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Coupled cytoplasmic transcription-and-translation—a method of choice for heterologous gene expression in *Xenopus* oocytes

Alexander A. Tokmakov[a,*], Eiko Matsumoto[a], Mikako Shirouzu[a], Shigeyuki Yokoyama[a,b,c]

[a] *Genomic Sciences Center, RIKEN Yokohama Institute, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa, Yokohama 230 0045, Japan*

[b] *RIKEN Harima Institute at SPring-8, Hyogo, Japan*

[c] *University of Tokyo, Tokyo, Japan*

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Abstract

We demonstrate here that the intracellular environment of *Xenopus* oocytes is quite compatible with the requirements of T7 RNA polymerase (T7 RNAP)-mediated transcription. This reaction runs robustly in the oocyte cytoplasm for many hours. The coinjection of a T7 promoter-driven luciferase-encoding plasmid DNA and purified T7 RNAP into oocytes results in the prolonged production of luciferase protein. Thus, the efficient coupling of T7 RNAP-mediated transcription with the intrinsic oocyte translation machinery occurs in the oocyte cytoplasm. The coupled protein synthesis generates high expression yield, displays little variation in the expression level between individual oocytes, requires very limited amounts of DNA template and T7 RNAP, and does not affect the oocyte viability and functional status. Our detailed, quantitative comparison of the existing expression methods in *Xenopus* oocytes highlights the advantages of the technique based on the cytoplasmic coinjection of T7 RNAP and T7 promoter-driven plasmid DNA and demonstrates that it is greatly superior to the alternative methods of heterologous gene expression.
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1. Introduction

Expression of heterologous genes in *Xenopus* oocytes is used to investigate the function, localization, and posttranslational modifications of synthesized proteins (see Shiokawa et al., 1997 for a review). Due to the high capacity of oocytes for translocation and posttranslational modifications, heterologous proteins usually retain their functional properties when expressed in oocytes. Heterologous genes are commonly delivered into oocytes either by cytoplasmic injections of in vitro synthesized specific mRNAs or by nuclear injections of

* Corresponding author. Tel.: +81 45 503 9204; fax: +81 45 5039201.
E-mail address: tokmak@gsc.riken.go.jp (A.A. Tokmakov).

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cDNAs, encoding the proteins of interest. Cytoplasmic injections of mRNA are used most often, because they omit the step of intracellular transcription and provide fairly reproducible, high expression levels in individual oocytes. However, this technique requires in vitro synthesis and manipulation of mRNA, which is readily degraded by contaminating RNases. Therefore, an RNase-free environment is a prerequisite for using this expression technique, which represents a serious disadvantage for users. On the other hand, the nuclear injection of cDNA, which does not require RNA manipulations, is unreliable and less robust technique. Although the nucleus represents about a quarter of the total oocyte volume, the thick pigment layer on the animal hemisphere of oocytes prevents its visual detection. The inability to deliver a normalized amount of cDNA reliably into the nucleus results in the poor reproducibility of this method. As a rule, protein synthesis by this technique is less efficient than the synthesis induced by cytoplasmic injections of mRNA, presumably due to the low capacity of nuclear transcription. The performance of this technique often benefits from the use of expression vectors under the control of eukaryotic promoters; however, promoter-independent transcription carried out by the endogenous RNA polymerase II present in the oocyte nucleus also takes place (Roeder, 1974).

Recently, a novel technique has been described for the expression of ion channels in *Xenopus* oocytes, which is based on the cytoplasmic coinjection of T7-driven plasmids and purified T7 RNAP (Geib et al., 2001). This method allows simultaneous gene transcription-and-translation (TnT) in the oocyte cytoplasm. Importantly, it does not involve the cumbersome in vitro preparation of mRNA for cytoplasmic injection and the unreliable nuclear injection of cDNA. Earlier, T7 RNAP was successively used to express heterologous genes in mammalian cells (Elroy-Stein et al., 1989; Deng et al., 1991; Alexander et al., 1992; Chen et al., 1994). The enzyme was exclusively localized in the cytoplasmic compartment and did not enter the nucleus (Dunn et al., 1988; Elroy-Stein and Moss, 1990). Thus, the observed gene expression was completely cytoplasmic and did not involve a nuclear translocation event. Moreover, T7 RNAP-mediated transcription did not require any additional transcription factors (Davanloo et al., 1984). Bypassing nuclear transcription resulted in the rapid and robust expression of a reporter T7-driven gene co-delivered with T7 RNAP (Gao and Huang, 1993). In oocytes, the cytoplasmic coinjection of T7-driven plasmids and purified T7 RNAP allowed the highly efficient expression of potassium and sodium channels, with characteristics indistinguishable from those of the channels expressed by the RNA injection method (Geib et al., 2001). However, a detailed characterization of this expression technique and its quantitative comparison with the existing expression methods have not been performed. Most notably, the measurement of in ovo synthesized heterologous mRNA levels has not been carried out, and the efficiency of T7 RNAP-mediated transcription in the oocyte cytoplasm has not been quantitatively estimated.

In the present study, we sought to compare different expression techniques, using the gene encoding firefly luciferase as a reporter of heterologous gene expression in *Xenopus* oocytes. It was previously shown that the luciferase gene can be transcribed and translated in *Xenopus* oocytes into a functionally active protein product from SV40 promoter-driven plasmids (Jin et al., 1991). This gene was chosen by considering the convenience of luciferase detection, since its activity can be measured quantitatively, with high sensitivity and reproducibility, using commercially available kits. A real-time RT-PCR technique was employed to monitor the synthesis and degradation of the luciferase mRNA. The gene was delivered into oocytes using three different techniques: (a) cytoplasmic injection of luciferase mRNA; (b) nuclear injection of luciferase-encoding plasmid DNA lacking eukaryotic promoter; and (c) cytoplasmic coinjection of purified T7 RNAP protein and T7 promoter-driven luciferase-encoding plasmid DNA. All three methods produced enzymatically active luciferase protein, albeit with different yields and kinetics. Although the cytoplasmic injection of mRNA resulted in the most robust initial production of luciferase, the protein synthesis after the coinjection of cDNA and T7 RNAP continued for a longer time and eventually reached a much higher level. In ovo synthesis of luciferase was still evident 48 h after the injection of DNA and T7 RNAP, whereas the protein synthesis after the cytoplasmic injection of luciferase RNA stopped completely within 12 h, due to degradation of the injected RNA. Both cytoplasmic injection techniques displayed little variation in the luciferase expression levels between individual oocytes. As little as 0.5 ng of plasmid DNA and 1 ng of T7 RNAP

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