CAP ANALOGUES DO NOT INHIBIT mRNA TRANSLATION IN *XENOPUS LAEVIS* OOCYTES

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1. Introduction

$m^7G5'ppp5'N$, the cap of eukaryotic mRNA, is thought to serve a dual function. It protects the mRNA against 5'-exonucleolytic degradation [1,2] and it plays a role during the initiation of protein synthesis (reviewed [3]). Protection against nucleases has been demonstrated using microinjection into *Xenopus laevis* oocytes [1], which most closely resembles in vivo mRNA translation. On the other hand, requirement of the cap for initiation of protein synthesis has only been demonstrated in vitro [3]. However, in some cell-free systems the requirement of the cap is not absolute [4–7] and it has, therefore, been suggested that the cap only facilitates initiation of protein synthesis.

In this study an attempt was made to block initiation of protein synthesis in *Xenopus* oocytes by microinjection of cap analogues into the cell. Cap analogues can specifically inhibit translation of cap bearing mRNAs in a wide variety of cell-free systems [8–13], provided that the experiments are performed at physiological concentrations of K^+ [14,15]. However, in oocytes endogenous protein synthesis and translation of exogenous mRNA were unaffected by the presence of cap analogues.

2. Materials and methods

2.1. *Materials*

Cap analogues, $m^7G5'p$ and $m^7G5'ppp5'G$, were products from P-L Biochemicals, Milwaukee. Molarity of $m^7G5'ppp5'G$ was calculated from the absorbance assuming $\epsilon_{260} = 20 \times 10^3$ [6]. [^{35}S]Methionine (380 Ci/mmol) was purchased from the Radiochemical Centre, Amersham.

2.2. *Translation of mRNA in* Xenopus *oocytes*

Isolation of poly(A)$^+$-mRNA from calf lenses and of rabbit globin mRNA has been described [14]. Alfalfa mosaic virus RNA-4 was a generous gift of Dr L. van Vloten-Doting (State University of Leiden). Microinjection of mRNAs into *Xenopus laevis* oocytes and subsequent quantitation of the translation products have been described [16].

3. Results

3.1. *Effect of $m^7G5'p$ on oocyte protein synthesis*

At first an attempt was made to block initiation of endogenous protein synthesis of oocytes by injecting increasing volumes of an $m^7G5'p$ solution (fig.1). However, the interpretation of this type of experiment was complicated by a stimulation of the incorporation of radioactive methionine into oocyte proteins by the mere injection of fluid (fig.1A). This phenomenon was not restricted to methionine, but was also observed when other amino acids (leucine, histidine or a mixture of 15 amino acids) were used as precursor. Apparently the effect is primarily due to an increased uptake of radioactive amino acids by the oocytes, in particular since incorporation into protein, when expressed as % radioactivity present in oocytes, is stimulated much less (fig.1B).

The injections resulted in final $m^7G5'p \leqslant 1.5$ mM inside the living oocyte. However, no significant

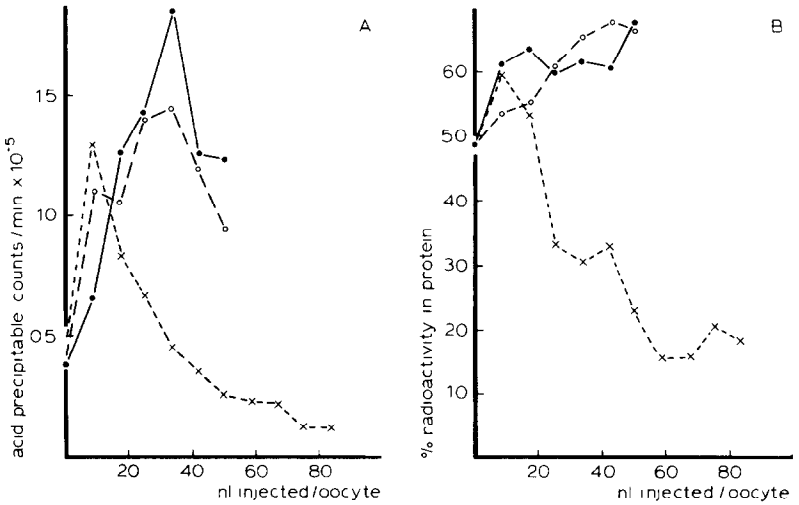

Fig.1. Influence of $m^7G5'p$ and aurintricarboxylic acid on *Xenopus* oocyte protein synthesis. (A) Trichloroacetic acid precipitable radioactivity (after alkaline stripping of tRNA) of oocytes injected with increasing volumes of water (•——•), 30 mM $m^7G5'p$ (o- - -o) and 20 mM aurintricarboxylic acid (X. . .X). (B) Acid precipitable radioactivity of oocytes expressed as % of total (i.e., acid soluble plus insoluble) radioactivity in order to correct for variations in the uptake of radioactive amino acid from the medium. Oocytes were labeled for 6 h immediately after injection.

inhibition of oocyte protein synthesis was observed, in contrast to the inhibition observed after injection of aurintricarboxylic acid (fig.1B).

3.2. *Effect of cap analogues on translation of injected mRNAs*

In a second series of experiments $m^7G5'p$ was injected together with calf lens mRNA. The synthesis of lens proteins (crystallins) can be measured very accurately by quantitative immunoprecipitation [16]. In these experiments the injection volume was kept constant. However, with 4 mM $m^7G5'p$, there was no obvious inhibition of either total protein synthesis (fig.2A,C) or the synthesis of lens crystallins (fig.2B,D). Also with the larger cap analogue $m^7G5'ppp5'G$, which inhibits protein synthesis in cell-free systems at 5–10-times lower concentrations than $m^7G5'p$ [8,9,13], no significant inhibition was

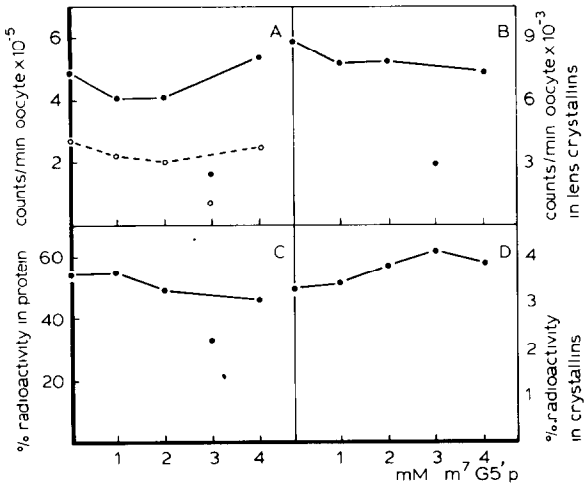

Fig.2. Influence of $m^7G5'p$ on translation of lens mRNAs in *Xenopus* oocytes. (A) Oocytes were injected with 25 nl water containing lens mRNA (0.2 mg/ml) and various amounts of $m^7G5'p$ and were labeled for 4 h immediately after injection. Intracellular concentration of the cap analogue was calculated assuming a 1 μl oocyte volume. Total (•——•) and acid-precipitable (o- - -o) radioactivity of oocytes was measured. (B) Radioactive lens crystallins were quantitated using subsequent immunoprecipitations with antisera against αA-, β- and γ-crystallins, respectively. The sum of the radioactivity in the 3 immunoprecipitates is depicted in the figure. (C) Acid precipitable radioactivity of oocytes was expressed as % total oocyte radioactivity. (D) Newly synthesized lens crystallins as % acid-precipitable radioactivity of oocytes.

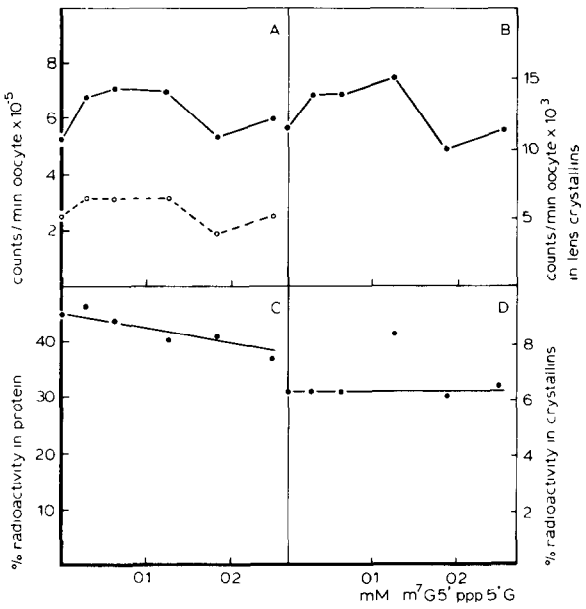

Fig.3. Influence of m⁷G5'ppp5'G on translation of lens mRNAs in *Xenopus* oocytes. See legend of fig.2. Data were obtained from a different batch of oocytes.

observed (fig.3). Inhibition of mRNA translation was also not observed, when any possible leakage of m⁷G5'p out of the oocytes was prevented by the presence of an equal concentration of cap analogue in the oocyte culture medium (table 1). Translation of mRNAs coding for rabbit globin and alfalfa mosaic virus coat protein, which is inhibited completely by such concentrations of cap analogues in cell-free systems [8–13,17], was in the oocyte unaffected by m⁷G5'p (table 1) and m⁷G5'ppp5'G (not shown).

4. Discussion

The cap analogue concentrations used in this study were sufficient to block the translation of all these mRNAs in an mRNA-dependent reticulocyte lysate completely, as shown [13]. Further, it has been demonstrated that in an untreated reticulocyte lysate (still containing the endogenous globin mRNA) α-crystallin synthesis was ~80% inhibited by 4 mM m⁷G5'p and globin synthesis ~60% inhibited [18]. In contrast, these concentrations of cap analogues

Table 1
Effect of m⁷G5'p on translation of different mRNA species

Exp.	mRNA injected/ oocyte	mM m⁷G5'p	Acid-precip. radioact.		Foreign translation product	
			cpm	% total	cpm	% acid-precip. radioact.
A	–	0.0	79 270	52.0	–	–
A	–	1.0	81 480	51.3	–	–
A	5 ng lens mRNA	0.0	97 745	52.0	6590	6.74
A	5 ng lens mRNA	1.0	68 255	50.2	4220	6.48
A	5 ng globin mRNA	0.0	87 290	55.8	2890	3.30
A	5 ng globin mRNA	1.0	71 970	53.5	2070	2.88
B	25 ng AMV RNA 4	0.0	73 655	n.d.	18 265	24.8
B	25 ng AMV RNA 4	1.0	55 815	n.d.	14 125	25.3
C	5 ng globin mRNA	0.0	148 930	n.d.	22 575	15.2
C	5 ng globin mRNA	5.0	151 598	n.d.	24 560	16.2

Water, 25 nl, or mRNA solution, sometimes also containing m⁷G5'p, was injected into oocytes, which were then labeled for 6 h with [^{35}S]methionine (exp. A,B) or [^{3}H]histidine (exp. C). Synthesis of lens crystallins was assayed as in fig.2 legend and synthesis of rabbit globin and alfalfa mosaic virus (AMV) coat protein was measured by determination of the radioactivity present in the appropriate region of an SDS–polyacrylamide gel [16]. Intracellular concentration of m⁷G5'p was calculated assuming an oocyte vol. 1 μl. In exp. A,C, m⁷G5'p was also included in the oocyte culture medium at the supposed intracellular concentration to neutralize possible diffusion of cap analogue out of the oocytes

do not affect the translation of either exogenous or endogenous mRNA inside living oocytes. Aurintricarboxylic acid, which inhibits the initiation of protein synthesis at low concentrations in vitro and, at higher concentrations, elongation also [19], strongly inhibited oocyte protein synthesis (fig.1). However, from our experiments it can not be concluded at which step oocyte protein synthesis in vivo is inhibited.

One could make a number of speculations as to why no inhibition was observed. For instance, it cannot be excluded that the cap analogues are degraded rapidly in the oocytes. Another possibility is, that even more cap analogue is needed to inhibit translation in oocytes. It should be noted that, as a rule, more cap analogue is needed to inhibit translation in a cell-free system when translation of mRNA is more efficient. Since *Xenopus* oocytes perform translation with the supreme efficiency of a living cell, the insensitivity to cap analogues might simply be a consequence of this efficiency. In the nuclease-treated reticulocyte lysate some mRNA apparently 'escapes' inhibition during prolonged incubation and reinitiation of protein synthesis is less inhibited by cap analogues [18]. Likewise mRNAs microinjected into living oocytes might have better chances of 'escaping' the initial inhibition by the cap analogues and the greater translational efficiency will then ensure a frequent reinitiation of translation.

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References

[1] Furuichi, Y., LaFiandra, A. and Shatkin, A. J. (1977) Nature 266, 235–239.
[2] Shimotohno, K., Kodama, Y., Hashimoto, J. and Miura, K. I. (1977) Proc. Natl. Acad. Sci. USA 74, 2734–2738.
[3] Shatkin, A. J. (1976) Cell 9, 645–653.
[4] Rose, J. K. and Lodish, H. F. (1976) Nature 262, 32–37.
[5] Shih, D. S., Dasgupta, R. and Kaesberg, P. (1976) J. Virol. 19, 637–642.
[6] Lodish, H. F. and Rose, J. K. (1977) J. Biol. Chem. 252, 1181–1188.
[7] Held, W. A., West, K. and Gallagher, J. F. (1977) J. Biol. Chem. 252, 8489–8497.
[8] Canaani, D., Revel, M. and Groner, Y. (1976) FEBS Lett. 64, 326–331.
[9] Groner, Y., Grosfeld, H. and Littauer, U. Z. (1976) Eur. J. Biochem. 71, 281–293.
[10] Hickey, E. D., Weber, L. A. and Baglioni, C. (1976) Proc. Natl. Acad. Sci. USA 73, 19–23.
[11] Shafritz, D. A., Weinstein, J. A., Safer, B., Merrick, W. C., Weber, L. A., Hickey, E. D. and Baglioni, C. (1976) Nature 261, 291–294.
[12] Weber, L. A., Feman, E. R., Hickey, E. D., Williams, M. C. and Baglioni, C. (1976) J. Biol. Chem. 251, 5657–5662.
[13] Asselbergs, F. A. M., Peters, W. H. M., Van Venrooij, W. J. and Bloemendal, H. (1978) Biochim. Biophys. Acta 520, 577–587.
[14] Weber, L. A., Hickey, E. D., Nuss, D. L. and Baglioni, C. (1977) Proc. Natl. Acad. Sci. USA 74, 3254–3258.
[15] Kemper, B. and Stolarsky, L. (1977) Biochemistry 16, 5770–5775.
[16] Asselbergs, F. A. M., Van Venrooij, W. J. and Bloemendal, H. (1978) Eur. J. Biochem. 87, 517–524.
[17] Van Vloten-Doting, L., Bol, J., Neeleman, L., Rutgers, T., Van Dalen, D., Castel, A., Bosch, L., Marbaix, G., Huez, G., Hubert, E. and Cleuter, Y. (1977) in: Nucleic acids and protein synthesis in plants (Borograd, L. and Weil, J. H. eds) Plenum, New York.
[18] Asselbergs, F. A. M., Peters, W., Van Venrooij, W. J. and Bloemendal, H. (1978) Eur. J. Biochem. 88, 483–488.
[19] Lodish, H., Housman, D. and Jacobsen, M. (1971) Biochemistry 10, 2348–2356.