Published in final edited form as:

Methods. 2010 May ; 51(1): 101–105. doi:10.1016/j.ymeth.2010.01.031.

Preparation of a highly active cell-free translation system from immature *Xenopus laevis* oocytes

Catherine A. Pratt and Kimberly L. Mowry *

Department of Molecular Biology, Cell Biology & Biochemistry, Brown University, Providence RI, USA

Abstract

Understanding mechanisms of post-transcriptional control of gene expression has come under much scrutiny in recent years. A key question in this field is how the translation of specific mRNAs is activated or repressed both spatially and temporally in a given cell. In oocytes of the frog *Xenopus laevis* a number of mRNAs are localized early in oogenesis and subsequently translated at later stages. We have developed a highly active cell-free translation system from oocytes in the early stages of oogenesis that is applicable to the study of translation and translational control of both endogenous and exogenous mRNAs.

Keywords

RNA; *Xenopus*; oocyte; translation; cell-free

1. Introduction

The study of eukaryotic translation has been greatly aided by the generation of cell-free systems that are based upon a variety of organisms and tissues [1–3]. In addition, *in vitro* translation systems have been reconstituted from purified components of both *Saccharomyces cerevisiae* and rabbit reticulocytes [4,5]. These systems offer unique advantages to the study of translation: while *in vitro* systems reconstituted from purified factors have been extensively used to detail the mechanisms underlying general translation [6,7], cell-free systems have been extremely useful in analyzing cell-specific gene expression at the level of translation [2,8]. For example, regulatory components present in germ cells are likely absent or modified in somatic tissues, and *vice versa*.

Cell-free systems from eggs and fully grown oocytes (stage VI; [9]) of the frog *Xenopus laevis* have been widely used to study a variety of cellular processes including translation, translocation of secretory proteins, nuclear envelope assembly, DNA replication and repair, and cell cycle [10–13]. The wide use of *Xenopus* oocytes and eggs for the preparation of these systems stems in part from the ease of acquisition of large amounts of material from these animals. A single female frog can yield thousands of oocytes that, due to their large size (50–1300 μm in diameter), can be easily sorted by stage (I–VI; [9]).

*Corresponding author: Kimberly Mowry, Box G-L268, Brown University, Providence RI, 02912, 401-863-3636 (phone), 401-863-1201 (FAX), Kimberly_Mowry@Brown.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

During oogenesis, numerous maternal mRNAs are stored within the oocyte in preparation for fertilization and early embryogenesis when the zygotic genome is transcriptionally inactive [14,15]. The production of protein from these mRNAs is stringently regulated, since all cellular processes in the early stages of embryogenesis are reliant on maternally loaded transcripts and proteins. In view of the fact that these mRNAs are stored in the oocyte but not translated until after maturation of the fully-grown oocyte or upon fertilization of the egg, they must be maintained in a translationally silent, or “masked” state. A number of studies have elucidated the mechanisms underlying the translational regulation of key mRNAs in the late stages of oogenesis [16,17], and this work has been much assisted by the *in vitro* translation systems developed for eggs and fully-developed *Xenopus* oocytes [13].

A number of mRNAs in *Xenopus* oocytes are localized early in oogenesis (stages I–III; [18, 19]). mRNA localization results in a local enrichment of a particular mRNA, and ultimately of the encoded protein, and this spatial restriction of translation is crucial for the development of the embryo [20]. It is therefore critical that no protein be produced from these mRNAs until they have reached their cellular destination. The mechanisms controlling translation of localized mRNAs in early oogenesis remain largely unknown, although a number of *cis*- and *trans*-acting factors have been identified through *in vivo* injection approaches [16,21]. In order to conduct mechanistic analyses of translational control mechanisms acting during early oogenesis, we have developed a cell-free translation system from stage I–III *Xenopus* oocytes. The lysate produced using this protocol is highly active, and can translate an exogenously supplied reporter mRNA with an efficiency equal to or greater than the commercially available rabbit reticulocyte lysate system. Given the high activity of this oocyte lysate and the ready availability of large amounts of *Xenopus* oocytes, this cell-free translation system represents a useful tool both for general studies of translational regulation and for understanding mechanisms of translational control in oocytes.

2. Description of Method

2.1 Materials

2.1.1 Isolation of oocytes—For the preparation of an active translation lysate from stage I–III oocytes, it is necessary to isolate a large number of oocytes from the early stages of oogenesis. First, the entire ovary of a young *Xenopus laevis* female (6.25–7.5 cm juveniles; Nasco LM00714MX) is surgically removed [22] and placed in MBSH buffer [88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 0.82 mM MgSO₄•7H₂O, 0.33 mM Ca(NO₃)₂•4H₂O, 0.41 mM CaCl₂•6H₂O, 10 mM HEPES (pH 7.6)]. The ovaries of frogs of this age contain predominately oocytes in the early stages of oogenesis, and therefore the majority of the harvested ovary can be used to generate translation lysate (see section 2.1.2). While older frogs can be used, it should be noted that more than one may be required to yield sufficient oocytes from stages I–III of oogenesis, and the task of removing older oocytes will be more laborious.

Next, the ovary is cut into small pieces and defolliculated by incubation in a (pH solution of collagenase [3 mg/ml collagenase (Sigma-Aldrich # C0130), 0.1 K₂HPO₄ 7.4]) for 15 minutes at 18°C with gentle shaking (~200 rpm). At this point, the oocytes should be liberated from the ovary. If necessary, however, the incubation time can be increased by an additional five minutes. The defolliculated oocytes are then washed three times with MBSH buffer. To remove oocytes from the later stages of oogenesis (late stage IV, stages V and VI), the oocytes are passed through a 500 µm sieve. The sieve is constructed from nylon mesh (Small Parts Inc. #CMN-0500-B) inserted into a circular hole cut into the lid of a plastic petri dish (Fig. 1). Some early stage IV oocytes may pass through the sieve, and these can be manually removed to yield a population of stage I–III oocytes (Fig. 1). The isolated stage I–III oocytes should occupy a volume of 100 µl or more when settled in a 1.5 ml microcentrifuge tube to ensure effective lysate production (see section 2.1.2).

2.1.2 Preparation of oocyte lysate—The ultimate outcome of the method described in this section is the production of a concentrated, translationally active oocyte lysate (Fig 1). It is critical that the entire protocol be performed at 0–4°C, either by maintaining all components on ice or conducting the protocol in a cold room. In addition, all glassware should be clean and sterilized by autoclaving, and care should be taken to avoid any RNase contamination.

Approximately 30 minutes before beginning the oocyte lysis procedure (below) the Hypotonic Lysis Buffer [HLB; 10 mM HEPES-KOH (pH 7.5), 15 mM KCl, 1.5 mM Mg(CH₃COO)₂, 2 mM DTT, 0.5 mM Pefabloc (Roche Diagnostics # 1429876)] should be prepared and chilled on ice. Aliquots of this buffer can be made in advance without DTT and Pefabloc, and stored at –20°C for up to six months. However, the DTT and Pefabloc should always be added fresh.

Place the stage I–III oocytes isolated above (section 2.1.1) in a 1.5 ml microcentrifuge tube and estimate the settled volume (at least 100 µl). Wash the oocytes twice with five volumes of cold HLB, allowing them to settle fully after each wash by placing the tube on ice for 2–5 minutes. Before the last wash, the oocytes in HLB should be transferred to a tightly fitting 1–2 ml dounce (Wheaton # 358029 or similar). After washing the oocytes, remove all the HLB with a drawn off Pasteur pipette and replace with one volume of HLB.

To lyse the oocytes use no more than three slow passes with the dounce. Excessive douncing is detrimental and will reduce the translational activity of the resulting lysate. The oocyte homogenate is then transferred to a chilled 1.5 ml microcentrifuge tube and centrifuged at 10,000 × g for 10 minutes at 4°C. After centrifugation the supernatant is carefully removed into a fresh chilled 1.5 ml microcentrifuge tube using a clean drawn off Pasteur pipette. Care should be taken to avoid the thin lipid and yolk layer that forms on top of the supernatant. This supernatant comprises the Xenopus Translation Lysate (XTL).

The translational activity of XTL as prepared in section 2.1.2 can vary from batch to batch, with variables including oocyte health and technical proficiency. For this reason it is advisable to test the activity of the lysates before they are used for further experiments, either by [³⁵S]-methionine incorporation (Fig. 2A) or by luciferase assay (Fig. 2B; section 2.2.1). For assaying [³⁵S]-methionine incorporation, the following reaction is set up in triplicate for each time point:

6 µl	XTL
0.6 µl	CTX [1M KCl, 16 mM Mg(CH ₃ COO) ₂ , 20 mM DTT]
1 µl	CPK [200 mM creatine phosphate, 4 mg/ml creatine kinase (Sigma)]
0.2 µl	amino acid mix minus Met (Promega)
10 µCi	[³⁵ S]-methionine (EasyTag, Perkin Elmer)
0.5 µl	recombinant RNasin (Promega)
1 µl	DEPC-dH ₂ O

To stop the reaction, 1 ml of ice cold 5% TCA is added. The reactions are then stored on ice until the time course is complete. [³⁵S]-methionine incorporation is assayed by binding to 24 mm glass microfibre filters (Whatman GF/A). After binding of the precipitated reaction, each filter is washed 10 times with 1 ml ice cold 5% TCA and dried. The filters are placed into individual scintillation vials with 3 ml scintillation fluid and counted.

If necessary, XTL can be flash frozen in liquid nitrogen and stored in aliquots at –80°C, but a reduction in translation efficiency (20–50 %) is usually observed after only a single freeze-thaw cycle. Protein concentration should be determined using a Bradford Assay (Bio-Rad Protein Assay substrate # 500-0006). Optimal XTL translation activity requires a total protein concentration of at least 20 mg/ml. Activity of the lysate decreases dramatically below this concentration.

2.1.3 Constructs for production of luciferase reporter mRNAs—XTL can be programmed with any mRNA to generate the encoded protein. Of note, it is particularly tractable for quantitatively assaying translation from a firefly luciferase reporter gene. For this, we have generated a series of constructs that are diagrammed in Fig. 3. pSP64-X β LucX β contains the firefly luciferase gene flanked by *Xenopus* β -globin 5'- and 3'-untranslated region (UTR) sequences (Fig. 3A). The β -globin 5'- and 3'-UTRs can be replaced with other UTR sequences of interest to test for a potential function in translation regulation using the 5' or 3' tester constructs pSP64-LucX β and pSP64-X β Luc (Figs. 3B, 3C). Each of these luciferase reporter plasmids contains an SP6 transcriptional promoter to allow for *in vitro* mRNA production (see section 2.1.4).

The dual luciferase assay (section 2.2.2) also makes use of an internal control in the form of the Renilla luciferase mRNA. The Renilla luciferase gene is available in the pRL-null plasmid from Promega (# E2271). This plasmid contains a T7 viral transcriptional promoter, and thus control reporter mRNA can be generated in a similar manner as from pSP64-X β LucX β (see section 2.1.4).

2.1.4 Preparation of mRNA—In order to generate capped mRNA for *in vitro* translation, template DNA is first linearized using an appropriate restriction enzyme to allow for runoff transcription [23,24]. In the case of the pSP64-Luc plasmids (Fig. 3) EcoRI is used, and BamHI is used for pRL-null (Promega). The linear DNA is purified and concentrated by extraction with phenol:chloroform:isoamyl alcohol (25:24:1) and ethanol precipitation. The template DNA is resuspended in nuclease-free deionized water (dH₂O). Nuclease-free dH₂O (DEPC-dH₂O) can be prepared as follows: 100ml dH₂O is incubated for 30 minutes with 1–2 drops DEPC (Sigma-Aldrich), swirled, and autoclaved. *In vitro* transcription is carried out using a suitable (SP6 or T7, depending on the promoter sequence present in the template DNA) mMessage mMachine *in vitro* transcription kit (Ambion). RNA yield from all templates is quantified by incorporation of [α -³²P]UTP (1 μ Ci/ μ l, Perkin Elmer) according to the manufacturers instructions. The resulting mRNA is diluted to 500 nM and can be stored in small (~5 μ l) aliquots at –80°C for several months.

2.2 Using XTL to analyze translation of exogenous RNAs

2.2.1 The *in vitro* translation assay—XTL can be effectively and quantitatively used to assay translation from reporter mRNAs in the following way. RNAs used to program translation in XTL must first be incubated at 70°C for three minutes to disrupt any secondary structure, and placed on ice until added to the reactions. The translation reactions are set up at room temperature as follows:

6 μ l	XTL
0.6 μ l	CTX [1M KCl, 16 mM Mg(CH ₃ COO) ₂ , 20 mM DTT]
1 μ l	CPK [200 mM creatine phosphate, 4 mg/ml creatine kinase (Sigma)]
0.1 μ l	amino acid mix minus Leu (Promega)
0.1 μ l	amino acid mix minus Met (Promega)
0.7 μ l	DEPC-dH ₂ O
0.5 μ l	recombinant RNasin (Promega)
1 μ l	luciferase reporter RNA [75nm (see section 2.1.4)]

For comparison of activity (see Fig. 2B), translation of reporter RNAs can also be performed using commercially available rabbit reticulocyte lysate (RRL; Promega # L4960), according to the manufacturers guidelines. Translation is allowed to proceed for 90 minutes at room temperature (XTL) or at 30°C (RRL). Luciferase activity is assayed as in section 2.2.2 to quantify translation efficiency.

2.2.2 The luciferase assay—Firefly luciferase activity can be accurately measured upon addition of enzyme substrate (reviewed in [25]). We routinely use commercially available reagents (Promega Luciferase Assay System # E1500) to measure luciferase activity. For the Dual-Luciferase™ assay, where Renilla mRNA is used as an internal control (see below), we use a similar system (Promega # E1910). Of note, due to the high efficiency of translation in XTL, the substrates provided in these kits (LARII and Stop and Glo®) should be diluted 1:2.5 into dH₂O prior to use. 5 µl of the translation reaction (see 2.2.1) is added to 25 µl diluted LARII (firefly luciferase substrate) in a 12×75 mm glass tube (Corning #99445-12) and light emission is measured on a Lumat 9501 luminometer for 5 seconds. As an internal control for translation in each assay, mRNA encoding the Renilla luciferase gene (see sections 2.1.3 and 2.1.4) can be used. For the Dual-Luciferase™ assay, each translation reaction therefore contains 5 nM Renilla luciferase mRNA in addition to 75 nM firefly luciferase reporter mRNA. Since each luciferase enzyme utilizes a different substrate, the activity of both can be measured in a single sample. After measuring the firefly luciferase activity, the reaction is removed from the luminometer, 25 µl diluted Stop and Glo® (Renilla luciferase substrate) is added, and the tube is replaced in the luminometer to measure Renilla luciferase activity. A ratio of firefly:Renilla luciferase activity is then generated to assess the levels of translation from different reporter constructs. Due to the sensitivity of the assay, small errors in pipetting accuracy can drastically affect the final readout. We therefore perform five individual replicates of each reaction to generate accurate, reproducible data.

2.2.3 Testing putative translational control RNA elements—In a variety of different cell types in eukaryotic organisms, mRNAs are known to be translationally regulated [26]. Frequently this regulation is controlled by elements contained in the 5'- and/or 3'-UTRs, and can result in either the activation or repression of translation [27,28]. Understanding the mechanisms of UTR-mediated translational control is a complex problem, and there have been countless studies in recent years that focus on this question (reviewed in [26]). The XTL system provides a useful tool for teasing apart mechanisms of translational control, in particular with regards to the myriad mRNAs that are regulated in early oogenesis [18,19]. In order to test a UTR in this system, the putative regulatory sequence(s) can be inserted into either the pSP64-LucXβ (Fig. 3B) or pSP64-XβLuc (Fig. 3C) constructs using the indicated restriction sites. mRNA generated through *in vitro* transcription, as described in section 2.1.4, is assayed as in section 2.2.2. Translation from pSP64-Luc constructs containing UTR sequences of interest, potentially including engineered mutations, can then be compared to pSP64-XβLucXβ as a positive control since the β-globin 5' and 3' UTR sequences present in pSP64-XβLucXβ (Fig. 3A) are unregulated in this system. Similarly, overexpression or add-back of putative *trans*-acting factors into this system can be used to assay function in the same manner, however care must be taken to avoid dilution of the XTL.

3. Concluding Remarks

We have described the preparation of XTL, a cell-free translation system using oocytes from the early stages of oogenesis in the frog, *Xenopus laevis* (Fig. 1). The XTL system can be used to assay translation from an exogenously added reporter mRNA (Figs. 2, 3), and to analyze regulatory elements in the 3'UTR of similar reporters. The translation efficiency of XTL is extremely high, and is comparable with commercially available *in vitro* translation systems (Fig. 2). Previously developed systems from *Xenopus* eggs and oocytes, while supporting the translation of endogenous mRNAs, are reportedly deficient in the translation of exogenous transcripts [13]. This may reflect a difference in initiation efficiency between oocytes early in oogenesis versus their more mature counterparts, or may be a result of the differing methods of lysate production. It is unclear which of these possibilities may be responsible, since our protocol is unsuitable to produce active translation lysates from late oocytes (data not shown). Similarly, the established protocols [13] for generation of translation lysates from late stage

oocytes cannot be used to produce active lysates from stage I–III oocytes (data not shown). The XTL translation lysate has also been used in our lab to study the translational status of endogenous transcripts by sucrose density centrifugation (not shown), further emphasizing the versatility of this extract. Of particular interest is the ability to dissect mechanisms of translational control that are acting on localized mRNAs early in oogenesis. This *in vitro* system can complement the array of *in vivo* experiments already in place in the *Xenopus* oocyte model system.

Acknowledgments

Work in our laboratory on development of these methods was supported by NIH grant # R01GM071049 to KLM.

References

1. Beckler GS, Thompson D, Van Oosbree T. In vitro translation using rabbit reticulocyte lysate. *Methods Mol Biol* 1995;37:215–32. [PubMed: 7780506]
2. Castagnetti S, Hentze MW, Ephrussi A, Gebauer F. Control of oskar mRNA translation by Bruno in a novel cell-free system from *Drosophila* ovaries. *Development* 2000;127:1063–8. [PubMed: 10662645]
3. Van Herwynen JF, Beckler GS. Translation using a wheat-germ extract. *Methods Mol Biol* 1995;37:245–51. [PubMed: 7780507]
4. Acker MG, Kolitz SE, Mitchell SF, Nanda JS, Lorsch JR. Reconstitution of yeast translation initiation. *Methods Enzymol* 2007;430:111–45. [PubMed: 17913637]
5. Pisarev AV, Unbehaun A, Hellen CU, Pestova TV. Assembly and analysis of eukaryotic translation initiation complexes. *Methods Enzymol* 2007;430:147–77. [PubMed: 17913638]
6. Fan-Minogue H, Du M, Pisarev AV, Kallmeyer AK, Salas-Marco J, Keeling KM, Thompson SR, Pestova TV, Bedwell DM. Distinct eRF3 requirements suggest alternate eRF1 conformations mediate peptide release during eukaryotic translation termination. *Mol Cell* 2008;30:599–609. [PubMed: 18538658]
7. Pisarev AV, Hellen CU, Pestova TV. Recycling of eukaryotic posttermination ribosomal complexes. *Cell* 2007;131:286–99. [PubMed: 17956730]
8. Thermann R, Hentze MW. *Drosophila* miR2 induces pseudo-polysomes and inhibits translation initiation. *Nature* 2007;447:875–8. [PubMed: 17507927]
9. Dumont JN. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J Morphol* 1972;136:153–79. [PubMed: 4109871]
10. Lohka MJ, Masui Y. Formation in vitro of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. *Science* 1983;220:719–21. [PubMed: 6601299]
11. Matthews G, Colman A. A highly efficient, cell-free translation/translocation system prepared from *Xenopus* eggs. *Nucleic Acids Res* 1991;19:6405–12. [PubMed: 1754376]
12. Murray AW. Cell cycle extracts. *Methods Cell Biol* 1991;36:581–605. [PubMed: 1839804]
13. Patrick TD, Lewer CE, Pain VM. Preparation and characterization of cell-free protein synthesis systems from oocytes and eggs of *Xenopus laevis*. *Development* 1989;106:1–9. [PubMed: 2627883]
14. Dworkin MB, Shrutkowski A, Dworkin-Rastl E. Mobilization of specific maternal RNA species into polysomes after fertilization in *Xenopus laevis*. *Proc Natl Acad Sci U S A* 1985;82:7636–40. [PubMed: 2415967]
15. Woodland HR, Flynn JM, Wyllie AJ. Utilization of stored mRNA in *Xenopus* embryos and its replacement by newly synthesized transcripts: histone H1 synthesis using interspecies hybrids. *Cell* 1979;18:165–71. [PubMed: 509520]
16. Otero LJ, Devaux A, Standart N. A 250-nucleotide UA-rich element in the 3' untranslated region of *Xenopus laevis* Vg1 mRNA represses translation both in vivo and in vitro. *RNA* 2001;7:1753–67. [PubMed: 11780632]
17. Stebbins-Boaz B, Cao Q, de Moor CH, Mendez R, Richter JD. Maskin is a CPEB-associated factor that transiently interacts with eIF-4E. *Mol Cell* 1999;4:1017–27. [PubMed: 10635326]

18. Melton DA. Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocytes. *Nature* 1987;328:80–2. [PubMed: 3600777]
19. Zhou Y, King ML. Localization of Xcat-2 RNA, a putative germ plasm component, to the mitochondrial cloud in *Xenopus* stage I oocytes. *Development* 1996;122:2947–53. [PubMed: 8787767]
20. Birsoy B, Kofron M, Schaible K, Wylie C, Heasman J. Vg 1 is an essential signaling molecule in *Xenopus* development. *Development* 2006;133:15–20. [PubMed: 16308332]
21. Colegrove-Otero LJ, Devaux A, Standart N. The *Xenopus* ELAV protein ElrB represses Vg1 mRNA translation during oogenesis. *Mol Cell Biol* 2005;25:9028–39. [PubMed: 16199879]
22. <http://www.xlaevis.com/oocyte.html>
23. Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K, Green MR. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res* 1984;12:7035–56. [PubMed: 6091052]
24. Yisraeli JK, Melton DA. Synthesis of long, capped transcripts in vitro by SP6 and T7 RNA polymerases. *Methods Enzymol* 1989;180:42–50. [PubMed: 2559301]
25. Kain SR, Ganguly S. Overview of genetic reporter systems. *Curr Protoc Mol Biol* Chapter 2001;9 (Unit9):6.
26. Besse F, Ephrussi A. Translational control of localized mRNAs: restricting protein synthesis in space and time. *Nat Rev Mol Cell Biol* 2008;9:971–80. [PubMed: 19023284]
27. de Moor CH, Meijer H, Lissenden S. Mechanisms of translational control by the 3' UTR in development and differentiation. *Semin Cell Dev Biol* 2005;16:49–58. [PubMed: 15659339]
28. Pickering BM, Willis AE. The implications of structured 5' untranslated regions on translation and disease. *Semin Cell Dev Biol* 2005;16:39–47. [PubMed: 15659338]

Figure 1.

Procedure for preparation of a translationally active oocyte lysate (XTL). Stage I–III oocytes are separated from stage IV–VI oocytes by passage through a 500 μ m sieve, followed by manual removal of any pigmented oocytes that pass through the sieve (black arrow heads). Stage I–III oocytes are washed into a hypotonic lysis buffer (HLB), disrupted by dounce homogenization, and centrifuged to remove cellular debris. The supernatant (XTL) is removed and used for in vitro translation.

Figure 2.

Activity of the XTL translation lysate. **(A)** Time course of incorporation of [³⁵S]-methionine into protein using XTL prepared as described in section 2.1.2. Translation assays were performed as described in section 2.1.2. At each time point, incorporation of the radiolabel was measured by TCA precipitation of a 10 μ l reaction and subsequent binding to a 24 mm GF/A Whatman glass microfiber filter. [³⁵S]-methionine incorporation (cpm, y-axis) is plotted against time (minutes, x-axis). **(B)** Translation efficiency of the *Xenopus* translation lysate (XTL) was compared to that of Rabbit reticulocyte lysate (RRL). XTL and RRL were programmed with RNA transcribed from pSP64-XLucX β (Fig. 3A), and incubation of XTL without RNA (no RNA) served as a negative control. Analysis of luciferase activity demonstrates that XTL translation activity is greater than that of the commercially available (Promega) RRL.

Figure 3.

Schematic of pSP64-Luc reporters for testing UTR-mediated translation regulation. **(A)** pSP64-XβLucXβ contains the firefly luciferase gene (white arrow) flanked by *Xenopus* β-globin 5' (black) and 3' (gray) untranslated region (UTR) sequences. The positions of key restriction sites are marked. **(B)** 5' UTR sequences of interest can be inserted into the HindIII site (marked by a white arrowhead) of pSP64-LucXβ. **(C)** 3' UTR sequences of interest can be inserted into the XbaI site (marked by a white arrowhead) of pSP64-XβLuc. For the production of run-off transcripts all plasmids (A–C) are linearized with EcoRI and transcribed using SP6 RNA polymerase; the SP6 promoter regions are marked at the left.