Translational capacity of sheep oocytes microinjected with messenger RNA

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Summary. Sheep oocytes were microinjected with tobacco mosaic virus RNA (TMV-RNA) and isotopically labelled with \( \ell \)-\( [\text{\textsuperscript{35}}\text{S}] \)methionine. Total incorporation of labelled methionine was similar in TMV-RNA-injected and in carrier-injected control oocytes, whether injections were performed during the period of high protein synthesis at maturation or during the period of reduced synthesis at a time equivalent to the mid-cleavage transition (48 h after germinal vesicle breakdown). Varying the amount of TMV-RNA injected from 2.5 to 10 pg had little effect on the overall level of amino acid incorporation. Furthermore TMV-RNA appeared to be very stable in oocytes and eggs; the proportion of total polypeptide synthesis directed by TMV-RNA did not diminish during the first 48 h after injection. Synthesis of most endogenous proteins was uniformly reduced to compensate for the synthesis of TMV-polypeptides. Our results suggest, therefore, that the translational capacity of sheep oocytes is fully saturated during maturation.

Keywords: oocyte; mRNA translation; mRNA stability

Introduction

Post-transcriptional control mechanisms regulate protein synthesis in oocytes at fertilization and in embryos during the first cleavage divisions (Davidson, 1976; Johnson, 1981). Subsequently, maternal messenger RNA (mRNA) translation declines and a major wave of transcriptional activity signals the onset of embryonic genetic activity. The means by which these post-transcriptional controls are imposed differ at the various stages of early embryonic development in mammals. In oocytes, re-programming of protein synthesis involves the translation of stored mRNA and the post-translational modification of newly synthesized proteins (reviewed by Wasserman et al., 1981). The mechanisms involved in the translation of stored mRNA raise a number of interesting questions including that of the inherent capacity of the oocyte to translate new mRNA as it becomes available during maturation.

Microinjection of mRNA into amphibian oocytes has provided important information on the translational capacity of these cells (Lane & Knowland, 1975; Laskey et al., 1977; Richter & Smith, 1981, 1984). In mammals it has been shown that mouse eggs will translate microinjected mRNA and that the protein product can be exported (Brinster et al., 1980, 1981). More recently Ebert & Brinster (1983) reported that the injection of a globin mRNA neither altered total protein synthesis nor affected the translation of endogenous mRNA in fertilized mouse eggs.

In the present experiments we report on the translational capacity of sheep oocytes during the phase of maturation that immediately precedes the translation of stored maternal mRNA.

Materials and Methods

Preparation and injection of oocytes. Oocytes were obtained from non-atretic follicles dissected from the ovaries of FSH-treated sheep. Before microinjection all but the last 2 or 3 layers of associated follicle cells were removed from
each oocyte using a series of accurately graded micropipettes. The medium in which the oocytes were held during microinjection consisted of phosphate-buffered saline (PBS: Paul, 1970) supplemented with BSA (4 mg/ml), cytochalasin-D (5 µg/ml) sodium pyruvate (0-4 mm) and kanamycin sulphate (50 µg/ml). After injection all oocytes were washed repeatedly (5 times) in the PBS medium devoid of cytochalasin D before culture.

Micropipettes were pulled to a uniform size (P-77 Brown-Flaming microelectrode puller), bevelled, oil-filled and autoclaved before use to eliminate RNase contamination. Injections were carried out on oocytes maintained in hanging drops in an oil-filled microinjection chamber. The volume of fluid injected was calculated from a calibration curve constructed by correlating the length of columns of fluid in different micropipettes with their volume using tracer dilution techniques. With standardized pipettes this system provides a rapid and accurate means of volume measurement during injection since the length of the column of medium in the pipette can be continuously monitored using a calibrated eye-piece. Moreover, by expelling a known length of fluid under oil and measuring the diameter of the resultant droplet, adjustments can be made for each pipette before use. The volumes of fluid injected into the cytoplasm of oocytes in this study ranged from 2.5–10 pl.

In preliminary experiments mRNA's of mammalian (globin), plant (α amylase) and viral (Avian influenza A and Tobacco Mosaic Virus) origin were tested by microinjection into oocytes. Although each mRNA species was translated by the oocytes, TMV-RNA was selected primarily because it coded for a major polypeptide which was readily distinguished from all endogenous oocyte polypeptides. Moreover, the TMV-RNA used in our experiments (generously provided by Dr T. Hunt, University of Cambridge) was of particularly high quality and integrity when tested by in-vitro translation using the reticulocyte lysate system. The TMV-RNA was stored in sealed glass capillaries under liquid nitrogen.

Isotopic labelling, fractionation and quantitation of proteins. Oocytes were divided into two groups after injection. One group was cultured for 36 h (Staigmiller & Moor, 1984) before being labelled. These oocytes were at metaphase II when L-[35S]methionine was added. The second group of oocytes was placed in label directly after microinjection and before germinal vesicle breakdown had occurred. Both groups were labelled for 12 h in incubation medium containing 500 µCi L-[35S]methionine/ml (sp. act. > 1000 Ci/mmol: Radiochemical Centre, Amersham, Bucks, UK).

After incubation, single oocytes were totally denuded of follicle cells, washed extensively and collected in 2 µl ice-cold 10 mm-Tris-HCl (pH 7-4), lyophilized and frozen at −70°C until required for electrophoresis. Lyophilized samples were resuspended in 30 µl of sample buffer (O'Farrell, 1975), heated to 100°C and 3 µl samples were used to measure L-[35S]methionine incorporation into TCA-precipitable protein. Parallel incorporation measurements showed that a negligible proportion of the incorporated radioactivity was soluble in TCA at 90°C but insoluble at 0°C.

Labelled proteins in individual samples were resolved by applying equal numbers of TCA-precipitable counts to an 8–15% linear gradient SDS polyacrylamide gel using the discontinuous SDS-glycine-Tris buffer system of Laemmli (1970). Fluorography was carried out using the technique of Bonner & Laskey (1974). After drying, gels

![Fig. 1. Incorporation of L-[35S]methionine into sheep oocytes injected with TMV-RNA (1 mg/ml) at the beginning of maturation. (a) Total incorporation (±s.e.m.) in individual oocytes labelled, after injection, for 12 h in L-[35S]methionine. Each separate experiment has three groups of 10–20 oocytes which represent an un.injected control group, carrier-injected control group, a TMV-RNA injected group (10 pg TMV-RNA/oocyte). (b) The effect of varying the volume of TMV-RNA (1 mg/ml) on the incorporation of methionine during the first 12 h after injection. To illustrate the trend towards a reduction in incorporation the results in TMV-RNA injected oocytes are expressed as a percentage of total incorporation in controls injected with an equal volume of carrier alone.](image-url)
Fluorogram of \(^{35}\)S-labelled polypeptides from individual carrier-injected (tracks 1 and 2) and TMV-RNA-injected oocytes (tracks 3–7). Oocytes were injected with 10 pl carrier or TMV-RNA (1 mg/ml). They were then labelled for 12 h in the presence of 500 \(\mu\)Ci/ml \(\text{L-}[^{35}\text{S}]\text{methionine, and run on 8–15\% SDS-gradient gels as described in the ‘Methods’} \). Equal numbers of TCA-precipitable counts were loaded onto each track of the gel. The oocyte in track 5 failed to translate the injected TMV-RNA; such oocytes have been excluded from further analysis. The major TMV polypeptide \((M_r = 110\,000)\) is indicated by an arrow head.

Results

Effect of exogenous mRNA on amino acid incorporation

Laskey et al. (1977) demonstrated that globin mRNA injected into *Xenopus* oocytes slightly reduces overall incorporation of \(\text{L-}[^{35}\text{S}]\text{methionine into total TCA precipitable protein. The results summarized in Fig. 1 show that a similar effect on methionine incorporation was obtained when TMV-RNA was injected into mammalian oocytes. Injection of a constant amount of mRNA reduced incorporation in one experiment \((P < 0.05)\) but was without significant effect in the remaining 4 experiments (Fig. 1a).

The amount of RNA injected was varied over a 5-fold range; the incorporation in RNA-injected and buffer-injected control oocytes did not differ significantly (Fig. 1b). However, the
Fig. 3. Analysis of the effect of injecting TMV-RNA (10 pg/oocyte) on the electrophoretic profile of labelled proteins in individually injected and control sheep oocytes. The relative amount of protein in each band is expressed as a percentage of total amount of labelled protein loaded onto each track (see Fig. 2 for experimental details). The histogram has been constructed from an analysis of 20 oocytes in each of the TMV-RNA- and carrier-injected groups.

Fig. 4. The comparative stability of TMV-RNA measured by its capacity to code for the major viral polypeptide (Mr, 110 000) during the first 12 h or between 36 and 48 h after injection into sheep oocytes. Oocytes were injected with TMV-RNA (10 pg) or carrier and labelled directly in L-[35S]methionine (12 h group) or cultured for 36 h before isotopic labelling for an equivalent period of time (48 h group).

reported trend (Laskey et al., 1977) towards a lower rate of amino acid incorporation in mRNA-injected *Xenopus* oocytes was also apparent in mammalian oocytes within 12 h of injection.

**Synthesis of protein in mRNA-injected oocytes**

The profile of labelled proteins in oocytes injected with carrier or TMV-RNA is shown in Fig. 2. The effect of exogenous mRNA on the synthesis of endogenous proteins has been analysed by densitometry and these quantitative results are presented in Fig. 3. Synthesis of the dominant
encoded protein of \( M_r \) 110 000 was accompanied by a significant decline in the synthesis of most classes of endogenous proteins. However, synthesis of a small number of endogenous proteins remained unaffected by exogenous mRNA translation. Several possible explanations for these unaltered rates of synthesis are discussed below.

To examine the possibility that exogenous mRNA competes with endogenous mRNA only above a certain threshold level, different amounts of TMV-RNA were injected into oocytes (2.5–10 pg mRNA per oocyte). The results, which were similar to those in Fig. 2, indicated that, at each of these concentrations of mRNA, synthesis of TMV proteins was accompanied by a decline in the synthesis of endogenous proteins.

**Stability of TMV RNA in oocytes**

Brinster *et al.* (1980) reported that the half-life of \( \alpha \)-globin mRNA in mouse eggs is short (about 9 h) and hence differed greatly from that in *Xenopus* oocytes (>2 weeks; Gurdon *et al.*, 1973). In the current series of sheep oocyte injection experiments, we found that stability of TMV-RNA was much greater than that found for globin mRNA in mouse eggs. Figure 4(a) shows that the total amount of labelled methionine incorporated into control and TMV-RNA-injected eggs was high during maturation but by 48 h started to decline in a manner similar to that described by Crosby *et al.* (1988) for cleaving embryos. However, the results presented in Fig. 4(b) show that the proportion of total protein synthesis represented by the TMV polypeptide of \( M_r \) 110 000 was very similar, irrespective of whether oocytes were examined 12 h or 48 h after injection with TMV-RNA. It is therefore clear that TMV-RNA is relatively stable in sheep oocytes and unfertilized eggs.

**Discussion**

It has been firmly established that amphibian oocytes and eggs can translate a wide variety of mammalian and viral mRNAs (Lane, 1981). It is now clear that mammalian oocytes, eggs and embryos can be similarly undiscriminating in their ability to translate mRNA without regard to species, phylum or even kingdom of the donor organism. Brinster *et al.* (1980, 1981) provided evidence to show that fertilized mouse eggs effectively translate mRNA from a variety of mammalian species. In our current experiments we found that sheep oocytes, eggs and embryos translate both viral and plant mRNAs (\( \alpha \)-amylose: R. M. Moor, unpublished observations).

In the present experiments TMV-RNA is stable in the sheep oocyte for a period of at least 48 h, and must compete with the existing cellular mRNAs for a limited protein synthetic capacity (see below). Published work on other mammalian species has concentrated on the fertilized mouse egg (Brinster *et al.*, 1980; Chen & Brinster, 1982), which, like the sheep oocyte, appears to have little if any spare translational capacity (Ebert & Brinster, 1983). Globin mRNA is unstable in the fertilized mouse egg, displaying a half life of about 9 h and becoming undetectable at 48 h after injection (Brinster *et al.*, 1980; Chen & Brinster, 1982). This observation, however, probably results from the switch in gene expression that occurs at the 2-cell stage in the mouse, when embryonic mRNAs are synthesized and maternal mRNAs, presumably together with injected globin mRNA, are degraded. Preliminary data indicate that TMV-RNA is stable in fertilized sheep eggs for over 50 h and probably also persists until the switch from maternal to embryonic gene expression which occurs between the 8- and 16-cell stage (Crosby *et al.*, 1988).

Our results do, however, bear comparison with those obtained in the *Xenopus* oocyte. First, we find that the injected mRNA is very stable, insofar as the rate of translation of the injected mRNA changes only slightly during a 48 h culture period. Furthermore, mRNA injected into sheep oocytes or into amphibian oocytes caused a decline in endogenous protein synthesis. It therefore appears that in both systems injected mRNA is translated only because it competes directly with endogenous mRNA. In *Xenopus*, it has been established that this competition is for a limiting component which is not associated with peptide chain initiation but rather is a component of
polysomes (Lingrel & Woodland, 1974; Laskey et al., 1977). Our findings are fully consistent with the concept that the sheep oocyte translation capacity is normally fully saturated.

Our results also clearly indicate that the translation of the majority of endogenous mRNA species is uniformly reduced by the oocyte to enable it to translate the newly injected TMV-RNA. During maturation, when the oocyte is similarly exposed to a large quantity of new, endogenous mRNA, about 30% of the total pool of stored maternal mRNA is released during a short period of early metaphase (M-phase) of the cell cycle and becomes available for translation (Bacharova et al., 1985). As with the injected oocyte, there is little change in the overall rate of polypeptide synthesis. However, a clear difference between the two systems is in the selectivity displayed by the maturing oocyte. While the TMV-injected oocytes respond by uniformly reducing translation of most RNAs, in the maturing oocyte translation of some mRNA species ceases almost entirely while translation of others remains unaffected by the newly liberated maternal mRNA (Wasserman et al., 1981; Moor & Crosby, 1986). One of the clearest examples of this selectivity is the cessation of actin synthesis during reprogramming events in the M-phase oocyte (see Moor & Osborn, 1983). Moreover, experiments on the mechanism of selectivity within the oocyte indicate that the surrounding follicle cells play a directive role in the translation of actin mRNA. This characteristic selectivity applies also to amphibians in which oocytes display a more defined specificity for the translation of endogenous as compared to exogenous mRNA.

How might such selectivity be effected? The failure of TMV-RNA to compete with a small number of endogenous mRNAs may be caused by the high efficiency of translation of these mRNAs as a result of their affinity for some component of the translation apparatus (for a discussion see Lodish, 1976). Although the availability of ribosomes is reduced such mRNAs would still compete well for the limited resources (Laskey et al., 1977). An alternative explanation is that the TMV-resistant RNAs are in a privileged environment: membrane-bound and free polysomes form separate compartments and there is no competition between mRNAs in each compartment (Richter & Smith, 1981). We have not yet performed the competition experiments required to distinguish between these explanations, but such studies are now possible.

Developmental changes in mRNA translatability are well established in invertebrate (Powell et al., 1984; Ruzdijic & Pedersen, 1987; Rosenthal & Ruderman, 1987) and vertebrate (Gurdon et al., 1973; Woodland & Witt, 1980; Kidder & Pedersen, 1982; Galili et al., 1988) systems. It appears that long poly(A) tails are required to stimulate both translation and stability of mRNA during early development of the surf clam, Spisula (Rosenthal & Ruderman, 1987). Galili et al. (1988) reached a similar conclusion by microinjecting mRNA into Xenopus oocytes. Moreover, sequences within the body of the mRNA, such as the AU-rich sequence found in many short-lived mRNAs, may be partly responsible for defining turnover rates (Merill et al., 1984; Shaw & Kamen, 1986; Graves et al., 1987). It would be of interest to determine whether the instability detected in the cultured cells in these experiments by the addition of AU-rich sequences operates in the oocyte or whether the special nature of translational control in the oocyte involves other mechanisms.

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