A Translation Regulatory Particle Containing the Xenopus Oocyte Y Box Protein mRNP3+4

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In oocytes, nontranslated maternal mRNAs are packaged by protein into messenger ribonucleoprotein particles (mRNPs) that are masked from translation by protein-RNA interactions. Proteins associated with such masked states of mRNAs are particularly abundant in amphibian oocytes. One of these mRNPs from Xenopus oocytes, mRNP3+4 (also called FRG Y2a/b or p54/p56), binds to diverse mRNAs independent of their sequence and is the germ line member of the evolutionarily conserved Y box protein multigene family. Xenopus oocytes contain soluble pools of mRNP3+4 6 S oligomers, probably dimers, and larger ~15 S particles containing mRNP3+4 and additional proteins. Here we report the purification of this larger form as an ~320-kDa particle that contains mRNP3+4 and nine additional polypeptides, including mRNA-binding polypeptides of 34 and 36 kDa and a doublet of 110/105 kDa that proved to be nucleolin. The particle has a protein kinase activity that phosphorylates its own mRNP3+4, nucleolin, and a 31-kDa polypeptide component and exhibits translational inhibition in both the wheat germ extract and rabbit reticulocyte lysate systems. The presence of mRNP3+4 and nucleolin in this large translation regulatory particle suggests that it participates in an early step of mRNP assembly and masking.

The packaging of mRNA into stable nontranslated cytoplasmic messenger ribonucleoprotein particles (mRNPs) is an essential mechanism of post-transcriptional regulation of gene expression utilized by both male and female germ cells of vertebrates and invertebrates. Both the nontranslated state and the stability of these translationally masked mRNAs are at least partly mediated by the protein component (1–3), but little is known about the mechanism of translational regulation. Xenopus oocytes provide a rich source of masked mRNPs that can be biochemically characterized, manipulated by microinjection studies, and readily followed during early embryogenesis (4, 5). These mRNA-associated proteins inhibit the translation of β-globin mRNA either in vivo following oocyte microinjection or in vitro in a wheat germ extract (WGE) translation system (6), thereby demonstrating their ability to repress translation.

Recent studies of the most abundant Xenopus oocyte mRNP polypeptides have begun to address the structure and function of mRNP proteins. These polypeptides were originally named mRNP3+4 (4) and subsequently p54 and p56 (5, 7, 8), pp56 and pp60 (9), or FRG Y2 (mRNP4) (10). Polypeptides mRNP3 and mRNP4 are highly related, probable pseudoalleles (8, 11), and therefore, because their simplest native form appears to be an ~6 S dimer (7), they are referred to as a single protein, mRNP3+4. mRNP3+4 particles have also been shown to be the germ line members of an evolutionarily conserved multigene family of nucleic acid-binding proteins, called the Y box proteins (12, 13), that are associated with masked mRNPs during oogenesis and spermatogenesis (8, 14–16). In various somatic cells, a closely related Y box protein is associated with non-translated mRNPs as well as with polyribosomal mRNP (17–20). In addition to their ability to bind RNA (21, 22), Y box proteins can also bind DNA and function as transcription factors that interact with specific promoter regions of genes containing a Y box element (23–27). It seems that these proteins can effect both functions: Ranjani et al. (28) have shown that the expression of mRNP4 (FRG Y2) in cultured somatic cells is associated with increased Y box-dependent reporter gene transcription as well as with a general reduction in translation, indicating that the germ line Y box protein can confer masking in somatic cells.

Recent studies have also indicated that a nuclear step governs translational masking in Xenopus oocytes. This was suspected due to the paradoxically efficient translation of oocyte microinjected mRNA in the presence of a vast excess of endogenous nontranslated mRNA (29). In fact, Bouvet and Wolffe (30) demonstrated that Xenopus oocyte masking requires transcription and that masked mRNA is bound by mRNP3+4 in the cytoplasm. Nuclear determination of masking is also suggested to require mRNP3+4 (31). Previous attempts to assemble in vitro translationally repressed mRNPs using purified mRNP3+4 have demonstrated translational repression in the relatively inefficient WGE translation system (32), but have not succeeded in repressing translation either in the rabbit reticulocyte lysate (RL) in vitro system (32) or upon microinjection into Xenopus oocytes (30) except when used at a high protein/mRNA ratio (33). We therefore sought to purify a native form of mRNP3+4 that could inhibit translation in the RRL system, and we have concentrated on the particle-bound form of mRNP3+4. In addition to assembled mRNPs and the soluble ~6 S mRNP3+4 oligomers, Xenopus oocytes also contain a pool of distinct ~15 S mRNP3+4 particles containing additional mRNA-binding polypeptides and a protein kinase activity (7). Here we report the isolation and characterization of mRNP3+4.
of an ~320-kDa mRNP3+4 particle that is closely related to the previously reported ~15 S mRNP3+4 particle, and we demonstrate its ability to inhibit the translation of particle-bound mRNA in both the WGE and RRL in vitro translation systems.

EXPERIMENTAL PROCEDURES

Particle Purification—All chemicals were reagent-grade and RNase-free. Solutions were made with diethyl pyrocarbonate (0.1%-treated water and autoclaved. Equipment that could not be autoclaved was cleaned with RNase Zap (Ambion Inc., Austin, TX). A 100,000 × g × 1-h supernatant (S100) was prepared from homogenized ovaries of adult Xenopus laevis toads and dialyzed against a modified buffer D (20 mM HEPES, pH 7.9; 0.1 mM KCl, 0.2 mM EDTA, 0.5 mM phe- nylmethylsulfonyl fluoride, and 1 mM dithiothreitol) (34). Protease inhibitors, leupeptin, and aprotinin were included in all steps of the purification (at standard concentrations). CaCl2 was added to 1 mM, and the extract was treated with micrococcal nuclease (30 mg/ml, room temperature) for 15 min, at which time EDTA was added to 3 mM, and the extract was aliquoted and frozen at −70 °C.

For protein purification, the micrococcal nuclease-treated S100 extract was filtered through a 0.45-μm filter and fractionated by anion exchange on a DEAE membrane (MemSep 1500 cartridge) with a 0–1.0 M NaCl gradient in 20 mM Tris, pH 7.3 (flow rate = 15 ml/min), using a ConSep chromatography unit (PerSeptive Biosystems, Framingham, MA). The 0.4 M KCl DEAE peak containing mRNP3+4 was isolated and combined from multiple runs, concentrated using molecular mass cutoff filtration units (30 kDa; Millipore Corp., Bedford, MA), and fractionated using a Superose 6 Pre-grade column (1 × 30 cm; Pharmacia Biotech Inc.) in buffer D. Representative DEAE and GFC fractionations are shown in Figs. 1 and 2; similar results were obtained in repeated purifications of the 320-kDa particle (n > 10). Alternatively, the 0.4 M KCl DEAE peak was dialyzed against buffer D and applied to poly(G)-agarose, and protein was eluted with a KCl gradient (35).

GFC molecular mass standards were aldolase, catalase, ferritin, thyroglobulin, and dextran blue (Pharmacia). The particle mass was determined from its elution volume in GFC, from three different preparations, to be 320 kDa ±30% (29 kDa). The integrity of the 320-kDa particle following a 2-h incubation with 1 mM ATP and 2 mM MgCl2 was analyzed by GFC using a smaller Superose 6 Pre-grade column (0.5 × 10 cm) with the same molecular mass standards and was found to be unchanged. For density gradient centrifugation, the DEAE/GFC-purified 320-kDa particle was applied to a 5-ml 4–15% (w/v) glycerol gradient in 130 mM NaCl and 10 mM Na2HPO4, pH 7.2. Parallel gradients contained 0.5 mg of aldolase, catalase, ferritin, and thyroglobulin as standards. Gradients were centrifuged for 2.5 h at 46,000 rpm and fractionated using a Beckman fractionator. The positions of the markers were determined by monitoring the absorbance at 280 nm.

Protein Analysis—Proteins were fractionated by SDS-PAGE using 18% polyacrylamide gels with 30:1.5 cross-linking or 10% polyacrylamide gels with 30:0.8 cross-linking. Particles containing mRNPs 3+4 were detected using mRNP3+4 (ps54/p56) antibodies for immunoblots as described previously (7). R2D2 nucleolin antibodies (35) were generously provided by Dr. Patrick DiMario (Louisiana State University, Baton Rouge, LA). RNA binding/photocross-linking and protein phosphorylation were carried out as described (7, 10). Alternatively, the 0.4 M KCl GFC-purified 320-kDa particle was applied to a 5-ml 4–15% (w/v) glycerol gradient in 130 mM NaCl and 10 mM Na2HPO4, pH 7.2. Parallel gradients contained 0.5 mg of aldolase, catalase, ferritin, and thyroglobulin as standards. Gradients were centrifuged for 2.5 h at 46,000 rpm and fractionated using a Beckman fractionator. The positions of the markers were determined by monitoring the absorbance at 280 nm.

Translation Inhibition Assay—The translation inhibition assay consisted of an incubation period when the protein was allowed to bind to substrate mRNA, followed by addition of either WGE or RRL. In all cases, 200 ng of in vitro synthesized mRNA was used to give a final concentration of 8 μg/ml in the translation system so that the translation components were in excess in both RRL and WGE systems. In vitro transcriptions incorporated 7-methyl-GTP/5′ppp5′GTP (New England Biolabs Inc., Beverly, MA) in the transcription mixture at 0.5 mM in the presence of 0.1 mM GTP to yield 5′-capped mRNA when desired. Substrate mRNAs were purified by phenol extraction and ethanol precipitation. Lamin LI mRNA was transcribed as described (7), and NO38 mRNA was transcribed from an NO38 cDNA plasmid linearized with BamHI (36); cDNA was generously provided by Dr. Marion Schmidt-Zachmann (German Cancer Research Center, Heidelberg, Germany).

The mRNA/protein incubation included mRNA with either the purified 320-kDa particle or no protein. The conditions for WGE allowed incubation of larger volumes than those for RRL, so the initial incubations were carried out in different volumes. Thus, for WGE (Boehringer Mannheim), the incubations contained RNA and protein particles at 20 μg/ml each; for RRL (Promega, Madison, WI), RNA and protein were included at 40 μg/ml each. Based on a density of 320 kDa, this is a 1.5:1 protein/mRNA ratio. Except in the case of controls, the mRNA/protein incubation included 1 mM ATP and 2 mM MgCl2. Following incubation of mRNA and protein at 16 °C for 2 h, the translation components were added with [35S]methionine (DuPont NEN), and translation was performed in 25 μl under the standard conditions recommended by the manufacturer. The final concentration of mRNA and 320-kDa particle was 15 and 20 nm, respectively. In some controls, the mRNA/protein mixture was phenol-extracted following the addition of RRL, and the RNA was recovered by ethanol precipitation and tested for translation. Translation was terminated by the addition of probe buffer; the sample was heated at 95 °C for 5 min and separated by SDS-PAGE; and the products were visualized by direct autoradiography.

RNase activity was evaluated by incubating 200 ng of 320-kDa particle with 1 × 105 cpn of a 204-nucleotide RNA (22) in the presence of 1 mM ATP and 2 mM MgCl2 for 2 h at 32 °C. Samples were heated to 95 °C, fractionated on 8% urea and 12% polyacrylamide gels, and visualized by autoradiography.

RESULTS

Purification of a 320-kDa Particle Containing mRNP3+4 and Nucleolin—A common set of proteins is associated with Xenopus oocytes mRNPs (4, 5), the most abundant being mRNP3+4. The free soluble pool of mRNP3+4 is composed of simple ~6 S oligomers, probably dimers, and larger particles of ~15 S containing mRNP3+4 and an additional RNA-binding polypeptide of ~100 kDa (7). Based on these observations, we set out to purify the large particle form of mRNP3+4 as a first step toward identifying an mRNP assembly particle. An S100 extract was prepared from adult Xenopus oocytes and treated with micrococcal nuclease to remove all free RNA. The micrococcal nuclease-treated S100 extract was then fractionated by various modes of ion exchange chromatography, and the profiles were analyzed for mRNP3+4 by immunoblotting. We found that anion exchange using a DEAE resin fractionated mRNP3+4 particles from the majority of the micrococcal nuclease-treated S100 protein (Fig. 1, mRNP3+4 first eluted at 0.4 M KCl and continued to elute late in the salt gradient as detected by immunoblots of the DEAE fractions (Fig. 1c). The late eluting peaks had a high A260/280 ratio and contained little protein, although mRNP3+4 is predominant (Fig. 1b, fractions 16–22). These late eluting peaks are likely to represent naturally occurring RNPfs; due to their inherent RNA content, they were not investigated in this study. Only the peak at 0.4 M KCl contained mRNP3+4 and relatively high amounts of protein (Fig. 1, a, doubly underlined peak; b, fractions 12–14).

As a next step in the purification, the 0.4 M KCl DEAE peak of mRNP3+4 protein was combined from multiple DEAE chromatographic runs, dialyzed, and concentrated for subsequent GFC. GFC separated two large particles (peaks I and II) from smaller proteins (Fig. 2). GFC peak I proved to be an aggregate that formed during concentration of the DEAE fraction; repeated concentration and fractionation of purified peak II material yielded a small amount of peak I particles. Assuming that the particles in peak II are near-globular, the mean predicted peak mass of peak II particles is 320 kDa.

SDS-PAGE and silver staining determined that the 320-kDa particle contains polypeptides of 110, 105, 65.5, 65, 48, 46, 36, 34, and 31 kDa as well as mRNP3+4 (Fig. 2b). Several of these polypeptides appear as doublets and will be referred to as such (e.g. 110/105 and 36/34 kDa). The composition of the 320-kDa particle was constant in repeated preparations (n > 10); in some preparations, however, a slight degradation of the 110/ 105-kDa doublet to 95/90 kDa was observed. In addition, the
ular mass markers are shown in the underlined region in (a) were analyzed for protein content after Coomassie Blue staining (b; 500 μl of each fraction, as indicated below each lane) and for mRNPs by immunoblotting (c; fraction number as indicated below each lane). The peak eluting at 0.4 M KCl (Fig. 3 lane 1, 4) was taken for subsequent fractionation. The late eluting peak at 0.4 M KCl (a, arrowhead, doubly underlined region; b and c, fractions 12–14) and containing mRNPs was taken for subsequent fractionation. The late eluting peaks (0.5–0.6 M KCl; fractions 15–22) contain endogenous RNA and were therefore not examined in this study. The positions of the molecular mass markers are shown in (b) on the right (116, 66, 45, 36, 29, 24, 20, and 14 kDa).

doublet of 36/34 kDa was sometimes unresolved by SDS-PAGE (Fig. 3a, lane 1). RNA binding/photocross-linking analysis demonstrated that the polypeptides of 110/105 and 36/34 kDa and mRNPs bind RNA (lane 2). The 320-kDa particle also contains a protein kinase activity that acts on the 110/105- and 36/34-kDa polypeptides (lane 3). The characteristics of the 110/105-kDa polypeptides, particularly their cleavage to 95/90 kDa, were suggestive of the RNA-binding protein nucleolin. Immunoblotting of the DEAE/GFC-purified 320-kDa particle with nucleolin-specific antibodies demonstrated that the 110/105-kDa polypeptides are indeed nucleolin (lane 5).

The observation that the 320-kDa particle contained both nucleolin, a nuclear and nucleolar phosphoprotein (57), and mRNPs was unexpected because the vast majority of mRNPs is cytoplasmic (5). Nucleolin was present at roughly equal proportion to mRNPs in the selected DEAE fractions as in the final GFC-purified particle. We questioned whether these two polypeptides are present in the same particle, and not two different, yet co-isolating particles. First, we attempted to separate nucleolin from mRNPs using poly(G)-agarose affinity purification of the 0.4 M KCl DEAE peak (35), but found all the polypeptides of the 320-kDa particle to copurify (data not shown). To prove that nucleolin and mRNPs are present in the same particle, we utilized co-immunoprecipitation analysis. Immunoprecipitation of the 320-kDa mRNPs with antibodies to mRNPs demonstrated that nucleolin and mRNPs co-immunoprecipitated (Fig. 3b). Earlier studies using the fractionated 320-kDa mRNPs demonstrated co-immunoprecipitation of an 100-kDa RNA-binding phosphoprotein with mRNPs (58). Although we did not suspect it at the time, it is likely that this 100-kDa mRNA-binding phosphoprotein is nucleolin (7). Density gradient centrifugation of the 320-kDa particle determined that it has an approximate value of 14 S. We conclude the DEAE/GFC-purified 320-kDa particle is closely related to the earlier observed 15 S mRNPs particle (7).

The 320-kDa mRNPs Particle Represses Translation in Vitro—The ability of the 320-kDa particle to modify the translation of a substrate mRNA was tested using a coupled in vitro binding and translation assay. For this assay, the 320-kDa particle and lamin LI substrate mRNA were incubated at a 1.5:1 molar ratio in the presence or absence of MgATP (2 mM MgCl2 and 1 mM ATP) at 16 °C for 2 h. WGE and translation components were then added directly to the 320-kDa particle/mRNA mixture and incubated under standard translation conditions (60 min, 30 °C). Translation was evaluated by the incorporation of [35S]methionine into lamin LI protein. The 320-kDa particle almost completely inhibited the translation of...
the lamin LI mRNA when incubated in the presence of MgATP for 2 h (Fig. 4, lane 4). Translation of endogenous mRNA in WGE was unaffected. Preincubation of the 320-kDa particle with mRNA prior to the addition of translation components and the presence of MgATP during the 320-kDa particle/mRNA incubation were essential for translational inhibition (lanes 2 and 3). A time course of shorter particle/mRNA incubation times yielded intermediate levels of translational inhibition at 30–90 min, with full inhibition observed at 2 h under the conditions used (data not shown). Translation of either capped or uncapped mRNA was similarly inhibited by the 320-kDa particle (data not shown).

A similar incubation of purified mRNP3+4 (11) at a 1.5:1 protein/mRNA ratio had no effect on translation (data not shown), suggesting that mRNP3+4 alone does not account for the activity of the 320-kDa particle. Translational inhibition by the 320-kDa particle utilized a relatively long incubation with mRNA (2 h, 16 °C). It was therefore important to demonstrate that the 320-kDa particle remained intact during the 2-h incubation. Thus, the mass of the 320-kDa particle was determined by GFC following a 2-h incubation at 16 °C in the presence of MgATP and was found to be unchanged. Furthermore, the polypeptide profile was determined following 320-kDa particle incubation under standard conditions with lamin LI mRNA in the presence of MgATP for 2 h at 16 °C. No proteolysis was observed (Fig. 5). Therefore, the 320-kDa particle/mRNA incubation had no effect on the integrity of the particle or its polypeptides, and the translational inhibition activity can be attributed to the 320-kDa particle.

Incubation of the 320-kDa particles with mRNA in the absence of MgATP had no effect on its translation (Fig. 4, lane 3), suggesting that the 320-kDa particle is free of RNase activity. However, it was necessary to directly demonstrate the absence of RNase activity under the translational inhibition assay conditions employed. To test this, a small radiolabeled RNA was incubated with the 320-kDa particle for 2 h at 22 °C in the presence of MgATP, and the RNA products were detected by autoradiography following denaturing gel electrophoresis (Fig. 6a). This assay is very sensitive because a large molar excess of 320-kDa particle was incubated with the radioactive RNA, thereby allowing low levels of RNase activity to be detected. No RNase activity was detected in the 320-kDa particle preparation (Fig. 6a, lane 2). An unrelated DEAE fraction provided a positive control for RNase activity (lane 3). This assay was routinely performed to evaluate each 320-kDa particle preparation.

A further concern was that the 320-kDa particle may covalently modify the mRNA, rendering it incompetent for translation. To demonstrate that the 320-kDa particle neither modified nor cleaved the substrate mRNA, mRNA was purified from the 320-kDa particle following standard 320-kDa particle/mRNA incubation and tested for translation. The mRNA recovered from the 320-kDa particle was efficiently translated (Fig. 6b, lane 3). Together, these controls demonstrate that the 320-kDa particle neither degrades mRNA nor modifies it to an inactive form.

While the 320-kDa mRNP3+4 particle efficiently inhibited translation of a substrate mRNA when tested in the WGE translation system (Fig. 4), the failure of previous studies to demonstrate translational inhibition of assembled mRNA/protein particles in an animal system (30, 32) prompted us to test the activity of the 320-kDa particle in a well defined animal translation system. For this we chose the RRL because it mimics the translation rate of mammalian cells (38). Lamin LI mRNA was incubated with 320-kDa particles under our standard conditions (2 h, 16 °C, in buffer D plus 2 mM MgCl2 and 1 mM ATP).
The 320-kDa particle with substrate mRNA in the presence of WGE system, translational inhibition required incubation of had no effect on the translation of RRL globin mRNA. As in the under standard conditions (Fig. 7, lane 2), translation products target an mRNA for mRNP3 assembly and masking of nuclear RNA into RNPs (43–45). While the transport of mRNA from the nucleus to the cytoplasm is coupled with a general exchange of hnRNPs proteins for cytoplasmic mRNP proteins (46, 47), the demonstration of selective hnRNP protein shuttling between the nucleus and cytoplasm (48) suggests that the exchange of nuclear for cytoplasmic RNA-binding proteins continues in the cytoplasm. Analysis of hnRNP-like proteins of lower eukaryotes proves that a continuum of nuclear/cytoplasmic RNP exchange exists, so specific hnRNP-like proteins either become polyribosomal (49) or co-fractionate with nontranslated mRNP (50). The recent demonstration of a nuclear step governing the cytoplasmic masking of mRNA in Xenopus oocytes suggests that specific nuclear RNP interactions target an mRNA for mRNP3+4 assembly and masking (30, 31).

To gain insight into the mechanism of nontranslated masked mRNP assembly and translational regulation, we systematically searched for a complex mRNP3+4-containing particle with the ability to assemble mRNA into a nontranslated mRNP. Using standard protein purification methods, we developed a purification scheme yielding an mRNP3+4 particle of 320 kDa related to the earlier identified ~15 S particle (7). Analysis of this particle has proved that it has the ability to repress the translation of mRNA when tested in a highly active in vitro translation system. Therefore, we call this particle the 320-kDa Translation Regulatory Particle (TRP).

Characteristics of the 320-kDa Translation Regulatory Particle—The 320-kDa TRP shares some of the characteristics of the partly fractionated ~15 S particle, including the presence of mRNP3+4, an RNA-binding protein of ~100 kDa that proved to be nucleolin, and a protein kinase activity that phosphorylates both mRNP3+4 and nucleolin. Co-immunoprecipitation of these polypeptides with mRNP3+4 antibodies demonstrates that these RNA-binding polypeptides are constituents of a single large particle species. Seven other
polypeptides consistently copurify with the 320-kDa TRP in the same relative concentration, suggesting that they too are components of the particle. These seven polypeptides may represent as few as four different gene products if each of the observed doublets arises from pseudoallelic variation, which is characteristic for the species X. laevis (51) and noted for nucleolin and mRNP3 + 4. Among these seven not yet fully characterized polypeptides are a pair of RNA-binding polypeptides (34/36 kDa) and a 31-kDa polypeptide that is also phosphorylated by the 320-kDa TRP kinase activity.

However, the 320-kDa TRP and the earlier described ~15 S particle (7) are demonstrably different in their inherent mRNA binding characteristics. For the ~15 S particle, RNA binding was only observed for nucleolin, unless the particle was treated with alkaline phosphatase, which promoted RNA binding by mRNP3 + 4 (7). While we have not yet undertaken similar studies with the 320-kDa TRP, the RNA binding of mRNP3 + 4 in the absence of any prior treatment shows that the 320-kDa TRP differs from the ~15 S particle. The ~15 S particle has also been reported to contain an ~60-kDa RNA-binding polypeptide as detected by RNA binding/UV cross-linking. While no such RNA binding activity has been observed in the 320-kDa TRP, the 65/65.5-kDa polypeptides of the 320-kDa TRP are close in size to the ~15 S particle 60-kDa polypeptide. We speculate that these two notable changes in RNA binding activity between the ~15 S particle and the 320-kDa TRP are due either to changes in modification and/or conformation or to polypeptide loss during the purification procedure.

Purification of the 320-kDa TRP determined that the protein kinase activity cofractionating with the ~15 S particle is an integral feature of the 320-kDa TRP. While a casein kinase type II-like activity has been identified both in large mRNP3 + 4 particles (7, 9) and in mRNPs (52, 53), none of the 320-kDa TRP polypeptides correspond to the subunits of Xenopus oocyte casein kinase type II (54). The ability of the protein kinase to phosphorylate mRNP3 + 4, nucleolin, and the 31-kDa polypeptide suggests that it functions in mRNP masking or unmasking. Further support for this hypothesis comes both from our observation of an MgCl2 and ATP requirement for the translational inhibition by the 320-kDa TRP and from the demonstration by Sommerville and co-workers (32) that derepression of mRNP translation in vitro takes place following alkaline phosphatase treatment. Further studies are underway to characterize the 320-kDa TRP kinase activity.

Translational Inhibition by the 320-kDa TRP—The 320-kDa TRP efficiently inhibits in vitro translation of substrate mRNA in both the WGE and RRL translation systems, using a 1.5:1 molar ratio of particle to substrate mRNA. This is indicative of an avid and effective binding of 1–2 TRP molecules/mRNA molecule. Translational repression in the RRL system is particularly impressive because of the high activity of this system. Translation repression in the RRL system is particularly impressive because of the high activity of this system. Translational inhibition only at a 30:1 molar ratio with mRNA (33). While we have not yet observed any translational inhibition of purified mRNP3 + 4 under our standard conditions. This suggests that translational inhibition by the 320-kDa particle is not solely attributable to its mRNP3 + 4 component. Future analysis of mRNPs assembled with the 320-kDa particle will determine its mechanism of translational inhibition.

Both an in vivo masked mRNA (lamin LI) and an mRNA that is efficiently translated in the oocyte (protein NO38) were translationally repressed by the 320-kDa TRP. This lack of specificity is open to alternative interpretations, depending on the in vivo mechanism of masking. Masking has been suggested to be a default process in which all mRNAs are initially masked, with selective activation of translated mRNAs (15, 30). A second mechanism proposes a specificity for pre-mRNAs that are intronless (31). A third speculative explanation depends on masked mRNAs having specific masking elements that target them for translational repression (55). In either the first or second mechanism, the observed lack of 320-kDa TRP specificity would be consistent with the masking process, while in the third mechanism, it would suggest that additional factors are needed for specificity. Because mRNP3 + 4 particles are abundant proteins, they are likely to be general masking proteins that can potentially repress any mRNA (28, 30). However, their localization and structural integration in vivo may be critical for the selection of pre-mRNAs for masking.

Nucleolin and Compartmentalization of the mRNP Masking Process—While nucleolin has been shown to continuously shuttle between the cytoplasm and the nucleus (56, 57), it is also clear that in the steady state, the vast majority of nucleolin is nuclear, actually bound to nucleolar pre-rRNA (58). In contrast, the majority of mRNP3 + 4 is cytoplasmic (8), but its ability to promote transcription indicates that significant nuclear translocation occurs (28, 59). At this point, it is also unknown whether the 320-kDa TRP is itself located in the nucleus or cytoplasm or in both compartments. While previous immunolocalization of mRNP3 + 4 in the cytoplasm would speak for the occurrence of most of the 320-kDa TRP in the cytoplasm, this does not exclude a nuclear pool of such mRNP3 + 4-containing particles (for the occurrence of a distinct form of large particles in both oocyte compartments, see, for example, Ref. 60). Obviously, the identification of a required nuclear step in TRP masking (30, 51) would be consistent with a nuclear localization of the 320-kDa TRP. To clarify this topological question, future experiments will need to address the possibility that a nuclear form of TRP might differ slightly from the cytoplasmic one and that the transition between these forms might also be regulated.

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Translation Regulatory Particle

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