Temporal regulation of the *Xenopus* FGF receptor in development: a translation inhibitory element in the 3′ untranslated region

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SUMMARY

Early frog embryogenesis depends on a maternal pool of mRNA to execute critical intercellular signalling events. FGF receptor-1, which is required for normal development, is stored as a stable, untranslated maternal mRNA transcript in the fully grown immature oocyte, but is translationally activated at meiotic maturation. We have identified a short cis-acting element in the FGF receptor 3′ untranslated region that inhibits translation of synthetic mRNA. This inhibitory element is sufficient to inhibit translation of heterologous reporter mRNA in the immature oocyte without changing RNA stability. Deletion of the poly(A) tract or polyadenylation signal sequences does not affect translational inhibition by this element. At meiotic maturation, we observe the reversal of translational repression mediated by the inhibitory element, mimicking that seen with endogenous maternal FGF receptor mRNA at meiosis. In addition, the activation of synthetic transcripts at maturation does not appear to require poly(A) lengthening. We also show that an oocyte cytoplasmic protein specifically binds the 3′ inhibitory element, suggesting that translational repression of *Xenopus* FGF receptor-1 maternal mRNA in the oocyte is mediated by RNA-protein interactions. These data describe a mechanism of translational control that appears to be independent of poly(A) changes.

Key words: translational control, 3′ untranslated region, maternal mRNA, FGF receptor, oogenesis, meiosis, RNA binding protein

INTRODUCTION

The frog oocyte contains a pool of maternally derived mRNA transcripts that are stored as nontranslated ribonucleoproteins. Translational recruitment of masked mRNAs provides a means for rapid gene expression during early embryonic development in the frog and a variety of vertebrate and invertebrate species (Davidson, 1986). Since transcription of embryonic genes does not begin until after the 4,000-cell mid-blastula stage (Newport and Kirschner, 1982), critical steps in frog embryogenesis depend on temporal and spatial translational control of key maternal genes. At various points in development, beginning with meiotic maturation, maternal transcripts are recruited for translation (reviewed by Richter, 1991). Translational regulation of maternal mRNAs involved in embryonic patterning is now clearly established in organisms, such as *Drosophila* and *Caenorhabditis elegans*, that rely on a maternal pool of mRNA to carry out early developmental events (Evans et al., 1994; Gavis and Lehmann, 1994). The mechanisms employed and the specificity involved in the regulation of these mRNAs are poorly understood.

A critical step in vertebrate embryogenesis is the induction of mesoderm. In *Xenopus laevis*, mesoderm induction commences prior to zygotic transcription (Woodland and Jones, 1987) and is dependent on the action of peptide hormones in the fibroblast growth factor (FGF) and activin families (Amaya et al., 1991; Hemmata and Melton, 1992). Inhibition of FGF signalling in *Xenopus* embryos leads to deficient mesoderm formation and the inhibition of mesoderm-specific gene expression (Amaya et al., 1993). Recent work has demonstrated that FGF signalling is also required for activin mediated induction of mesoderm (Cornell and Kimmelman, 1994; LaBonne and Whitman, 1994). Early frog development, therefore, is dependent on the regulated expression of the FGF receptor as an integral component of the signalling pathway. Competence to respond to peptide growth factors requires that, at a minimum, specific blastomeres display appropriate cell surface receptors at the correct time and location coincident with the appropriate ligand. This characteristic of embryonic cells, which is established prior to the onset of zygotic transcription, is dependent on the temporal and spatial expression of maternal genes under translational regulation.

The *Xenopus* FGF receptor-1 (XFGFR-1) is a maternal mRNA that is present throughout early embryogenesis (Musci et al., 1990; Friesel and Dawid, 1991). The XFGFR-1 mRNA, while present in the immature oocyte, is not translated at this stage of oogenesis (Musci et al., 1990). In this paper, we investigate the mechanisms that control the translation of XFGFR-1 protein. We show that the inhibition of XFGFR-1 mRNA translation in the immature oocyte is mediated by a cis-acting element in the 3′ untranslated region.
transcription inhibitory element (TIE) in the 3’ untranslated region (UTR). In addition, our data suggests that both TIE-mediated inhibition and subsequent release of inhibition at oocyte maturation does not depend on polyadenylation. We show that the TIE specifically binds to oocyte cytoplasmic protein, suggesting that translational repression of XFGFR-1 maternal mRNA in the oocyte may be mediated by RNA-protein interactions in the 3’ UTR.

**MATERIALS AND METHODS**

**Egg and oocyte manipulation**

Eggs were stripped from ovulating females and collected in buffer as previously described (Newport and Kirschner, 1982). Stage VI oocytes were manually defolliculated from freshly obtained tissue and maintained in culture as previously described (Amaya et al., 1991).

**Library screening and cDNA cloning**

A *Xenopus* stage 17 lambda gt10 library (Kintner and Melton, 1987) was screened with a 0.8 kb fragment of previously cloned FGFR-1 cDNA (Muscil et al., 1990). A clone representing the 1.2 kb full-length 3’ untranslated sequence was obtained, designated as clone 3.1 and was inserted into pBluescript II/KS (Stratagene), designated p3.1. The first 500 nucleotides were amplified by PCR and ligated into pBSII/KS, called p500/3. The first 500 nucleotides were amplified by PCR and ligated into pBSII/KS, called p500/3UTR, and modified pSP64T vectors (see below).

**Plasmid construction**

All constructs were made in a modified pSP64T vector containing 5’ and 3’ untranslated β-globin sequences as previously described (Amaya et al., 1991) with the addition of a NotI site in the downstream polylinker. All chimeric cDNA constructs were confirmed by direct double-stranded DNA sequencing. The XFGFR cDNA construct (XFR) used was described previously (Amaya et al., 1991). To make XFR3’UTR (coding sequence + complete 3’ UTR), the full 3’ UTR from clone 3.1 was joined to a previously isolated partial cDNA (2.9 kb) (Muscil et al., 1990). XFR + 180 (+ first 180 nts of 3’ UTR) was generated by blunt ligation of the 180 bp HindII fragment from p3.1 immediately downstream of the XFR coding sequence.

The human EGF receptor cDNA (gift of H. Ingraham) was used as a template for the PCR amplification of the first 2.0 kb of coding region to include the extracellular and transmembrane domain, called HED. The ATG start site was modified by PCR mutagenesis to create a Ncol restriction site at the start codon and a downstream Xbal site enabling insertion of the clone into the modified pSP64T with *Xenopus* β-globin 5’ and 3’ UTR as previously described (Amaya et al., 1991). The primers for this PCR amplification were: sense: 5’-GCGCCATGGGACCCTCCGGGACGGCCG-3’, antisense: 5’-CGTTCTGATGTAGAGAGGCCGATCCCCAGGGC-3’.

**Protein analysis**

Synthetic transcripts were translated in wheat germ extract with [35S]methionine labeling (Promega) as previously described (Muscil et al., 1990). For metabolic labeling, microinjected oocytes and non-injected controls were incubated in buffer solution A (Amaya et al., 1991) for 16 hour at 16°C with 0.25 mCi/ml of [35S]methionine (final concentration) per sample. Oocyte lysate was either analyzed immediately or frozen at −80°C for subsequent immunoprecipitation. Lysates were diluted 1:5 with immunoprecipitation buffer as previously reported (Amaya et al., 1991). Antibodies used were: 1 μg/ml of anti-human EGF receptor monoclonal antibody (Oncogene Science), 2 μl/ml of anti-XFGFR immune serum (antibody R#1, Amaya et al., 1993), and 200 ng/ml of anti-β-galactosidase antibody (Boehringer Mannheim).

**Polyadenylation assay**

To measure poly(A) length of endogenous XFGFR mRNA, a modified PCR-based ‘poly(A) test’ was used (Salles et al., 1992). Oocyte and egg poly(A) selected RNA (50 ng) was reverse transcribed (Superscript reverse transcriptase, BRL, Inc.) using an oligo(dT) primer adapter (Salles et al., 1992). cDNA was amplified using 25 pmoles each of a specific XFGFR 3’ UTR primer (5’-GGCTGTTAGGTGTTCAATGTC-3’) and the oligo(dT) primer/adapter in a 50 μl reaction (PCR buffer and AmpliTag, Perkin-Elmer) as follows: 1 cycle of 5 minutes, 94°C, then 35 cycles of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C. An aliquot was separated on 1.5% agarose gel and transferred to Duralon-UV membrane (Stratagene).
agene, Inc.) and incubated overnight at 42°C in hybridization solution (50% formamide, 5x SSC, 5x Denhardt's, 1% SDS, 100 μg/ml herring sperm DNA) with 1 million cts/minute/ml of a 32P-labelled 3' UTR cDNA probe (Oligolabeling Kit, Pharmacia, Inc.). Blots were washed under stringent conditions (0.2x SSC, 0.1% SDS at 42°C) and exposed to film at -80°C.

Oocyte extract preparation
Fresh ovarian tissue was treated with collagenase (Boehringer Mannheim) to yield oocytes freed from ovarian stoma, using published protocols (Smith et al., 1991). Viable stage VI oocytes were used to prepare S100 extract (Dignam et al., 1983).

RNA gel shift and UV crosslinking experiments
RNA mobility shift assays were performed as described by Leibold and Munro (1988) except that 1.5 pmoles of probe was used and incubations were carried out in 50 mM KCl. Crosslinking reactions were performed with the same conditions as the mobility shift assay with a 15 minute RNase A/T1 digestion of the reactions (0.1 mg/ml and 750 U/ml respectively, 37°C) after UV irradiation for 10 minutes at 254 nm at a distance of 18 cm. After RNase digestion, reactions were resolved by 12.5% SDS-PAGE. Probes were transcribed using 5-bromo UTP (Sigma) with reagents supplied in Maxiscript T3 Kit (Ambion) and 32P-labelled CTP (800 Ci/mmole, Amersham) All probes were gel purified on a 6% denaturing polyacrylamide gel.

RESULTS

Inhibition of FGF receptor-1 translation in the immature oocyte
The XFGFR-1 mRNA is present at a constant level in the immature oocyte, the unfertilized egg and during early embryonic stages (Friesel and Dawid, 1991; Musci et al., 1990). We have shown, however, that functional protein is not detectable in the immature oocyte (Musci et al., 1990), suggesting that maternal XFGFR-1 mRNA is translationally repressed in the oocyte. To test this hypothesis, we first determined when endogenous maternal FGF-1 translation was initiated. We used an affinity-purified polyclonal anti-XFGFR-1 antibody (Amaya et al., 1993) to probe lysate from immature oocytes and eggs by western analysis. XFGFR protein was undetectable in the immature oocyte but was found to be present in the egg (Fig. 1A). These results demonstrate that translation of XFGFR is initiated sometime between late oogenesis and the egg. To correlate initiation of translation with meiotic maturation, we treated explanted oocytes with progesterone. The amount of XFGFR protein from progesterone-matured oocytes was equivalent to that seen in the egg (Fig. 1A). Thus, the store of maternal mRNA for XFGFR is translationally repressed in the pre-egg stage of oogenesis, and inhibition of translation is released upon meiotic maturation.

The translational activation of masked maternal mRNAs is frequently associated with poly(A) lengthening (reviewed by Jackson, 1993). We reasoned that repression of XFGFR-1 translation might involve regulatory regions located

XFGFR mRNA is activated at oocyte maturation and this activation is coincident with a lengthening of the poly(A) tail.

The 3' untranslated region inhibits translation of maternal FGF receptor mRNA
Since cis-acting RNA elements have been shown to function as inhibitory regulators of translation in other organisms (reviewed by Jackson, 1993), we reasoned that repression of XFGFR-1 translation might involve regulatory regions located
within the RNA transcript itself. We tested the potential of the 3′ UTR to regulate translation of XFGFR mRNA by injecting in vitro synthesized XFGFR mRNA in immature oocytes. We used synthetic XFGFR mRNA that contained either the entire 3′ UTR (XFR+3′ UTR) or only the coding sequence (XFR). All transcripts were capped and contained both 5′ and 3′ Xenopus β-globin sequences to enhance stability of the transcripts (Fig. 2A). The 3′ β-globin sequence included a poly(A)23 track followed by a poly(C)30 tail in order to effectively eliminate poly(A) tail modification following injection (Wormington, 1991). When synthetic XFR lacking the 3′ UTR was injected into immature oocytes, high levels of XFGFR protein were detected. In contrast, when identical amount of XFR+3′ UTR RNA was injected, no protein was detected (Fig. 2B). This inhibition of XFR+3′ UTR translation was seen over a 20-fold range of injected amounts of synthetic RNA (Fig. 2B). At high injection quantities, protein product from XFR+3′ UTR mRNA was detected, but always at significantly lower levels when compared to transcripts without the XFGFR 3′ UTR (Fig. 2B).

To demonstrate further that inhibition was not due to factors intrinsic to the synthetic constructs, we tested translation in a wheat germ lysate. Both XFR+3′ UTR and XFR were translated to yield mature proteins (Fig. 2C; also see Fig. 2B, lane 6). Therefore, factors present in the oocyte lead to translational inhibition of transcripts possessing the XFGFR 3′ UTR. Taken together, these data suggest that the repression of XFGFR mRNA translation during oogenesis is regulated by sequences located within the 3′ UTR.

**The 3′ UTR of XFGFR inhibits translation of heterologous protein**

We then tested whether the inhibitory effect of the XFGFR 3′ UTR on translation might be dependent on an interaction between the XFGFR coding sequence and the 3′ UTR. We created chimeric mRNA molecules by joining a truncated human EGF receptor coding sequence (HED) to the XFGFR 3′ UTR and analyzed the ability of the 3′ UTR to inhibit translation of HED (Fig. 3A, schematic). This reporter protein was chosen because of its similarity to the XFGFR: both are equivalent in size; both are transmembrane proteins and both are glycosylated. Additionally, overexpression of this truncated human EGF receptor does not interfere with cellular function.

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**Fig. 2.** The 3′ UTR of the XFGFR-1 inhibits translation in the oocyte. (A) Schematic of FGFR constructs used for oocyte microinjection. (B) Immunoprecipitation of metabolically labelled XFGFR protein from oocyte lysate after microinjection with XFGFR synthetic message. Lanes 1,3,5: XFR; lanes 2,4,6: XFR+3′ UTR. Injection amounts were, 0.75 fmol/oocyte (lanes 1 and 2), 1.5 fmol/oocyte (lanes 3 and 4), 15 fmol/oocyte (lanes 5 and 6). Immunoprecipitates of 14 oocytes/sample were separated on 7.5% SDS-PAGE. (C) In vitro translation using wheat germ lysate of equimolar amounts of XFGFR synthetic RNA, with and without XFGFR 3′ UTR. 35S-labelled translation products were separated on 7.5% SDS-PAGE and exposed to X-ray film.
or induce maturation of the oocyte (data not shown). The presence of the XFGFR 3’ UTR was sufficient to inhibit translation of HED+3’UTR (Fig. 3B, lane 3). The absence of detectable protein from HED+3’UTR was not due to RNA degradation, as mRNAs with and without the XFGFR 3’ UTR were equally stable after injection and incubation (Fig. 3B). As a control for the capacity of any two groups of oocytes to translate protein equally, all experimental mRNAs were co-injected with transcripts for β-galactosidase and lysates were analyzed for the presence of this protein (Fig. 3B). These results demonstrate that the inhibitory effect of the XFGFR 3’ UTR is cis-acting and can be transferred to a heterologous mRNA. Inhibition of translation, therefore, is not related to upstream coding sequences and thus appears to be due to an interaction between the UTR and some component in the oocyte.

**Deletion analysis of the 3’ UTR defines a translation inhibitory element**

Our next experiments determined whether translational inhibition was localized to a specific region of the UTR. We performed truncation and deletion analyses of the entire XFGFR 3’ UTR and determined that a 180 nucleotide region was sufficient to inhibit translation (Fig. 3A). We refer to this region of the 3’ UTR as a translation inhibitory element or TIE. (C) 180 nt element is sufficient for translational inhibition in the oocyte. Immunoprecipitation of HED and XFR protein from oocyte lysate after injection of synthetic mRNA (1.5 fmol/oocyte), with and without the TIE. NI, non-injected oocyte lysate. Bottom panel (lanes 1, 2, 4, 5): immunoprecipitation of protein product of co-injected β-galactosidase control mRNA.
the XFGFR (Fig. 3C, lane 5). When the TIE was deleted from the entire XFGFR 3’ UTR (HED3’Δ180), complete translation was restored (Fig. 3B, lanes 2). Protein product from HED3’Δ180 was equivalent to that translated from HED (Fig. 3B). These results demonstrate that a deletion of 180 nucleotides from the 3’ UTR completely removes translational inhibition.

Inhibition by the TIE appears to be independent of poly(A) changes

We next considered whether the TIE might regulate changes in poly(A) tail length in the immature oocyte and thereby inhibit translation. We tested constructs with truncations in the XFGFR 3’ UTR that removed both the polyadenylation consensus hexanucleotide and the poly(A) tail (Fig. 5A), thereby eliminating the possibility of poly(A) modification after injection. Only HEDΔ180 transcripts were translated,

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**Fig. 4.** Sequence of XFGFR 180 nucleotide TIE. Nucleotides are numbered from the first nucleotide following the termination codon to the end of the inhibitory element. The uridine (U) content of this stretch of 3’ UTR is 35%, which is similar to the U-content of the entire XFGFR 3’ UTR (submitted to Genbank). The black boxes represent two poly(A) addition signal (AAUAAA) sequences found at the end of the UTR.

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**Fig. 5.** Translational inhibition by TIE without synthetic poly(A) tail. (A) Schematic of 3’ UTR showing site of truncation to remove hexanucleotide polyadenylation elements (AAUAAA). (B) Top panel: immunoprecipitation of HED protein from immature oocyte lysate (28 oocytes/sample). HEDΔ180-A, 180 nt deletion of XFGFR 3’ UTR truncated at site indicated in schematic A; HED+3’UTR, is the XFGFR 3’ UTR truncated as indicated; NI, non-injected oocyte lysate. Middle panel: immunoprecipitation of co-injected β-galactosidase control mRNA. Bottom panel: dot blot of injected RNAs isolated from oocytes after injection and overnight incubation, probed with 32P-labelled HED cDNA. (C) Northern analysis of injected HED synthetic mRNAs before and after oocyte injection and isolation. Lane 1: HED with truncated 3’ UTR lacking 180nt TIE, lane 2: HED with truncated 3’ UTR. Northern probe is the same cDNA as in B, bottom panel.
demonstrating that the TIE was inhibiting translation in the absence of a poly(A) tail (Fig. 5B).

Although these experiments utilized constructs with poly(A) tails and poly(A) addition sites deleted, the formal possibility existed that uncharacterized regulatory elements could be present in the remaining UTR which mediate poly(A) addition. To address this, we isolated the synthetic poly(A) minus RNAs following injection and analyzed them for changes in size. Northern analysis of postinjection RNAs revealed no apparent increase in size compared to preinjection synthetic transcripts except for a faint slower mobility smearing (Fig. 5C). This change could either represent residual cytoplasmic proteins bound to the injected RNA or that a small fraction of synthetic mRNA becomes elongated. However, the latter possibility is unlikely since there is no polyadenylation site in the UTR of this construct. In summary, these results suggest that the 3′ UTR TIE can function independently of a poly(A) tail or changes in the adenylated state of the transcript.

Inhibition by the TIE is reversed by progesterone maturation

Based on the above experiments, we suggest that inhibition of endogenous XFGFR mRNA prior to oocyte maturation is mediated by the TIE. This hypothesis makes some predictions about the regulation of XFGFR translation. Since endogenous XFGFR is translated at maturation, the inhibitory effects of the TIE should be reversed upon maturation. To test this hypothesis, we injected oocytes with transcripts containing the TIE (XFR+180), treated them with progesterone and then assayed for protein. We demonstrate that XFGFR transcripts were not translated when oocytes were in the immature state, but, upon maturation, transcripts containing the TIE were readily translated (Fig. 6A). Transcripts that did not have the TIE showed no difference in their protein levels in immature versus mature oocytes (Fig. 6A). Collectively, these results demonstrate that synthetic mRNA containing the TIE was translationally regulated in a manner indistinguishable from that of endogenous XFGFR mRNA and that if the TIE is removed, regulation of XFGFR translation through maturation is lost.

Unmasking of XFGFR RNA at maturation is independent of adenylated changes

We then determined whether the translation of synthetic XFR+180 mRNA seen at meiotic maturation had taken place without adenylated. Several investigators have shown that polyadenylation is required for the translational activation of several Xenopus maternal mRNAs (Richter, 1993; Sheets et al., 1994). Translational activation of endogenous XFGFR-1 at maturation is associated with poly(A) tail lengthening (Fig. 1B). The choice of the 3′-end used in our experiments was made so that adenylated changes in the poly(A) stretch of synthetic transcripts would be unlikely. Yet, while we had seen translational activation under these conditions, we wished to confirm this experimentally. Following injection and oocyte maturation, we assayed the size of re-isolated synthetic XFR+180 RNA. We observed no apparent increase in the size of the transcripts containing the TIE following meiotic maturation with progesterone (Fig. 6B). A faint smear of higher molecular weight RNA is observed at greater than 4.0 kb from RNA re-isolated from oocytes with and without progesterone treatment (Fig 6B). This likely represents probe hybridization to endogenous XFGFR-1 mRNA. Therefore, the release of translational inhibition imposed by 3′ UTR inhibitory sequence appears to be independent of a change in adenylated of the synthetic transcript. Taken together, these results suggest that activation of XFGFR translation at oocyte maturation appears to not require polyadenylation.

TIE binds specifically to oocyte protein

We postulated that repression of XFGFR translation by the TIE involved the specific interaction between the TIE and oocyte cytoplasmic protein. To detect RNA-protein binding, we performed a gel retardation assay. Radiolabelled TIE RNA formed complexes with protein from oocyte S100 extracts resulting in a shift in mobility (Fig. 7A). In contrast, a different pattern was seen using a labelled control RNA of a similar size (Fig. 7A). This suggests that the two RNA segments bind different protein(s) from cytoplasmic extract. To explore the specificity of RNA-protein interaction and to determine the size of the protein(s) bound, we used UV crosslinking of radiolabelled RNA-protein complexes. The predominant protein visualized runs at approximately \(4 \times 10^3 M_r \) (Fig. 7B, lane 1). Binding of the \(4 \times 10^3 M_r \) protein to the labelled TIE RNA is sensitive to competition with molar excess unlabelled TIE but
not sensitive to competition by molar excess of unlabelled control RNA (Fig. 7B, lane 4, 5).

We wanted to know whether this apparent 43×10^3 M_r protein represented an oocyte Y-box RNA binding protein (Murray et al., 1992). We utilized antibodies to the abundant Y-box proteins, p54/56, to exclude these as candidates. Western analysis of a TIE-S100 crosslinking reaction demonstrated that the 43×10^3 M_r protein is not likely to represent one of these proteins (Fig. 7B). The 54 and 56×10^3 M_r bands observed on Western analysis correspond only to faintly labelled bands between 50 and 60×10^3 M_r in the UV crosslinking reactions (Fig 7B, lane 1). These RNA binding proteins are known to be associated with nontranslated mRNA in the oocyte (Murray et al., 1992) and are thought to bind non-specifically (Marello et al., 1992). Therefore, while the TIE binds p54/56, it also specifically binds to an oocyte cytoplasmic protein of approximately 43×10^3 M_r (including residual ribonucleotide). We are currently pursuing the identity and function of this protein in order to determine its role in translational repression.

**DISCUSSION**

The storage of maternal mRNA in a non-translated state poses several biological problems for the oocyte and early embryo. In the immature frog oocyte, a wide array of messages are translationally silent in the context of a translationally competent cytoplasmic environment. How specific mRNAs remain repressed and then selectively become released into the translational machinery remains poorly understood.

In this report, we focused on the translational inhibition of the XFGFR maternal mRNA in the immature oocyte. Like many dormant mRNAs in the frog oocyte, the XFGFR becomes recruited for translation during progesterone-induced meiotic maturation, but how this is achieved is unknown. Our experimental system took advantage of both the translationally promiscuous state of the immature oocyte, and the conditions that maintain dormancy of endogenous XFGFR transcripts. Injection of synthetic reporter mRNAs into immature oocytes allowed the analysis of XFGFR 3' untranslated sequences for their ability to inhibit translation. We demonstrate that a small region of the XFGFR 3' UTR, the translation inhibitory element or TIE, is sufficient to inhibit translation of upstream coding sequences of synthetic XFGFR mRNA. A heterologous reporter mRNA was also inhibited from translation by the inclusion of the TIE, demonstrating that this repression is not due to characteristics imposed by the coding sequence. Instead, translational repression appears to be due to a factor, or factors, supplied by the oocyte which interact with the TIE.

We have shown that TIE-mediated repression of translation is reversed at meiotic maturation, mimicking the repression and subsequent activation of endogenous XFGFR mRNA that occurs during oogenesis. In addition, translational activation is not dependent on changes in the adenylation state of the synthetic message. Furthermore, we provide evidence for specific protein binding to the TIE, and propose that transla-

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**Fig. 7.** TIE binds oocyte cytoplasmic protein. (A) Gel shift analysis of TIE with immature oocyte S100 extract. S100 extract was incubated with 32P-labelled probe and digested with RNase T1. Reactions were separated on 6% non-denaturing PAGE and exposed to X-ray film. XFR control, 180 nt stretch of coding sequence. (B) UV crosslinking of radiolabelled TIE to bound protein from oocyte S100 extract. Cytoplasmic extracts were incubated with 32P-labelled TIE with or without unlabelled competitor RNA. Left panel, 0, 10 and 100-fold molar excess non-labelled TIE RNA. Middle panel, 10 and 100-fold molar excess of unlabelled XFR control. Right panel, immunoblot of transferred TIE-S100 extract crosslinking reaction (from lane 1, left panel) and control oocyte S100 extract (25 μg) alone, probed with anti-p54/56 antibody (Murray et al., 1992).
tional repression of the XFGFR mRNA in the developing oocyte is achieved through RNA-protein interactions.

**How does the TIE inhibit translation?**

An emerging theme in the regulation of translation is the involvement of cis-acting control elements contained within the mRNA transcript. Elements located in the 3' UTR of mRNAs have been found to participate in the orchestrated control of both temporal and regional translation from a wide spectrum of organisms (reviewed in Wickens, 1992). For example, the switch from spermatogenesis to oogenesis in the C. elegans hermaphrodite germ line is regulated by the translational inhibition of the sex-determining gene fem-3. The 3' UTR of fem-3 mRNA is thought to bind a translational inhibitory protein and this repression is associated with deadenylation of the poly(A) tail (Ahringer and Kimble, 1991). Recent work on the regulation of glp-1, an important regulator of nematode embryonic patterning, has shown the presence of both temporal and spatial translational control elements within the 3' UTR of the maternal mRNA (Evans et al., 1994).

Several mechanisms are likely to be involved in translational repression or activation by elements in the 3' UTR (reviewed by Spirin, 1994). In frogs, as well as several other species, changes in the adenylation state of mRNA is associated with translational regulation. The regulation of poly(A) tail lengthening during oocyte maturation is dependent on the presence of specific sequences in the 3' UTR (Fox et al., 1989; Paris and Richter, 1990). For example, translation of Xenopus c-mos and cyclins, critical regulators of meiosis, requires 3' UTR mediated polyadenylation (Sheets et al., 1994). In addition, control of translation may involve the subcellular sequestration or regional localization of transcripts (Gavis and Lehmann, 1994; Mowry and Melton, 1992), and message-specific RNA degradation (Brown et al., 1993). However, a characteristic feature of frog maternal mRNAs is their association with protein (reviewed by Sommerville, 1992). These nontranslated messenger ribonucleoproteins (mRNPs) are thought to remain dormant because some property of the complex prevents entry into the translational pathway. The abundant Y-box proteins (or p54/56) are nonspecific and appear to be associated with all dormant mRNAs in the oocyte (Murray et al., 1992; Tafuri and Wolffe, 1993). Although expression of these proteins in a frog somatic cell line has led to overall translational inhibition (Ranjjan et al., 1993), merely the association of oocyte Y-box proteins with dormant RNAs may not be sufficient for translational inhibition, since in the germ line these proteins are associated with both translationally active and inactive mRNAs (Tafuri and Wolffe, 1993).

We have demonstrated, in addition to Y-box proteins, the specific binding of a 43x10^3 M_r protein to the 180 nucleotide TIE of the XFGFR 3' UTR. These findings suggest that, in addition to the abundant Y-box proteins, other factors are likely to be involved in XFGFR translational repression. These data are consistent with the general concept that specific mRNA-protein interactions mediate the masking/unmasking process in the oocyte and early embryo. Recent work by Bouvet and Wolffe (1994) has shown that Y-box proteins facilitate complete translational repression of in vivo transcribed mRNAs in oocytes compared to injected synthetic mRNAs. This suggests that packaging of nascent mRNAs with RNA-binding proteins takes place more efficiently during the process of transcription. Therefore the incomplete repression of TIE-mediated translation that we observed following injection of in vitro transcribed message (Fig. 6), may reflect incomplete or inefficient interaction with Y-box proteins and potentially the 43x10^3 M_r protein, which specifically binds the TIE. We hypothesize that the TIE inhibits XFGFR translation by one of two mechanisms. Either the TIE binds specific protein(s) that maintain an mRNP form of XFGFR mRNA that is inaccessible to the translational apparatus, or the TIE targets XFGFR mRNA to a specific cytoplasmic compartment that limits access to translation. Recently, a protein interaction with a 3' UTR element of rabbit erythroid lipoygenase mRNA has been shown to repress the initiation of translation in reticulocytes (Ostareck-Lederer et al., 1994). How this interaction causes inhibition of translation is unknown.

Poly(A) removal during frog oocyte maturation is associated with the inactivation of certain maternal mRNA transcripts (Varnum and Wormington, 1990). Whether prevention of adenylation, or active deadenylation, of maternal mRNAs causes translational repression in frogs remains an open question. In mouse oocytes, for example, deadenylation of nascent tissue plasminogen activator mRNAs renders these transcripts dormant until meiosis (Huae et al., 1992). Therefore, TIE-mediated deadenylation or prevention of adenylation of XFGFR, in the immature oocyte, remained a potential mechanism by which to inhibit premature XFGFR translation. We tested synthetic transcripts that were minus poly(A) tracts, and known poly(A) addition signals, and therefore, not likely to be deadenylated following injection into the immature oocyte. In these transcripts, translation was still regulated by the presence or absence of the TIE. Therefore, the state of translational repression, mediated by the negative regulatory element, appears to be independent of message adenylation.

**Is there a link between translational inhibition and activation?**

Cytoplasmic poly(A) lengthening of several maternal mRNAs in frog oocytes and embryos accompanies their translational activation and is dependent on 3' UTR regulated polyadenylation (Sheets et al., 1994). However, exceptions to this general scheme have also been observed. For example, the poly(A) tail of Xenopus histone mRNA undergoes shortening during translational activation in early development (Ruderman et al., 1979). During mouse spermiogenesis, poly(A) shortening is associated with translational activation of several repressed mRNAs (Kleene, 1989). In contrast, translational activation of nanos maternal mRNA during Drosophila embryogenesis, is not associated with polyadenylation (Salles et al., 1994). In addition the possibility exists that either certain messages are activated through another mechanism entirely or, as suggested by others (Sheets et al., 1994), polyadenylation might be a consequence of activation, activation that is initiated by an independent mechanism.

In this report, we have shown that the activity of the XFGFR TIE mimics the state of endogenous maternal XFGFR mRNA during oogenesis. Inhibition of translation prior to oocyte maturation is followed by translational activation at meiosis. We observed that the translational activation of synthetic XFGFR mRNA occurred without apparent changes in the adenylation state of the message. This suggests that the release of inhibi-
tion imposed by the XFGFR TIE may be independent from the eventual poly(A) lengthening that endogenous XFGFR mRNA undergoes at maturation. Perhaps the extent of translation that we observed at oocyte maturation with synthetic message was limited by the inability of released transcripts to undergo poly(A) lengthening (Fig. 6A). Further experiments aimed at determining the relationship between these two events, release of inhibition and polyadenylation, would be required to answer this question. However in the case of XFGFR-1 mRNA, reversing the action of the TIE appears to be necessary before maturation-dependent translation occurs. The role that polyadenylation plays in this activation, if any, requires further investigation.

Translational regulation in embryogenesis

Previous work has established that FGF signalling is involved in mediating early patterning events during embryogenesis (Amaya et al., 1991, 1993). In animals, such as frogs, that depend on maternal mRNA stores, key events in development, therefore, depend on the coordinated translational control of the mRNA components of signalling systems such as FGF. The embryonic regulation of specific FGF signalling components may depend on other elements of control. For example: besides temporal regulation during oogenesis, regional specific translation of receptor mRNA may determine FGF competence in temporal regulation during oogenesis, regional specific trans-


Ostareck-Lederer, A., Ostareck, D. H., Standart, N. and Thiele, B. J. (1994). Translation imposed by the XFGFR TIE may be independent from the eventual poly(A) lengthening that endogenous XFGFR mRNA undergoes at maturation. perhaps the extent of translation that we observed at oocyte maturation with synthetic message was limited by the inability of released transcripts to undergo poly(A) lengthening (Fig. 6A). Further experiments aimed at determining the relationship between these two events, release of inhibition and polyadenylation, would be required to answer this question. However in the case of XFGFR-1 mRNA, reversing the action of the TIE appears to be necessary before maturation-dependent translation occurs. The role that polyadenylation plays in this activation, if any, requires further investigation.

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