Inducers of Erythroleukemic Differentiation Cause Messenger RNAs That Lack Poly(A)-binding Protein to Accumulate in Translationally Inactive, Salt-labile 80 S Ribosomal Complexes*

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Translation has an established role in the regulation of cell growth. Posttranslational modification of translation initiation and elongation factors or regulation of mRNA polyadenylation represent common means of regulating translation in response to mitogenic or developmental signals. Induced differentiation of Friend virus-transformed erythroleukemia cells is accompanied by a rapid decrease in the translation rate of these cells. Although inducers do not alter initiation factor modifications, characterization of their effect on mRNA translation provides evidence that this is mediated by the poly(A)-binding protein (PABP). Inducer exposure results in an increase in the amount of mRNA that sediments at 80 S and a decrease in the amount in polysomes. Although these 80 S ribosomes have characteristics previously attributed to “vacant ribosomal couples,” including lability in 500 mM KCl and an inability to incorporate amino acids into protein, we provide evidence that these 80 S complexes are not vacant but contain mRNA that is stably bound to the 40 S subunit, whereas the 60 S subunit is dissociated from the complex by high salt. The absence of eukaryotic initiation factor 2 from these complexes suggests that translation has proceeded through subunit joining. Immunoblotting demonstrates that the mRNAs in these 80 S ribosomal complexes do not contain bound PABP and that this protein is found to be almost exclusively associated with translating polysomes. These data suggest that the PABP plays a role in the accumulation of these 80 S ribosomal-mRNA complexes and may facilitate the formation of translationally active salt-stable ribosomes.

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† The abbreviations used are: eIF, eukaryotic initiation factor; PABP, poly(A)-binding protein; MEL, murine erythroleukemia; hsc, heat shock protein; cognate; CAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; r-protein, ribosomal protein; RNP, ribonucleoprotein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
ing agents (reviewed in Ref. 24). An early and common effect of inducer exposure is to decrease the rate of protein synthesis (25). Inducers do not affect initiation factor phosphorylation8 but do decrease the amount of polysomes while increasing the amount of 80 S ribosomes, similar to the effect observed upon loss of PABP in yeast mutants (22). In yeast, these 80 S ribosomes were ascribed to be “vacant ribosomal couples” based upon their ability to be dissociated into 40 S and 60 S subunits by increasing the monovalent cation concentration in density gradients (26, 27). In this study, we demonstrate that the 80 S ribosomes in MEL cells exhibit characteristics of vacant ribosomal couples, including lability in 500 mM KCl and an inability to incorporate amino acids into protein. However, these complexes are not vacant but contain mRNA that is stably bound to the 40 S subunit, whereas the 60 S subunit is dissociated from the complex by high salt. Furthermore, immunoblotting demonstrates that the mRNAs in these 80 S ribosomal complexes do not contain bound PABP, and this protein is found to be almost exclusively associated with translating polysomes. These data are consistent with the conclusion that the PABP plays a role in the accumulation of these 80 S ribosomal–mRNA complexes and may facilitate the formation of translationally active salt-stable ribosomes.

EXPERIMENTAL PROCEDURES

Materials—Tissue culture supplies were from Life Sciences. Fetal bovine serum was obtained from Intergen. A23187 was from Calbiochem. Biochemicals were from Sigma, and molecular biological reagents were obtained from New England BioLabs, Promega, and Boehringer Mannheim. Nitrocellulose membranes were from Schleicher and Schuell, and Immobilon membranes were from Millipore. Radiolabeling reagents were from DuPont NEN, and reagents for Western blotting were from Amersham Corp.

Cell Culture—MEL cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 12% fetal bovine serum and were maintained at densities that ensured logarithmic growth (0.5–10 × 10^6 cells/ml). Differentiation was induced by the addition of calcium ionophore A23187 (0.75 μM/ml) or MeSO (1.5%, v/v), as described previously (28).

Density Gradient Sedimentation—Cytosolic extracts were prepared from MEL cells by lysis in 1% Triton X-100/0.5% deoxycholate, 100 mM KCl, 10 mM Hepes, pH 7.4, 0.2% Tween 20, and 0.05% Antifoam A and incubated with a polyclonal rabbit antibody to the PABP (38) (a kind gift of R. Moon, University of Washington) for 3 h at room temperature. Bound antibody was detected by a goat anti-rabbit IgG horseradish peroxidase conjugate (Cappel) and developed by chemiluminescence with ECL reagents (Amersham Corp.), as described by the manufacturer. For detection of eIF-2, the separated proteins were transferred to nitrocellulose membranes by electroblotting in 192 mM glycine, 25 mM Tris, 0.1% SDS, and 10% methanol for 2 h at 450 mA. Membranes were blocked as described, incubated with polyclonal goat antibody to rabbit eIF-2 (a gift of W. Merrick, Case Western Reserve University) and detected with 125I-labeled protein A and autoradiography. The purified rabbit eIF-2 that was used as a reference standard was also a gift of W. Merrick.

Protein Synthesis in Density Gradient Fractions—MEL cells were grown in the presence of A23187 for 8 h and, for the final 10 min of incubation, either [3H]leucine (100 Ci/mM) or [35S]methionine (500 μCi/ml) was added as indicated in the text. Extract preparation and gradient sedimentation through 10–50% gradients containing 100 mM KCl was performed as described above. The gradient fractions were collected, and [3H]leucyl-tRNAs were decaysed by incubation in 1 M sodium hydroxide and 1.85% H2O2 for 30 min. Peptides were precipitated on ice with trichloroacetic acid (final concentration, 10%) and collected onto Whatman GF/C glass fiber filters by vacuum filtration. Amino acid incorporation into acid-precipitable material was determined by scintillation counting in a Beckman LS8000 SE liquid scintillation counter.

For electrophoretic analysis of the 35S-labeled peptides, the extracts were sedimented through 10–25% sucrose gradients containing 100 mM KCl, as described previously. Proteins were precipitated from the 80 S fractions with 10 mM Tris, pH 7.4, and the proteins precipitated with 10% trichloroacetic acid. The pellets were washed with 10 mM Tris, pH 8.0, 5 v/v volumes of acetone, and then solubilized by boiling in Laemmli sample buffer. Equal volumes from each fraction were separated by gel electrophoresis in SDS-containing gels, as described by Laemmli (37).

To detect PABP, the separated proteins were transferred to polyvinylidene difluoride membranes by electrophobbing in 0.01 M CAPS buffer at 450 mA for 45 min in a minigel transfer apparatus ( Hoefer). The membranes were blocked by incubation in 5% milk, phosphate-buffered saline, 0.2% Tween 20, and 0.05% Antifoam A and incubated with a polyclonal rabbit antibody to the PABP (38) (a kind gift of R. Moon, University of Washington) for 3 h at room temperature. Bound antibody was detected by a goat anti-rabbit IgG horseradish peroxidase conjugate (Cappel) and developed by chemiluminescence with ECL reagents (Amersham Corp.), as described by the manufacturer.

RESULTS

Inducer Exposure of MEL Cells Decreases the Amount of Polyosome mRNA and Increases the Amount of 80 S mRNA—Inducers of erythroleukemic differentiation decrease the protein synthesis rate in exposed cells (25). To investigate

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The mechanism regulating this decrease, initial experiments determined the kinetics of this decrease for the MEL subclones used in these experiments. Consistent with previous reports (25), Me2SO produced a 50% decrease in methionine incorporation, with the full extent of the decrease evident by 8–10 h of exposure (data not shown). This time coincides with the initiation of commitment to differentiation for these subclones (32) and precedes the increase in transcription of β-globin by 15–20 h (39). For cells exposed to A23187, similar kinetics were observed, although the extent of the decrease was greater, to 25% of control levels (Fig. 1, for example). Thus, in subsequent experiments, the effects of these agents were examined following 7.5–9 h of exposure.

Since initiation factor phosphorylation is a common means of regulating translation (3), we used isoelectric focusing or two-dimensional gel electrophoresis and immunoblotting to assess the effects of Me2SO and A23187 on phosphorylation of eIF-2α, eIF-2β, eIF-4B, eIF-4E, or ribosomal protein S6. These studies demonstrated that modification of these proteins was unaffected by inducer exposure.2 Therefore, density gradients were used to analyze translation in MEL cells. In control cells, most ribosomes sedimented as polysomes (Fig. 1). By 7.5 h of Me2SO exposure, the amount of RNA in polysomes had decreased by 50%, concurrent with an increase in 80 S ribosomes. Identical changes were seen in cells exposed to the chemically distinct inducer, hypoxanthine (data not shown). A23187 caused a qualitatively similar effect, but the extent of the change was more pronounced (Fig. 1B, left and middle panels). This effect was rapidly reversible; within 7 min following transfer to normal growth medium, the amount of 80 S ribosomes had decreased by 50%, and polysomes had begun to reform (Fig. 1B, compare middle and right panels). Cells removed from Me2SO demonstrated a similarly rapid restoration of translation (data not shown); thus, the translational effect of inducers is acutely sensitive to their continued presence in the medium. Since the effect of inducers was greatest in cells exposed to A23187, this agent was used in subsequent experiments investigating the basis of this regulation.

The effect of A23187 on translation of several representative mRNAs was determined. The mRNAs examined included those encoding the ribosomal proteins (r-proteins) S12 and L26, hsc70, PABP, β-actin, GAPDH, and the transcription factor Spi-1 (PU.1). RNA extracted from gradient fractions of control and A23187-exposed cells was analyzed by Northern blotting. Since this approach assessed changes in distribution of RNA in gradient fractions and not in total abundance, parallel experiments determined the effect of A23187 on the expression of each RNA, and these data were included in the graphic quantitations. Fig. 2A demonstrates the effect of A23187 exposure on translation of r-protein L26 mRNA. For control growth conditions, approximately two-thirds of this mRNA cosedimented with polysomes, with the remainder in subpolysomal regions (80 S and smaller). Following 8.5 h of A23187 exposure, the bulk of this mRNA had shifted out of polysomes and was found in two discrete peaks: the largest, which cosedimented with 80 S ribosomes; and the remainder, with 40 S ribosomes (see Fig. 3B for quantitation). This latter, more slowly sedimenting peak of material likely represents either uninitiated mRNP or preinitiation (mRNA/40 S ribosome) complexes. Two other mRNAs were also observed to substantially increase in 80 S fractions following A23187 exposure. mRNAs encoding r-protein S12 and the PABP were largely unassociated with polysomes under control growth conditions (Fig. 2B). Whereas S12 mRNA appeared equally distributed between mRNP and 80 S fractions, PABP mRNA was found almost exclusively in mRNP (fractions 2 and 3), sedimenting more slowly than the 80 S peak of ribosomes in fraction 4. However, both of these mRNAs shifted into 80 S fractions following exposure to A23187.

The remaining mRNAs examined were found primarily in polysomes under control growth conditions (Fig. 3, A and B). For GAPDH mRNA, A23187 caused a shift into smaller polysomes concurrent with a modest increase in the amount of mRNA sedimenting at 40 S and 80 S. For β-actin mRNA, a shift into smaller polysomes was also noted, although nearly all of this mRNA continued to be found in polysomal fractions. For hsc70 and Spi-1 mRNAs, A23187 exposure resulted in a decrease in the amount of polysomal mRNA, whereas the absolute amount of these mRNAs that sedimented at 80 S was unchanged. However, since both of these mRNAs decreased in total abundance, the relative percentage of Spi-1 and hsc70 mRNA that sedimented at 80 S was increased by A23187 exposure.

To ensure that the 80 S accumulation observed for these mRNAs was representative of the majority of mRNAs, the fractionated RNA was bound to slot-blot and hybridized with 32P-labeled cDNA, which had been synthesized from cytosolic RNA obtained from either control or inducer-exposed cells. This experiment demonstrated that in control cells, mRNA was equally distributed between polysomal and subpolysomal fractions (Fig. 3C). However, following A23187 exposure, only 20% of the RNA remained in polysomes, with the rest distributed into the subpolysomal fractions. Thus, the translational effect of inducers was to decrease the amount of mRNA in polysomes...
while increasing the amount that sedimented at 80 S and as more slowly sedimenting mRNPs.

Sedimentation in 500 mM KCl Dissociates the 60 S Subunit but not the 40 S Subunit from 80 S mRNAs—Previous investigators have suggested that accumulated 80 S ribosomes, such as those observed in Fig. 1, are “vacant couples” that do not contain mRNA. However, since inducers caused a concurrent increase in the amount of mRNA and of ribosomes in the 80 S fractions, this suggested that, in MEL cells, these ribosomes were not “vacant.” Vacant couples can be dissociated by increasing the concentration of monovalent cations in density gradients (26, 27). To determine if the 80 S ribosomes in MEL cells were similarly affected, extracts prepared from A23187-exposed cells were fractionated in density gradients containing 500 mM KCl. As demonstrated in Fig. 4A, the 80S ribosomes were completely dissociated into 40 S and 60 S subunits when centrifuged in 500 mM KCl. Although the bulk of the polysomes were stable under these conditions, it was noted that some of the smaller polysomes (2–3 ribosomes/mRNA) also dissociated under these conditions.

To determine if the increased KCl concentration had a similar effect on the sedimentation of mRNAs, RNA was extracted from the gradient fractions and analyzed by Northern blotting. β-Actin was used as a representative polysomal mRNA, whereas S12 was used as a representative 80 S mRNA. The data shown in Fig. 4B demonstrates that the sedimentation of S12 mRNA was sensitive to salt concentration since, in contrast to sedimentation in 100 mM KCl where the bulk of this mRNA was in an 80 S particle (see Fig. 2), in 500 mM KCl, S12 mRNA sedimented as a 40 S particle. Similar behavior was observed for L26 mRNA (data not shown). In contrast, the increased KCl concentration had no effect on the sedimentation of β-actin mRNA because all of this mRNA sedimented in polysome fractions, irrespective of the KCl concentration.

To determine if the salt lability of the 80 S particles observed for S12 mRNA was a general property of 80 S mRNAs, the RNAs extracted from the gradient fractions were bound to membranes by slot-blotting and hybridized with 32P-labeled cDNA that had been synthesized from whole cytosolic RNA obtained from A23187-treated cells. As previously shown for A23187-treated cells, the majority of the mRNAs were found in the 80 S fractions when centrifuged in gradients containing 100 mM KCl. However, when the extracts were centrifuged through gradients containing 500 mM KCl, the bulk of the 80 S mRNA sedimented more slowly, and the largest peak of material sedimented at approximately 40 S (Fig. 4C). Thus, salt lability appeared to be a general property of the 80 S complexes, and sedimentation of both ribosomes and mRNAs appeared to be similarly affected by an increase in the concentration of KCl in the gradients.

To more carefully assess this characteristic of the 80 S complexes, 10–25% sucrose gradients were used to optimize the separation of these smaller particles. As demonstrated in Fig. 5A, 40 S, 60 S, and 80 S ribosomes were easily resolved into distinct fractions using these gradients. In A23187-treated cells, approximately 50% of the ribosomes sedimented at 80 S (Figs. 1 and 2), and in the 10–25% gradients, 80 S ribosomes were the most abundant ribosomal complex detected. When the KCl concentration in these gradients was increased to 500 mM, near complete dissociation of the 80 S ribosomes into individual subunits occurred (a small amount of 18 S and 28 S RNA was still detectable in this region by ethidium bromide staining of the RNAs extracted from the 80 S fractions). Sedimentation in gradients containing EDTA also dissociated the 80 S ribosomes, although under these latter conditions, the subunit peaks were further increased since, as opposed to 500 mM KCl, EDTA also dissociated the polysomes.

The effects of 500 mM KCl and EDTA on the sedimentation of S12 mRNA in these gradients were analyzed by Northern blotting of RNA extracted from the gradient fractions. In addition to the high accumulation of S12 mRNA in the 80 S complexes, its small size (approximately 500 nucleotides excluding poly(A) tail (33)) facilitates the resolution of nonribosomal com-
plexes (mRNP) of this mRNA from those bound to 40 S ribosomes in preinitiation complexes. In gradients containing 100 mM KCl, S12 mRNA sedimented in two discrete peaks (Fig. 5B). The greatest amount of this mRNA was found in the 80 S fractions, although a significant amount sedimented at 40 S. However, in gradients containing 500 mM KCl, S12 mRNA was completely absent from the 80 S fractions, and although there appeared to be a slight increase in mRNA that was unassociated with ribosomes (mRNP), the bulk of this mRNA cosedimented with the 40 S ribosomal subunits. Similar results were obtained for L26 mRNA (data not shown).

The preceding data suggested that the increase in KCl concentration caused dissociation of the 60 S subunit from the 80 S complex but did not affect the 40S/mRNA interaction. However, to ensure that the 80 S mRNAs were not a distinct mRNP particle that was also dissociated in 500 mM KCl, gradient fractionation was performed in the presence of EDTA, since this destabilizes ribosomes but has little effect upon protein/RNA interactions (40). As expected, this resulted in sedimentation of the ribosomes as individual subunits. There was also a complete loss of S12 mRNA from the 80 S fractions, and these mRNAs sedimented near the top of the gradient rather than with the 40 S subunits, as had occurred in 500 mM KCl (Fig. 5B). Thus, EDTA not only dissociated the 80 S ribosomes but also resulted in the dissociation of mRNAs from both 80 S and 40 S fractions, consistent with the conclusion that the 80 S mRNAs were associated with ribosomes and were not a distinct mRNP.

Since the 60 S ribosomal subunit only weakly interacted with the 40 S ribosome and the mRNA in the 80 S complexes, we determined if the 80 S complex was the result of a normal subunit joining reaction or if the interaction of the 60 S ribosome with the 40 S/mRNA complex was nonspecific. Initiation factor eIF-2 binds initiator met-tRNA and is present on 40 S ribosomes and 40S/mRNA “preinitiation” complexes but is extruded at the time that subunit joining takes place (3). The 80 S complexes were, therefore, examined for the presence of eIF-2 by immunoblotting with an antibody that recognizes the β-subunit of eIF-2. This factor was present in 40 S gradient fractions, demonstrating that its interaction with ribosomes was stable under the conditions of gradient sedimentation (Fig. 6). However, eIF-2 was not detected in the 80 S fractions. This finding suggests that the 80 S complex had initiated translation through subunit joining and that inducers affected a step in translation that was subsequent to subunit joining.

The 80 S Ribosomal/mRNA Complexes Are Not Translationally Active—In reticulocyte lysates, 80 S ribosomes do not incorporate amino acids into proteins (41). In MEL cells, leucine incorporation into ribosomal proteins is uniformly decreased by 70% following A23187 exposure,3 suggesting that the accumulated 80 S r-protein mRNAs (S12 and L26) were not translated despite their association with ribosomes. To determine if the accumulated 80 S mRNAs were being translated, A23187-exposed MEL cells were incubated for 10 min with [3H]leucine; then extracts were prepared and fractionated on density gradients. As determined by scintillation counting of trichloroacetic acid-insoluble radioactivity, the 80 S ribosomal/mRNA complexes are not translationally active.

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Fig. 3. The accumulation of mRNAs in 80 S fractions is selective. Cells were exposed to A23187 for 8 h, and extracts were prepared and fractionated in 10–50% sucrose gradients containing 100 mM KCl, as described in the text. The position of the 80 S ribosomal peak, as determined by UV absorbance, is indicated above the autoradiographs and the graphic representations of the densitometric quantitations. A, RNA was extracted from the gradients and analyzed by Northern blot hybridization with 32P-labeled cDNAs encoding β-actin, GAPDH, hsc70, and Spi-1. Blots from control (C) cells are mounted directly above the corresponding blot from the A23187-treated cells (A). Densitometric quantitation of the results are shown in B. These quantitations include the effect of A23187 on mRNA abundance, determined in separate experiments performed in triplicate. (A23187 resulted in the following changes in expression of each mRNA, relative to control: actin, 54%; GAPDH, 31%; hsc70, 22%; and Spi-1, 77%). C, RNA extracted from the gradient fractions of control (C) and A23187-treated (A) cells was slot blotted and hybridized with 32P-labeled cDNA that had been synthesized from whole cytoplasmic RNA obtained from either control or A23187-treated cells. Graphic quantitations are shown just below the autoradiographs.
tic acid-insoluble material, even with this brief incubation most of the incorporated radioactivity was found at the top of the gradient in proteins that had been released from the ribosomes (Fig. 7A). For the radioactivity associated with ribosomal fractions, 85% of the acid-precipitable counts were found in poly-some fractions, whereas the remaining 15% were in the 80 S fractions. Since the amount of RNA (determined by UV absorbance) was equally distributed between 80 S and polysome fractions, leucine incorporation per ribosome was substantially reduced in the 80 S fractions when compared to the polysomes.

To determine if the radioactivity detected in the 80 S fractions was due to ongoing peptide synthesis or represented completed peptides that had become associated with the ribosomes, MEL cells were pulse-labeled as above, except that [35S]methionine was used to facilitate the detection of individual peptides by gel electrophoresis and fluorography. Cell extractswerefractionatedon10–25%sucrosegradients, and the 80 S fractions were pooled and compared with the polysomes that had been obtained from the pelleted material of these same gradients (Fig. 7B). Gel electrophoresis and fluorography of the trichloroacetic acid-precipitable polysomal material demonstrated a diffuse smear, consistent with the incorporation of [35S]methionine into incompletely synthesized peptides of random length. In contrast, the radiolabeled proteins in the 80 S fractions were present as discrete bands, suggesting that the radioactivity detected in these fractions was due to proteins that had completed synthesis and become associated with ribosomes. Thus, the 80 S ribosomal complexes appear to be completely translationally inactive.
sedimented in 10–50% sucrose gradients containing 100 mM KCl. The 10-min prior to the preparation of cytosolic extracts. The extracts were fractions were collected, and labeled tRNAs were deacylated with 1M peptides were labeled as above except that [35S]methionine (500 sodium hydroxide and 1.875% H2O2. The peptides were then precipi-
ated with 10% trichloroacetic acid, and the incorporated radioactivity incorporated amino acids into peptides.

The PABP Is Not Present in Salt-labile Ribosomal/mRNA Complexes—In separate experiments, we identified a protein of approximately $M_\text{r}$ 75,000 that was significantly reduced in abundance in the 80 S fractions when compared to polysomal fractions. This protein comigrated during two-dimensional gel electrophoresis with an abundant protein that was present in preparations of MEL cell PABPs, suggesting that it was the PABP. Since the PABP has been implicated to play a role in mRNA translation (20–22), we assessed the distribution of this protein in gradient fractions by Western blotting and determined the effect of A23187 on its distribution. These experiments demonstrated that nearly all of the PABP in these cells was found in polysomes and confirmed that the PABP was markedly reduced in 80 S fractions (Fig. 8A), compared to the amount of mRNA (see Figs. 3C and 4C, for example). Longer exposures detected only a small amount of this protein in the subpolysomal fractions (Fig. 8B), and no immunoreactive material was detected in the pelleted nuclear material, excluding the possibility that this protein had been stripped off mRNAs during extract preparation (data not shown). In addition, in cells exposed to A23187, there was no change in the distribution of the PABP, despite the significant redistribution of mRNAs out of polysomes and into subpolysomal fractions.

Since polysomal mRNAs were unaffected by sedimentation in 500 mM KCl (26, 27) (Fig. 4), this suggested that mRNAs that bound the PABP might be identified by their stability in 500 mM KCl. Since binding of the PABP to poly(A) is unaffected at the salt concentrations used in these experiments, we determined the effect of KCl on sedimentation of the PABP by Western blotting of gradient fractions, as above. These experiments demonstrated that the sedimentation of the PABP was largely unaffected by the increase in KCl concentration (Fig. 8B). A slight decrease in the amount of PABP in the 80 S...
fraction (fraction 5) was noted, and this was associated with a slight increase in the amount of PABP in the 40 S fraction (fraction 3). However, this change was negligible when compared to that observed for both the mRNAs and ribosomes in the previous experiments and suggests that only a small subset of the 80 S mRNAs bound the PABP. Thus, although 500 mM KCl had substantial effects on sedimentation of 80 S mRNAs, the sedimentation of the PABP was largely unaffected, and this protein continued to be preferentially associated with polysomes.

DISCUSSION

The data in this study demonstrate that the inducer of MEL cell differentiation, A23187, causes an accumulation of mRNAs in an 80 S ribosomal complex. This complex appears to represent a distinct ribosomal/mRNA complex with characteristic properties. High concentrations of KCl displace the 60 S ribosomal subunit from the complex while leaving the 40 S subunit/mRNA interactions intact. Amino acid incorporation into protein is markedly reduced in these complexes, indicating that these ribosomes are not actively translating mRNA. Immunoblotting demonstrates that the 80 S mRNAs do not appear to bind the PABP, because this protein is almost exclusively associated with polysomes. These data not only identify these 80 S ribosome/mRNAs as a unique complex but provide further evidence supporting a translational function for the PABP and also suggest that the affected translational mechanism follows ribosomal subunit joining.

The previous determination that accumulated 80 S ribosomes were “vacant ribosomal couples” was based on the observation that amino acid incorporation was markedly reduced in reticulocyte lysate 80 S gradient fractions (41). In addition, unlike polysomes, the 80 S ribosomes were dissociated by 500 mM KCl; thus, salt lability became a surrogate determinant for translationally inactive ribosomal couples (26, 42). By these criteria, the accumulated 80 S ribosomes in inducer-exposed MEL cells are vacant couples. However, the concurrent accumulation of mRNAs and ribosomes in 80 S fractions suggested that these ribosomes were not truly vacant. The similar effects of KCl and EDTA on sedimentation of both mRNA and ribosomes supported the conclusion that these components existed in a common complex and were not in distinct, cosedimenting particles. Although the salt lability and translational inactivity of these 80 S ribosomal complexes is consistent with the previous suggestion that the synthesizing peptide in the peptide channel of the ribosome stabilizes translating ribosomes (43), the reason for the differential effect of 500 mM KCl on sedimentation of 80 S ribosomes and polysomes remains conjectural.

The accumulation of salt-labile 80 S ribosomes is not a unique effect of A23187, since we have observed similar effects in cells exposed to the inducers MeSO and hypoxanthine. Furthermore, these 80 S ribosomal complexes also accumulate to a lesser extent in control cells. Thus, inducers do not exert a unique effect upon translation but rather appear to affect a step that is a normally occurring intermediate in translation. Following withdrawal of inducers, the number of 80 S ribosomes rapidly decreases, concurrent with an increase in polysomes. Similar observations have been made for ascites tumor cells shifted from an amino acid-poor medium to a rich medium (44) and in ribosomes prepared from animals following forced feeding after a period of starvation (45). Thus, regulation of the amount of mRNA in this complex is not limited to inducer-exposed MEL cells but appears to be a more general process. Although the mechanisms regulating this process remain to be identified, the absence of the PABP from 80 S mRNAs suggests that this protein may mediate these events.

mRNAs in 80 S ribosomes do not appear to contain bound PABP. Exposure of MEL cells to inducers causes an absolute increase in these 80 S mRNAs and a decrease in polysomal mRNAs, without altering the distribution of the PABP in the gradients. Since inducers also decrease the abundance of the PABP, this demonstrates that there is a net loss of PABP from the poly(A) tails of polysomal mRNAs and suggests that this results in the accumulation of these mRNAs in translationally inactive 80 S ribosomes. This is consistent with a translational function for the PABP and is supported by the data that the PABP stimulates translation in reticulocyte lysates (21) and that translation is inhibited in PABP-deficient yeast while decreasing polysome numbers (22). More recently, overexpression of the PABP in oocytes has been shown to prolong the association of certain mRNAs with polysomes (46). Thus, it is likely that either the PABP or a protein recruited to ribosomes by the PABP is responsible for the translational effect of inducer exposure.

The data here suggest that ribosome binding to mRNAs is unaffected by the PABP. Munroe and Jacobson (20) also concluded that the binding of 40 S subunits to mRNA is unimpaired for poly(A)− mRNAs in reticulocyte lysates but that translation was inhibited at subunit joining (20). However, since eIF-2 is released from ribosomes at subunit joining and is not immunologically detectable in MEL cell 80 S ribosomes, the data presented here are more consistent with an effect exerted following subunit joining. The different conclusions of these experiments may have resulted from differences in magnesium concentration used during gradient sedimentation. Since magnesium plays a significant role in stabilizing mRNA structure, the lower concentration used in the experiments reported previously (20) may have contributed to the dissociation of the subunits during sedimentation. Although it may also be argued that the higher concentration of magnesium used in our experiments caused nonspecific association of the subunits, this appears inconsistent both with the absence of eIF-2 from the 80 S particles and with our unpublished observations that other murine cell lines centrifuged under identical conditions contain large amounts of free 40 S and 60 S ribosomal subunits and small amounts of these 80 S complexes.

Whereas these results suggest that translation through subunit joining is unaffected by inducer exposure, the exact mechanism that results in the accumulation of 80 S ribosome/mRNAs remains undefined. It has been suggested that the translational function of the PABP may be to enhance reinitiation on mRNAs (47). Although this would reduce ribosome number per mRNA, this interpretation is not consistent with the observation that the 80 S ribosomes appear to be translationally inactive. Inhibition of elongation rate, as might occur by elongation factor modification (3), would result in the accumulation of polysomes, as seen with cycloheximide. Although this is inconsistent with the data presented, it is possible that elongation could be “locally” inhibited on individual ribosomes. Alternatively, mRNAs that lack the PABP may arrest translation prior to formation of the first peptide bond or at an early stage in peptide synthesis. This latter possibility is consistent with genetic evidence from yeast that deletion of the SIS1 gene, a heat shock protein 40 chaperone homolog, is complemented by mutations in the same genes that complement deletion of the PABP (22, 48). Finally, these ribosomes may have terminated translation and are interacting with elements within the 3′-untranslated region or the poly(A) tail of these mRNAs. Further characterization of this unique subset of ribosomal/mRNA complexes will be necessary to explain the reasons for their accumulation.

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The average length of the poly(A) tracts on mRNAs in 80 S fractions and in mRNP is 25–60 adenosines, and for both S12 and L26 mRNAs, poly(A) tail lengths in these fractions range from approximately 25–200 adenosines. Thus, the exclusion of the PABP from nonpolysomal mRNAs in MEL cells is not due to the lack of poly(A) tracts of sufficient length to bind this protein. This demonstrates that in MEL cells, the PABP is present in limiting abundance, consistent with observations in Xenopus oocytes (38) but not with published reports for HeLa cells (49). Since the PABP is excluded from a specific subset of mRNAs in these cells, it is evident that its interaction with mRNAs cannot be governed solely by its affinity for poly(A), and mechanisms must exist to regulate the addition and/or removal of this protein from poly(A) tails. Interpretations that are consistent with the distribution of the PABP observed in MEL cells are that: 1) mRNAs that bind PABP have a selective translational advantage; or 2) PABP is only loaded onto poly(A) mRNAs (52). If this results from the presence of PABP on mRNAs that are newly transported to the cytoplasm, then their resultant accumulation in 80 S ribosomes may be subsequent to the cotranslational removal of the PABP. The data presented here suggest that inducers increase the rate of this process, and thus, that this is a regulable process. The proposed role of the PABP in regulating both mRNA translation and stability suggests that these changes have a significant role in regulating gene expression in inducer exposed cells.

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