A Monoclonal Antibody to an Oocyte-specific Poly(A) RNA-binding Protein*

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Xenopus oocyte-specific poly(A) RNA-binding proteins were isolated and used to prepare monoclonal antibodies. One antibody was used to characterize one particular antigen by immunoblot analysis. The antigen had a molecular weight of 56,000, was oocyte-specific, and decreased in amount during oogenesis. The antigen was localized in the cytoplasm throughout oogenesis as 15% of the total cell mass at 40–60 S. The antigen also was shown to bind poly(A) RNA following chromatography of ribonucleoprotein particles on oligo(dT)-cellulose. The antibody was used to immunoadsorb nontranslating ribonucleoprotein particles. Fifty-five per cent of the poly(A) RNA sedimenting between 40–60 S was shown to be bound by the antigen. The further use of this antibody in attempting to examine other components of the ribonucleoprotein particle is discussed.

Eucaryotic messenger RNAs exist in the cytoplasm of cells in either a polysomal or nonpolysomal form. The species of protein associated with polysomal mRNA differs from that associated with nonpolysomal RNA (1). In addition, certain proteins have been shown to bind specific regions of the mRNA such as 5'-terminal 7-methylguanosine (CAP) (2) and the 3'-terminal poly(A) tail (3–5). The exact function of these or other mRNA-binding proteins, however, remains to be elucidated.

The microinjected Xenopus oocyte has proven to be an excellent surrogate system for studying translational (reviewed in Ref. 6) and transcriptional (reviewed in Ref. 7) control. Aside from their ability to carry out diverse metabolic processes on injected molecules, oocytes also display interesting characteristics with respect to their metabolism of endogenous mRNA. Essentially the entire mass of the mRNA is synthesized very early during oogenesis (Dumont (8) stage 1–2 oocytes). Although poly(A) RNA continues to be synthesized throughout the remainder of oogenesis, the steady state mass is maintained by an approximately equal rate of turnover (9, 10). Mitochondrial poly(A) RNA, which accounts for as much as 16% of the total cellular poly(A) RNA (11), does not display the same steady state level as does cytoplasmic mRNA, but rather continually accumulates throughout oogenesis (10). These two classes of mRNA constitute a pool of mostly (95%) nontranslating mRNA. Such mRNA is referred to as masked or maternal mRNA.

It appears that at least two elements preclude the precocious translation of maternal mRNA. The first is that oocytes have the ability to translate only a limited amount of mRNA regardless of the amount of translatable mRNA present. Thus, the injection of increasing amounts of globin mRNA results in increasing amounts of globin synthesis, but also a reciprocal decrease in endogenous protein synthesis (12, 13); total cellular protein synthesis remains constant. The second is due to the unusual primary structure of much of the mRNA. It has been shown that up to two-thirds of the cytoplasmic mRNA contain covalently attached single copy and repetitive RNA sequences (11). The mRNAs which contain the repetitive RNA sequences are poor templates for protein synthesis (14).

In further attempts to define the translational capacity of the oocyte, it was demonstrated that the oocyte's ability to translate injected mRNA also was dependent upon the site of translation of the mRNA. Messenger RNAs which normally are translated in the free cytoplasm, e.g. globin, are translated relatively efficiently at all amounts of mRNA injected. On the other hand, mRNAs translated on the rough endoplasmic reticulum membrane, e.g. ovalbumin and zein, are translated efficiently only at low amounts of mRNA injected. However, when ovalbumin or zein mRNA-injected oocytes are additionally injected with heterologous rough endoplasmic reticulum or rough endoplasmic reticulum-specific proteins, enhanced synthesis of ovalbumin and zein takes place (13, 15). Thus, mRNA translation in oocytes can be influenced by organelle-specific proteins.

The observation described above led us to speculate that oocytes also might contain proteins which could alter the translatability of injected and endogenous mRNAs (16). We have identified a group of poly(A) RNA-binding proteins which also are oocyte-specific and which decrease in amount as oogenesis progresses (16). We have used these proteins to generate a series of monoclonal antibodies. In experiments described in this paper, one particular antibody was used to demonstrate that the antigen is oocyte-specific, is developmentally regulated during oogenesis, and binds poly(A) RNA.

In addition, the antibody was used to immunoadsorb nontranslating RNP particles and the amount of poly(A) RNA in such particles was quantitated. The antibody may further be used to isolate ribonucleoprotein particles to examine their RNA and protein components.

MATERIALS AND METHODS
Collection of Oocytes and Preparation of Antigen—Female Xenopus laevis were obtained from Nasco (Ft. Atkinson, WI) and oocytes

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1 The abbreviations used are: RNP, ribonucleoprotein; SDS, sodium dodecyl sulfate.
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Dispersed with collagenase and sized with multiple pore nylon screens (8). Dumont (8) stage 1–2 oocytes were collected and washed with polysome buffer (20 nm Tris-HCI, pH 7.5, 5 mM MgCl₂, 100 mM NaCl, 50 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml of polyvinylsulfuric acid, 10 μg/ml of diethyl pyrocarbonate, and 10 μg/ml of cycloheximide). Oocytes then were homogenized in the same buffer containing 1% Triton X-100 and 1% deoxycholate and centrifuged for 15 min at 4°C at 12,000 x g. The supernatant was layered over a 15–40% sucrose gradient in the same buffer in an SW 41 tube and centrifuged for 4 h at 34,000 rpm at 4°C (17). The 40–60 S fraction was collected and dialyzed against 10 mM Tris-HCl, pH 7.5, 50 mM NaCl, and 1 mM EDTA for 20–24 h with several changes of the buffer. The proteins in these fractions were applied to a phosphocellulose column (Whatman P-11), and the proteins eluting between 1 and 2 M NaCl were collected (16). These proteins were used to immunize mice.

Generation of Monoclonal Antibodies—BALB/c mice were injected subcutaneously with 50 μg of protein in Freund’s complete adjuvant once every 2 weeks for 6 weeks. A fourth dose of 15 μg of protein in adjuvant was injected intraperitoneally followed in 7 days by a fifth injection into the tail vein of 10 μg of protein in phosphate-buffered saline. The spleen was excised 5 days later and used for cell fusions. Spleen cells were fused with SP/2 myeloma cells (18, 19). Positive colonies were detected with a solid phase immunosassay essentially as described (19) using [125I]-rabbit anti-mouse IgG as the second labeled antibody. Cells positive in this assay were diluted and subcloned onto soft agar. Cells were grown, reassayed by solid phase immunosassay, and subsequently were grown in 30 ml culture flasks. Antibodies further were characterized by SDS-gel electrophoresis (20) and a protein blot analysis using [125I]-rabbit anti-mouse IgG as the second labeled antibody (19).

Oocyte Enucleation and SDS-Gel Electrophoresis—Oocytes of various stages were collected in cold Barth’s saline (21). Oocytes then were manually enucleated with watchmaker forceps, and the isolated nuclei and cytoplasts were immediately frozen on solid CO₂. The nuclei and cytoplasms were immediately frozen on solid CO₂. The nuclei were disrupted directly in SDS-gel buffer (20), and the cytoplasms were centrifuged at 2000 rpm to remove yolk. In some experiments, the cytoplasm was centrifuged further at 12,000 x g for 15 min at 4°C to pellet mitochondria. Protein was resolved by SDS electrophoresis in 10 or 12% polyacrylamide gels.

Oligo(dT)-cellulose Chromatography of Ribonucleoproteins—Stage 1–2 oocytes were homogenized, and centrifuged as described previously. The 40–60 S regions of the sucrose gradient were collected and dialyzed against 10 mM Tris-HCl, pH 7.5. To this solution was added an equal volume of 100 mM Tris-HCl, pH 7.8, 20 mM EDTA, and 600 mM NaCl. The mixture then was passed over an oligo(dT)-cellulose column (Type T, P-L Biochemicals) equilibrated in the final concentration buffer. Ribonucleoproteins were then sequentially eluted as described (16), with 0.5 × dialysis buffer, the same buffer plus 25% formamide (Bethesda Research Laboratories, ultratrace), buffer plus 50% formamide, and finally 10 mM Tris-HCl, pH 7.8. The protein in the eluted fractions then was used for immunoblot analysis. Identical experiments were carried out on RNP’s from the sucrose gradient which were incubated with 0.5 μg/ml of RNase A and 0.1 μg/ml of RNase P₁ before loading onto the oligo(dT)-cellulose column (16).

Ribonucleoprotein Immunodot Adsorption—Ribonucleoproteins were collected from the 40–60 S fraction from a sucrose gradient as described above. This fraction was diluted with an equal volume of polysome buffer and incubated with antibody previously made Rnase- free by passage through a protein A-Sepharose column (Pharmacia Fine Chemicals) at a ratio of 0.1 A₂₆₀ unit of RNP/μg of antibody. The RNPs were incubated for 2 h at 4°C with the antibody and then incubated for another 12–15 h at 4°C with 5 μg of swollen protein A-Sepharose 4B with constant mixing. The resin then was packed into a Pasteur pipette and washed with several volumes of polysome buffer until no A₂₆₀ absorbing material could be detected in the eluate. The bound material was eluted with 0.2 M glycine, pH 3.5. The bound and unbound fractions were divided into two parts. To one part was added 4 volumes of 100% ethanol which then was stored at −20°C for about 12 h. The precipitated protein was collected by centrifugation and dissolved in SDS-gel buffer (20) for SDS-gel electrophoresis and immunoblot analysis. The RNA was extracted from the other fraction by phenol and chloroform (16) and precipitated with 0.1 volume of NaCl and 2 volumes of absolute ethanol at −20°C for about 12 h. The RNA was centrifuged in an SW 50.1 tube at 33,000 rpm for 1 h at 4°C. The pellet RNA then was suspended in 2 × SSC (1 × SSC is 0.15 mM NaCl and 0.015 M Na citrate) for hybridization studies with [³H]poly(U). Protein electrophoribbing, RNA radioiodination, [³H]poly(U) hybridization, and RNA binding studies have been described (16).

RESULTS

Growing Xenopus oocytes accumulate vitellogenin (yolk) by endocytosis from the bloodstream. Prevalent nonyolk proteins, however, are synthesized throughout oogenesis (16). When proteins from various stage oocytes are resolved by SDS-gel electrophoresis, electrophoribbed onto nitrocellulose paper, and reacted with globin 125I-mRNA, a different pattern is observed. Several proteins which bind the labeled RNA decrease in amount of oogenesis progresses (16). This observation is confirmed in Fig. 1A. Eight stage 1–2 and stage 5–6 oocytes were homogenized in low salt (50 mM NaCl), electrophoribbed, electrophoribbed, and reacted with globin 125I-mRNA. It is readily apparent that many RNA-binding proteins are present in stage 1–2 oocytes and are absent (or diminished) in stage 5–6 oocytes. Many of the high molecular weight proteins (>80,000) which are present predominantly in small (stage 1–2) oocytes sediment in sucrose gradients between 100 and 200 S (16). Another protein of apparent molecular weight 56,000 (denoted by the arrow) is also most prevalent in small oocytes. This protein sediments between 40 and 60 S in sucrose gradients, elutes from phosphocellulose only under high ionic strength conditions (at least 1 M NaCl), and is retained by oligo(dT)-cellulose via a poly(A) RNA intermediate (16).

The proteins eluting from phosphocellulose with at least 1 M NaCl were used as antigens to make monoclonal antibodies. One such antibody was found to be directed against an antigen which has all of the characteristics of the Mr = 56,000 RNA-binding protein. Fig. 1B shows an immunoblot of total low salt (50 mM NaCl) soluble protein from oocytes of various stages and from four-cell Xenopus embryos. The antibody reacts with only one cellular protein, which has an apparent molecular weight of 56,000. This protein declines in amount

![Fig. 1. Detection of RNA-binding proteins. Oocytes were collected, staged, and homogenized in low salt (50 mM NaCl). Total soluble protein was collected following ethanol precipitation and resolved by SDS-10% polyacrylamide gel electrophoresis. The proteins were electrophoribbed from the gel onto nitrocellulose paper and reacted with globin 125I mRNA. A, autoradiogram of the proteins reacting with labeled globin mRNA. Total soluble protein from eight oocytes (stage 1) was electroblotted onto nitrocellulose gel electrophoresis and immunoblot analysis. The RNA was extracted from the other fraction by phenol and chloroform (16) and precipitated with 0.1 volume of NaCl and 2 volumes of absolute ethanol at −20°C for about 12 h. The RNA was centrifuged in an SW 50.1 tube at 33,000 rpm for 1 h at 4°C. The pellet RNA then was suspended in 2 × SSC (1 × SSC is 0.15 mM NaCl and 0.015 M Na citrate) for hybridization studies with [³H]poly(U). Protein electrophoribbing, RNA radioiodination, [³H]poly(U) hybridization, and RNA binding studies have been described (16).](http://www.jbc.org/)
through oogenesis and early embryogenesis.

Using the RNA binding assay described in Fig. 1A, we have determined previously the relative amount of the \( M_r = 56,000 \) protein present in oocytes (16). These data are portrayed in Fig. 2 in order to compare them with the data obtained from Fig. 1B after quantitating the amount of radioactivity present in each lane. Using either the RNA binding assay or the immunoblot assay, it is apparent that the \( M_r = 56,000 \) binding protein decreases with stage. Relative to the amount present in stage 1–2 oocytes, the binding protein decreases by 32% between stages 1–2 and 3–4 using the RNA binding assay and by 48% using the antibody. A further decrease of 39% by the RNA binding assay and 16% by the antibody occurs between stages 3–4 and 5–6. When determined with the antibody, another decrease of 26% occurs between oocyte stages 5–6 and the four-cell embryo. It should be noted that although the \( M_r = 56,000 \) binding protein has been shown always to decrease during oogenesis, the amount of decrease varies between different groups of oocytes (e.g. compare Fig. 1A with Fig. 2).

The presence of the antigen in adult frog tissues also was assessed (Fig. 3). One hundred \( \mu g \) of total low salt soluble protein from frog brain, heart, liver, and muscle, as well as oocytes, were probed for the presence of the antigen by an immunoblot. Similar to what was determined with the RNA binding assay (16), the binding protein is present almost exclusively in oocytes. Upon overexposure of the radiogram, that found in oocytes.

... was localized strictly in the cytoplasm (Fig. 4). Since oocytes also contain an abundance of mitochondria (and mitochondrial poly(A) RNA), they too were tested for the presence of the antigen. No antigen could be detected in mitochondria, at least in stage 6 oocytes.

Using an RNA binding assay, it previously was determined that the \( M_r = 56,000 \) RNA-binding protein sedimented at 40–60 S. However, other binding proteins with a similar molecular weight sedimented throughout the gradient (16). It therefore was difficult to determine with certainty the quantitative distribution of the \( M_r = 56,000 \) protein with respect to the 80 S monosome. In order to determine more precisely the sedimentation of the antigen, oocyte RNPs again were centrifuged through a sucrose gradient except that the monoclonal antibody was used as a probe for the presence of the binding protein (Fig. 5). The majority (60%) of the protein sedimented between 40 and 60 S and another 30% sedimented at around 80 S. The remaining amount sedimented only slightly into the polysome region of the gradient. It thus appears that the antigen sediments mainly to a region of the gradient where nontranslating RNPs would be expected to sediment, i.e. between 40 and 80 S.

The Antigen is an RNA-binding Protein—The data presented above demonstrate that the antigen has several characteristics of the previously identified RNA-binding protein: molecular weight, oocyte specificity, oogenetically regulated, and sedimentation in sucrose gradients. However, it is important to determine whether the antigen does, in fact, bind poly(A) RNA. We have chosen to demonstrate this by two methods. The first method involves oligo(dT)-cellulose chromatography, and the second involves isolation of RNPs by immunoaffinity chromatography.

... RNPs in this fraction were incubated with the antibody described in this study or a monoclonal antibody directed against fibronectin. The fibronectin antibody was used to control for nonspecific sticking of the antigen or RNA to the column. The antigen-antibody complexes were then immunoadsorbed to protein A-Sepharose column and determined the poly(A) RNA content of such RNPs. Small oocytes were homogenized and centrifuged, and the 40–60 S fraction was collected. The RNPs in this fraction were incubated with the antibody described in this study or a monoclonal antibody directed against fibronectin. The fibronectin antibody was used to control for nonspecific sticking of the antigen or RNA to the column. The antigen-antibody complexes were then immunoadsorbed to protein A-Sepharose. The bound and voided fractions were collected and 1) probed for the presence of the antigen and 2) used to determine the amount of poly(A) RNA present. Fig. 7 shows that essentially all of the antigen (i.e. RNA-binding protein) was bound by the immunoaffinity column when the anti-RNA-binding protein was the antibody employed. When anti-fibronectin or mouse IgG was used in place of the anti-RNA-binding protein, no frog antigen was retained by the column (Fig. 7 and data not shown).
protein which reacts with a monoclonal antibody and 125I-labeled second antibody.

Fig. 4 (center). **Subcellular location of the antigen.** Oocytes of different stages were collected and manually enucleated. The isolated nuclei (N) and cytoplasms (C) and, from stage oocytes, mitochondria (M) were probed for the presence of the antigen as described in the legend to Fig. 1. Shown is an autoradiogram of the protein reacting with a monoclonal antibody and 125I-labeled second antibody.

Fig. 5 (right). **Sedimentation characteristics of the antigen.** Stage 1–2 oocytes were homogenized, and the postmitochondrial supernatant was layered over a 15–40% sucrose gradient. The protein was extracted from each fraction and applied to a SDS-10% polyacrylamide gel. Following electrophoresis, the presence of the antigen was determined as described in the legend to Fig. 1. Shown is an autoradiogram. The 80 S monosome was located in fraction 4.

**Fig. 6 (left).** Oligo(dT)-cellulose chromatography of RNP particles. Stage 1–2 oocytes were homogenized and centrifuged as described in the legend to Fig. 5. The 40–60 S fraction was collected, and the RNPs were applied to an oligo(dT)-cellulose column without further modification (A) or after exposure to ribonuclease (B). The unbound protein (lane 1) and protein eluting in 25% formamide (lane 2), 50% formamide (lane 3), or buffer without salt (lane 4) were collected, electrophoresed, electroblotted, and probed for the presence of the antigen as described in the legend to Fig. 1. Shown are autoradiograms.

**Fig. 7 (right).** Immunoadsorption of ribonucleoprotein particles. Stage 1–2 oocytes were collected, homogenized, and centrifuged as described in the legend to Fig. 5. The 40–60 S fraction was incubated with the anti-RNA-binding protein monoclonal antibody (1) or anti-fibronectin monoclonal antibody (2) and passed over a protein-A Sepharose column. The RNA was extracted from each fraction and then hybridized to [3H]poly(U). The quantitation of poly(A) was determined by comparison to a standard curve constructed using [3H]poly(U) and poly(A).

**Table I.** Amount of poly(A) retained by immunoaffinity chromatography

| Exp. 1 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 |
|--------|--------|--------|--------|--------|--------|
| Poly(A) hybridized to [3H]poly(U) | % | ng |
| Anti-fibronectin binding protein | Applied to column | 400 | 364 | 320 |
| Void | 46 | 49 | 40 | 163 | 152 | 114 |
| Retain | 54 | 51 | 60 | 196 | 159 | 170 |
| Total recovered | 100 | 100 | 100 | 350 | 311 | 284 |
| Anti-fibronectin | Applied to column | 400 | 351 |
| Void | 94 | 88 | 291 | 298 |
| Retain | 6 | 12 | 19 | 37 |
| Total recovered | 100 | 100 | 310 | 305 |
| Mouse IgG | Applied to column | 320 |
| Void | 92 | 273 |
| Retain | 8 | 24 |
| Total recovered | 100 | 297 |

The poly(A) RNA from the bound and voided fractions from several immunoadsorption experiments was quantitated by [3H]poly(U) hybridization (Table I). Between 80 and 94% of the input RNAs were recovered from the various immunoaffinity columns. Fifty-one to 54% of the recovered poly(A) RNA was retained by the column when the anti-fibronectin protein antibody was used. In contrast, less than 15% of the poly(A) RNA was retained by the column in the presence of anti-fibronectin and only 8% in the presence of mouse IgG. These low background levels observed with anti-fibronectin and mouse IgG most probably represent nonspecific association of the RNPs to the column. The mean size of the RNA obtained from the immunoaffinity column was approximately 2000 nucleotides (data not shown), in agreement with a previously published value of the size of oocyte poly(A) RNA (11). Thus, no apparent RNA degradation took place as a result of chromatography. Taken together, these experiments lead us to conclude that we have isolated a monoclonal antibody directed against a Xenopus oocyte poly(A) RNA-binding protein which may further be used for ribonucleoprotein purification.

**Discussion**

Ribonucleoprotein particles have been described by a number of investigators. Perhaps the most well studied examples are those which contain RNAs such as 5 S rRNA (Refs. 24 and 25; see Ref. 6 for review), tRNA (26), and small nuclear RNAs (27, 28). Poly(A) RNAs also are known to exist in
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ribonucleoprotein form (29-31). One protein in particular (M, = 75,000) has been shown to bind the poly(A) portion of cytoplasmic mRNA in a periodic fashion (4, 5). However, the exact in vivo function of this or other poly(A) RNA-binding protein remains to be elucidated.

Oocytes are particularly well suited for the study of the function of ribonucleoproteins since the majority of their mRNA exists in nonpolyosomal form (6, 9). In Xenopus oocytes, several proteins which bind poly(A) RNA have been identified (16, 23, 32). Some of these proteins are most prevalent in particles which sediment less than the 80 S monosome, strongly suggesting they are associated with nontranslating mRNAs. In order to determine a possible translational regulatory role of these proteins, the following experiments were performed. The proteins which sediment between 40 and 60 S and which elute from phosphocellulose in high salt (at least 1 m NaCl) were reconstituted with globin mRNA in vitro. This reconstituted RNP was not translatable in the injected oocyte. However, when this reconstituted RNP was deproteinized prior to its injection, globin synthesis was apparent. Thus, it would appear that at least one of those proteins was involved in mRNA sequestration. Other proteins might have functions unrelated to mRNA sequestration as well.

The monoclonal antibody described here is directed against one of the RNA-binding proteins described above. The criteria we have used for its identification include: molecular weight, sucrose gradient sedimentation, tissue specificity, prevalence in small oocytes, and chromatography characteristics on oligo(dT)-cellulose. Experiments presently are being conducted to determine whether the antigen is responsible for mRNA sequestration.

The antigen described in this study and the RNA-binding proteins described elsewhere (16, 23, 32) are oocyte-specific and decrease during oogenesis. If at least some of the proteins do have an in vivo role in translational regulation as suggested (16, 23), then their gradual disappearance during oogenesis might suggest that a changing array of protein synthesis also takes place during oogenesis. In this connection, the pattern of polypeptide synthesis during oogenesis has been examined and been found to change only slightly (33). On the other hand, total protein synthesis increases about 8-fold between stage 3 and stage 6 Xenopus oocytes (6), even though the steady state mass of poly(A) RNA remains constant (9, 10).

It is possible that as the RNA-binding proteins turnover, more mRNA is released for translation. This would imply that the proteins are synthesized early in oogenesis and turnover thereafter. Experiments designed to determine the synthesis and turnover rates of these proteins are underway. Dixon and Ford (32) have conducted experiments which show that certain proteins are synthesized in early oogenesis and turnover thereafter.

We have shown that nontranslating RNPs may be isolated by immunoaffinity chromatography. Of the poly(A) mRNA sedimenting between 40 and 60 S, 55% is bound by the antigen. This is in close agreement with previous experiments which demonstrated that 52% of the poly(A) RNA present in the 40-60 S region eluted from oligo(dT)-cellulose only in the presence of 25 and 50% formamide. These same conditions are required to elute the antigen from oligo(dT)-cellulose (16). Thus, we conclude that about one-half of the poly(A) RNA sedimenting between 40 and 60 S is indeed bound by the antigen. The data presented in Fig. 4 clearly demonstrate that this RNA is non-mitochondrial and non-nuclear. It presently is not known whether this RNA also contains the covalently attached single copy repeat RNA described earlier (11), whether it is a subset of the species of RNA present in oocytes, or whether it contains other small cytoplasmic RNAs previously implicated in translational control (34, 35). The ribonucleoprotein particle, however, is likely to contain proteins, in addition to the antigen described here.

The antibody described in this study and others that presently are being examined may be used to isolate nontranslating RNPs. Furthermore, if it is assumed that all cellular (translating and nontranslating) mRNAs are associated with protein, then the antigen described here is a constituent of only a fraction of the nontranslating RNP. Other antibodies should be useful in isolating other RNPs. Different RNPs might be expressed (i.e. translated) at different times during development. We believe that the immunoadsorption experiments described here will be further useful in isolating and characterizing ribonucleoprotein particles.

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Additions and Corrections

Vol. 259 (1984) 975–979

β-Substituted ethylamine derivatives as suicide inhibitors of lysyl oxidase.

Shiow-Shih Tang, Diane E. Simpson, and Herbert M. Kagan

Page 976, Fig. 1:

The label on the ordinate should read “Per Cent Inhibition of Activity.”

Vol. 259 (1984) 1622–1628

Citrate activation of NAD-specific isocitrate dehydrogenase from bovine heart.

Jerome L. Gabriel and Gerhard W. E. Plaut

Page 1627, Table II, second line below the heading, “Bovine heart”:

“V decreased” should be changed to “V unaffected.”

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A monoclonal antibody to an oocyte-specific poly(A) RNA-binding protein.

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Page 2193, legend to Fig. 3. Part of the figure legend was omitted. The figure with the complete legend is shown below.

Fig. 3. The detection of the antigen in different frog tissues. Total low salt soluble protein from stage 1–2 oocytes (O), frog brain (B), heart (H), liver (L), or muscle (M) was collected, and 100 μg of each were probed for the presence of the antigen as described in the legend to Fig. 1. Shown is an autoradiogram of the protein which reacts with a monoclonal antibody and 125I-labeled second antibody.

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