

Translational inactivation of ribosomal protein mRNAs during *Xenopus* oocyte maturation

Linda E. Hyman¹ and W. Michael Wormington

Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254 USA

Ribosomal protein synthesis ceases upon maturation of *Xenopus* oocytes. We find that this cessation results from the dissociation of ribosomal protein mRNAs from polysomes and is accompanied by the deadenylation of these transcripts. A synthetic mRNA encoding ribosomal protein L1, microinjected into stage VI oocytes, is deadenylated and released from polysomes upon maturation. Our results indicate that sequences located within 387 bp of the 3' terminus of L1 mRNA direct both the deadenylation and polysomal release of this ribosomal protein mRNA. The proper translational regulation of an exogenous ribosomal protein mRNA in microinjected oocytes provides a basis for determining the sequence specificity for the differential utilization of maternal mRNAs during oocyte maturation.

[Key Words: Ribosomal proteins; *Xenopus*; oocyte maturation; synthetic mRNAs; deadenylation; translational control]

Received January 28, 1988; revised version accepted March 21, 1988.

The synthesis of ribosomal proteins during *Xenopus* oogenesis contributes to the production of a maternal ribosome pool that is sufficient to support protein synthesis throughout embryogenesis. This elevated accumulation of ribosomal proteins is due largely to the preferential translation of ribosomal protein mRNAs (rp-mRNAs) in vitellogenic stage III oocytes when ribosomal proteins comprise ~20% of total protein synthesis (Dixon and Ford 1982; Baum and Wormington 1985; Cardinali et al. 1987). In contrast, synthesis of the majority of ribosomal proteins during embryogenesis is not observed until development of the tail-bud embryo. Previous studies by Pierandrei-Amaldi and colleagues (1982) and Baum and Wormington (1985) have shown that two principal factors contribute to the absence of ribosomal protein synthesis in early embryonic development. First, maternal rp-mRNAs are degraded following fertilization. Second, although zygotic rp-mRNAs begin to accumulate during early gastrulation, these transcripts are excluded from polysomes until the tail-bud stage. Thus, the onset of ribosomal protein synthesis in both oogenesis and embryogenesis is regulated by selective rp-mRNA utilization.

We are interested in determining the mechanisms that direct the transition from active ribosomal protein synthesis during oogenesis to the translational inactivity and instability of these maternal mRNAs following fertilization. The process of oocyte maturation comprises

several events likely to participate in the cessation of ribosomal subunit assembly. The induction of maturation initiates a series of physiological changes, including the resumption of meiosis, breakdown of the nuclear envelope, chromosome condensation, and repression of transcription by all three classes of nuclear RNA polymerase (reviewed in Maller 1985). The absence of both rp-mRNA and rRNA transcription and precursor rRNA processing (Busby and Reeder 1982), in particular, precludes the assembly of ribosomal subunits in mature oocytes.

In this paper we have examined the regulation of ribosomal protein synthesis during oocyte maturation. Our results indicate that the majority of ribosomal proteins are not synthesized in mature oocytes, despite a twofold increase in overall protein synthesis observed upon induction of maturation (Woodland 1974; Richter et al. 1982). This translational inactivity results from the release of rp-mRNAs from polysomes and is accompanied by the deadenylation of these transcripts. An SP6-derived, polyadenylated mRNA encoding ribosomal protein L1 is actively translated upon microinjection into stage VI oocytes. Our results show that this SP6 L1 mRNA is deadenylated and released from polysomes upon oocyte maturation in parallel with the translational inactivation of endogenous rp-mRNAs. This indicates that the exogenous rp-mRNA is subject to proper translational regulation during oocyte maturation. Our results show that sequences located within 387 bp of the 3' terminus of L1 mRNA, when fused to a heterologous mRNA, direct both deadenylation and polysomal dissociation during oocyte maturation.

¹Present address: Department of Molecular Biology and Microbiology, Tufts University, School of Medicine, Boston, Massachusetts 02111 USA.

Results

Ribosomal proteins are not synthesized in mature oocytes

The induction of oocyte maturation results in an increased recruitment of mRNAs onto polysomes, leading to a twofold increase in protein synthesis (Woodland 1974; Richter et al. 1982). Therefore, we determined whether the synthesis of ribosomal proteins is elevated in parallel with overall translation in mature oocytes. Fully grown stage VI oocytes and oocytes matured in vitro with progesterone were metabolically labeled by incubation in modified Barth's solution (MBS) containing [³⁵S]methionine. Total protein was extracted and analyzed by electrophoresis in a two-dimensional gel system that resolves basic proteins (Baum and Wormington 1985). The autoradiograms in Figure 1A and B, show the patterns of basic proteins synthesized in stage VI and mature oocytes, respectively. Ribosomal proteins comprise a relatively significant fraction of the proteins synthesized in stage VI oocytes. In contrast, the overwhelming majority of these proteins are not among the basic proteins synthesized in mature oocytes. Only two 40S ribosomal proteins, S2 and S4, continue to be synthesized in mature oocytes. None of the other ³⁵S-labeled basic proteins shown in Figure 1B comigrate with proteins purified from ovarian ribosomes in this two-dimensional gel system. The absence of ribosomal protein synthesis in mature oocytes does not reflect a generalized reduction in the production of basic proteins. For example, the synthesis of Xlo 63, a basic, nonribosomal protein, increases at least twofold in response to maturation. Figure 1B also shows clearly that maturation results in a marked translational recruitment of core histone mRNAs, as has been reported previously (Adamson and Woodland 1977). These results indicate that the majority of rp-mRNAs do not contribute to the elevated level of protein synthesis in mature oocytes. The absence of ribosomal protein synthesis, in conjunction with the cessation of both rRNA transcription and processing of precursor rRNA sequences (Busby and Reeder 1982), effectively terminates the de novo assembly of ribosomal subunits in mature oocytes.

rp-mRNAs dissociate from polysomes in mature oocytes

Previous studies have shown that the levels of maternal rp-mRNAs decline after fertilization (Pierandrei-Amaldi et al. 1982; Baum and Wormington 1985). However, the behavior of these transcripts during maturation was not examined. Therefore, we compared the levels of these mRNAs and the extent of their polysomal association in stage VI and mature oocytes. RNA was extracted from polysomal and nonpolysomal [ribonucleoprotein (RNP)] fractions, prepared as described in detail elsewhere (Baum et al. 1988), and the relative distribution of rp-mRNAs L1, L14, L15, and S1 was determined by Northern blot analyses. As shown in Figure 2, these four rp-mRNAs are about evenly distributed between RNP

and polysomal fractions isolated from stage VI oocytes. Upon maturation, the levels of these four transcripts are not significantly reduced. However, all four rp-mRNAs

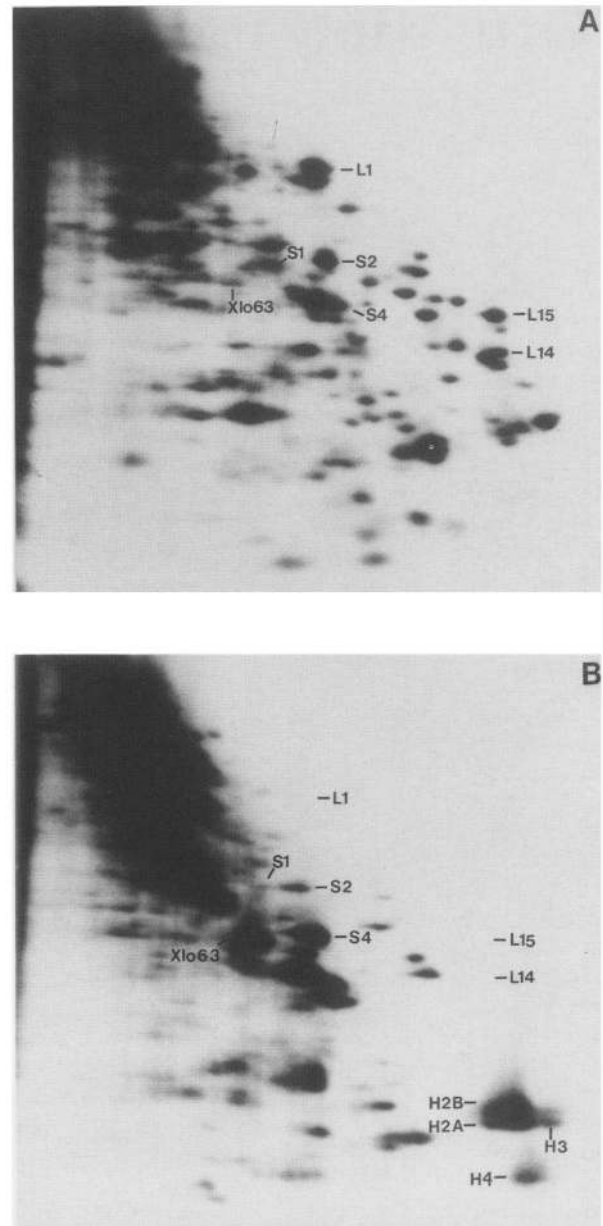
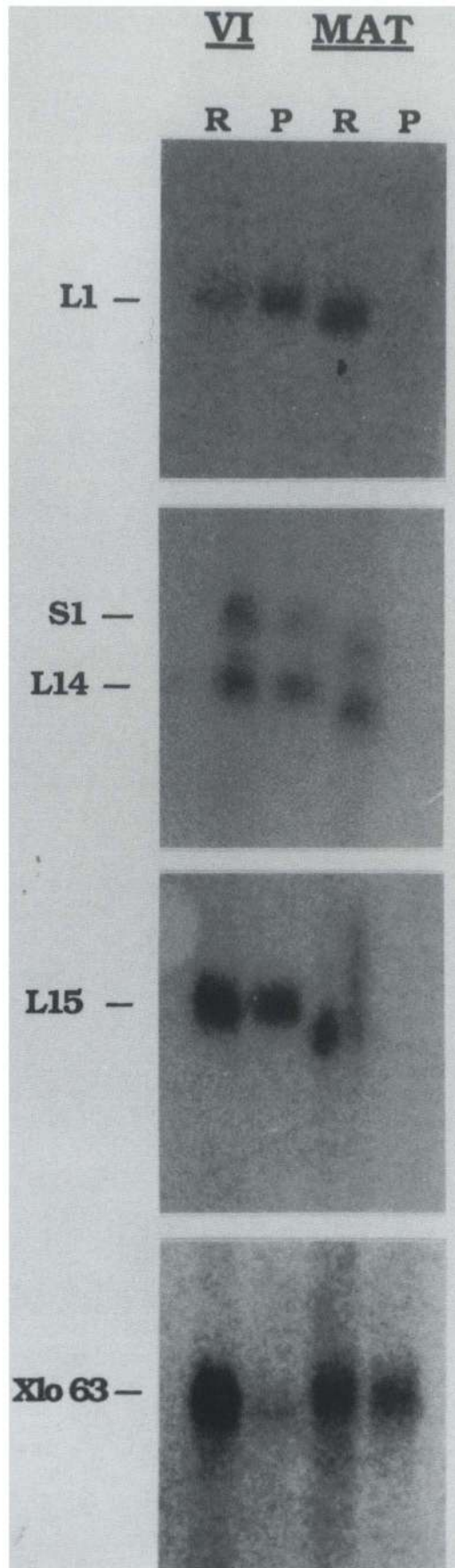


Figure 1. Ribosomal proteins are not synthesized in mature oocytes. Two-dimensional gel electrophoresis of basic proteins synthesized in stage VI (A) and mature (B) oocytes. Each autoradiogram represents 10⁶ cpm of ³⁵S-labeled proteins equivalent to the total protein of one stage VI, or 0.5 mature oocytes. Conditions for two-dimensional gel electrophoresis have been described previously (Baum and Wormington 1985). Ribosomal proteins were identified by their comigration with proteins extracted from ribosomal subunits and stained with Coomassie blue. The positions of representative ribosomal proteins L1, S1, S2, S4, L14, and L15 are indicated. Nonribosomal protein Xlo 63 and the core histones are also indicated. Electrophoresis in the first dimension acid-urea gel is from left to right, and in the second dimension SDS-polyacrylamide gel is from top to bottom.



are dissociated from polysomes and have been quantitatively shifted to the nontranslating RNP fraction (Fig. 2). To exclude the possibility that polysomes isolated from mature oocytes are unusually susceptible to degradation, we examined the same RNA preparations to determine the RNP-polysome distribution of Xlo 63 mRNA, which is actively translated in both stage VI and mature oocytes. As shown in Figure 2, the mRNA corresponding to Xlo 63 is associated with polysomes from both oocyte stages. Indeed, it appears that the polysomal association of this mRNA has increased upon maturation. The release of rp-mRNAs from polysomes in mature oocytes contrasts with the increased translational recruitment of the overall oocyte mRNA population and illustrates the distinct translational regulation encompassing this class of maternal mRNAs.

Deadenylation of rp-mRNAs in mature oocytes

The results in the previous section indicate that the dissociation of rp-mRNAs from polysomes does not lead to a concomitant reduction in the levels of these transcripts in mature oocytes. Examination of the Northern blots shown in Figure 2, however, reveals that in contrast to Xlo 63 mRNA, rp-mRNAs isolated from mature oocytes migrate more rapidly in denaturing formaldehyde gels, consistent with a reduction in their size. This increased electrophoretic mobility approximates the shift predicated for removal of the poly(A) tracts from the 3' termini of these rp-mRNAs. Previous studies have demonstrated that the total amount of poly(A) is reduced in unfertilized eggs relative to stage VI oocytes (Darnbrough and Ford 1976; Sagata et al. 1980). Therefore, we determined whether the altered mobility of these transcripts is due to their deadenylation. Total RNA extracted from stage VI and mature oocytes was fractionated by oligo(dT)-cellulose chromatography, and the distribution of L1 and Xlo 63 mRNAs in the poly(A)⁺ and poly(A)⁻ fractions was determined by Northern blot analysis. As shown in Figure 3, L1 mRNA from stage VI oocytes is found exclusively within the poly(A)⁺ fraction. An equivalent amount of L1 mRNA is recovered from mature oocytes but is now found almost entirely within the poly(A)⁻ fraction. This result is also observed for other rp-mRNAs (data not shown). L1 mRNA in mature oocytes appears to have undergone extensive deadenylation because it is not retained on oligo(dT)-cellulose under conditions in which a minimal poly(A) tail consisting of 20–30 residues is capable of quantitative binding (Nudel et al. 1976). L1 mRNA isolated from mature oocytes also does not bind to

Figure 2. Ribosomal protein mRNAs are dissociated from polysomes in mature oocytes. RNA was extracted from nonpolysomal (R) and polysomal (P) fractions from stage VI and mature oocytes, electrophoresed on a 1% agarose-formaldehyde gel, and blotted onto nitrocellulose. Filters were hybridized with ³²P-labeled RNA probes specific for mRNAs L1, S1, L14, L15, and Xlo 63. Each lane contains the RNA equivalent to the polysomal or nonpolysomal fraction of two oocytes.

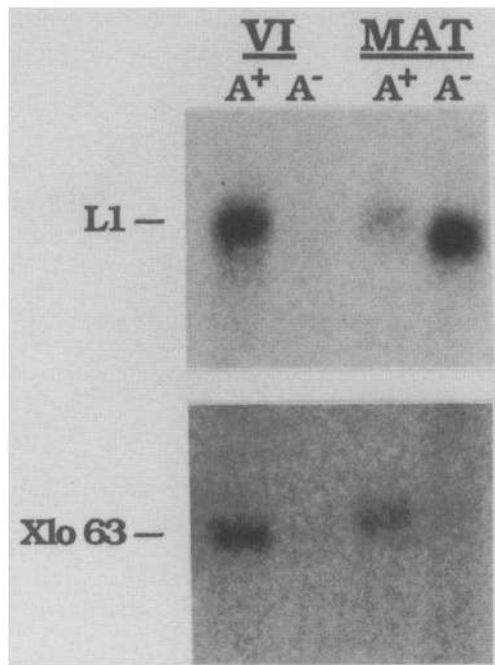


Figure 3. Deadenylation of rp-mRNAs in mature oocytes. Total RNA was extracted from stage VI and mature oocytes and fractionated by oligo(dT)-cellulose chromatography. RNA present in the flow-through (A⁻) and bound (A⁺) fractions was electrophoresed on a 1% agarose-formaldehyde gel, transferred to nitrocellulose, and hybridized with ³²P-labeled RNA probes specific for mRNAs L1 and Xlo 63. Each lane contains the RNA equivalent to the (A⁻) or (A⁺) fraction of two oocytes.

poly(U)-Sepharose (data not shown), providing additional evidence for the loss of the poly(A) tract from this transcript. Interestingly, Xlo 63 mRNA, which is efficiently translated in both stage VI and mature oocytes, undergoes an apparent lengthening of its poly(A) tract upon induction of maturation (Fig. 3). These results indicate that a strong correlation exists between the dissociation of rp-mRNAs from polysomes during maturation and the loss of poly(A) tracts from these transcripts.

Translational inactivation of an exogenous rp-mRNA during oocyte maturation

To determine the involvement of *cis*-acting sequences in the translational inactivation of rp-mRNAs, we have examined the regulation of exogenous transcripts during maturation of microinjected oocytes. Polyadenylated L1 and β -globin mRNAs were transcribed *in vitro*, using SP6 RNA polymerase (Fig. 4). Previous studies have shown that each of these SP6 transcripts is translated in a dosage-dependent manner in microinjected stage VI oocytes (Baum et al. 1988). As shown in Figure 5, SP6 L1 mRNA is predominantly associated with polysomes following its microinjection into stage VI oocytes. *In vitro* maturation of these microinjected oocytes results in dissociation of the majority of SP6 L1 mRNA from polysomes (Fig. 5). The SP6 L1 mRNA that is associated with RNPs in mature oocytes has also undergone a reduction in length that is consistent with its deadenylation. Interestingly, the very small proportion of SP6 L1 mRNA

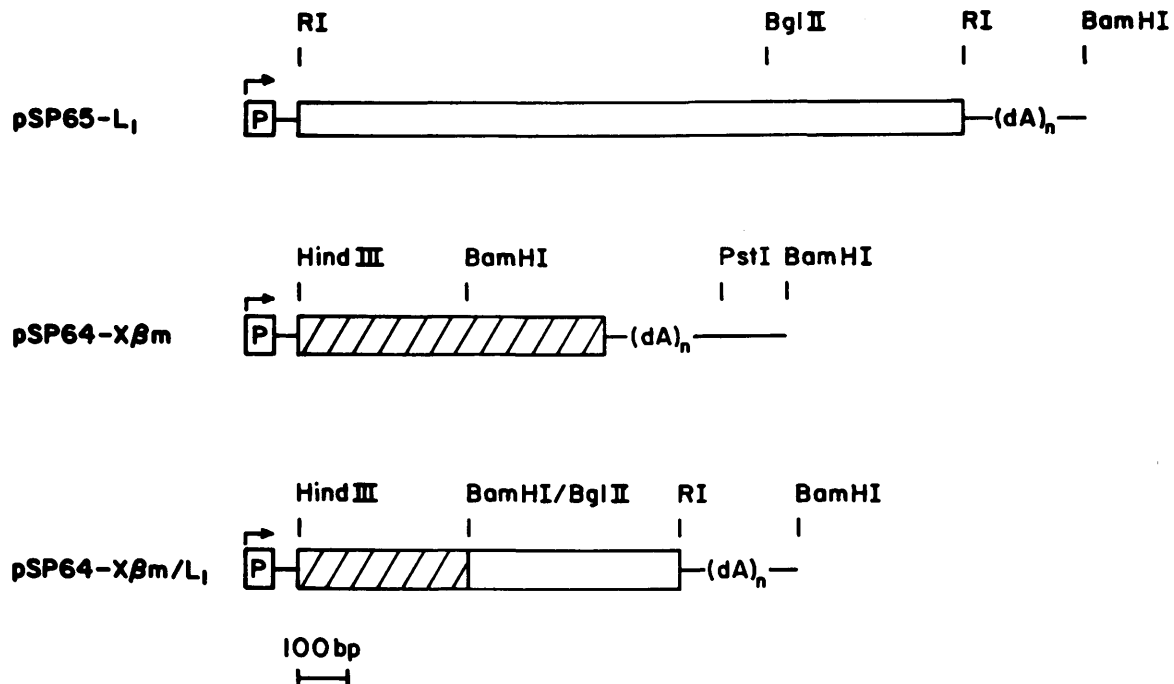


Figure 4. SP6 plasmids used as templates for mRNA synthesis. (P) The SP6 promoter; (→) the direction of transcription. L1 and globin sequences are indicated by the open and hatched boxes, respectively. The construction of the fusion plasmid pSP64-X β m/L1 is described in Methods.

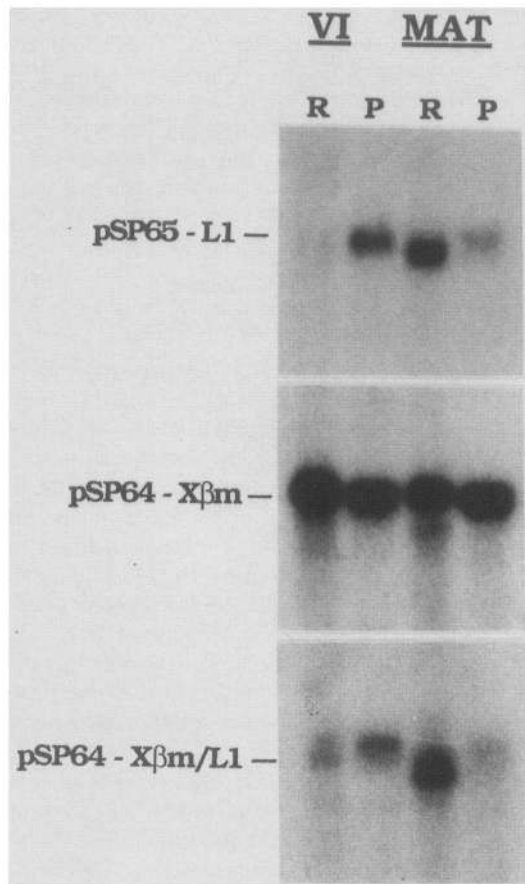


Figure 5. Polysomal association of SP6 mRNAs in stage VI and mature oocytes. Five nanograms of each ^{32}P -labeled RNA was microinjected into the cytoplasm of stage VI oocytes. Oocytes were incubated for 8 hr in MBS following microinjection. Injected oocytes were then incubated for an additional 12 hr in either the absence or presence of 1 $\mu\text{g}/\text{ml}$ of progesterone. RNA was extracted from nonpolysomal (R) and polysomal (P) fractions of stage VI and mature oocytes and was electrophoresed on a 1% agarose-formaldehyde gel, which was dried prior to autoradiographic exposure. Each lane contains the RNA equivalent to the polysomal or nonpolysomal fraction of two oocytes.

that remains polysomal in mature oocytes is not reduced in length, providing further evidence that deadenylation is associated with the polysomal release of these transcripts.

To confirm that the reduced size of the SP6 L1 mRNA following maturation is, in fact, due to its deadenylation, total RNA extracted from microinjected stage VI and mature oocytes was hybridized with oligo(dT)₁₂₋₁₈ and digested with RNase H to remove the poly(A) tracts. As shown in Figure 6, *in vitro* deadenylation with RNase H reduces the length of SP6 L1 mRNA recovered from stage VI oocytes but does not alter the size of the same transcript isolated from mature oocytes. The size of SP6 L1 mRNA isolated from mature oocytes is virtually identical to that observed for stage VI oocyte RNA digested with RNase H, indicating that deadenylation accounts for the size reduction of the exogenous L1 transcript during maturation. This result indicates that the

exogenous SP6 L1 mRNA is subject to both polysomal release and deadenylation, as is the cognate endogenous L1 mRNA.

To ascertain the selectivity of the inactivation of microinjected mRNAs during maturation, we examined the behavior of the SP6 β -globin mRNA. The results shown in Figure 5 indicate that SP6 β -globin mRNA is associated with polysomes in both stage VI and mature oocytes, in agreement with previous studies of native globin mRNA by Richter et al. (1982). Furthermore, no reduction in the length of SP6 β -globin mRNA is observed upon maturation. As shown in Figure 6, RNase H digestion of RNA isolated from microinjected stage VI and mature oocytes indicates that the SP6 β -globin mRNA remains polyadenylated following maturation. Thus, in contrast to the SP6 L1 mRNA, the SP6 β -globin transcript is neither released from polysomes nor deadenylated in response to oocyte maturation.

To localize sequences within L1 mRNA that are responsible for its translational inactivation during oocyte maturation, we constructed a hybrid SP6 template containing sequences derived from the L1 and β -globin cDNAs. Figure 4 shows the structure of pSP64-X β m/L1, which directs synthesis of a hybrid transcript containing 300 bp of the 5' end of the β -globin mRNA fused in frame to 387 bp of the 3' end of L1 mRNA. Figure 5 shows that this fusion transcript is efficiently recruited onto polysomes upon microinjection into stage VI oocytes. The induction of maturation results in the polysomal dissociation of the fusion mRNA, just as is observed for the SP6 L1 mRNA (Figs. 5 and 6). Moreover, the fusion transcript is deadenylated in mature oocytes analogous to the behavior of SP6 L1 mRNA (Fig. 6). These results indicate that sequences present within 387 bp of the 3' terminus of L1 mRNA are sufficient to direct translational inactivation during oocyte maturation when fused to a heterologous mRNA.

Discussion

Deadenylation and the translational inactivation of rp-mRNAs during oocyte maturation

The translation of rp-mRNAs comprises a significant proportion of overall protein synthesis during oogenesis (Baum and Wormington 1985; Cardinali et al. 1987). The results presented here demonstrate that the induction of oocyte maturation results in the cessation of ribosomal protein synthesis. The absence of these proteins, the generalized repression of transcription, and the inability to process preexisting rRNA precursors effectively terminates the further assembly of ribosomal subunits in mature oocytes. Our results show that the absence of ribosomal protein synthesis in mature oocytes is due to the translational inactivation of these mRNAs. Although rp-mRNAs are efficiently translated in stage VI oocytes, these transcripts are quantitatively released from polysomes and stably sequestered as nontranslating RNPs in mature oocytes. This loss of translational activity is accompanied by the extensive deadenylation of rp-mRNAs. Additional studies are clearly required in

order to determine the causal relationship between these two events. The deadenylation of these mRNAs does not lead to their immediate degradation. The instability of deadenylated rp-mRNAs is not apparent until after

fertilization and continues throughout early embryogenesis preceding the onset of new rp-mRNA synthesis (Pierandrei-Amaldi et al. 1982; Baum and Wormington 1985).

Previous studies by Darnbrough and Ford (1976) and Sagata et al. (1980) showed that the total amount of poly(A) is reduced by ~40% upon oocyte maturation, whereas the level of total RNA remains unchanged. Colot and Rosbash (1982) demonstrated that this reduction is due to the selective deadenylation of a subset of oocyte mRNAs. Taking into account the levels of individual rp-mRNAs (Baum and Wormington 1985), our results indicate that these transcripts as a group may comprise as much as 25% of the total deadenylated maternal mRNA population. Interestingly, abundant proteins such as cytoskeletal actins (Sturgess et al. 1980; Hyman et al. 1982) and translation elongation factor 1 α (P. Krieg, D. Melton, S. Varnum, and M. Wormington, unpubl.), which are actively synthesized in stage VI oocytes, are no longer produced after maturation. The mRNAs encoding these proteins are also deadenylated in mature oocytes. It appears that deadenylation may be a central mechanism in the translational inactivation of these 'housekeeping' maternal mRNAs. The relationship between deadenylation and translational inactivation of specific maternal mRNAs is not unique to *Xenopus* oocyte maturation. Extensive studies of mRNAs in *Spisula* oocytes by Ruderman and colleagues have shown that an excellent correlation exists between the translation of individual maternal mRNAs and their polyadenylation states (Rosenthal et al. 1983; Rosenthal and Ruderman 1987).

Proper regulation of an exogenous rp-mRNA during oocyte maturation

To investigate the translational inactivation of rp-mRNAs during maturation, we have demonstrated that an exogenous L1 mRNA is correctly regulated in microinjected oocytes. An SP6 L1 mRNA is efficiently translated upon microinjection into stage VI oocytes (Baum et al. 1988). In vitro maturation of these oocytes results in both the deadenylation and polysomal dissociation of the SP6 L1 transcript that parallels the inactivation of endogenous L1 mRNA. This represents the first demonstration that an injected RNA is subject to the same translational regulation that controls the cognate, endogenous oocyte mRNA. Our results indicate that sequences present within 387 bp of the 3' end of L1 mRNA specify both deadenylation and polysomal dissociation when fused to a heterologous, β -globin mRNA. This suggests that the RNA processing activity that deadenylates rp-mRNAs in mature oocytes recognizes *cis*-acting sequences present within this region of the L1 mRNA. As is observed for endogenous rp-mRNAs, the release of the SP6 L1 and SP6 globin/L1 fusion mRNAs from polysomes appears to be tightly associated with the removal of the 3' poly(A) tracts from these transcripts.

The deadenylation of the SP6 L1 mRNA upon maturation of injected oocytes provides an *in vivo* assay to char-

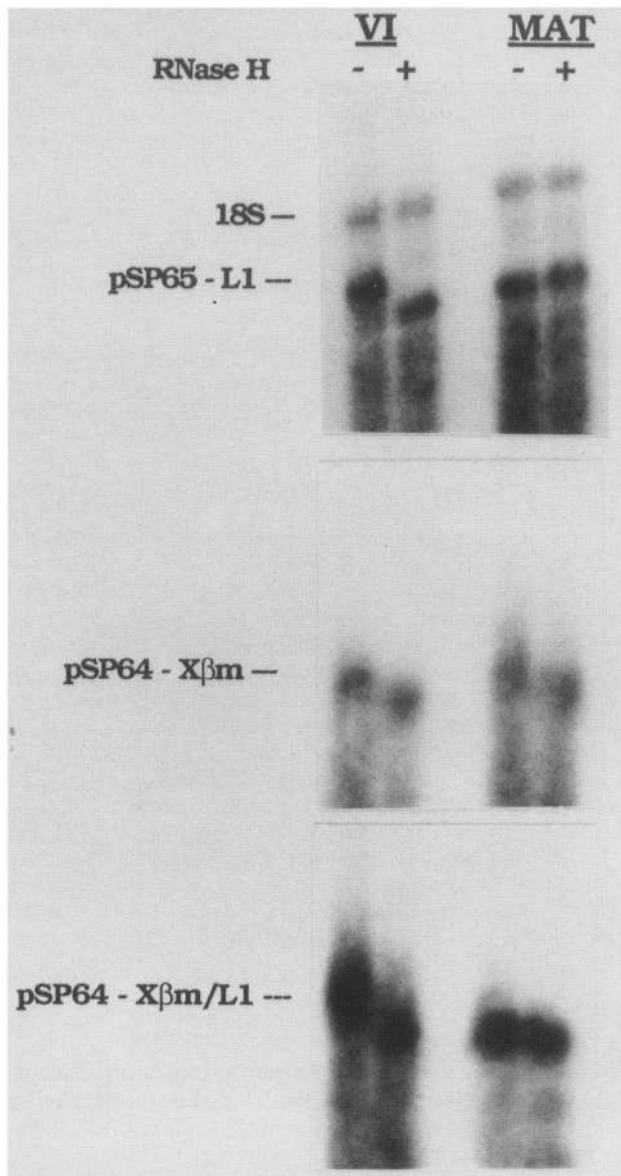


Figure 6. Deadenylation of exogenous L1 and globin/L1 fusion mRNAs during oocyte maturation. Five nanograms of each ^{32}P -labeled RNA was microinjected into the cytoplasm of stage VI oocytes. Oocytes were incubated for 8 hr in MBS following microinjection. Injected oocytes were then incubated for an additional 12 hr in either the absence or presence of 1 $\mu\text{g}/\text{ml}$ of progesterone. Total RNA was extracted from stage VI and mature oocytes. The RNA equivalent to two oocytes was hybridized to oligo(dT) $_{12-18}$ and incubated with or without RNase H. RNAs were electrophoresed on a 1% agarose-formaldehyde gel, which was dried prior to autoradiographic exposure. The position of 18S rRNA is indicated in samples recovered from oocytes microinjected with pSP65-L1 RNA. The endogenous rRNA was labeled by incorporation of residual [α - ^{32}P]UTP present in this RNA sample.

acterize the requirements for this RNA processing event. The mechanism for deadenylation of eukaryotic mRNAs has not been defined. This is in contrast to a number of studies that have clearly demonstrated that 3'-end formation of mammalian mRNAs is a nuclear event involving an endonucleolytic cleavage and subsequent polyadenylation. The hexanucleotide sequence AAUAAA is essential in specifying the site of 3'-end formation (reviewed in Birnstiel et al. 1985). The possible role of this conserved sequence, or other *cis*-acting sequences present within the 3'-terminal region of L1 mRNA in the deadenylation reaction, remains to be determined. It is important to note that although 3'-end formation and polyadenylation of primary transcripts occurs in the nucleus, the deadenylation of rp-mRNAs during oocyte maturation is a cytoplasmic event. Preliminary experiments indicate that deadenylation of rp-mRNAs cannot be detected prior to germinal vesicle breakdown (L.E. Hyman, unpubl.). One consequence of nuclear envelope dissolution could be the release of a putative RNA processing activity into the cytoplasm where it would selectively deadenylate transcripts containing the appropriate recognition sequences.

Two potential, although not mutually exclusive, mechanisms can be postulated to address the contribution of deadenylation to the translational inactivation of rp-mRNAs. First, the role of polyadenylation in enhancing the translational efficiency of heterologous mRNAs (Drummond et al. 1985), as well as ribosomal protein L1 mRNA (Baum et al. 1988), in microinjected oocytes has been well documented. Jacobson and colleagues have proposed that the poly(A) tract has a positive function by interacting with ancillary proteins which, in turn, facilitate efficient translation initiation (Jacobson and Favreau 1983; Palatnik et al. 1984). Thus, the loss of the poly(A) tract and its associated factors could reduce drastically the translational efficiency of deadenylated rp-mRNAs relative to polyadenylated transcripts recruited onto polysomes during maturation. Second, translational inactivation may not be due to removal of the poly(A) tract per se but, instead, reflects the potential contribution of negative *cis*-acting elements whose influence would be circumvented by the normal presence of a poly(A) tract. *Cis*-acting sequences that reduce translational efficiency have been identified recently by Krays et al. (1987) within the 3'-untranslated region of the human interferon- β mRNA. It is interesting to note that highly conserved sequences have been identified within the 3'-untranslated regions of the *Xenopus* ribosomal protein genes sequenced to date (Lorenz et al. 1985; Beccari et al. 1986). The presence of these conserved sequences renders them initial candidates to be assayed for their contribution to the translational inactivation of these mRNAs during maturation.

Methods

Oocyte microinjection, in vitro maturation, and protein analysis

Fully grown stage VI oocytes were isolated from mature

Xenopus laevis ovaries, defolliculated with collagenase, and maintained in MBS (Gurdon 1976) at 18°C. Maturation was induced by the addition of progesterone to a final concentration of 1 μ g/ml. Maturation was ascertained by the presence of a white spot in the animal hemisphere indicative of germinal vesicle breakdown. Newly synthesized proteins were metabolically labeled by incubation of stage VI and mature oocytes in MBS containing [³⁵S]methionine at 2 mCi/ml for 6 hr. Proteins labeled with ³⁵S were isolated from oocytes and analyzed by two-dimensional gel electrophoresis, as described previously (Baum and Wormington 1985). SP6 mRNAs were dissolved in 88 mM NaCl and microinjected into the cytoplasm of stage VI oocytes. Microinjected oocytes were incubated in MBS for 8 hr prior to the addition of progesterone in order to allow the exogenous transcripts to reach a steady-state level of polysomal association in stage VI oocytes (Baum et al. 1988).

Plasmid DNAs and in vitro transcription with SP6 RNA polymerase

pSP65-L1, containing a cDNA corresponding to ribosomal protein L1a (Lorenz et al. 1985), inserted at the *EcoRI* site of pSP65AT (Baum et al. 1988) was linearized with *Bam*HI for use as a template for in vitro transcription. pSP64-X β m, containing a *Xenopus* β -globin cDNA (Melton et al. 1984), was linearized at the *Pst*I site, and the 3'-protruding ends were removed by digestion with T4 DNA polymerase prior to in vitro transcription. To construct the fusion plasmid pSP64-X β m/L1, pSP65-L1 DNA was digested with *Bgl*II and *Bam*HI to isolate a 570-bp fragment containing 387 bp of the 3' end of the L1 cDNA and the dA-dT insert from pSP65AT. This fragment was ligated to pSP64-X β m DNA digested previously with *Bam*HI to remove a 300-bp fragment containing the corresponding 3' region of the β -globin cDNA. The resulting plasmid, pSP64-X β m/L1, was linearized with *Bam*HI for use as a template for in vitro transcription. A schematic representation of each template is shown in Figure 4. The RNAs produced by SP6 transcription of pSP65-L1 and pSP64-X β m/L1 have poly(A) tracts of 111 residues. The pSP64-X β m transcript has a poly(A) tract of 23 residues. In vitro synthesis of capped transcripts with SP6 RNA polymerase was performed as described (Krieg and Melton 1984). SP6 RNAs were labeled by inclusion of [α -³²P]UTP in the transcription reaction. RNAs were stored under ethanol at -20°C.

Isolation and analysis of RNA

Total oocyte RNA was extracted, as described (Wormington 1986), and fractionated by oligo(dT)-cellulose chromatography, as described (Maniatis et al. 1982). RNA was isolated from polysomal and nonpolysomal fractions, prepared as described in detail by Baum et al. (1988). Electrophoresis of RNAs in denaturing formaldehyde-agarose gels, Northern blot transfer to nitrocellulose, and hybridization to ³²P-labeled RNA probes have been described previously (Baum and Wormington 1985; Wormington 1986). RNA yields were quantitated by measurements of rRNA content. In vitro deadenylation of total oocyte RNA by digestion with RNase H was performed by modification of a procedure described by Swartwout et al. (1987). Briefly, 8 μ g of total RNA (two oocyte equivalents) was preincubated with 2 μ g of oligo(dT)₁₂₋₁₈ for 30 min at 37°C in a 20- μ l reaction containing 20 mM HEPES-KOH (pH 8.0), 50 mM KCl, 10 mM MgCl₂, 2 mM dithiothreitol, and 20 units of RNasin (Promega). Digestions were started by the addition of 1.8 units RNase H (BRL) and incubated for an additional 1 hr at 37°C. Reactions were terminated by the addition of 10 mM EDTA, and samples

were precipitated with ethanol. Samples were analyzed by formaldehyde-agarose gel electrophoresis and autoradiography of the dried gels.

Acknowledgments

We thank Brett Keiper, Claire Moore, Michael Rosbash, Susan Varnum, and Pieter Wensink for improving the manuscript with helpful comments. This work was supported by National Institutes of Health grant HD-17691 and March of Dimes Basic Research grant 1-1040 to W.M.W.

References

- Adamson, E.D. and H.R. Woodland. 1977. Changes in the rate of histone synthesis during oocyte maturation and very early development of *Xenopus laevis*. *Dev. Biol.* **57**: 136–144.
- Baum, E.Z. and W.M. Wormington. 1985. Coordinate expression of ribosomal protein genes during *Xenopus* development. *Dev. Biol.* **111**: 488–498.
- Baum, E.Z., L.E. Hyman, and W.M. Wormington. 1988. Post-translational control of ribosomal protein L1 accumulation in *Xenopus* oocytes. *Dev. Biol.* **126**: 141–149.
- Beccari, E., P. Mazzetti, A. Mileo, P. Pierandrei-Amaldi, and F. Amaldi. 1986. Sequences coding for the ribosomal protein L14 in *Xenopus laevis* and *Xenopus tropicalis*; homologies in the 5' untranslated region are shared with other r-protein mRNAs. *Nucleic Acids Res.* **14**: 7633–7646.
- Birnstiel, M.L., M. Busslinger, and K. Strub. 1985. Transcription termination and 3' processing: The end is in site! *Cell* **41**: 349–359.
- Busby, S.J. and R.H. Reeder. 1982. Fate of amplified nucleoli in *Xenopus laevis* embryos. *Dev. Biol.* **91**: 458–467.
- Cardinali, B., N. Campioni, and P. Pierandrei-Amaldi. 1987. Ribosomal protein, histone and calmodulin mRNAs are differentially regulated at the translational level during oogenesis of *Xenopus laevis*. *Exp. Cell Res.* **169**: 432–441.
- Colot, H.V. and M. Rosbash. 1982. Behavior of individual maternal pA⁺ RNAs during embryogenesis of *Xenopus laevis*. *Dev. Biol.* **94**: 79–86.
- Darnbrough, C. and P.J. Ford. 1976. Cell-free translation of messenger RNA from oocytes of *Xenopus laevis*. *Dev. Biol.* **50**: 285–301.
- Dixon, L.K. and P.J. Ford. 1982. Regulation of protein synthesis and accumulation during oogenesis of *Xenopus laevis*. *Dev. Biol.* **93**: 478–497.
- Drummond, D.R., J. Armstrong, and A. Colman. 1985. The effect of capping and polyadenylation on the stability, movement and translation of synthetic messenger RNAs in *Xenopus* oocytes. *Nucleic Acids Res.* **13**: 7375–7394.
- Gurdon, J.B. 1976. Injected nuclei in frog oocytes: Fate, enlargement and chromatin dispersal. *J. Embryol. Exp. Morphol.* **36**: 523–540.
- Hyman, L.E., H.V. Colot, and M. Rosbash. 1982. Accumulation and behavior of mRNA during oogenesis and early embryogenesis of *Xenopus laevis*. In *Molecular aspects of early development* (ed. G.M. Malacinski and W. Klein), pp. 142–151, Plenum Press, New York.
- Jacobson, A. and M. Favreau. 1983. Possible involvement of poly(A) in protein synthesis. *Nucleic Acids Res.* **11**: 6353–6368.
- Krieg, P.A. and D.A. Melton. 1984. Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs. *Nucleic Acids Res.* **12**: 7057–7070.
- Kruys, V., M. Wathélet, P. Poupard, R. Contreras, W. Fiers, J. Content, and G. Huez. 1987. The 3' untranslated region of the human interferon- β mRNA has an inhibitory effect on translation. *Proc. Natl. Acad. Sci.* **84**: 6030–6034.
- Loreni, F., I. Ruberti, I. Bozzoni, P. Pierandrei-Amaldi, and F. Amaldi. 1985. Nucleotide sequences of the L1 ribosomal protein gene on *Xenopus laevis*: Remarkable sequence homology among introns. *EMBO J.* **4**: 3483–3488.
- Maller, J. 1985. Oocyte maturation in amphibians. *Developmental biology: A comprehensive synthesis* (ed. L.W. Browder.), vol. 1, pp. 289–311, Plenum Press, New York.
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Melton, D.A., P.A. Krieg, M.R. Rebagliati, T. Maniatis, K. Zinn, and M.R. Green. 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* **12**: 7035–7056.
- Nudel, U., H. Soreq, U.Z. Littauer, G. Marbaix, G. Huez, M. Leclercq, E. Hubert, and H. Chantrenne. 1976. Globin mRNA species containing poly(A) segments of different lengths: Their functional stability in *Xenopus* oocytes. *Eur. J. Biochem.* **64**: 115–121.
- Palatnik, C.M., C. Wilkin, and A. Jacobson. 1984. Translational control during early *Dictyostelium* development: Possible involvement of poly(A) sequences. *Cell* **36**: 1017–1025.
- Pierandrei-Amaldi, P., N. Campioni, E. Beccari, I. Bozzoni, and F. Amaldi. 1982. Expression of ribosomal protein genes in *Xenopus laevis* development. *Cell* **30**: 163–171.
- Richter, J.D., W.J. Wasserman, and L.D. Smith. 1982. The mechanism for increased protein synthesis during *Xenopus* oocyte maturation. *Dev. Biol.* **89**: 159–167.
- Rosenthal, E.T. and J.V. Ruderman. 1987. Widespread changes in the translation and adenylation of maternal messenger RNAs following fertilization of *Spisula* oocytes. *Dev. Biol.* **121**: 237–246.
- Rosenthal, E.T., T.R. Tansey, and J.V. Ruderman. 1983. Sequence-specific adenylations and deadenylations accompany changes in the translation of maternal mRNA after fertilization of *Spisula* oocytes. *J. Mol. Biol.* **166**: 309–327.
- Sagata, N., K. Shiokawa, and K. Yamana. 1980. A study on the steady-state population of poly(A)⁺ RNA during early development of *Xenopus laevis*. *Dev. Biol.* **77**: 431–438.
- Sturgess, E.A., J.E.M. Ballantine, H.R. Woodland, T.R. Mohun, C.D. Lane, and G.J. Dimitriades. 1980. Actin synthesis during the early development of *Xenopus laevis*. *J. Embryol. Exp. Morphol.* **58**: 303–320.
- Swartwout, S.G., H. Preisler, W. Guan, and A.J. Kinniburgh. 1987. Relatively stable population of c-myc RNA that lacks long poly(A). *Mol. Cell. Biol.* **7**: 2052–2058.
- Woodland, H.R. 1974. Changes in the polysome content of developing *Xenopus laevis* embryos. *Dev. Biol.* **40**: 90–101.
- Wormington, W.M. 1986. Stable repression of ribosomal protein L1 synthesis in *Xenopus* oocytes by microinjection of antisense RNA. *Proc. Natl. Acad. Sci.* **83**: 8639–8643.



Translational inactivation of ribosomal protein mRNAs during *Xenopus* oocyte maturation.

L E Hyman and W M Wormington

Genes Dev. 1988, **2**:

Access the most recent version at doi:[10.1101/gad.2.5.598](https://doi.org/10.1101/gad.2.5.598)

References

This article cites 28 articles, 3 of which can be accessed free at:
<http://genesdev.cshlp.org/content/2/5/598.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

horizon
a PerkinElmer company

Streamline your research with
Horizon Discovery's ASO tool

The advertisement features a dark blue background with a glowing DNA double helix structure on the left. The 'horizon' logo and 'a PerkinElmer company' tagline are in white. The main text 'Streamline your research with Horizon Discovery's ASO tool' is in white, with 'Horizon Discovery's ASO tool' in bold.