly (A)⁺</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>SP6 chym 1me cap</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>SP6 chym 1me cap, poly (A)⁺</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>61</td>
<td>90</td>
</tr>
</tbody>
</table>

Embryos

<table>
<thead>
<tr>
<th>RNA type</th>
<th>Amount of RNA after specified time</th>
<th>Stage 1 (0h)</th>
<th>Stage 7 (4h)</th>
<th>Stage 8 (5h)</th>
<th>Stage 9 (7h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP6 lys, 1me cap</td>
<td>100</td>
<td>28</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SP6 lys, H4 and hsp 70, all 1me cap</td>
<td>[100]</td>
<td>—</td>
<td>[35]</td>
<td>[26]</td>
<td>—</td>
</tr>
</tbody>
</table>

Oocytes or fertilized eggs were injected with 5 or 50 ng per cell of the RNAs indicated above. After the indicated times, the cells were frozen, extracted and the RNA analysed and stability values obtained as described in the text except for the values in parentheses. In these instances microdensitometry readings from the autoradiographs and acid insolubility estimates on total extracted nucleic acid were used. Further details can be found in Drummond et al. (1985a,b). Abbreviations: lys, chick lysozyme; chym, calf prochymosin; H4, Xenopus histone H4; hsp 70, Xenopus 70×10³ M, heat-shock protein.

shown in Fig. 1, the synthetic transcripts all contained a methyl group only on the first nucleotide. We have compared the stability of transcripts containing methyl groups on the first (monomethyl cap), the first and second (dimethyl cap) or on neither (uncapped). The results are shown in Table 1. In contrast to a previous report where uncapped transcripts were undetectable 15 min after injection (Krieg & Melton, 1984), we found 61% of uncapped transcript present after 6 h. However, the possession of some form of cap was essential for longer-term stability.

(2) Polyadenylation

The role of poly (A) in stabilizing mRNAs injected into oocytes is controversial with claims for a significant stabilizing effect for some mRNAs (globin, Marbaix et al. 1975; histone, Huez et al. 1978) being countered by claims for no effect at all on the stability of other mRNAs (interferon, Sehgal, Soreq & Tamm, 1978; α-2u globulin, Deshpande, Chatterjee & Roy, 1979). We have directly tested the effect of polyadenylation on mRNA stability by post-transcriptionally adding poly (A) tails to synthetic RNAs, and then assessing the effect on stability in oocytes of this modification. As shown in Fig. 1C and Table 1 polyadenylation had only a slight effect on stability over the first 24 h. However, after 48 h, the polyadenylated...
transcripts were clearly more stable irrespective of RNA type. Further experiments demonstrated that this stability was unrelated to mRNA translation (Drummond et al. 1985b).

In summary, we have demonstrated that all the RNAs tested were at least 50% stable over the first 24 h so long as a 5-terminal cap structure was present. For the synthetic transcripts, stability was increased in the long term by the possession of a poly (A) tail.

**Stability of RNA in embryos**

We have tested the stability of synthetic transcripts in embryos over the first 4 h of development (i.e. from the 1-cell to the early- or mid-blastula stage). Three different transcripts, encoding the *Xenopus* hsp 70 heat-shock protein, *Xenopus* histone H4 and chicken lysozyme, were coinjected into fertilized eggs, and the RNA recovered from the blastulae and analysed. The results shown in Fig. 1D and Table 1 show that all the RNAs are far less stable in embryos than oocytes over similar periods of incubation. Again, as in oocytes, degradation seems to be an all-or-none process with no detectable shift in radioactivity to gradually smaller molecular weights. This indicates that degradation is either a progressive or a cooperative process involving one or more nucleases respectively. We have not yet investigated the effect of polyadenylation on the stability of these RNAs.

**Movement of injected RNAs in oocytes**

We have used two methods to examine the movement of injected RNA in oocytes. Common to both methods was the injection of RNA (natural or synthetic) at the animal or vegetal poles. In one method the oocytes were frozen to -70°C at various times after injection and bisected into animal and vegetal halves. RNA was then extracted from pooled halves and analysed electrophoretically etc., as described above (see Fig. 1). In the second method oocytes were fixed, embedded in wax, sectioned and then exposed to photographic emulsion (Fig. 3); this latter procedure has so far been restricted to the analysis of radioactive RNA movement.

The experiments shown in Fig. 2A,B indicate that the rates of movement of natural poly (A)$^+$ and poly (A)$^-$ (reo mRNAs) are slow and differ according to the site of injection. We see that movement away from the animal halves appears to be much slower than movement in the opposite direction. This same trend was found after injection of synthetic RNAs (Drummond et al. 1985b; also Fig. 2B) with striking confirmation of the slow movement coming from the *in situ* autoradiography (Fig. 3). However, when the appropriate quantification was derived from the gels it appeared that the synthetic RNAs moved more rapidly than the natural mRNAs. This difference was confirmed by coinjecting radioactive natural poly (A)$^-$ mRNAs (reo) and SP6 transcripts (lysozyme) as shown in Fig. 2B. These relative rates of movement of the injected templates were not influenced by RNA concentration nor by size in the range of 500–1800 bases (Drummond et al. 1985a,b).
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Fig. 2. Movement of RNA in oocytes and embryos. RNAs were injected into the animal or vegetal pole of oocytes (A,B) or fertilized eggs (C). At the times indicated total RNA was extracted from animal or vegetal halves and analysed on denaturing gels. (A) Globin mRNA; (B) lysozyme (Lys) and reovirus 12S (S) and 18S (M) RNAs; panel (C) SP6 lysozyme RNA (Lys) and SP6 Xenopus histone H4 RNA (H4). Abbreviations: A, animal halves; V, vegetal halves; A inj, injection into animal pole; V inj, injection into vegetal pole. Marker tracks (m) as in Fig. 1. Arrows point to the positions of 18 and 28S ribosomal RNAs. For further details see Drummond et al. (1985a,b).
Fig. 3. *In situ* autoradiography of injected oocytes. $^{32}$P-Lys$^+$ dimethyl cap RNA was injected into oocytes at the vegetal (A,B,C,E and G) or the animal pole (D,F, and H) and the oocytes fixed within 10 min (A–D), 6 h (E,F) or 24 h (G,H) and sectioned. After mounting on slides the oocyte sections were autoradiographed. The autoradiographed sections were visualized by phase-contrast (A) or dark-field (B–H) illumination. Panels A,B show the same oocyte section. The bright rim around the oocytes in panels B–H is due to the light scattering by dense pigment granules (not silver grains) distributed around the periphery of the oocytes. The nucleus of the oocyte is indicated (gv, germinal vesicle).
One explanation for the difference in mobility between the synthetic templates and natural mRNAs included in Fig. 2 could be the absence in the former of a poly (A) tail. Although this could not be the only factor since the reovirus mRNAs are also poly (A)+, we compared the movement rates of synthetic templates with and without added poly (A) tails (Drummond et al. 1985b). Polyadenylation did reduce the rate of diffusion, however, the data were not sufficiently accurate for any unequivocal interpretation as to the quantitative contribution of polyadenylation to the new, slower rate. Other contributing factors may be the binding of the 3' or 5' regions of the natural poly (A)+ and poly (A)− mRNAs with components of the cytosol. Precedents for such interactions exist in observations of the binding of mRNAs to poly (A) (Richter & Evers, 1984) or ‘cap’-binding proteins (Zumbe, Stahli & Trachsel, 1982). The synthetic transcripts used in our diffusion study lacked much of their normal 5' and 3' regions.

Two main conclusions emerge from the experiments outlined above. First, diffusion is generally slow irrespective of the source of the RNA. This behaviour contrasts with the more rapid equilibration shown by the other, much smaller RNAs (e.g. transfer RNAs, 5S, 7S and small nuclear RNAs, De Robertis et al. 1982). We presume that the slow rate can be explained by the size and conformation of these RNA molecules combined with the increased diffusional pathlength imposed by the crowded cytoplasm. The second conclusion is that diffusion away from the animal half is much slower than that in the opposite direction. This might reflect the volumes of ‘accessible’ cytoplasm in the two halves (see Drummond et al. 1985a). We measured the relative volumes accessible to tritiated fucose and found the ratio to be 60:40 (animal:vegetal). This is probably not a sufficient difference to account for our observations, however, regions accessible to a small sugar molecule might be unavailable to the high molecular weight RNAs.

How do these observations affect the interpretation of the competition experiments cited earlier? Clearly with diffusion being so slow, the competition for translation observed between injected and endogenous mRNAs can only reflect events in those localized regions populated by both RNA types. This makes attempts to compare relative translational efficiencies or to measure maternal mRNA pool sizes (Richter & Smith, 1981) even more difficult to interpret. However, our results do not alter the conclusions regarding spare translational capacity or different translational pools (Laskey et al. 1977; Richter & Smith, 1981) except that a small (≤5 %) translational capacity in large oocytes might be obscured if only a limited proportion of the oocyte’s endogenous protein synthesis is exposed to competition due to slow mRNA diffusion.

The slow diffusion observed has implications for the design of experiments where the desired aim is to ‘neutralize’ endogenous mRNA sequences by hybridization to injected anti-sense RNA (Melton, 1984; Harland & Weintraub, 1985). In order to ensure sufficient hybridization we would suggest (a) injection of supersaturating concentrations of anti-sense RNA and (b) injection of small (i.e. = 100 bases) anti-sense RNA molecules generated either by transcription of
truncated genes or by alkali digestion (see Cox, Deleon, Angerer & Angerer, 1984). In both cases we would recommend delaying the assay until 24 h (at least) after injection, although it is clear that satisfactory results have been obtained with shorter incubation periods (Melton, 1984; Harland & Weintraub, 1985).

A second problem area highlighted by these results concerns studies investigating the localization of mRNAs in oocytes. Rebagliati et al. (1985) have recently found four RNAs to be localized to either the animal or vegetal regions of the *Xenopus* oocyte. It is conceivable that these RNAs contain sequence(s) that target the RNAs in some way to the appropriate region. One test of this possibility would be to inject oocytes with a synthetic version of the localized RNA and look for relocalization. If relocalization occurred then the sequences responsible could be identified using a systematic mutagenesis/RNA-injection strategy. Our results would indicate that such injection experiments would require many days during which time pathological changes may begin to occur in the oocytes. An alternative strategy might be to inject the gene encoding the localized RNA into the germinal vesicle of the oocyte. In this way the oocyte itself will produce the studied transcript. We have reported tentative evidence that transcripts produced in this way are more rapidly distributed within the cytoplasm (Drummond et al. 1985a).

Movement of injected RNAs in embryos

We have examined the movement of synthetic RNAs in fertilized embryos by similar methods to those used in oocytes. Embryos were injected at the 1-cell stage in either the animal or vegetal pole, with a mixture of *Xenopus* histone H4 and chicken lysozyme RNAs. At the 8-cell stage, the embryos were frozen, bisected and analysed as shown in Fig. 2C. The results are highly reminiscent of those obtained in oocytes 48 h after injection in that there is very little detectable movement animal to vegetal in contrast to considerable movement in the opposite direction. However, it should be emphasized that the 8-cell stage is reached within 2 h. This increased rate of movement might reflect the greater fluidity of the cytoplasm seen after maturation and fertilization as well as the bulk redistribution of the yolk shortly after fertilization (Wilt & Phillips, 1984).

Extra information was obtained when *in situ* autoradiography experiments were performed. We coinjected chicken lysozyme and *Xenopus* β-globin transcripts and fixed the embryos at stage 7. Sample results are shown in Fig. 4. We anticipated that cleavage would pose an additional obstacle to RNA movement and this turned out to be the case. Clearly the distribution is localized at stage 7 even after injection at the 1-cell stage. Localization can occur to animal (Fig. 4C–F) and vegetal (Fig. 4G,H) areas. We cannot reconcile these data with the observations of Capco & Jeffery (1981) who found that RNA extracted from the vegetal pole of eggs would relocalize to this area within 2 h of injection, regardless of the site of injection. Froehlich, Bowder & Schutz (1977) reported that rabbit globin mRNA injected into fertilized embryos became sixfold more concentrated in the animal regions by the gastrula stage. However, these results are consistent with our
Fig. 4. *In situ* autoradiography of injected embryos. Fertilized eggs were injected at the 1-cell stage with $^{32}$P-labelled SP6 globin RNA and fixed immediately (A,B) or at the blastula stage (C–H) and processed as in Fig. 3. The sites of injection were vegetal (A,B,G,H) or animal (C–F). The sections were visualized by phase-contrast (B,D,F,H) or dark-field illumination (A,C,E,G). Standard negatives were used to print A–F whilst G,H were prepared from colour transparencies.
observations since their rabbit globin mRNA was injected into the animal hemisphere.

In summary then, we find that movement of injected RNA in embryos appears much faster than that in oocytes, however, the rapid divisions ensure segregation of injected RNAs. This poses problems for experiments involving injections of sense or anti-sense RNA since homogenous distribution throughout the embryo may be a desired objective. However, small reductions in temperature (i.e. 5°C fall) have dramatic effects on the cell division rate and this might not be accompanied by comparable effects on RNA diffusion. The best compromise would therefore appear to involve injections into the vegetal hemisphere of a fertilized egg followed by incubation at 15°C or below until the 8-cell stage when segregation of animal and vegetal hemispheres becomes finally complete.

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REFERENCES


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