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Use of Frog Eggs and Oocytes for the Study of Messenger RNA and its Translation in Living Cells

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Injected frog eggs and oocytes provide a very sensitive assay system for the identification of messenger RNA and permit the study of translational control in living cells. The translation of each haemoglobin messenger RNA molecule once every 5–10 minutes for at least 24 hours makes it possible to recognize less than 1 ng of this messenger RNA.

EXPERIMENTS described elsewhere have shown that oocytes injected with haemin and purified 9S RNA from rabbit reticulocytes will synthesize haemoglobin; the effect is not obtained with other kinds of reticulocyte RNA[1,2]. This raises the possibility that the microinjection of messenger RNA (mRNA) into frog oocytes and eggs may constitute a generally useful experimental system for identifying and studying the translation of different kinds of mRNA. This system has the unique advantage of enabling purified RNA to be translated and the control of this process to be studied in a normal living cell. In this article we discuss several of the conditions associated with the successful translation of injected mRNA. In particular we are concerned with the treatment of recipient cells, with the duration and efficiency of haemoglobin (Hb) message translation, and with the potential use of this experimental system for translating messages other than that for Hb.

9S mRNA for haemoglobin has been prepared by SDS treatment of the 14S messenger ribonucleoprotein released from reticulocyte polyribosomes by EDTA[1]. For all experiments described here, oocytes and eggs were taken from *Xenopus laevis*. Except where stated, oocytes were taken from frogs which had been induced by hormone treatment to ovulate between 2 and 4 weeks previously. This was done to ensure that the larger oocytes were growing actively. The eggs used were unfertilized, but underwent the usual activation response to penetration. Oocytes were injected dry but without the removal of follicle cells[2], and eggs were irradiated with ultraviolet light to facilitate penetration of the jelly and vitelline membrane[3].

Micropipettes 10–15 μm in diameter were calibrated to deliver a volume of 50–70 mμl., the actual amount being kept constant in each related series of experiments. Samples for injection were kept on ice, as well as under oil to avoid concentration by evaporation. All samples were taken up in, or dialysed into, the following injection medium: 88 mM NaCl, 1.0 mM KCl, 15 mM Tris-HCl, *p*H 7.6. Injected cells were incubated at 19° C in the saline medium described by Gurdon[2].

Labelling of Eggs and Oocytes

Labelled amino-acids can be introduced into cells either by injection or by addition to the culture medium. The choice of labelling procedure is determined chiefly by: (*a*) the rate at which label in the culture medium penetrates incubated cells; (*b*) the rate at which injected label leaks out, and (*c*) the duration of the labelling period.

We have investigated the labelling of cells by ^{3}H-histidine, with the results shown in Fig. 1*A–D*. Labelled amino-acids leak out of injected oocytes rapidly, so that less than 50% remains after 1 h, but leakage from injected eggs takes place at a much lower rate (Fig. 1*A* and *C*). In contrast, labelled histidine in the medium penetrates eggs very poorly, but is rapidly taken up by oocytes (Fig. 1*A* and *C*). The incorporation of intracellular amino-acid into protein is shown in Fig. 1*B* and *D*. The general conclusion from experiments of this kind is that when use is made of short labelling periods of up to 1 h or of unfertilized eggs (which do not normally remain metabolically active for more than a few hours), the highest yield of labelled protein is obtained by injection. When oocytes are used, especially for labelling periods of more than 2 h, it is best to label by incubation (at least for ^{3}H-histidine). A minor disadvantage of labelling oocytes by incubation is that the follicle cells and very small oocytes (usually present in ovarian tissue containing large oocytes) will take up label and synthesize labelled "endogenous" protein, but not the protein coded for by added mRNA because they are too small to be conveniently injected. When it is important to eliminate totally the background contributed by follicle cells and small oocytes, unfertilized eggs (which have no follicle cells) should be used.

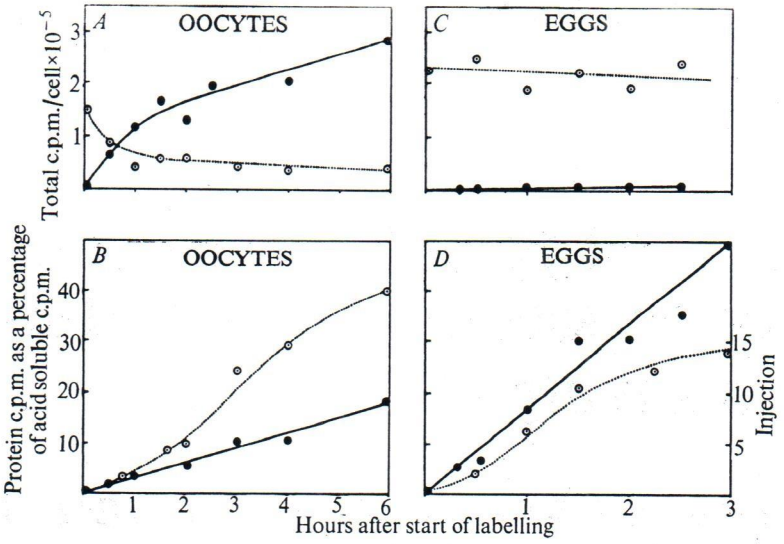

Fig. 1 *A* and *B*, Full size oocytes; *C* and *D*, ultraviolet irradiated unfertilized eggs. For incubation experiments (●) cells were injected with saline solution and incubated at 19° C in culture medium containing ^{3}H-histidine at about 1 mCi/ml. For injection experiments (⊙) each cell received 50 nl. of ^{3}H-histidine at about 6 mCi/ml. At the end of the labelling period, samples of ten cells were frozen. After homogenization in 0.5 M KCl 15 mM Tris, 0.1% unlabelled histidine, *p*H 7.6, an aliquot was dissolved in 90% formic acid and counted to give total counts per sample. The rest of the sample was precipitated in 10% trichloroacetic acid (TCA), and centrifuged. The pellet was taken up in 10% TCA, heated at 90° C for 30 min, pelleted, rewashed in cold TCA, then with alcohol and ether, and finally dissolved in 90% formic acid for counting. From this was determined the amount of labelled protein in the original sample. In *A* and *C*, it is important to note that the total radioactivity includes protein as well as acid soluble c.p.m. The proportion of total radioactivity which is protein can be determined by reference to *B* and *D*.

Translation of Hb Message

The accumulation of labelled haemoglobin in conditions of continuous labelling is shown for injected eggs in Fig. 2*A*. The slow initial rise in the rate of Hb synthesis probably reflects the time needed (10–15 min) for the injected message to become fully incorporated into the structure of the cell in such a way as to serve for protein synthesis; the reduced rate at which labelled Hb is accumulated after 2 h from the time of injection can be partially accounted for by the substantial reduction in the amount of unincorporated labelled amino-acid which remains in eggs injected 2 h before (compare Fig. 1*C* and *D*). The translation of Hb mRNA is compared with that of endogenous mRNA in continuously labelled oocytes in Fig. 2*B*. The amount of Hb mRNA injected was sufficient in these experiments for the labelled Hb to amount to about half that of the labelled "endogenous" protein. It is clear from Fig. 2*B* that both Hb and endogenous protein accumulate at approximately constant rates during the 9 h period of these experiments.

The relative rates of Hb and endogenous message translation have been further examined by pulse labelling oocytes injected with Hb mRNA. Fig. 3 shows that the ratio of Hb to endogenous protein synthesis is similar up to 21 h after injection. Only within the next 10 h is some decrease in this ratio observed. Because no steps were taken to prevent RNA synthesis which takes place in cultured injected oocytes[2], this last result does not necessarily indicate that the rate of translation or stability of Hb mRNA differs from that of the endogenous mRNA already present at the time of injection.

Table 1 summarizes some other respects in which injected and endogenous mRNA translation have been compared. Injected message translation is at least as sensitive as that of endogenous message to the inhibition of protein synthesis by puromycin. The intracellular location of injected message translation is interesting because the nucleus of normal oocytes always has a higher concentration of labelled protein than the cytoplasm, even after short labelling periods (discussed in ref. 4). It has not yet been clearly established whether proteins are synthesized in the nucleus or whether they pass from the cytoplasm to the nucleus very soon after synthesis. From the experiments summarized in Table 1, it seems that injected message is translated in the same part of the cell as most of the endogenous message. The fact that haemoglobin synthesis takes place in oocytes enucleated before RNA injection, as well as in unfertilized eggs which possess no nucleus but only ultraviolet-irradiated chromosomes, shows that Hb is synthesized largely, if not entirely, in the cytoplasm.

We conclude that injected Hb mRNA becomes rapidly associated with components of egg and oocyte cytoplasm so as to be translated in the same way as resident mRNA molecules already in use for protein synthesis. Once started, Hb

Table 1 Inhibitor Sensitivity and Intracellular Location of Hb Synthesis by Injected Oocytes

Recipient cells *	Materials injected		Protein synthesis as % of acid soluble radioactivity		Synthesis ratio: haemoglobin / endogenous
			Endogenous protein †	Haemoglobin	
Large oocytes	9S RNA at 800 µg/ml. and haemin		14.6	43.9	3.01
Small oocytes (half max. diam.)	9S RNA at 800 µg/ml. and haemin		12.5	41.6	3.33
Large oocytes	9S RNA at 800 µg/ml. and haemin	Puromycin at 700 µg/ml.	2.4	3.5	1.46
Large oocytes, enucleated after incubation ‡	9S RNA at 800 µg/ml. and haemin		10.1	33.3	3.30
Large oocytes, enucleated before injection ‡	9S RNA at 800 µg/ml. and haemin		1.5	6.4	4.27

* Each sample, which consisted of thirty oocytes, was labelled by incubation in ^{3}H-histidine for 6 h at 19° C.

† Calculated from 'Sephadex G-100' fractionations as described by Moar *et al.*[13].

‡ Enucleation involved manual removal of the germinal vesicle, which is squeezed out of oocytes after an animal pole incision. The oocytes injected after enucleation did not heal satisfactorily, and incorporation into protein was very low.

mRNA and endogenous mRNA are translated at the same relative rate for at least 21 h; Hb mRNA continues to be translated, possibly at a lower rate, for several hours after this.

Efficiency of Hb mRNA Translation

We wish to know the frequency with which each injected mRNA molecule is translated per minute; this information will permit an estimate of the total number of times an mRNA molecule is translated during its life in an egg or oocyte. The frequency of message translation can be determined from a knowledge of the number of mRNA molecules present in a host cell, and of the specific activity of the intracellular histidine pool (hence the number of Hb molecules synthesized).

The size of the histidine pool in eggs and oocytes has been determined by fractionating on an automatic amino-acid analyser the supernatant of cells homogenized in 0.5 N perchloric acid. One sample of 300 oocytes, from which the follicle cells and ovarian tissue were not removed, contained 36 pmol of histidine per large oocyte. Another sample of 400 oocytes, from which were removed all small oocytes and ovarian tissue except the follicle cells immediately surrounding each large oocyte, contained 23 pmol of histidine per oocyte. A sample of 350 unfertilized eggs, analysed in the same way, contained 91 pmol of histidine per cell. These values for the histidine pool may be compared with the figure of 146 pmol per egg obtained by Kutsky *et al.*[5], using unfertilized eggs of *Rana pipiens*, and 330–700 pmol per egg obtained by Ecker and Smith[6] for eggs of this same species. Since *Rana pipiens* eggs are three times the volume of *Xenopus laevis* eggs, our measurements suggest that a similar or slightly smaller intracellular concentration of histidine exists in *Xenopus* than in the only other amphibian species tested.

Oocytes from the same frogs as those used for estimating the histidine pool size have been injected with Hb mRNA, and the efficiency of message translation has been calculated according to the procedure outlined in Table 2.

To test whether the results summarized in Table 2 are representative of our normal experience, we have calculated in Table 3 the efficiency of translation of the same sample of mRNA used in fifteen other experiments with cells whose histidine pool size was not measured but assumed to be similar to that of other oocytes or eggs. The two samples of oocytes which gave the results in Table 2 were evidently worse in one case, and better in the other case, than is typical. As might be expected, the efficiency of translation is greater at the lowest intracellular concentrations of injected mRNA. This effect is clearly seen in Fig. 4 and is consistent with the observation, reported elsewhere[13], that there is a substantial decrease in translational efficiency at intracellular concentrations of message which exceed 8 μg/ml.

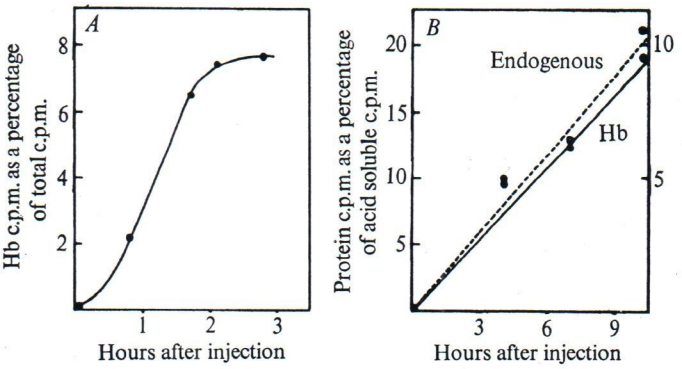

Fig. 2 *A*, Each point is based on a 'Sephadex' analysis of thirty unfertilized eggs injected with 9S RNA at about 800 μg/ml., haemin, and ^{3}H-histidine, and frozen at the times shown. *B*, Each point is based on a 'Sephadex' analysis of twenty large oocytes injected with 9S RNA at about 800 μg/ml. and haemin, incubated in medium containing ^{3}H-histidine, and frozen at the times shown. "Endogenous" refers to protein synthesized from endogenous mRNA, as opposed to the injected 9S mRNA. The ordinate on the left refers to endogenous and that on the right to Hb radioactivity.

In estimating the efficiency of translation, we have made certain assumptions which affect the accuracy of our calculations in the following ways. In determining the specific activity of the histidine pool, our measurements of the extractable free histidine constitute an upper limit to the size of the pool that is actually used for protein synthesis; this could have been

Table 2 Efficiency of Hb mRNA Translation in Injected Oocytes of Measured Histidine Pool Size

	Oocytes of frog A	Oocytes of frog B			
1 Concentration of Hb mRNA in sample for injection (μg/ml.)	200	250	125	62.5	31.25
2 Injected Hb mRNA (ng/cell) *	14,000	17,500	8,760	4,360	2,180
3 „ „ „ (pmol/cell) †	0.070	0.087	0.043	0.022	0.011
4 Number cells/sample	30	20			
5 c.p.m. ^{3}H-histidine incorporated into Hb/cell/h ‡	4,480	6,428	8,490	6,439	3,155
6 Pool size of histidine (pmol/cell)	23.2	35.9			
7 Specific activity of intracellular histidine pool (c.p.m./pmol) §	2,845	758	1,392	2,654	1,186
8 Globin chain synthesis‖ (pmol globin/cell/h)	0.157	0.848	0.610	0.243	0.266
9 Number globin molecules synthesized/h/9 S RNA molecule (row 8 ÷ row 3)	2.24	9.77	14.0	11.1	24.35

* These values disregard leakage from injected oocytes, though this is believed to occur (see text).

† Assumes that the molecular weight of 9S mRNA is 200,000. Various published and unpublished measurements by different methods have estimated it to be between 175,000 and 225,000 (for example, ref. 8).

‡ All samples were labelled by incubation for 10 h at 19° C in ^{3}H-histidine of specific activity 52.1 Ci/mmol.

§ Calculated from the histidine pool size and the acid soluble c.p.m./cell recovered from columns of 'Sephadex'.

‖ Assumes 9.5 histidine residues per average (α and β) globin chain.

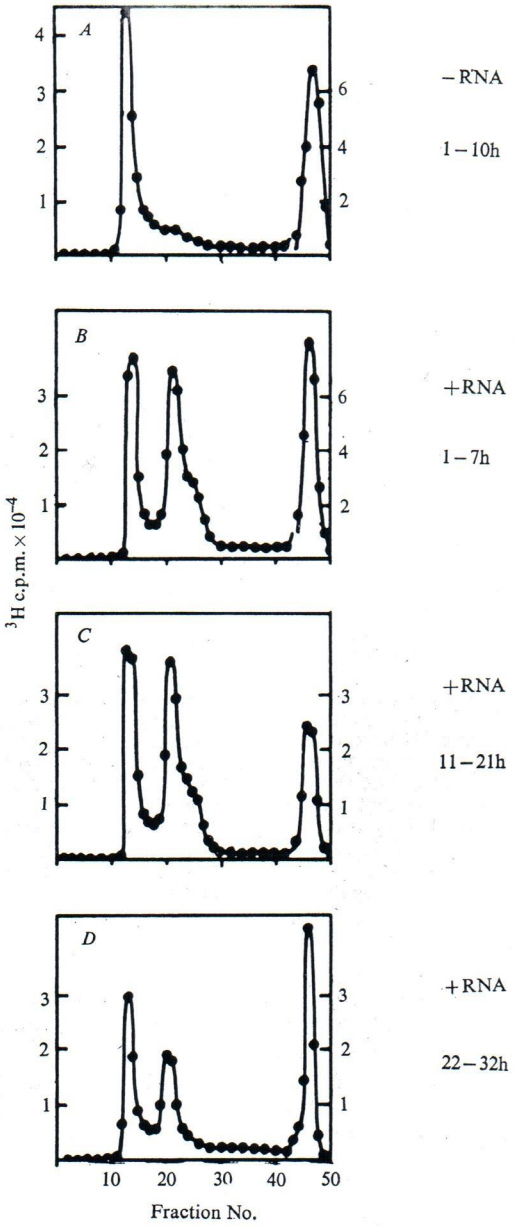

Fig. 3 'Sephadex' analysis of injected oocytes. Each sample of twenty oocytes was injected with haemin, and in the cases of *B*, *C* and *D* with 9S RNA at 400 μg/ml. (about the saturating concentration for these oocytes). Samples were labelled for the periods shown, and frozen at the end of the labelling period; samples *C* and *D* were incubated in unlabelled culture medium until the beginning of the labelling period. The radioactivity on the left hand ordinate of each figure refers to the first two 'Sephadex' peaks; that on the right hand ordinate refers to the right hand 'Sephadex' peak of ^{3}H-histidine.

much smaller, and if negligible would mean that we have overestimated the efficiency of translation by about six times. On the other hand, we have assumed that the same amount of acid-soluble radioactivity as was extracted from oocytes at the end of the labelling period was present throughout the incubation. It is clear from our study of histidine penetration (Fig. 1*A*) that the cell content of ^{3}H-histidine increases during the labelling period, and this will have led to our underestimating the efficiency of message translation. Another factor which could have led to a two-fold underestimate of translational efficiency is the leakage of injected mRNA. We know that about half of all injected ^{3}H-histidine leaks out of oocytes within an hour (Fig. 1*A*). Finally, it is not certain that every RNA molecule within the 9S preparation injected was a potentially functional message. By assuming that every molecule within the injected sample is able to serve as a normal Hb message, we can only have underestimated the efficiency of translation.

In Table 4 we compare the efficiency of Hb mRNA translation in different conditions. It is evident that Hb mRNA is translated 100–1,000 times more efficiently in frog oocytes than in a good cell-free system, and if, as seems possible from the above arguments, we have underestimated the efficiency of injected message translation, then the translation of Hb mRNA in frog oocytes compares very favourably with that in normal living reticulocytes.

Can Other mRNAs be Translated?

The results so far reported do not permit the conclusion that every mRNA could be translated in frog oocytes. The successful translation of rabbit 9S RNA in these cells might depend on its possession of one or both of the following properties: (*a*) it is prepared from a species different from that of the translational system; (*b*) it codes for a protein never normally synthesized (in detectable amounts) by the host cells.

In the absence of a purified mRNA which codes for proteins normally synthesized in *Xenopus* eggs and oocytes, we have not been able to test the importance of these conditions. But the results of injecting three other types of mRNA into frog eggs and oocytes permit us to conclude that (i) rabbit 9S RNA is not the only heterologous RNA capable of being translated in frog oocytes; (ii) some kinds of artificial polymer cannot be translated, (iii) at least one kind of heterologous mRNA cannot be translated. The three types of mRNA referred to are the mRNA for mouse myeloma protein, AUGpolyU, and f2 bacteriophage virus mRNA.

A 9–13S fraction of RNA extracted from the K-41 mouse myeloma promotes the synthesis of the expected myeloma

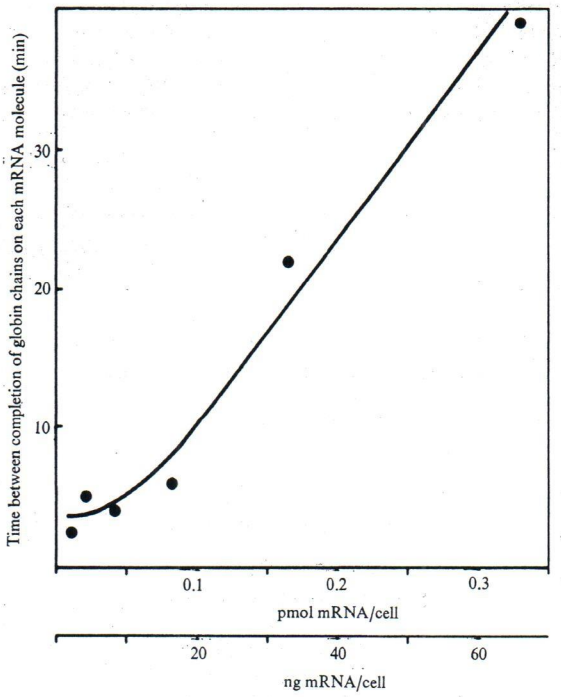

Fig. 4 Each point is based on the 'Sephadex' analysis of twenty oocytes injected with 9S mRNA and haemin, and incubated in medium containing ^{3}H-histidine for 10 h. The various assumptions made in the calculations in Table 2 are also made here. It is assumed that all molecules in the injected RNA sample were translated with the same efficiency. If it is assumed that there is only one ribosome working on each mRNA molecule at a time, then the ordinate scale shows the time taken to synthesize one globin chain.

light chain immunoglobulins when added to a cell-free system[7]. When this RNA is injected into frog oocytes, 2.5–4% of the proteins labelled by ^{3}H-serine in one experiment, and by ^{3}H-threonine in another experiment, were precipitated by K-41 antiserum; this constitutes a four to six-fold increase above the 0.5% precipitation observed in controls. When the precipitated material was dissociated and passed through a column of 'Sephadex', the experimental but not control samples contained radioactivity which migrated with carrier light chain monomer (Stavnezer, Huang, Gurdon and Lane, in preparation).

Table 3 Efficiency of Hb mRNA Translation in Injected Cells of Unknown Histidine Pool Size

Intracellular concentration of injected mRNA (μg/ml. or ng/cell) *	c.p.m. ^{3}H-haemoglobin synthesized/cell /h × 10^{-3} †	Duration of labelling after injection (h)	No. molecules Hb synthesized/h/mRNA molecule ‡
Oocytes			
25	13,340	10	3.4
13	16,940	10	4.8
12.5	8,170	10	4.6
11.8	15,310	10	5.4
11.0	4,250	3	4.8
6.3	5,500	10	4.4
6.3	6,590	10	7.6
6.2	7,050	10	10.4
5.0	15,310	10	5.4
3.12	8,200	10	12.0
1.66	4,690	10	5.8
0.56	4,520	10	8.6
Average of concentrations up to 13 μg/ml.			6.7
Eggs			
20	1,390	2	9.9
10	1,360	2	17.7
5	660	2	14.6
		Average	14.1

This table includes the results of five experimental series, carried out on oocytes or eggs of different frogs.

* Calculated from the known concentration of RNA in samples for injection and from the known volume injected into each cell. Leakage, which may occur on a substantial scale, is ignored. 1 egg or oocyte is assumed to contain 1 μl. of solution.

† Based on 'Sephadex G-100' analyses.

‡ The calculations for oocytes assume a pool size of 30 pmol per cell (average of the two values in Table 2), and those for eggs assume a pool size of 91.2 pmol per cell, this being the value obtained from an automatic amino-acid analyis of 350 unfertilized eggs. In other respects calculations were carried out as in Table 2.

The effect of injecting AUGpolyU and ^{3}H-phenylalanine into oocytes has been examined by Woodland and Browne (unpublished). AUGpolyU seems not to stimulate incorporation of ^{3}H-phenylalanine into polyphenylalanine, but it drastically inhibits the synthesis of proteins characteristic of the host cells.

The possibility that bacterial virus mRNA can be translated in frog cells has been examined in collaboration with Dr A. Garen. RNA was prepared from f2 phage by two cold phenol extractions. Immediately after alcohol precipitation it was injected into oocytes at a concentration of about 1 mg/ml. ^{3}H-tyrosine-labelled proteins were extracted from RNA-injected and control eggs and analysed by acrylamide gel electrophoresis. No difference between the two samples was observed, and in neither case was a peak of radioactive protein seen in the region of the gels that contained ^{14}C-labelled carrier coat protein and replicase. Total protein synthesis was unaffected. Although we have not determined the reason why f2 RNA fails to be translated in frog oocytes, this may be because it requires the translational machinery of bacterial as opposed to animal cells.

Identification of Unknown mRNAs

The opportunity which injected frog cells offer for translating purified mRNA with high efficiency in a normal living cell suggests the application of this system to the identification of unknown mRNAs and their products. The identification of the unknown message for a known protein can be undertaken by separating total RNA into different fractions, injecting these into oocytes or eggs, and assaying for the synthesis of the protein. It is essential to know that the message product is not degraded in oocyte cytoplasm or rendered unrecognizable by complexing with oocyte materials. We have failed to find such effects with several different proteins which had been labelled with ^{125}I.

Table 4 Comparative Efficiency of Hb mRNA Translation

Translational systems	Temperature of incubation (°C)	No. globin chains synthesized per mRNA molecule/h	Half-life of translational system	Total No. translations/half-life of system
Cell-free system (lysate using added mRNA)	25 *	0.8 *	60 min *	0.8 *
Rabbit reticulocytes in culture (using endogenous mRNA)	37	800 †	2–3 h ‡ (in culture)	1,840
	20	80 †	?	?
Injected frog cells:				
Oocytes (typical)	19	7 §	26 ¶	180
Oocytes (best)	19	24 ‖	26 ¶	625
Eggs	19	14 §	?	?

The estimates for the efficiency in frog cells are based on various assumptions, one of which is that the amino-acid pool functional in protein synthesis is the same as the total extractable amino-acid. If this were not so, our estimate would be a maximum of six times too high (see the text for the other assumptions made).

* From ref. 9.
† From ref. 10.
‡ From ref. 11.
§ See Table 3.
‖ See Table 2.
¶ See Fig. 3.

If it is required to identify an mRNA and its product when neither have so far been identified, two procedures are available. The first is to look for an increase in the total proportion of intracellular labelled amino-acid that is incorporated into protein. mRNA as pure as our 9S RNA for Hb raises the overall rate of protein synthesis of recipient cells about two-fold when injected at saturating concentrations[13]. The second procedure is to test the ability of a potential message-containing fraction of RNA to compete with a known message (such as Hb mRNA) for a limited supply of the host cells' translational apparatus. Hb mRNA, at a saturating concentration and supplemented by increasing amounts of potential messenger RNA, is injected into a series of test cells; to keep the total RNA injected per cell constant, the increasing amounts of potential mRNA are supplemented with proportionately decreasing amounts of a translationally inert RNA such as 28S rRNA (see below). The existence of mRNA in the potential message fraction is indicated by a reduction in the amount of Hb synthesis when related to acid-soluble radioactivity in the injected cells. Table 5 illustrates the use of this procedure in respect of cultured mouse cell polysomal RNA. Further experiments would be required to prove that the cultured cell RNA reduces Hb synthesis by competition for translational apparatus rather than by some unspecific inhibitory effect; however, this design of experiment can

provide a useful means of screening the potential message content of RNA fractions.

For either of these tests to work satisfactorily it is necessary to know that none of the major classes of cellular RNA (that is, 28S, 18S, 4+5S) with which mRNA is likely to be contaminated will affect Hb mRNA translation. The results in Table 5 and other unpublished experiments have shown that 28S rRNA does not inhibit Hb mRNA translation, and it has been demonstrated elsewhere[12] that neither 28S, 18S nor 4+5S RNA from reticulocytes promotes Hb synthesis when injected on their own. We have found that very high concentrations of RNA, especially of 4+5S RNA, are toxic to some samples of eggs and oocytes; therefore the total amount of injected RNA should, whenever possible, be kept below 50 ng per cell, and the total amount of RNA in all samples to be compared should be kept constant. By attention to these details, we believe that oocytes and eggs can provide a valuable assay system for identifying different kinds of mRNA and their products.

Advantages of Oocyte System

For the study of messenger RNA and its translation, the microinjection of *Xenopus* eggs and oocytes has three special merits. First, the translation of purified mRNA is undertaken in a normal living cell, and is therefore less likely to be affected by artefacts than a cell-free system. Second, injected oocytes are capable of translating mRNA with a high efficiency for long periods. Using our normal labelling procedures, we have obtained, over 24 h, as much as 10^6 d.p.m. of labelled Hb from 1 ng of injected mRNA. By increasing the amount and range of labelled amino-acids, it would be possible with this system to recognize as little as 1 pg of Hb mRNA. Finally, *Xenopus* oocytes seem to show very little species specificity with respect to the type of mRNA which they can translate. It seems possible that all kinds of eukaryotic and animal virus mRNA may be capable of translation in *Xenopus* oocytes. The large size, resistance to microinjection, and easy availability of *Xenopus* oocytes have relieved us of the incentive to test the translational usefulness of eggs and oocytes from other animal species.

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Table 5 Messenger RNA Competition Experiment

Intracellular concentration of injected RNA (ng/cell)			Protein synthesis as % of acid-soluble c.p.m.	
Hb mRNA	28S rRNA	3T6 RNA	Hb	Endogenous
10	30	0	61.5	111
10	27.5	2.5	35.8	80.1
10	25	5	20.4	35.4
10	17.5	12.5	19.8	45.5
10	10	20	18.4	25.3
10	0	30	12.6	33.2
10	0	0	55.3*	103.1*

The injected cells were large oocytes, which were incubated for 9 h in ^{3}H-histidine before freezing. Analysis was by 'Sephadex'. 3T6 RNA was obtained from polysomes of cultured mouse cells. The variation in values for endogenous protein synthesis is probably due to the variable amounts of uninjected ovarian material in each sample (see text).

* These are average values for three experiments of this kind.

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