Growing *Xenopus* oocytes have spare translational capacity

(translational control/mRNA injection/protein synthetic rates/mRNA stability/mRNA availability)

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Communicated by Joseph G. Gall, June 3, 1985

ABSTRACT Previous studies have shown that exogenous mRNAs injected into full-grown (stage 6) *Xenopus* oocytes are translated only at the expense of endogenous messages; translational capacity is limited. In this report, we demonstrate that injection of globin mRNA into small, stage 4 oocytes results in an increase in total protein synthesis without a concomitant decrease in the translation of endogenous mRNAs. The absence of competition with endogenous messages in stage 4 oocytes injected with globin mRNA, compared with stage 6 oocytes, was not due to differential turnover of the injected mRNA. Hybridization of RNA from mRNA-injected oocytes at both stages revealed that similar amounts of globin mRNA were present. These results are interpreted to mean that protein synthesis in growing oocytes is limited by the availability of mRNA and not components of the translational machinery. This conclusion, however, does not apply to all mRNA classes. The capacity of stage 4 oocytes to translate a mRNA (zein) on membrane-bound polysomes is as restricted as reported previously for stage 6 oocytes. This result suggests that putative membrane binding sites or rough endoplasmic reticulum content are limited in oocytes at both stages. The possible role of association of injected mRNA with endogenous proteins that prevent translation is discussed.

The induction of maturation in *Xenopus laevis* oocytes results in at least a 2-fold increase in the rate of protein synthesis (1), and this increase involves the recruitment of additional mRNA onto polysomes (2). The mechanism(s) that prevents translation of this maternal mRNA prior to the induction of maturation remains unknown. However, current approaches to the question have been influenced greatly by the observation, reported by Laskey *et al.* (3) and confirmed by Richter and Smith (4), that the capacity for translation in Dumont (5) stage 6 oocytes is limited by some component of the translational apparatus other than mRNA. Both groups observed that while exogenous mRNA injected into oocytes was translated, protein synthesis from endogenous mRNAs decreased, with the net result being no change in total protein synthesis.

Recently, in experiments designed to quantitate protein synthesis during oogenesis, we observed that although a significant percentage of the maternal mRNA mass was found on polysomes in stage 6 oocytes, substantially less mRNA was translated in smaller oocytes (6). In other studies (7), the observation was made that growing oocytes contained a class of oocyte-specific proteins, extractable from nontranslatable ribonucleoprotein particles, that could reversibly suppress the translation of mRNA when the message was reconstituted with them *in vitro* and injected back into oocytes. Taken together, these data suggested the possibility that the bulk of the maternal mRNA in small oocytes might be associated with proteins that restricted its availability for translation.

Stated differently, the data suggested the possibility that mRNA might be limiting for translation in growing oocytes.

In the current study, we have tested the above hypothesis directly by injecting exogenous mRNA into stage 4 oocytes in which the endogenous protein synthetic rate is about one-third that of stage 6 oocytes (6). We observed not only that the injected mRNA was translated but also that protein synthesis in such oocytes could be increased to the level usually seen at stage 6. These results suggest that growing oocytes do contain spare translational capacity.

MATERIALS AND METHODS

Animals and Collection of Oocytes. Large adult *X. laevis* females were purchased from Snake Farm (Fish Hoek, Cape Province, South Africa), Kelly Evans (Ann Arbor, MI, 716 Northside), or Nasco (Fort Atkinson, WI). The animals were maintained as described by Webb *et al.* (8). Ovaries were surgically removed from hypothermically anesthetized females and early stage 4 (diameter 0.650 mm) or stage 6 oocytes (diameter 1.20 mm) were manually defolliculated with watchmaker's forceps and cultured in OR-2 medium (9).

Isolation of Heterologous mRNAs. Globin mRNA (containing approximately equal amounts of α - and β -globin message) was prepared (10) from rabbits made anemic by subcutaneous injections of phenylhydrazine (11). For zein mRNA, polysomes bound to endoplasmic reticulum were prepared from maize and the message was isolated as described by Larkins and Hurkman (12). The purity of these RNAs was verified by *in vitro* translation of the messages in a wheat germ lysate and NaDodSO₄/PAGE of the products. All RNAs were suspended in sterile water at a concentration of 0.45–2 mg/ml.

Injection of Oocytes. Oocytes were injected with 7.5–30 nl of the RNA suspension and were incubated for 10 hr before injection of [³H]leucine (15 nl, 90,000–160,000 cpm, 55 Ci/mmol; Schwarz/Mann; 1 Ci = 37 GBq). The leucine content of globin is about the same as that estimated for endogenous proteins, whereas that of zein is no more than twice that of endogenous proteins (4, 13). After a 1-hr culture period, during which incorporation of [³H]leucine is linear (1), oocytes, in groups of three to five, were homogenized in 50 mM NaCl/0.5 mM phenylmethylsulfonyl fluoride and centrifuged (3000 rpm, 10 min, Sorvall HB-4 rotor) to remove yolk platelets. Recovery of total incorporated radioactivity for endogenous and all heterologous proteins through the extraction procedure was $\approx 75\%$. The supernatant protein was precipitated overnight at -20°C after addition of 10 volumes of absolute ethanol. The protein was collected by centrifugation, suspended in NaDodSO₄/PAGE sample buffer (14), and applied to 10.0% or 12.5% polyacrylamide gels for electrophoresis. The gels were then treated with EN-³HANCE (NEN) and exposed to preflashed x-ray film (XR-5, Kodak). Control oocytes were injected with 30 nl of distilled water.

Separation and Quantitation of Endogenous and Heterologous Protein. Globin and zein proteins were identified as distinct bands after NaDodSO₄/PAGE in 10% or 12.5% gels.

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The radioactivity in proteins separated by electrophoresis was determined by cutting out portions of the gel that corresponded in relative mobilities to the message-specific products (globin and zein); the remainder of the gel was assumed to contain translation products of endogenous mRNA. The samples were then oxidized in a Packard Tri-Carb sample oxidizer. Recovery of radioactivity after combustion was >80%.

Measurement of Protein Synthesis. At various times after injection of [3 H]leucine, groups of oocytes were processed as described by Wasserman *et al.* (1). Rates of protein synthesis were calculated from the initial slope of incorporated radioactivity, the expanded pool size after microinjection (6), and the leucine composition of the protein.

RNA Isolation and Blot-Hybridization Analysis. RNA was extracted from oocytes of known stage according to a modification of the phenol/cresol procedure (15). In control experiments, 14 C-labeled RNA mixed with oocytes was recovered with 80% efficiency after extraction from both stage 4 and stage 6 oocytes. For blot analysis, RNA was electrophoresed in 1.4% agarose in the presence of methyl-mercuric hydroxide (16), transferred to nitrocellulose, and hybridized to β -globin cDNA (17) that had been 32 P-labeled by nick-translation. Densitometric scanning of autoradiographs was done with a Quick Scan integrating densitometer (Helena Laboratories, Beaumont, TX).

RESULTS

Previous studies (4) showed that when stage 6 oocytes were injected with globin mRNA, incorporation of [3 H]leucine into globin increased with increasing amounts of injected mRNA. However, incorporation into endogenous proteins decreased as globin synthesis increased. The experiment shown in Fig. 1 *Left* confirms this observation qualitatively. Measurements of the radioactivity incorporated into globin and endogenous proteins showed that, at 60 ng of injected mRNA, endogenous protein synthesis decreased by 31%, which agrees well with the findings of Richter and Smith (4) and Laskey *et al.* (3).

In contrast to the above data, there was no obvious decrease in endogenous protein synthesis in small (650- μ m diameter) stage 4 oocytes injected with globin mRNA, even though these oocytes also synthesized globin (Fig. 1 *Right*).

FIG. 1. Example of synthesis of proteins after globin mRNA injection into oocytes. Oocytes were injected with globin mRNA, incubated in culture medium for 10 hr, and then injected with [3 H]leucine and incubated for an additional hour. Fluorograms show proteins, including α - and β -globin, synthesized after globin mRNA injection into stage 6 (*Left*) and stage 4 (*Right*) oocytes. Numbers above each lane indicate the amount (ng) of mRNA injected.

This was shown more quantitatively by measuring directly the radioactivity incorporated into the globin bands as well as into the endogenous proteins. Incorporation into endogenous proteins remained essentially constant at all mRNA concentrations injected, but incorporation into globin, even at the lowest mRNA concentration, was at least equal to that in endogenous proteins (data not shown). This result suggested that injection of exogenous mRNA could result in >2-fold stimulation in total protein synthesis. This is demonstrated more directly by the data in Fig. 2, obtained from stage 4 oocytes that were injected with various amounts of globin mRNA. The incorporation of [3 H]leucine into total proteins was monitored. Total incorporation approached a plateau about 200% above control values in oocytes injected with 20 ng or more of globin message.

The absolute rate of protein synthesis in stage 4 oocytes was measured after injection of "saturating" amounts of globin mRNA and compared with the rate measured in stage 6 oocytes not injected with message. For further comparison, a similar experiment was conducted on stage 3 oocytes (500- μ m diameter). These results are summarized in Table 1. Consistent with the data discussed above, this experiment shows that injection of the globin mRNA into stage 4 oocytes stimulated total protein synthesis 2.6-fold. Perhaps more important, the increased protein-synthetic rate essentially equaled that seen in stage 6 oocytes not injected with exogenous mRNA. In the latter case, total rates of protein synthesis are not appreciably different with or without injected mRNA, since stage 6 oocytes contain no spare translational capacity. Thus, these results show that stage 4 oocytes not only contain spare translational capacity, but that the total capacity is approximately equal to that seen in stage 6 oocytes.

The data in Table 1 show that stage 3 oocytes also contain spare translational capacity, although that capacity is not as great as seen at stage 4. Thus, total protein synthesis in stage 3 oocytes was increased almost 4-fold by the injection of the same amount of globin message as was injected into stage 4 oocytes. In this case, however, the rate of protein synthesis was only about half that seen in stage 6 oocytes. We have not performed experiments to determine whether injection of additional message into stage 3 oocytes would elevate the total rate of protein synthesis further.

In the study by Richter and Smith (4), stage 6 oocytes also were injected with zein mRNA, which is translated on rough endoplasmic reticulum. In that case, unlike the situation with globin mRNA, injection of increasing amounts of message did not result in increased synthesis of zein; rough endoplasmic reticulum was thought to be limiting (4). The data in Fig. 3 (see also Table 1) show that stage 4 oocytes injected with zein

FIG. 2. Effect of injecting various amounts of globin mRNA on total protein synthesis in stage 4 oocytes. The percent increase in incorporation of [3 H]leucine into total protein is expressed relative to the incorporation in control (no mRNA injected) oocytes.

Table 1. Protein synthesis in growing oocytes after mRNA injection

Oocyte stage	Injected RNA*	Total protein synthesis, ng/hr
6	None	22.0
6	Globin mRNA (20 ng)	20.9 [†]
6	Zein mRNA (20 ng)	17.5 [‡]
4	None	7.8
4	Globin mRNA (37 ng)	20.6
4	Zein mRNA (30 ng)	9.2
3	None	2.9
3	Globin mRNA (37 ng)	11.3

Protein-synthetic rates were determined according to Wasserman *et al.* (1) $\approx$ 10 hr after injection of material.

*Per oocyte, in 20 nl of distilled water.

[†]Data taken from ref. 18.

[‡]Data taken from ref. 2.

mRNA behave in a manner similar to that observed for stage 6 oocytes. Thus, the capacity of stage 4 oocytes to translate zein mRNA appears to be greatly restricted (about one-third that of stage 6 oocytes), and the injected message results in little increase in total protein synthesis. Presumably, stage 4 oocytes possess a limited number of putative membrane binding sites and these sites or the content of rough endoplasmic reticulum, continue to increase during oogenesis.

The limited capacity of stage 4 (and stage 6) oocytes to translate membrane-bound messages, coupled with the fact that such messages probably are a minority of total mRNAs translated, explains both the absence of a substantial increase in total protein synthesis and the apparent absence of competition with endogenous mRNAs after injection of mRNA into oocytes. In contrast, the data obtained by injecting globin mRNA show clearly that both stage 3 and stage 4 oocytes contain spare translational capacity for messages translated on free polysomes. Nevertheless, it also is clear that the capacity to translate globin message in stage 4 oocytes appears to be saturated at the lower amounts of message injected. One explanation for this apparent paradox is based on the report (4) that a large fraction of mRNA underwent degradation after injection into stage 6 oocytes, and that the fraction increased as the amount of message injected increased. Perhaps, degradation is even more ex-

FIG. 3. Fluorogram of proteins synthesized after zein mRNA injection into stage 4 oocytes. Numbers above each lane indicate the amount (ng) of mRNA injected per oocyte.

FIG. 4. The amount of globin mRNA stabilized by stage 4 and 6 oocytes as determined by RNA blot hybridization. Oocytes were injected with globin mRNA (20 ng each) and then were cultured for 10 hr; RNA was extracted and then electrophoresed in agarose gels, blotted onto nitrocellulose paper, and hybridized to ³²P-labeled β -globin cDNA. Lanes: 1, 4.5 ng of β -globin mRNA standard; 2 and 3, RNA from four stage 6 oocytes and from four stage 4 oocytes, respectively.

tensive in stage 4 oocytes, so that only a small (noncompeting) amount can ultimately be protected.

To test the above possibility, stage 4 and stage 6 oocytes were injected with globin mRNA (20 ng per oocyte) and, after 10 hr incubation, the RNA was extracted, electrophoresed in agarose gels, and transferred to nitrocellulose paper. The blots were probed with radioactive cDNA to the β -globin message. An autoradiograph of such an RNA blot is shown in Fig. 4. In three separate experiments of this type, we failed to detect any case in which the stability of globin mRNA was lower in stage 4 than in stage 6 oocytes; mRNA stability always was slightly greater in stage 4. Estimates of the actual quantity of stable message were made from densitometric scans of the autoradiographs, using known amounts of globin mRNA run on the same gels as standards. The percentages of globin mRNA that remained stable in stage 4 relative to stage 6 oocytes were 12.8%:5.8%, 3.2%:2.5%, and 98%:75%, respectively, for the three experiments. Coincidentally, these experiments resolve another paradox present in the literature: Drummond *et al.* (19) reported that the majority of globin mRNA injected into stage 6 oocytes is stable, in contrast to the study of Richter and Smith (4). Our current data show considerable variability among oocytes from different females, so that almost any percentage could be obtained, depending on the female donor used.

DISCUSSION

The induction of oocyte maturation in stage 6 oocytes by either steroid hormones or maturation-promoting factor results in a doubling of the protein-synthetic rate (1, 20). This increase requires the recruitment of mRNA onto polysomes (2), but message availability is not the only limitation to protein synthesis. That injected messages usually compete with endogenous mRNA for translation demonstrates that the stage 6 oocyte has a limited translational capacity (3, 4). The nature of the limiting component is not known. However, Richter *et al.* (21) have shown that injection of adenovirus type 5 mRNA into the oocyte results in a stimulation of endogenous protein synthesis, probably via a protein(s) translated from one or more of the adenovirus transcripts. The only other example of a nonhormonal stimulation of protein synthesis in *Xenopus* oocytes is the report of Wasserman *et al.* (22) that either injection of maturation-promoting factor or exposure to the weak base procaine results in a

2- to 3-fold increase in protein synthesis in large (800- μ m diameter) stage 4 oocytes.

The present data show that injection of globin mRNA into both stage 3 oocytes and small (650- μ m diameter) stage 4 oocytes results in an increase in protein synthetic rate. Hence, we conclude that oocytes at these stages of oogenesis have spare translational capacity; the availability of mRNA is limiting for protein synthesis. This is not to say that other components of the translational machinery are present in vast excess. Indeed, although the rate of protein synthesis in stage 3 oocytes is stimulated almost 4-fold by injection of globin mRNA, this elevated rate is only about half that seen in stage 6 oocytes (Table 1; ref. 6). On the other hand, the rate of protein synthesis in small, stage 4 oocytes can be stimulated <3-fold by exogenous mRNA, but this level approximates that seen in stage 6 oocytes (Table 1; ref. 6). These results suggest that the translational apparatus reaches its maximal capacity soon after oocytes progress to stage 4 and that the increased protein synthetic rate between stage 4 and stage 6 results solely from increased message availability.

Taylor and Smith (6) have measured directly the mRNA content of polysomes of stage 3 and stage 6 oocytes. Those values agree well with numbers calculated from the various translational rate parameters. On this basis, we estimate that stage 4 oocytes contain about 1.5 ng of mRNA in polysomes, whereas stage 6 oocytes contain as much as 4 ng of mRNA in polysomes. This means that the translational machinery of stage 4 oocytes should be able to handle about 4 ng of mRNA before competition for limiting components between endogenous and exogenous mRNAs becomes evident. Although much of the mRNA injected into oocytes is degraded, it is clear from our data that even at 20 ng of injected mRNA, sufficient message would have been stabilized to at least approach the limit of translational capacity. Yet competition was not observed when as much as 60 ng of globin mRNA was injected. Perhaps more significantly, synthesis of globin did not increase appreciably (if at all) with increasing amounts of injected message beyond the 20-ng level.

One explanation for the above discrepancy is that as the translational machinery becomes saturated with injected mRNA, additional messages would associate with endogenous proteins that prevent translation. To be consistent with other data obtained with stage 6 oocytes, we need only stipulate further that the putative endogenous proteins are present in greater amount at stage 4 than at stage 6. While this suggestion is speculative at this time, it is interesting that the oocyte-specific proteins associated with nontranslating endogenous poly(A)⁺ RNA from *Xenopus* oocytes (23–25)

are present in largest amount in small and mid-size (stages 3–4) oocytes but have decreased substantially by the time oocytes approach completion of their growth (stages 5–6).

We thank Dr. B. Larkins for the zein mRNA and Dr. J. Dixon for the globin mRNA and plasmid DNA. This work was supported by a grant from the National Institutes of Health (HD04229) awarded to L.D.S.

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