Inhibition of Translation and Progesterone-induced Maturation of *Xenopus* Oocytes by Expressing the Amino-terminal Portion of the Eukaryotic Translation Initiation Factor 4G

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The eukaryotic translation initiation factor 4G (eIF4G) plays a pivotal role in translation. EIF4G interacts with several other factors including eIF4E, which is a cap-binding protein, and the poly(A)-binding protein (PABP). In this work, we demonstrate that the expression of the amino-terminal one-third of eIF4G, which interacts with eIF4E and PABP, in *Xenopus* oocyte inhibits translation and progesterone-induced maturation.

Key words: eukaryotic translation initiation factor 4G; *Xenopus*; oocyte; maturation

The importance of translational regulation on the control of cell fate has emerged in the past decade. Mostly, the critical step of translation is initiation. Regulatory factors interact with the mRNA and the ribosome. The interaction of eIF4E with the cap-structure of the mRNA has been intensively studied. EIS4E is indispensable for the cap-dependent translation, and it is known that destruction of the eIF4E gene is lethal in yeast *Saccharomyces cerevisiae*. EIF4E interacts with eIF4G to make a complex called eIF4F. EIF4G binds several other initiation factors including eIF4A, which is an RNA helicase, and eIF3. Recently, it was shown that eIF4G contains the binding site for PABP, which binds to the poly(A) tail of the mRNA 3'-end, in its amino-terminal region. Thus, eIF4G could bridge the two ends of the mRNA by associating with eIF4E and PABP. *In vitro* as well as *in vivo* studies demonstrated that the eIF4G-PABP interaction facilitates translation of polyadenylated mRNAs. Furthermore, synergism of the effects of the cap and the poly(A) tail was observed. It has been shown that the 2A protease from poliovirus or coxsackievirus cleaves eIF4G at the downstream of the eIF4E-binding site, which results in the shut off of translation in host cells. In this work, the amino-terminal one-third of eIF4G containing the binding sites for eIF4E and PABP (4G-

![Diagram](image-url)
Fig. 2. Effects of the expression of 4G-Nt on translation and progesterone-induced oocyte maturation.
A. Inhibition of translation dependent on the amount of 4G-Nt mRNA. The indicated amount of each mRNA was injected into oocytes followed by injection of the luciferase mRNA, and luciferase activity was measured. The graph depicts the mean from the two pools of five oocytes collected for each sample.
B. Effects of the expression of 4G-Nt on progesterone-induced oocyte maturation. Twenty oocytes were injected for each sample. Control; no injection. Representative data of two independent experiments is given.

Nt) was expressed in *Xenopus* oocytes to examine its effects on translation.

A schematic diagram of the structure of eIF4G and 4G-Nt proteins is shown in Fig. 1A. The cDNA encoding the amino acids 1 to 641 of the human eIF4GI (4G-Nt) was constructed by PCR using the full-length eIF4GI clone as a template. It is known that the cleavage site of coxsackievirus 2A protease is located between the amino acids 641 and 642. This cDNA was placed downstream of the T7 promoter of the pSP36T derivative vector. The mRNA encoding 4G-Nt was transcribed with T7 RNA polymerase. The mRNA for the full length eIF4GI and the luciferase mRNA was prepared as described elsewhere. Stage VI oocytes were manually dissected from ovaries and incubated at 18–20°C in OR2 medium [82.5 mM NaCl, 2.5 mM KCl, 1.0 mM CaCl₂, 1.0 mM MgCl₂, 1.0 mM Na₂HPO₄, 5.0 mM HEPES-KOH, pH 7.8] containing 10 μg/ml streptomycin and 10 μ/ml penicillin.

The mRNA encoding eIF4GI or 4G-Nt was injected into oocytes and then the luciferase mRNA, which was capped as well as polyadenylated, was injected. EIF4GI modestly stimulates, while 4G-Nt reduced, translation of luciferase mRNA. A portion of the oocytes were labeled with [³⁵S] methionine to examine the expression of proteins. The expression of 4G-Nt seemed to be several-folds greater than that of eIF4GI (Fig. 1D). (Note: The slow migration of these proteins is probably because of the proline rich sequences in the amino terminal region of these proteins.) Then, various amounts of 4G-Nt mRNA was injected to see its effects on translation. As shown in Fig. 2A, the injection of 5 fmol of 4G-Nt mRNA was sufficient to reduce translation of luciferase mRNA. These results demonstrate that 4G-Nt inhibits translation in *Xenopus* oocytes.

Oocyte maturation is a process of meiosis, and is triggered by progesterone. Progesterone-induced oocyte maturation requires translation of a subset of maternal mRNAs present in oocytes. To examine if 4G-Nt could inhibit translation of endogenous mRNAs, oocytes expressing 4G-Nt were treated with progesterone, and GVBD was scored (Fig. 2B). Strikingly, none of the oocytes expressing 4G-Nt underwent GVBD, while 100% of the control oocytes and the oocytes expressing eIF4GI underwent GVBD after 18 hours of incubation in OR2 containing progesterone. As for the oocytes expressing 4G-Nt, even after 25 hours of incubation, only 50% underwent GVBD. These results demonstrate that 4G-Nt inhibits translation of endogenous mRNAs. The 4G-Nt may be applicable to study gene expression in *Xenopus* oocytes and embryos.
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References


