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Translational Capacity of Living Frog Eggs and Oocytes, as Judged by Messenger RNA Injection

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Growing oocytes and activated eggs of *Xenopus laevis* have been injected with increasing amounts of 9 s haemoglobin messenger RNA extracted from rabbit reticulocytes. At low concentrations of injected RNA, there is a linear relationship between the amount of RNA injected and the amount of haemoglobin synthesized. Higher concentrations of RNA saturate the translational capacity of oocytes and eggs, so that increasing amounts of injected RNA fail to stimulate increased haemoglobin synthesis. Under these conditions, the rate of protein synthesis promoted by endogenous messenger RNA is unaffected by the injection of 9 s RNA and the over-all rate of protein synthesis by message-saturated cells is nearly doubled. At very high concentrations of injected RNA and after long periods of incubation, the injected RNA competes with endogenous messenger RNA for a translational component which limits the over-all rate of protein synthesis in injected cells. The results show that frog eggs and oocytes possess an unused capacity for translating injected messenger RNA, and that the amount of this spare capacity can be determined by the construction of messenger RNA saturation curves. The results also suggest that the normal rate of protein synthesis in these cells is limited by their content of functional messenger RNA.

1. Introduction

When haemin and a 9 s fraction of RNA purified from rabbit reticulocytes are injected into frog oocytes, haemoglobin is synthesized (Lane, Marbaix & Gurdon, 1971); uninjected oocytes, and oocytes injected with haemin but no 9 s reticulocyte RNA, do not synthesize haemoglobin at a detectable rate. This type of experiment has been of considerable value in determining the identity and analysing the translational control of haemoglobin messenger RNA (Lane *et al.*, 1971; Gurdon, Lane, Woodland & Marbaix, 1971). An experimental system in which messenger RNA is translated after injection into living cells can also prove useful for analysing certain aspects of the recipient cells' translational machinery. In particular it should be possible to establish a relationship between the amount of messenger RNA injected and the amount of haemoglobin synthesized. If a point is reached at which increases in the amount of injected RNA yield no further increase in haemoglobin synthesis, the resulting "saturation curve" could be used to determine the amount of spare translational capacity in a sample of recipient cells, as well as to provide other information about the recipient cells' messenger RNA and translational system actively engaged

in protein synthesis. This paper demonstrates that saturation curves can be prepared for haemoglobin messenger RNA injected into the growing oocytes and activated eggs of frogs.

2. Materials and Methods

9 s RNA was prepared from reticulocytes by sodium dodecyl sulphate treatment of ribonucleoprotein detached from polysomes by EDTA (Chantrenne, Burny & Marbaix, 1967). RNA was mixed with haemin and handled for micro-injection as described by Lane *et al.* (1971). Except where stated, the *Xenopus laevis* females used to provide oocytes had been induced by hormone treatment to lay eggs between 2 and 4 weeks before the time when their oocytes were taken for RNA injection. Dissected oocytes were maintained in culture medium for up to 6 hr before injection. The injection medium used was 88 mM-NaCl, 1·0 mM-KCl, 15 mM-Tris·HCl, pH 7·6. The same calibrated micropipette was used for all samples of oocytes which concerned any one message-injection experiment. The culture medium, injection procedure, etc., were as described by Gurdon (1967,1968). The injection of RNA appeared to have no adverse effect on the viability of oocytes and eggs, since the injected cells were not distinguishable at the end of the incubation period from uninjected oocytes or spontaneously activated eggs. Oocytes and eggs were labelled by incubation and injection respectively (see Gurdon *et al.*, 1971), using L-[2,5-^{3}H]histidine of specific activity 52 Ci/m-mole (Radiochemical Centre, Amersham).

After incubation, all injected cells were washed in label-free medium and frozen. They were then homogenized in a solution containing 52·2 mM-Tris, 52·2 mM-glycine, 5 mM-L-histidine monohydrochloride and rabbit haemoglobin at 5 mg/ml., the pH of the whole solution being 8·9. The homogenate was centrifuged at 75,000 ***g*** for 30 min at 4°C and the supernatant was applied directly to a 140 cm × 1 cm G100 Sephadex column which was equilibrated and run in Tris–glycine buffer (as above, less haemoglobin and unlabelled histidine). Samples of 100 μl. were taken from each fraction collected from the column and added to an ethoxyethanol-containing scintillation medium for counting. The very slight variation in counting efficiency observed between fractions was ignored.

3. Results

(a) *Quantitation of haemoglobin synthesis by Sephadex fractionation of extracts*

Figure 1(c) and (f) illustrates the distribution of radioactivity in eggs and oocytes injected with saline solution but not with RNA. Figure 1(a), (b), (d) and (e) shows that the injection of 9 s RNA stimulates incorporation of label into molecules which co-chromatograph with added haemoglobin, and that the amount of this labelled material is reduced when less RNA is injected. The labelled molecules are indistinguishable from haemoglobin under many different conditions of fractionation and analysis (Lane *et al.*, 1971).

To quantitate the amount of haemoglobin synthesized, two methods have been used; both give similar results. For each method, the radioactivity recovered from Sephadex columns is divided into the three regions indicated in Figure 1(a): the front peak, the haemoglobin peak and the amino acid peak. The patterns of elution are very reproducible and the same groups of tubes have been pooled for each run to be compared. By the first method, it is assumed that the "endogenous" radioactivity within the haemoglobin peak is a constant proportion of the front peak material irrespective of the amount of haemoglobin synthesized. The actual proportion is determined from the saline-injected controls in each series of experiments; in such controls (Fig. 1(c) and (f)), the endogenous radioactivity in the haemoglobin peak constitutes from 35 to 40% of that in the front peak. The amount of haemoglobin

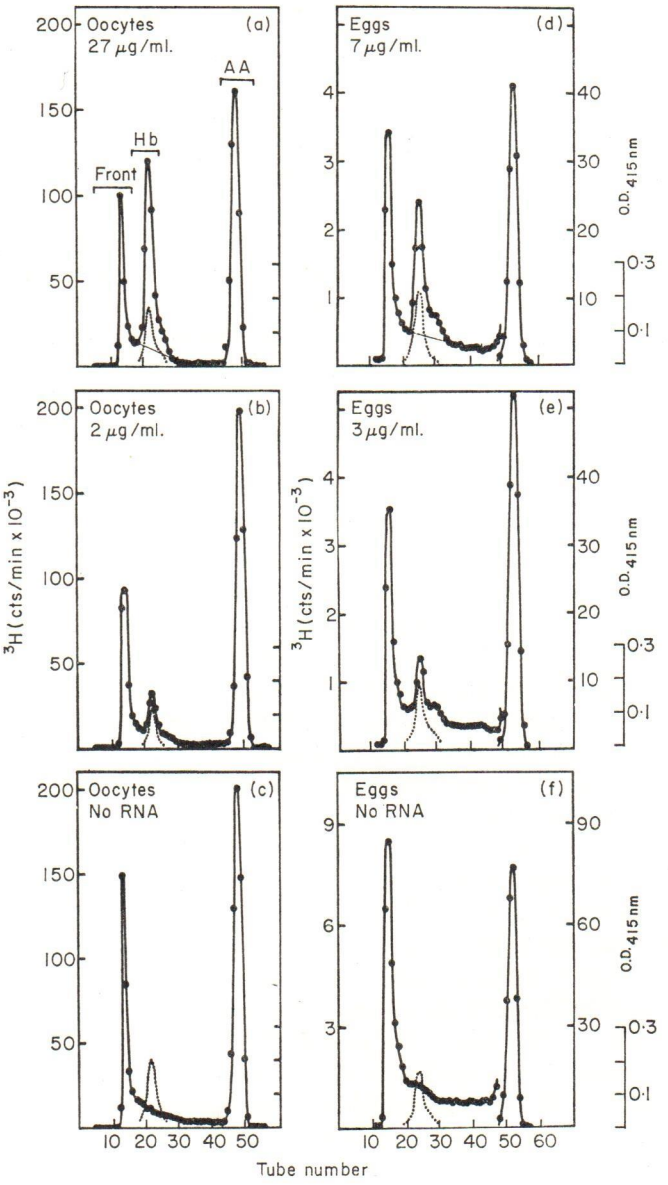

FIG. 1. Sephadex G100 fractionations of injected oocytes and eggs. Each sample consisted of 20 injected cells. The radioactivity shown represents cts/min/cell. Oocytes (taken from a recently ovulated female; see legend to Fig. 2) were labelled by addition of [^{3}H]histidine to their culture medium, and eggs by including the same label in the sample for injection (see Gurdon *et al.*, 1971). Oocytes were incubated for 10 hr after injection, and eggs for 2 hr. The concentrations of 9 s RNA shown in (a), (b), (d) and (e) represent the intracellular concentration, allowing for a 20-fold dilution of the injected sample by diffusion within injected cells, but ignoring leakage. Samples (c) and (f) were injected with injection-medium and haemin, but no RNA. Front, Hb, and AA in (a) refer to the front, haemoglobin, and amino acid peaks of radioactivity. —●—●—, Radioactivity, --------, O.D.

synthesized is represented by the radioactivity present in the haemoglobin peak in excess of endogenous radioactivity in that region. The second method involves drawing a line between the first and last tubes of the haemoglobin peak, as in Figure 1(a) and (d), and regarding all radioactivity above the line as that contained in haemoglobin. By this method, the value for haemoglobin synthesis in control samples is always very close to 0. The value for endogenous protein synthesis is that which remains after subtracting haemoglobin radioactivity from the sum of that in the first two Sephadex peaks.

To obtain a more accurate indication of the amount of haemoglobin synthesized by each sample of RNA-injected eggs or oocytes, allowance must be made for variation in the amount of labelled amino acid per cell (i.e. specific activity of the precursor pool), and for variation in the level of protein synthesis in individual eggs and oocytes. The simplest means of allowing for these variables is to relate the amount of haemoglobin synthesized to the amount of endogenous protein synthesis in the same sample (as in Tables 1 and 2, column 8). All our conclusions are based on the assessment of haemoglobin synthesis by this relative means.

(b) *The interpretation of message saturation experiments*

When mRNA is injected into a cell whose ribosomes and other translational components are partially or fully utilized by endogenous mRNA, the relationship of the amount of mRNA injected to the amount of its product synthesized will be most strongly influenced by the following factors: (1) the amount of spare translational apparatus (including ribosomes) not fully utilized by endogenous mRNA; (2) the extent to which the same translational component can, in principle, be shared or competed for by both endogenous and injected mRNA. Among the four possible combinations of these two variables, the absence of spare translational capacity combined with the inability to share components is excluded, because this would not permit the translation of injected mRNA, as is in fact observed. The sharing of, or competition for, translational components (whether there is spare translational capacity or not), would be revealed by a decline in endogenous protein synthesis at the same time as the rise in Hb synthesis observed with increasing amounts of injected mRNA. The existence of spare translational capacity, on the other hand, would be seen as an increase in Hb synthesis with no reduction in the rate of endogenous protein synthesis. In general, the longer the period of labelling after the time of mRNA injection, the greater is the extent to which translational components released by endogenous mRNA will be made available to the injected mRNA.

The following more technical considerations also concern the interpretation of mRNA saturation experiments. It is known that almost complete recovery of radioactivity is obtained during analysis, since that discarded with the yolk pellet is only 2 to 3% of the total radioactivity of each sample. Our Sephadex fractionations always show a shoulder of radioactivity on the right-hand side of the haemoglobin peak, as is also observed in the optical density of carrier haemoglobin (discussed by Lane *et al.*, 1971). This shoulder of radioactivity is included in the values for synthesized haemoglobin, because its presence is related to the injection of 9 s RNA, and because it is always present in a similar proportion to the main peak of haemoglobin. There is some variability in the values for endogenous protein synthesis because the amount of follicular ovarian tissue and small oocytes which accompany the large oocytes and which are not injected with RNA is not constant for each sample of injected oocytes.

TABLE 1

Sephadex analysis of oocytes injected with RNA

1 Intracellular concentration injected RNA (μg/ml.)†	2 Cts/min in Sephadex peaks $\times 10^{-3}$ Front	3 Haemoglobin	4 Amino acid	5 Cts/min in Hb $\times 10^{-3}$‡	6 Endogenous cts/min as % of acid-soluble cts/min‡	7 Haemoglobin cts/min as % of acid-soluble cts/min	8 Haemoglobin cts/min as % of endogenous cts/min	9 Haemoglobin synthesis per RNA injected§
27	220·5	444·5	476·6	350	65·8	73·4	111·4	4·1
13·3	227·2	411·3	404·9	329	76·8	81·2	105·8	7·9
6·7	185·6	325·6	331·3	250	79·1	75·5	95·4	14·3
3·3	218·7	311·1	521·3	212	61·0	40·7	66·7	22·2
1·1	256·7	162·1	552·8	88	60·0	15·9	27·4	24·9
0·37	244·1	154·3	875·1	34	41·6	3·9	9·34	25·2
0·09	240·5	85·7	440·1	6	72·7	1·4	1·87	20·8
None	320·2	111·0	588·2	0	73·3	—	—	—

† Calculated from the concentration of RNA injected, the volume of injectate, and assuming recipient cell volume to be 1 μl. The effect of leakage is ignored.

‡ Calculated as explained in the text.

§ These figures are derived by dividing column 8 by column 1.

TABLE 2

Sephadex analysis of eggs injected with RNA

1 Intracellular concentration injected RNA (μg/ml.)†	2 Cts/min in Sephadex peaks $\times 10^{-3}$ Front	3 Haemoglobin	4 Amino acid	5 Cts/min in Hb $\times 10^{-3}$‡	6 Endogenous cts/min as % of acid-soluble cts/min‡	7 Haemoglobin cts/min as % of acid-soluble cts/min	8 Haemoglobin cts/min as % of endogenous cts/min.	9 Haemoglobin synthesis per RNA injected§
27	38·2	48·5	136·0	33·2	39·7	24·3	61·1	2·26
13·3	7·4	12·4	25·0	7·64	48·7	30·6	62·9	4·72
6·7	9·5	10·6	33·6	6·48	39·9	19·1	49·9	7·49
3·3	10·8	7·7	38·5	3·40	40·0	8·8	22·5	6·76
1·1	12·5	6·2	45·5	1·26	38·5	2·8	7·2	6·55
None	29·2	11·5	245·5	0	16·5	—	—	—

†‡§ See notes beneath Table 1.

The absolute values for acid-soluble and protein radioactivity are not comparable for oocytes and eggs, because the method of labelling (hence leakage of label) and the duration of labelling were different for oocytes and eggs (Gurdon *et al.*, 1971).

(c) *Evidence for the existence of unutilized translational capacity in oocytes and eggs*

The translation of injected mRNA, as is observed, could be achieved either by the release of ribosomes, etc., from endogenous mRNA in the course of protein synthesis, or by the utilization of spare components in the recipient cells. A clear demonstration of spare translational capacity in eggs or oocytes requires that the amount of injected message and the amount of haemoglobin synthesized are both increased in proportion *without reducing* the rate of endogenous protein synthesis. These conditions have been satisfied in both oocytes and eggs. Tables 1 and 2 (columns 1 and 7) show that when increasing amounts of haemoglobin mRNA are injected up to an intracellular concentration of about 10 μg/ml., the amount of haemoglobin synthesized is increased proportionately. Figure 2 shows that an almost linear relationship exists at low mRNA concentrations. Furthermore, the rate of endogenous message translation does not change when the amount of injected message is increased up to nearly 30 μg/cell. Thus, endogenous protein radioactivity remains constant, while total radioactivity increases, as the amount of injected Hb mRNA is raised (Tables 1 and 2, columns 6 and 7). These results demonstrate that both oocytes and activated eggs of *Xenopus* possess an unutilized translational capacity, which includes ribosomes as well as all other cell components needed for protein synthesis. The results also suggest that in these cells, the over-all rate of protein synthesis is limited by the amount of functional mRNA, a conclusion which is substantiated with further evidence below.

(d) *The amount of unutilized translational capacity*

When large amounts of mRNA are injected into oocytes or eggs, there is no longer a proportionate increase in the amount of Hb synthesized. This effect is clearly seen

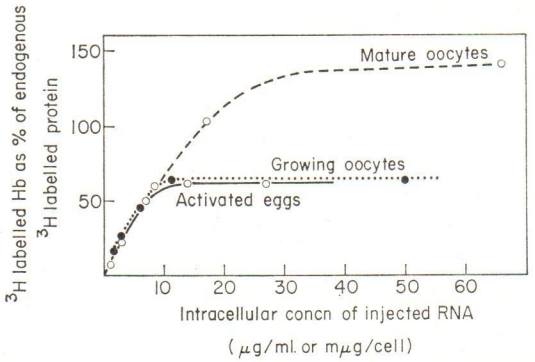

FIG. 2. 9 S mRNA saturation curves for injected oocytes and eggs which were labelled as described in the legend to Fig. 1. The values for Hb and endogenous proteins cts/min were calculated from the results of Sephadex fractionations as described in the text. Each point is based on the analysis of 20 injected cells. The intracellular concentration of RNA has been calculated assuming a 20-fold dilution of RNA due to injection, but ignoring leakage. Growing oocytes were the largest oocytes obtained from a female which had been induced to ovulate 10 days previously; mature oocytes were the largest contained in a female which had not ovulated for more than 1 year. Unfertilized eggs undergo activation as a result of injection.

in Tables 1 and 2 (columns 7 and 8) as well as in Figure 2 and may be most simply explained in one of the two following ways. The first possibility invokes a type of end-product inhibition in which all injected mRNA molecules would be translated at the normal rate until the amount of their products reaches a high enough concentration to inhibit further translation. The second possibility is that host cells possess a finite translational capacity which can provide for the translation of only a limited number of mRNA molecules; any injected mRNA molecules in excess of this number would not be translated. We consider the first of these possibilities to be largely excluded by the following arguments. If end-product inhibition exists, we would expect Hb mRNA translation to be strongly inhibited when injected with large amounts of Hb. In fact the ratio of Hb:endogenous protein synthesis is not reduced when mRNA is injected with Hb at 1 mg/ml. We should also expect Hb synthesis to have largely, or completely, ceased as soon as enough of the message product has accumulated to cause inhibition. In fact, oocytes injected with Hb mRNA at 700 μg/ml. (thereby resulting in an intracellular concentration of 35 μg/ml., which is well above the saturation level) synthesized Hb at a similar rate for the first three, and subsequent nine, hours after injection. For these reasons, we interpret the low efficiency of Hb synthesis at high message concentrations as a message-saturation effect.

Message-saturation experiments have been carried out on four different samples of cells (one for eggs and three for oocytes in various degrees of maturity). The following conclusions, most easily appreciated by reference to Figure 2, can be drawn. First, the linear portion of the curves changes in each case to an almost horizontal component after the saturation value for injected mRNA has been reached. The horizontal character of these curves shows that the translational component which limits the rate of Hb synthesis beyond the mRNA saturation level is not released during endogenous message translation in such a way that it is available to the injected mRNA, at least over labelling periods of up to nine hours at 19°C.

Another conclusion that can be drawn from message saturation curves concerns the amount of injected mRNA needed to saturate the recipient cells. This is estimated by extrapolating the inclined and horizontal components of each saturation curve. The point where the lines meet in Figure 2 corresponds to an intracellular Hb mRNA concentration of about 8 to 9 μg/ml. for the eggs and growing oocytes and of 15 to 20 μg/ml. for the mature oocytes. It would be necessary to carry out many more experiments to be sure that these values are reproducible. It is however interesting to note that, in respect of RNA synthesis, growing oocytes are very active, whereas mature oocytes and activated eggs are totally inactive. The very high capacity of mature oocytes to translate injected mRNA implies that their cells have a lower content of functional mRNA, or a much higher concentration of the component which normally limits protein synthesis, than have growing oocytes and eggs.

The third conclusion that emerges from message-saturation experiments concerns the approximate estimate that can be made of the amount of endogenous mRNA in translational use. Activated eggs and oocytes are saturated by 8 to 9 mμg of injected mRNA when the amount of Hb synthesized amounts to 60% of the amount of endogenous protein synthesis; the endogenous rate of protein synthesis is therefore 61% of that observed in eggs at the message-saturation level. If we make the assumption that endogenous mRNA is translated with the same efficiency as the injected mRNA at non-saturating conditions, and that endogenous and injected mRNA contain a

similar proportion of histidine codons, we can draw the conclusion that activated eggs and growing oocytes contain about 14 mμg of mRNA per cell in translational use. The same calculation carried out on the saturation curve for fully mature oocytes also indicates that 14 mμg of mRNA are in translational use in these cells. However, until the reproducibility of these saturation curves can be established, we prefer not to pursue the interpretation of the difference in saturation levels between growing and mature oocytes.

(e) *Exchange of translational components between endogenous and injected messenger RNA*

At the saturation concentration of injected mRNA, some translational component, such as ribosomes, limits the rate of protein synthesis. If this component can service both endogenous and injected mRNA's, and if, like ribosomes, it is released from time to time from each mRNA molecule in whose translation it participates, it should eventually come to be distributed between all mRNA molecules in the cell according to their relative abundance. The fact that a flat saturation curve for injected mRNA is obtained over nine-hour labelling periods (Fig. 2) shows that no very rapid exchange of the limiting translational component takes place between endogenous and injected mRNA during this period. This could be because the injected mRNA molecules come to occupy a different part of the cell from that already occupied by endogenous

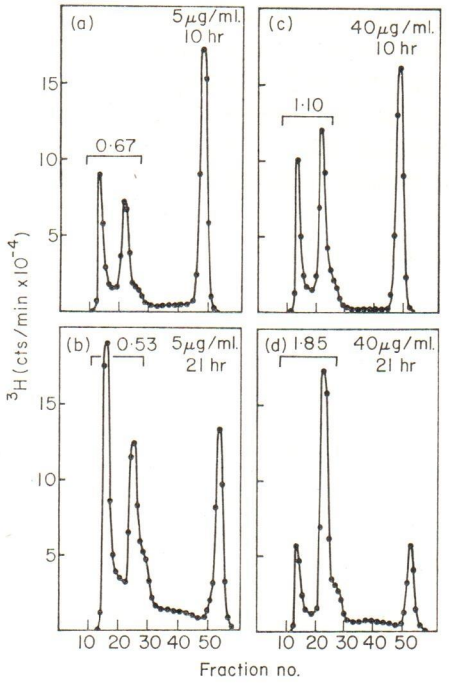

FIG. 3. Sephadex fractionations of oocytes incubated for the times shown and injected with 9 s mRNA so as to yield the stated intracellular concentrations which were calculated as stated in the legends to Figs 1 and 2. Oocytes were labelled by addition of [^{3}H]histidine to their culture medium. Each sample consisted of 20 oocytes taken from a recently ovulated female (see Fig. 2). The bracketed value above the first two peaks in each Fig. represents the ratio of Hb cts/min to endogenous protein cts/min; this was calculated as described in the text.

mRNA, so that ribosomes, etc. are unable, or unlikely, to pass from one kind of mRNA to another. An alternative explanation, that the limiting component *can never* be shared between two different messages (i.e. is mRNA-specific), could be disproved if it could be shown that under some conditions the limiting component is shared between (i.e. competed for by) endogenous and injected mRNA's.

Evidence for such an effect is provided by labelling injected cells for long periods of time or by using concentrations of mRNA which greatly exceed the saturation value. Under these conditions, the injected mRNA competes with endogenous mRNA and reduces the rate of its translation. Figure 3(a) and (b) shows that the ratio of Hb to endogenous protein synthesis is not significantly changed over long labelling periods when the amount of mRNA injected is much less than the saturating value, as has previously been found (Gurdon *et al.*, 1971). In contrast, the injection of mRNA at well above the saturation level results in a substantial increase in the ratio of Hb to endogenous protein synthesis over long incubation periods (Fig. 3(c) and (d)). Appropriate calculations reveal that endogenous protein synthesis is decreased, and Hb synthesis increased, by about 30% between 10 and 21 hours after injection. To draw the conclusion from these results that the injected Hb mRNA is competing with endogenous mRNA for translational components, it is necessary to show that the actual rate of endogenous protein synthesis is decreased. Calculations, based on the amount of free [^{3}H]histidine in the experiments shown in Figure 3(a) to (d) indicate that this is in fact the case. We know that protein synthesis does not normally decrease after injection, because pulse-labelling of oocytes with Hb mRNA at a *sub-saturation* concentration has failed to show any decrease in the rate of Hb or endogenous protein synthesis up to 21 hours after injection (Gurdon *et al.*, 1971). Further evidence for translational competition between Hb and endogenous mRNA's is provided when cells injected with mRNA at, and greatly above, the saturation level are compared after the same labelling periods (Fig. 4). In this case the very large amount of mRNA has reduced endogenous message translation, and increased Hb message translation,

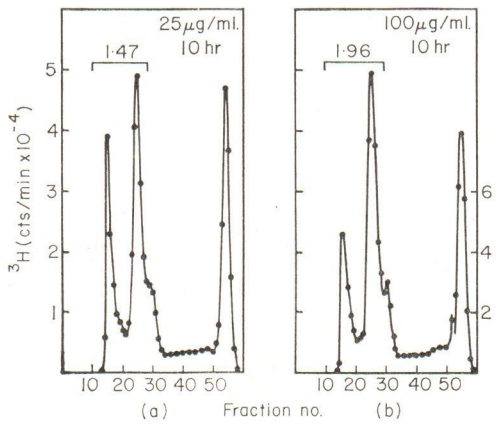

FIG. 4. Sephadex fractionations of 2 samples of 20 oocytes incubated for 10 hr, and labelled by addition of [^{3}H]histidine to the culture medium. Sample (a) was injected with 9 s mRNA so as to yield an intracellular concentration of about the saturation value for these oocytes (see "mature oocytes" in Fig. 2), and sample (b) with RNA at about 4 times the saturation value. The bracketed values above the first 2 Sephadex peaks represent the ratio of Hb cts/min to endogenous protein cts/min.

each by 10% compared to that expected at the normal message-saturation level (compare Fig. 4(a) and (b)).

4. Discussion

The experiments described here have clearly established that the injection of large amounts of mRNA saturates the translational capacity of living eggs and oocytes; a situation is thereby created in which the over-all rate of protein synthesis is limited by some cell component other than mRNA. At lower concentrations of injected RNA, the linear relationship between the amount of RNA injected and the amount of haemoglobin synthesized shows that,

(a) the quantity of RNA which is injected and which fails to leak out,

(b) the specific activity of the histidine pool, and

(c) the quantitation of the amount of haemoglobin synthesized,

can all be accurately controlled and estimated. An important point which emerges from these experiments is that enough purified RNA can be injected to reach and exceed the message saturation level without altering the normal rate of protein synthesis of recipient cells. Up to 50 mμg of Hb mRNA can be injected into each egg or oocyte without having any toxic effect.

The fact that very high concentrations of injected Hb mRNA compete with endogenous mRNA shows that both kinds of message can utilize the same limiting translational component. This demonstrates the lack of message-specificity of at least one component required for protein synthesis. Without the demonstration of message competition it would not have been possible to draw this conclusion, since it could have been argued that eggs and oocytes might contain some Hb message-specific components which would never normally be utilized during oogenesis and early development. The results also permit the conclusion that the over-all rate of protein synthesis in uninjected eggs and oocytes is limited by their content of functional mRNA or mRNA-specific factors. The demonstration of message competition shows that if endogenous message translation is limited by translational factors, then these cannot be the same as those which limit Hb mRNA translation.

All the conclusions stated here are based on experience with one particular type of injected mRNA. Although mRNA's for different proteins may well differ substantially in some properties, such as half-life, there is no obvious reason at present to believe that the conclusions drawn from the results of injecting Hb mRNA will not also prove to be valid for other kinds of injected mRNA.

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