

Uptake and Utilization of mRNA by Myogenic Cells in Culture

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ABSTRACT Primary chick myoblast cultures demonstrate the ability to take up exogenously supplied polyadenylated RNA and express the encoded information in a specific manner. This expression is shown to exhibit tissue specificity. Analysis of creatine kinase activity monitored at various times of incubation in the presence of either polyadenylated or nonpolyadenylated RNA indicates that only the poly(A)⁺ mRNA is capable of being actively translated. Radioactively labeled poly(A)⁺ mRNA is taken up by the cell cultures in a time-dependent manner and subsequently shown to be associated with polysomes. This association with polysomes does not occur in the presence of puromycin and is unaffected by actinomycin D. Thus, nonspecific interaction with polysomes and induction of new RNA synthesis are ruled out and the association of the exogenously supplied poly(A)⁺ mRNA with polysomes is indicative of its translation in the recipient cells. When heterologous mRNA (globin) is supplied to the myoblasts, it is also taken up and properly translated. In addition, exogenously supplied myosin heavy chain mRNA is found associated with polysomes consisting of 4-10 ribosomes in myoblast cell cultures while in myotubes it is associated with very large polysomes, thus reflecting the different translational efficiencies that this message exhibits at two very different stages of myogenesis.

The results indicate that muscle cell cultures can serve as an *in vitro* system to study translational controls and their roles in development.

A number of studies has suggested that not only purified DNA but also purified RNA can be taken up by cells (reviewed in references 3, 14, and 38). The first direct evidence arose from repeated observations of the infectivity of purified viral RNA and viroids (21, 35). The aminoacylation of bacterial tRNA by mouse fibroblasts in culture (37) and the ability of RNA extracted from human fibroblasts treated with poly(I) · poly(C) to elicit production of human interferon in chick fibroblasts as well as the production of mouse interferon by avian and simian cells incubated with mRNA extracted from mouse cells (26) serve to further demonstrate the uptake of functional RNA by cells.

The cellular uptake of RNA has been reported to result in specific immune responses (19), the synthesis of specific proteins (30), as well as the induction of membrane differentiation (27, 31). In most of the cases in which RNA has been demonstrated to enter cells, there is a lack of information concerning the manner by which it penetrates the cells and subsequently exerts its effects, and often the RNA itself has been poorly characterized. On the other hand, specific mRNA transcripts and their activities have been studied after entry into *Xenopus*

laevis oocytes by microinjection techniques (16, 32). These experiments have been used to determine the relationship between structural aspects of mRNA molecules and their function in living cells. In these studies, mRNA transcripts enter directly into association with ribosomes, resulting in the accumulation of the corresponding protein products, indicating that the exogeneously supplied polynucleotides are translated. In this experimental approach, it is important to note that oocytes are unique cells that contain a vast excess of components required for protein synthesis. Of further significance is the fact that the amount of specific mRNA transcripts injected per oocyte greatly exceeds that normally found within the cell at any developmental stage.

That exogenously supplied RNA molecules can enter into protein synthesis has been shown by the fact that when tRNA is added to cells it becomes aminoacylated and is subsequently utilized in protein synthesis within recipient cells (11). By the same token, mRNA should be found in association with ribosomes in functional polysomes. If, indeed, mRNA is capable of entering cultured myogenic cells and can be shown to be translated, it would allow a means by which the translatability

of specific mRNA transcripts, either in the presence or absence of translational control elements, could be determined at various stages of differentiation.

In this report we demonstrate the ability of myogenic cells to take up mRNA and faithfully translate it. The expression of the encoded information is independent of new RNA synthesis, and the exogenously supplied mRNA transcripts are found associated with ribosomes on functional polysomes. Nonpolyadenylated RNA (i.e., rRNA and tRNA), although taken up by the cells, is not found to be associated with polysomes.

MATERIALS AND METHODS

Myogenic Cell Cultures

Breast muscle cell cultures, essentially free of fibroblasts, were obtained from 12-d, pathogen-free, chick embryos. The breast muscle was mechanically dissociated as described by Tepperman et al. (40). The cells were plated on gelatin-coated 60-mm culture dishes at an initial density of 2.5×10^6 cells/plate and grown in F-10 medium supplemented with 10% horse serum and 3% chick embryo extract at 37°C in 5% CO₂. 24 h after the initial plating the cells were supplied with fresh growth medium. Normally, 12 h after this medium change the cells were washed 10–15 times with phosphate-buffered saline (PBS) at 37°C. RNA was subsequently added to the cells at the indicated concentrations in 0.5 ml of PBS and the cultures were allowed to incubate for 15 min at 37°C in 5% CO₂. After this initial 15-min incubation period the cells were supplemented with complete growth medium. Control cultures were treated in an identical manner without the addition of RNA. After the indicated incubation time the cultures were processed as required for each experiment. Where indicated, actinomycin D was added to the cultures 20 min before the addition of RNA.

Isolation of RNA

Leg muscle, liver, and brain from 14-d embryonic chicks were homogenized in 0.02 M Tris-HCl (pH 7.4), 0.005 MgCl₂, 0.25 M NaCl, and 0.006 M β -mercaptoethanol. The 12,000 g supernate was phenol extracted as previously described (20). Poly(A)⁺ containing RNA and poly(A)⁻ RNA were separated by oligo d(T)-cellulose chromatography as described by Aviv and Leder (1). Radioactive poly(A)⁺ mRNA and poly(A)⁻ RNA were prepared from chick embryo breast muscle cell cultures (initial plating density of 6×10^6 cells/100-mm plate). At 24 h the cultures were labeled with 10 μ Ci/plate of [³H]uridine for 24 h in fresh medium. At this time fresh medium was added with an additional 10 μ Ci [³H]uridine and the cells were harvested 24 h later. After lysing the cells by the procedure of Morse et al. (28), the 15,000 g supernate was phenol extracted, and [³H]poly(A)⁺ mRNA and [³H]poly(A)⁻ RNA were isolated as described above.

Radioactively labeled myosin heavy chain (MHC) mRNA was extracted from the 80–120S mRNPs of similarly labeled muscle cultures. The 80–120S mRNPs were separated from the cytoplasmic fraction by centrifugation on 10–30% sucrose density gradients as described by Dym et al. (8).

Globin mRNA (9S) was purified from the cytoplasm of rabbit reticulocytes (Gibco Diagnostics, Gibco Invenex Div., Chagrin Falls, Ohio).

Determination of Creatine Kinase Activity

The myoblast cultures were harvested in 0.5 ml of 0.05 M glycylglycine (pH 6.75) and the cell suspension was homogenized. The rate of ATP formation from ADP and creatine phosphate was determined by coupling the reaction to that of hexokinase and glucose-6-phosphate dehydrogenase (29, 34). Myokinase activity was suppressed by the addition of 200 mM AMP. The change in absorption at 340 nm was continually monitored on a Gilford recording spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, Ohio) with 1 U of enzyme activity defined as the amount of enzyme catalyzing the formation of 1 μ mol/min per ml of NADPH at 30°C.

Creatine Kinase Isoenzyme Composition

The creatine kinase isoenzyme composition in the different samples was determined by electrophoresis on 7% native polyacrylamide gels according to the methods of Levine et al. (23).

The muscle type (M-CPK) was also detected by specific antiserum. Cell cultures were scraped in 1.0 ml of PBS, pelleted at 5,000 rpm, brought up in 0.2 ml of PBS, and centrifuged at 12,000 rpm for 12 min. The supernates were centrifuged again at 45,000 rpm for 45 min. 25- μ l samples of the supernate were reacted with 25 μ l of antiserum and incubated for 1 h at 37°C. Protein A was

added to the mixture which was incubated for an additional 10 min. After storage at 4°C for 12 h, the antiserum-antigen-protein A complex was collected by a 30-min centrifugation in an Eppendorf microcentrifuge. The pellet was brought up in 30 μ l of SDS sample buffer and electrophoresed on the Laemmli gel system (22).

Myofibrillar Proteins

The myofibrillar proteins were extracted from the muscle cell cultures by three rounds of ionic precipitation. The [³⁵S]methionine-labeled myofibrillar proteins were analyzed by: (a) 7.5% SDS polyacrylamide cylindrical gel electrophoresis (8 mA/tube for 3 h) in which the gels were sliced and the radioactivity in each fraction was determined by scintillation counting. (b) Two-dimensional gel electrophoresis (33). Samples were brought up in 20 μ l of lysis buffer (9.5 M urea, 5% β -mercaptoethanol, 2.0% ampholyte, pH 5–7) and electrophoresed on isoelectric focusing gels. The isoelectric focusing gels were stored at –80°C. Upon thawing, the gels were equilibrated for 30 min in sample buffer (2% SDS, 5% β -mercaptoethanol, 0.06 M Tris, pH 6.8) and electrophoresed on a three-step (12.5, 10, 7.5%) SDS polyacrylamide slab gel (22).

Association of RNA with Ribosomes in Polysomes

The association of exogenously supplied [³H]uridine-labeled RNA with polysomes of recipient cells was monitored by sucrose density gradient centrifugation. The cell cultures were lysed by the procedure of Morse et al. (28), except that the buffer contained 50 μ g/ml of cycloheximide. The cell lysate was added to a 10,000 g supernate (prepared in the same buffer containing cycloheximide) of 14-d embryonic muscle tissue (0.5 mg wet weight muscle tissue/cell culture) and centrifuged at 10,000 g for 10 min. Subsequently, the combined 10,000 g supernates, containing the radioactive RNA, were layered on 10–30% sucrose density gradients prepared in the same buffer (0.25 M KCl, 0.005 M MgCl₂, 0.01 M Tris-HCl (pH 7.4), 50 μ g/ml cycloheximide) and centrifuged at 32,000 rpm for 1 h using an IEC SB283 head (Damon/IEC Div., Damon Corp., Needham Heights, Mass.). The gradients were continually monitored during collection of fractions, using a Gilford flow-through spectrophotometer. Radioactivity was determined by scintillation counting with a DPM counter (Tracor Analytic, Searle Analytic Inc., Des Plaines, Ill.).

Translation of Globin mRNA in Myoblast Cultures

Globin mRNA (10 μ g) was added to a myoblast culture and subsequently incubated with 10 μ Ci of [³⁵S]methionine. Globin was subsequently purified from the cultured cells and the α - and β -globin chains were analyzed by CM-cellulose chromatography by the procedures of Gurdon et al. (16).

RESULTS

Creatine Kinase Activity and Added RNA

When myoblast cell cultures are extensively washed free of growth medium and poly(A)⁺ mRNA from embryonic chick leg muscle is subsequently added, an increase in creatine kinase activity occurs over endogenous (control) levels (Fig. 1). Actinomycin D (2 μ g/ml, inhibiting not less than 97% of all RNA synthesis) does not prevent this increase in creatine kinase activity for up to 3 h. The subsequent drop of activity in the drug-treated cultures is a result of cell death. The addition either of poly(A)⁻ RNA, consisting mainly of rRNA and tRNA, or of polyadenylic acid (results not shown) does not result in an increase in creatine kinase activity (Fig. 1). In a number of experiments the poly(A)⁻ RNA was actually found to lower the activity of this enzyme below values obtained in the absence of any added RNA. The increase in creatine kinase activity is found to be directly correlated with the amount of the exogenous poly(A)⁺ mRNA added to the cultures (Fig. 2). Creatine kinase activity increases linearly when up to 30 μ g of RNA are added per culture. The dose-dependent response, the requirement for poly(A)⁺ mRNA, and the ability to increase the activity of creatine kinase in the presence of actinomycin

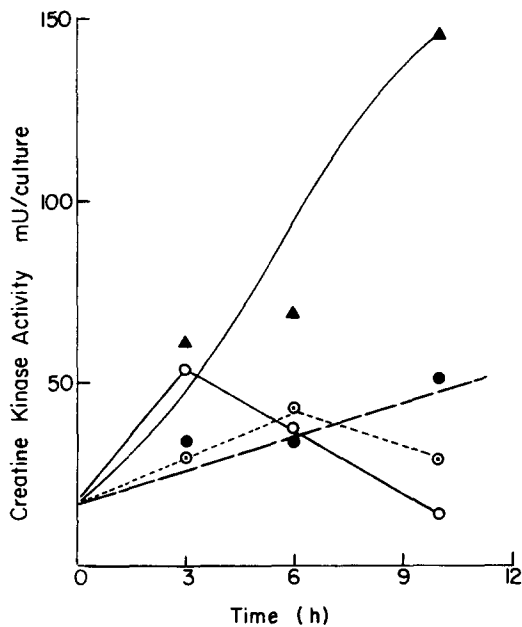


FIGURE 1 Effect of various exogenous RNA transcripts on creatine kinase activity. 36-h-old chick breast muscle cell cultures (myoblasts) were extensively washed with PB Saline after removal of the growth medium. Exogenous RNA was added at the concentration of 30 $\mu\text{g}/\text{culture}$ for 15 min, at which time the cultures were supplemented with fresh growth medium. At the indicated time points, plates were removed and assayed for creatine kinase activity: (●) control group without RNA, (▲) chick leg muscle poly(A)⁺ mRNA, (○) chick leg muscle poly(A)⁺ mRNA and actinomycin D (2 $\mu\text{g}/\text{ml}$), (⊙) chick leg muscle poly(A)⁻ RNA. These data are representative of one out of six experiments performed.

D, all suggest that the RNA is acting directly to increase the synthesis of new enzyme. The fact that protein synthesis is required is demonstrated by the observation that cycloheximide completely blocks this increase over endogenous levels resulting from the addition of poly(A)⁺ mRNA (Fig. 3).

Fidelity of Translation of Added mRNA

If, indeed, the poly(A)⁺ mRNA that is added to myoblast cell cultures is being directly translated after uptake into the cells, the source of the added mRNA should determine the isoenzyme pattern responsible for the increased creatine kinase activity in the recipient myoblasts. It is known that both liver and brain have the BB (nonmuscle) type of creatine kinase, whereas in developing muscle the pattern changes from the BB to the MM (muscle) type as the muscle differentiates from the myoblast to the multinucleated myotube (25, 9). When the isoenzyme pattern of creatine kinase was determined after the addition of poly(A)⁺ mRNA from liver, brain, and muscle, the following results were obtained; while the addition of mRNA from all these sources resulted in an increase in total creatine kinase activity above that of control levels, only the addition of muscle mRNA caused the appearance of the muscle type isoenzyme (Table I). Only the BB type could be detected in cultures when either brain or liver mRNAs were added. The appearance of the muscle-specific isoenzyme in myoblast cultures treated with muscle mRNA is further demonstrated by the precipitation of this protein by specific antiserum (Fig. 4). It is also evident that the addition of total poly(A)⁺ mRNA results in an overall stimulation of protein synthesis, not only of the M-type CPK isoenzyme. This is to be expected because

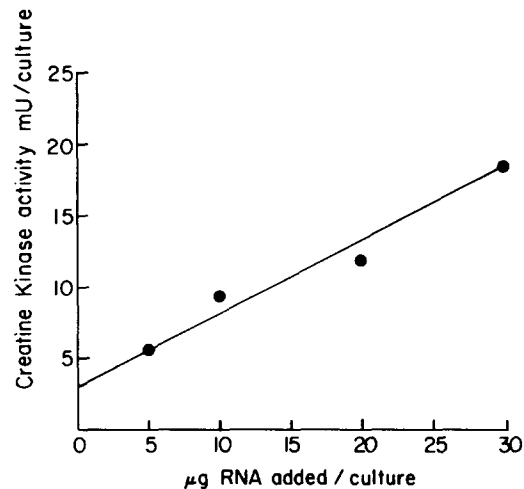


FIGURE 2 Effect of increasing amounts of added RNA on the increase of creatine kinase activity. Increasing amounts (5–30 μg) of chick leg muscle poly(A)⁺ mRNA were added to 24-h-old chick breast muscle cell cultures as described in Materials and Methods. 5.5 h later, the cultures were assayed for creatine kinase activity.

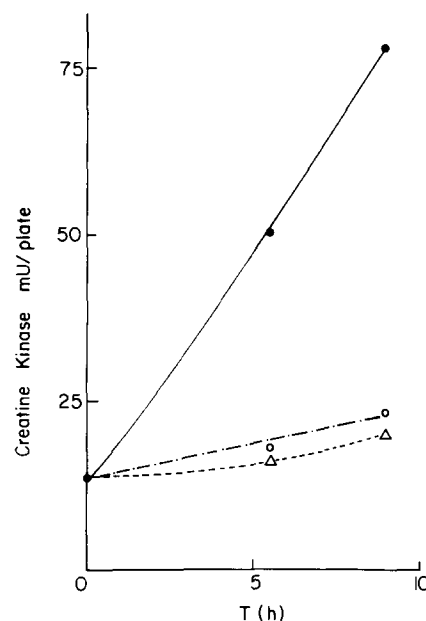


FIGURE 3 Effect of inhibition of protein synthesis on the increase of creatine kinase activity caused by addition of RNA. 30 $\mu\text{g}/\text{plate}$ of leg muscle poly(A)⁺ mRNA was added to 26-h-old chick breast muscle cell cultures in the presence (Δ) or absence (●) of cycloheximide (50 $\mu\text{g}/\text{ml}$), (○) control without added RNA. The cultures were assayed for creatine kinase activity at the times indicated.

TABLE I

Creatine Kinase Isoenzyme Composition in Cultures as Directed by RNA from Different Tissues

Source of RNA	CK activity	CK isoenzymes
	% of control	
Control (no RNA added)	100	BB
Liver	427.7	BB
Brain	126.9	BB
Muscle	411.1	MM, MB, BB

The different poly(A)⁺ mRNAs were prepared from brain, liver, or leg muscle taken from 14-d-old chick embryos. In each case, 30 $\mu\text{g}/\text{culture}$ of the appropriate RNA was added. 5.5 h later, the cultures were assayed for creatine kinase activity and the isoenzyme composition was determined as described in Materials and Methods.

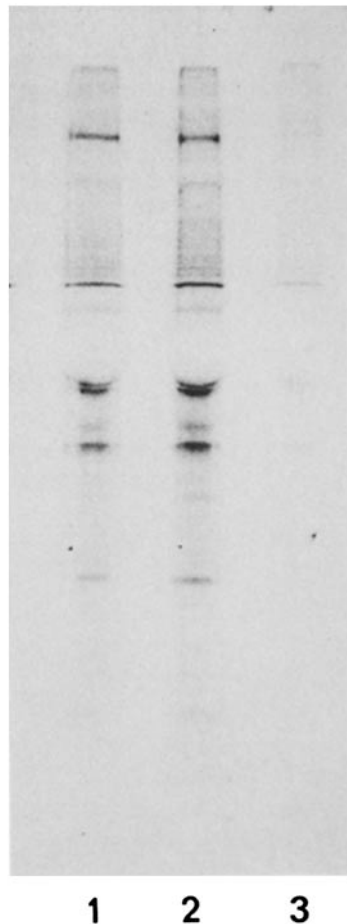


FIGURE 4 Effect of increasing amounts of added mRNA on the synthesis of M-type creatine kinase. Various concentrations of poly(A)⁺ RNA, prepared from 15-d embryonic muscle tissue, were added to 24-h-old myoblast cultures incubated in the presence of [³⁵S]methionine. M-type CPK was precipitated by specific antiserum and resolved by SDS polyacrylamide gel electrophoresis. Incorporation of label into protein was detected by autoradiography. Lanes 1, 2, and 3 represent the addition of 30, 45, and 0 μg of poly(A)⁺ RNA. The bar marks the position of M creatine kinase marker.

we are adding the total muscle cell complement of mRNA. The fact that the isoenzyme synthesized is directly determined by the source of the mRNA strongly favors the direct involvement of the added mRNA in protein synthesis.

The total leg muscle mRNA population should code for the majority of, if not all, the muscle-specific proteins (5). We therefore determined the effect of added muscle poly(A)⁺ mRNA on the synthesis of total myofibrillar proteins (Fig. 5). Addition of muscle mRNA resulted in enhanced synthesis of myofibrillar proteins in the general molecular weight region of the myosin heavy chain (mol wt 200,000), actin (mol wt 43,000), and tropomyosin (mol wt 36,000). We also assayed for, and found a specific increase in, the synthesis of α-actin, the muscle-specific actin (Fig. 6). Again, this demonstrates that exogenously supplied muscle mRNA is faithfully translated in the recipient cells.

To establish whether myogenic cells are capable of translating a totally heterologous messenger, 9S globin mRNA obtained from rabbit reticulocytes was supplied to cell cultures (Fig. 7). Globin mRNA is found to direct the synthesis of both α- and β-globin polypeptides, however, the ratio of α- to β-globin synthesized by the myoblasts is opposite that observed

in *Xenopus* oocytes and cell-free translation systems (16, 24). This reversed predominance in the synthesis of globin polypeptide chains is also observed in the mouse ovum injected with rabbit globin mRNA (4) and may serve as a reminder that each system has unique characteristics responsible for the different results cited. Nevertheless, the use of different systems should help to clarify our understanding of the various controls exerted on messenger stability and translation.

Association of Added RNA with Polysomes

To ascertain that the poly(A)⁺ mRNA added to the cells is taken up and subsequently translated on polysomes, the following experiments were performed: Radioactively labeled RNA obtained from muscle cell cultures was added to myoblast cultures. After 30 min the cultures were harvested and the polysomes were analyzed by sucrose density gradient centrifugation. The sedimentation profiles are very different for the poly(A)⁺ mRNA and the poly(A)⁻ RNA (Fig. 8A). Whereas the poly(A)⁺ mRNA enters the cells and is associated with polysomes, the poly(A)⁻ RNA, although entering the cells, is not associated with polysomes and is found only at the top of the sucrose density gradient. This, again, indicates that exogenously supplied mRNA is actively engaged in protein synthesis. To rule out induction of new RNA synthesis and nonspecific binding to polysomes, similar experiments were performed in the presence of actinomycin D and puromycin (Fig. 8B). The association of the radioactively labeled poly(A)⁺ mRNA with polysomes is unaffected by actinomycin D, ruling out the requirement for new RNA synthesis. On the other hand,

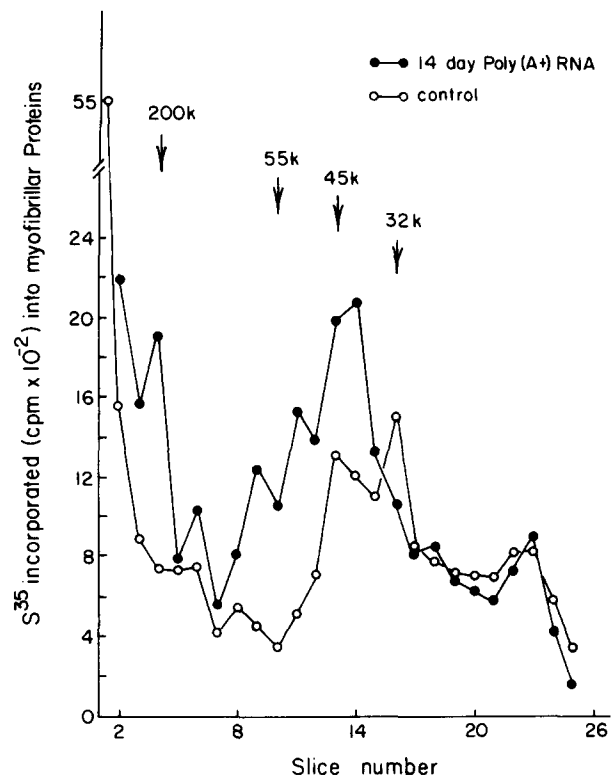


FIGURE 5 Synthesis of myofibrillar proteins in the presence and absence of exogenously added poly(A)⁺ RNA. Total mRNA from 14-d embryonic chick muscle was added to myoblast cell cultures in PB Saline for 30 min. Subsequently, incubation medium was added containing [³⁵S]methionine. After 2 h, the myofibrillar proteins were extracted by ionic precipitation (three times) and analyzed by 7.5% SDS polyacrylamide gel electrophoresis.

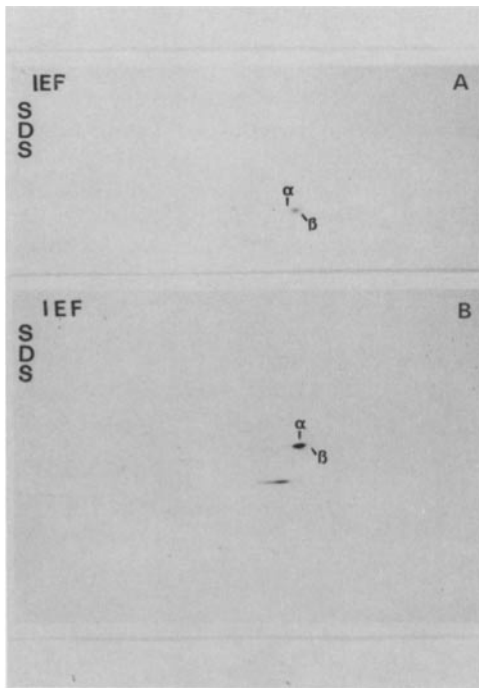


FIGURE 6 Synthesis of α -actin in the presence (B) and absence (A) of exogenously supplied poly(A)⁺ RNA. Myoblast cultures were labeled for 5 h with [³⁵S]methionine after the addition of muscle poly(A)⁺ RNA. The actins were resolved from cell homogenates by two-dimensional gel electrophoresis and detected by autoradiography. Each panel shows only the actin region of the gel with the more acidic proteins on the left and the more basic proteins on the right. Actin identifications were made by coelectrophoresis and by alignment of the respective patterns.

puromycin causes a total shift of the added mRNA from the polysomal region of the sucrose density gradient to the top of the gradient. In this manner we established that the sedimentation of added mRNA with polysomes is the result of a functional association with ribosomes. Experiments carried out for different time periods show the time-course of entry of the added mRNA transcripts and their incorporation into polysomes (Fig. 9). In this type of experiment it is usual that ~3–5% of the exogenously supplied mRNA is eventually incorporated into polysomes. Only 0.4–0.6% of the poly(A)⁺ or adenylic acid polymer enters the cells, and this small amount is not associated with polysomes. This difference also suggests that the cells may preferentially take up certain species of RNA, however, additional studies must be performed to ascertain this.

In addressing the question of whether certain mRNA transcripts are preferentially utilized by the recipient cells, it is also of interest to determine whether this utilization changes during cellular development. It has been shown that in myotubes MHCs are synthesized on very large polysomes while in replicating myoblasts mRNA transcripts coding for the very same proteins are translated less efficiently and on somewhat smaller polysomes (18, 41). To reexamine these different translational efficiencies, we added radioactively labeled MHC mRNA to both myoblast and myotube cell cultures (Fig. 10). Both cultured cell types are capable of taking up and translating this mRNA, however, the myotube cultures translate MHC mRNA on larger polysomes (Fig. 10B). Considering an average of 5% uptake in such experiments, it is calculated that each cell is incorporating ~25,000 MHC mRNAs. This number is some-

what higher than that found normally in these myogenic cells (8), but is still less than the number applied by injection to *Xenopus* oocytes (15, 16). This and the fact that muscle cells at different stages of development utilize mRNA with different efficiencies make this model system attractive for studying controls exerted on translation.

DISCUSSION

The studies reported here demonstrate that in primary muscle cultures exogenously supplied mRNA is capable of directing the synthesis of specific proteins. This is illustrated by the fact that only poly(A)⁺ mRNA is effective in elevating creatine kinase activity. This increase in activity occurs in the presence of actinomycin D but is completely inhibited by cycloheximide. Furthermore, the source of the poly(A)⁺ mRNA specifies the creatine kinase isoenzyme composition of the recipient cells.

Radioactively labeled mRNA added to cultures is found associated with polysomes, and neither the uptake nor the utilization of the labeled transcripts is inhibited by the presence of actinomycin D. These findings substantiate our claim that muscle cell cultures can serve as a model system for the study of post-transcriptional mechanisms effecting mRNA expression. Translational fidelity of the added mRNA is exemplified by the addition of globin mRNA, a totally heterologous message. Muscle cultures accurately direct the synthesis of both the α - and β -globin polypeptides. The synthesis of these polypeptides demonstrates a high α - to β -globin ratio which is atypical with respect to that found in reticulocytes.

The ability of myogenic cells to correctly translate exogenously supplied mRNA makes it possible to probe the mechanisms involved in the selective translation of mRNA transcripts at defined stages of differentiation. Applications of this system can be used to investigate the biochemical properties of specific mRNA molecules, i.e., their utilization, stability, and

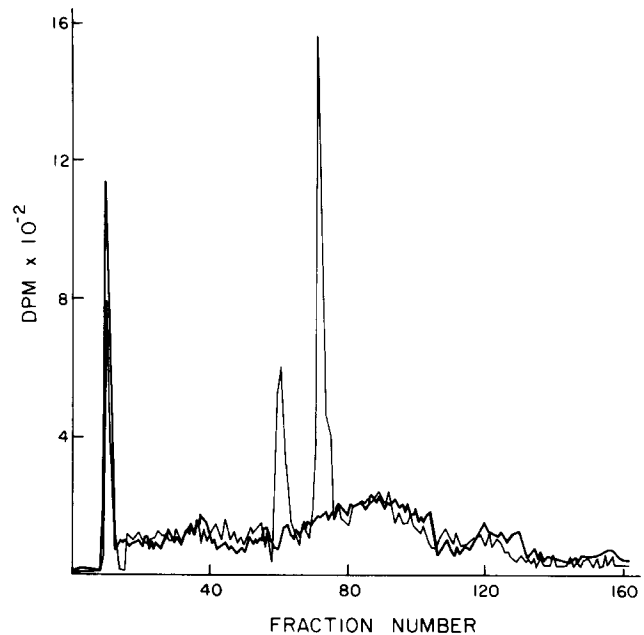


FIGURE 7 Translation of exogenously supplied globin mRNA in myoblast cultures. Globin mRNA (10 μ g) was added to the cultures which were subsequently incubated with [³⁵S]methionine for 5 h. Globin was purified as described in Materials and Methods from control (—) and mRNA-supplemented cultures (---). Fractions 60–65 represent β -globin, while fractions 70–75 represent α -globin.

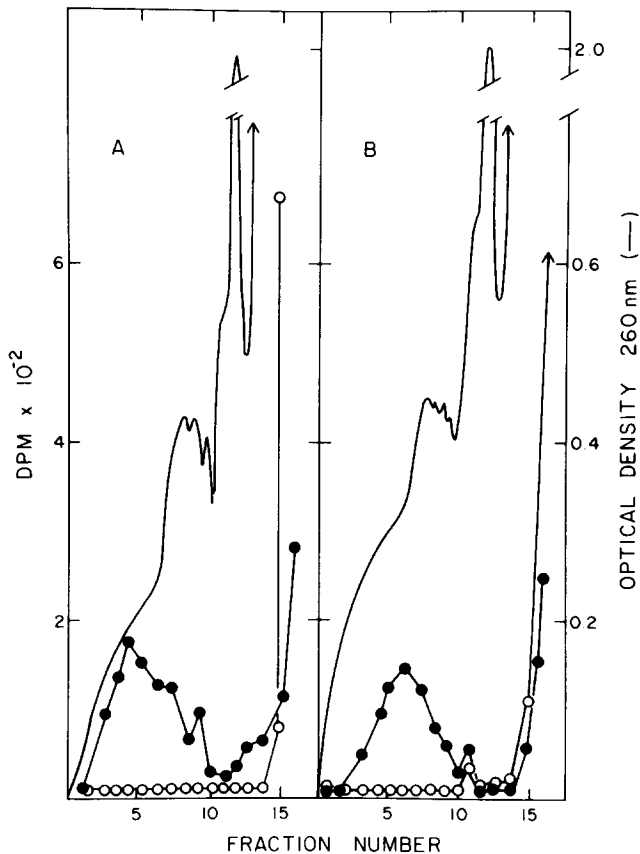


FIGURE 8 The association of exogenously added mRNA with polysomes. Myoblast cell cultures were incubated for 30 min with 60,000 dpm of [³H]uridine-labeled poly(A)⁺ mRNA. After cell lysis, the 10,000 g extract was combined with unlabeled embryonic muscle tissue extract and analyzed by sucrose density centrifugation. (A) [³H]poly(A)⁺ RNA (●), [³H]poly(A)⁻ RNA (○). (B) [³H]poly(A)⁺ RNA plus actinomycin D (●), [³H]poly(A)⁺ RNA plus puromycin (○).

association with control elements (17, 2, 10). We have demonstrated that the 26S MHC mRNA transcript displays different translational efficiencies at two separate stages in myogenesis. This is apparent by its differential association with recipient polysomes in myoblasts and myotubes. It is unlikely that MHC mRNA transcripts are degraded to a greater extent in myoblasts than in myotubes because (a) the total amount of radioactivity at the top of the sucrose density gradients in myotubes and myoblasts is identical, and (b) the MHC mRNA transcripts can be shifted to larger polysomes by the presence of eIF-3 associated proteins in myoblast cultures.¹ Recent studies (8, 7) have indicated that the MHC mRNA is stored as an inactive mRNA-protein complex in non-dividing myoblasts, a cell population at which exogenously supplied MHC mRNA is less efficiently translated. Later in development, these transcripts are transferred to polysomes where they serve to augment myosin synthesis. All of these studies argue for the involvement of translational controls in myogenesis.

The findings presented here further indicate that a critical reevaluation of the inductive effects of RNA added to embryonic systems is required (31, 27), especially if one views an inducer as a substance acting by directly derepressing the

¹ O'Loughlin, J., L. Lehr, and S. M. Heywood. Manuscript in preparation.

genome. Our data show that mRNA can influence cellular phenotype by the direct translation of transcripts added to cells *in vitro*. However, caution should be exercised as to whether similar events occur *in vivo*. Cellular mRNA has been shown to be translated upon penetration of membranes associated with such organelles as mitochondria (39) and chloroplasts (12). One can, therefore, speculate upon the existence of similar intercellular phenomena playing a role in differentiating systems.

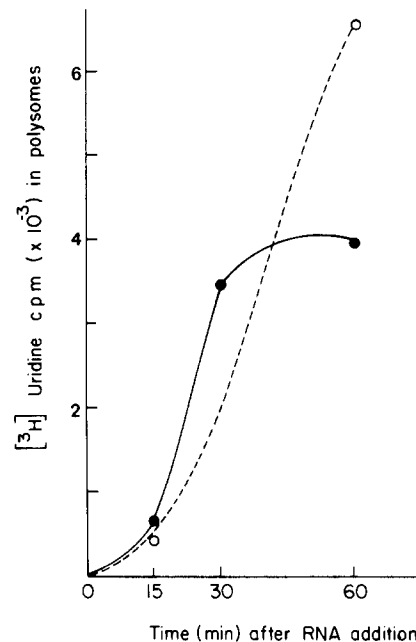


FIGURE 9 The kinetics of incorporation of added mRNA into polysomes. [³H]uridine-labeled mRNA was added to myoblast cell cultures in the presence (○) and absence (●) of actinomycin D. Analysis of radioactivity in polysomes was carried out as in Fig. 8.

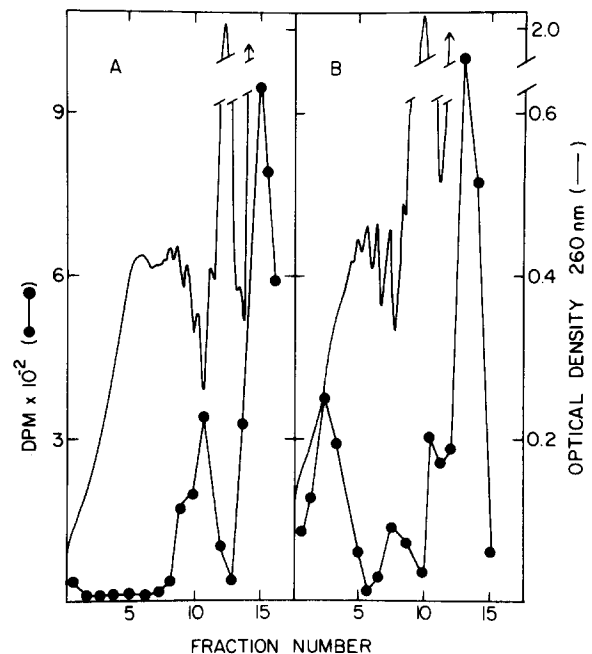


FIGURE 10 The differential association of 26S myosin mRNA with polysomes in myoblast cultures vs. polysomes in myotube cultures. [³H]myosin mRNA (50,000 dpm) was exogenously added to either myoblast (A) or myotube (B) cultures prepared from embryonic heart muscle as described in Materials and Methods.

The success of experiments described in this communication is contingent upon repeated rinsing of the cells before the addition of RNA. This is attributed to the abundance of nucleases in the sera used to enrich the basic growth medium. A method which overcomes this difficulty and which has the advantage of introducing RNA-protein complexes into cells involves the use of phospholipid vesicles (6, 36).² Initiation factor-associated proteins have been shown to discriminate between various mRNAs (13). The direct interaction between these proteins and mRNA transcripts leading to altered translational properties is being currently studied in this laboratory by employing liposomes as an experimental tool.

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