Studies on a Nonpolysomal Ribonucleoprotein Coding for Myosin Heavy Chains from Chick Embryonic Muscles*

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A messenger ribonucleoprotein (mRNP) particle containing the mRNA coding for the myosin heavy chain (MHC mRNA) has been isolated from the postpolysomal fraction of homogenates of 14-day-old chick embryonic leg muscles. The mRNP sediments in sucrose gradient as 120 S and has a characteristic buoyant density of 1.415 g/cm³, which corresponds to an RNA:protein ratio of 1:3.8. The RNA isolated from the 120 S particle behaved like authentic MHC mRNA purified from chick embryonic muscles with respect to electrophoretic mobility and ability to program the synthesis of myosin heavy chain in a rabbit reticulocyte lysate system as judged by multi-step co-purification of the in vitro products with chick embryonic leg muscle myosin added as carrier. The RNA obtained from the 120 S particle was as effective as purified MHC mRNA in stimulating the synthesis of the complete myosin heavy chains in rabbit reticulocyte lysate under conditions where non-muscle mRNAs had no such effect. Analysis of the protein moieties of the 120 S particle by sodium dodecyl sulfate-polyacrylamide gel electrophoresis shows the presence of seven distinct polypeptides with apparent molecular weights of 44,000, 49,000, 53,000, 81,000, 83,000, 86,000, and 98,000, whereas typical ribosomal proteins are absent.

These results indicate that the 120 S particles are distinct cellular entities unrelated to ribosomes or initiation complexes. The presence of muscle-specific mRNAs as cytoplasmic mRNPs suggests that these particles may be involved in translational control during myogenesis in embryonic muscles.

It is currently believed that mRNPs exist in the cytoplasm of a wide variety of eukaryotic cells as protein-bound complexes referred to as mRNP particles or informosomes (for a review see Refs. 1 and 2). Rapidly labeled RNAs, not associated with polysomes, have been detected in the cytoplasm of fish embryos (1), mouse L cells (3), sea urchin embryos (4), silk worm (5), HeLa cells (6), rat liver (7), duck erythrocytes (8), and embryonic muscle cells (9), and Ehrlich ascites tumor cells (10). Among the several mRNAs, coding for specific proteins, which have been identified in the postpolysomal supernatant of a variety of tissues, are the mRNAs coding for actin (11), ferritin (12), the α chain of rabbit globin (13); the β and γ chains of duck globin (8), histone (14), vesicular stomatitis virus proteins (15), and myosin heavy chains (16-18). Although cytoplasmic mRNP particles have been implicated in the translational control of long-lived eukaryotic mRNAs, neither their biochemical properties nor the precise nature of the regulatory processes in which they may participate have been studied in detail so far.

Recent studies from a number of laboratories suggest that translational control of gene expression may be involved during terminal differentiation in myogenesis of the skeletal and cardiac muscle fibers. In cultures of skeletal muscle cells the mononucleated rapidly proliferating myoblast cells undergo several cycles of cell division, then withdraw from the mitotic cycle and fuse to form a multinucleated embryonic myotube (for a review see Refs. 19 and 20). The time of cell fusion and the formation of the myotube is correlated with the onset of intensive synthesis of myosin and a number of muscle-specific proteins and enzymes. The addition of actinomycin D to cultures of rat myoblast cells at or just prior to fusion, does not
block the immediate appearance of myosin and other muscle-specific proteins (21). During the transition from nondifferentiated myoblasts to the differentiated myotube stage of cultures of fetal muscle cells, poly(A)-containing RNAs of different sizes are stabilized with a significant increase in their half-lives (9). A 26 S rapidly labeled RNA species, considered as the putative messenger for the large subunit of myosin, which is not associated with polysomes in the prefusion stage, is located in polysomes after cell fusion (9). When cultures of precardiac mesodermal cells, the progenitors of cardiac tissue, are treated with 5-bromodeoxyuridine, a thymidine analog, at a nondifferentiated stage of growth (stages 7 to 9), the subsequent expression of their differentiated phenotype, as judged by the beating of the fused cardiac mesoderm at stage 10, remained unaltered (22). In parallel experiments treatment of these cells at stages 7 to 9 with actinomycin D at a dose sufficient to block mRNA synthesis neither inhibited their differentiation into beating cardiac cells nor influenced the subsequent appearance of cross-striated myofilaments (22). These studies suggest that critical regulatory controls of gene expression may operate at a posttranscriptional level during the terminal differentiation of skeletal and cardiac muscle cells. The requirement of muscle-specific initiation factors for in vitro translation of muscle mRNAs (23, 24) and the identification of biologically active actin mRNA, not associated with polysomes in embryonic muscle cells (11), also support this view.

The biosynthesis of muscle proteins and its regulation during muscle development is currently being studied in this laboratory. We have chosen the 200,000-dalton myosin heavy chain as a suitable marker for muscle-specific proteins and systematically studied the isolation, in vitro translation and characterization of the mRNA coding for this myofibrillar protein (25-27). In order to probe for possible translational controls which may be involved in myogenesis, we have searched for untranslated forms of muscle-specific mRNAs in developing muscles. We report here the isolation of MHC mRNA from the nonpolysomal cytoplasmic fraction of chick embryonic muscles. The properties of this protein-bound form of mRNA are described and the possible significance of these observations in relation to some of the cellular regulatory processes in muscle cells is discussed.

**EXPERIMENTAL PROCEDURES**

Isolation of Subcellular Components—All preparative operations were carried out at 0–4°C except that the extraction of RNA with phenol/CHCl₃/isoamyl alcohol (11) was done at room temperature. Standard precautions were taken throughout all operations to prevent nuclease activity, e.g., use of acid-washed glassware and sterilized solutions (11, 25).

The scheme used for the isolation of subcellular components is shown in Scheme 1. Details of some of the preparative procedures are described under "Experimental Procedures" and in legends to Figs. 1 and 2.

**Scheme 1**

*Schematic representation of the isolation of cytoplasmic nonpolysomal myosin mRNP particles from chick embryonic muscles. Details are described under "Experimental Procedures" and in legends to Figs. 1 and 2.*

**Scheme 2**

*Schematic representation of the characterization of in vitro products programmed by purified MHC mRNA and RNA of 120 S particles in rabbit reticulocyte lysate. Details are described under "Experimental Procedures" and in legends to Figs. 4 and 5.*
used in this scheme have been previously reported (11, 25-27). The polysome-free supernatant fraction (100,000 × g, 1 h) of the embryonic leg muscle homogenates was used for the isolation of mRNP particles containing MHC mRNA. After dilution with 2 volumes of Buffer A, which contains 0.01 m Tris/HCl (pH 7.6), 0.25 m KCl, 0.01 m MgCl₂, 0.1 mm EDTA, 0.1 mm dithiothreitol, 500 μg/ml of heparin (used as RNAse inhibitor), and 0.5 mm phenethylmethylsulfonyl fluoride (used as protease inhibitor), the polysome-free supernatant was then centrifuged at 255,000 × g for 120 min. The pellet of postpolyosomal particles (11), thus obtained, was rinsed three times with ice-cold Buffer A and was further fractionated as described below.

The pellets pooled from three preparations were suspended in 200 ml of Buffer B (0.01 m Tris/HCl (pH 7.6); 0.05 m EDTA; 0.5 μg/ml of heparin and 0.5 mm phenethylmethylsulfonyl fluoride) by gentle over-night stirring with a magnetic stirrer at 4°C. The suspension was centrifuged at 12,000 × g for 15 min to remove any aggregate or denatured material and the clarified supernatant was further centrifuged at 55,000 × g for 210 min. The resulting pellet was suspended in 6 to 8 ml of Buffer B by the procedure described above. After centrifugation at 12,000 × g for 15 min to remove any denatured material, the supernatant was fractionated by sucrose density gradient centrifugation. About 60 to 80 A₂₅₀ units of postpolyosomal particles suspended in 1 ml were layered on 28 ml of linear 15 to 30% sucrose gradient in Buffer B and were centrifuged at 24,000 rpm for 17 h in a SW 25.1 Beckman-Spinco rotor at 4°C. Gradient fractions were collected in an ISCO gradient fractionator and their absorbance was monitored at both 260 and 280 nm. A typical absorbance profile is shown in Fig. 1. Fractions 1 to 17, as judged by their high A₂₆₀/A₂₈₀ ratios, were pooled and processed further for the isolation of mRNA.

Ribosomal subunits, 80 S monosomes and subribosomal particles containing actin mRNP were prepared as described previously (11). The final aqueous phase after three sucrose gradient runs (Fig. 1) were centrifuged through a 15 to 40% linear sucrose gradient in 20 mM Tris/HCl (pH 7.6), 0.5 M KCl, 5 mM magnesium acetate at 47,000 rpm for 90 min in a Beckman-Spinco SW 50.1 rotor. The gradients were fractionated and monitored for ultraviolet absorbance as described above. A typical profile is shown in Fig. 2. Fractions 16 to 25, sedimenting slower than 80 S monosomes used as markers, were pooled and processed for RNA isolation.

The precipitation step was repeated three times to remove all traces of sodium dodecyl sulfate and heparin (11, 25). MHC mRNA was isolated from myosin synthesizing polysomes prepared from chick embryonic leg muscles and was further purified by using a combination of Millipore filtration and oligo(dT)-cellulose chromatography according to the methods previously described (25-27).

Characterization of Products of in Vitro Translation – The rabbit reticulocyte lysate system, prepared by the method of Adamson et al. (29), and supplemented with exogenous RNA fractions was used to test for the de novo synthesis of the myosin heavy chain. The incubation mixtures contained, in a final volume of 0.5 ml: 0.2 ml of reticulocyte lysate; 10 mM Tris/HCl, pH 7.4; 2 mM magnesium acetate; 2 mM dithiothreitol; 1 mM ATP; 0.2 mM GTP; 15 mM creatine phosphate, 0.15 m KCl, 5 × 10⁻³ M leucine, indicated amounts of RNA (usually 5 to 20 μg); 8 μM concentrations of each of 20 different amino acids; and 15 μCi of L-[³⁵S]methionine or uniformly labeled "C-amino-acid mixture as indicated in the legend. After incubation at 30°C for 60 min reaction mixtures from 10 incubations were pooled and the KCl concentration was adjusted to 0.5 M. Four milligrams of chicken embryonic leg muscle myosin purified by chromatography on DEAE-cellulose (30), were added as carrier, and the in vitro products were copurified with the carrier myosin by the procedure outlined in Scheme 2.

The pooled reaction mixtures were dialyzed against 6 liters of Buffer E (50 mM Tris/HCl, pH 7.5; 0.5 mM KCl; 1 mM EDTA; and 0.2 mM phenethylmethylsulfonyl fluoride).
mm phenylmethanesulfonyl fluoride) to remove excess "C-amino-acids. The dialyzed sample was centrifuged at 160,000 × g for 120 min to pellet the ribosomes. The supernatant was then concentrated to 1 ml with Amicon ultrafiltration (Calbiochem, Los Angeles) and dialyzed 3 to 9 against 1 liter of Buffer E. Myosin was then slowly precipitated by dialyzing the sample overnight at 4° against 2 liters of a low ionic strength buffer which contained 5 mM Tris/HCl (pH 7.4), and 1 mM EDTA. The precipitate was carefully collected and gently homogenized in 1 ml of Buffer F (0.05 M KCl, pH 7.6, 0.1 mM dithiothreitol; and 0.1 mM EDTA) with 3 to 4 strokes in a 2-ml Dounce homog- nizer. The sample was then dialyzed against 500 ml of Buffer F for 2 to 3 h and the resulting clear solution of myosin was applied to a column (14 × 1 cm) of DEAE-cellulose (Whatman DE52) previously equilibrated with Buffer F. The column was thoroughly washed with Buffer F containing 0.35 mM NaCl, as previously described (25-27). The column fractions corresponding to the myosin heavy chain peak were pooled and concentrated by Aqueasate for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The sample was then dialyzed against 1 mM Tris/acetate, pH 8.5, containing 1% sodium dodecyl sulfate and 1% β-mercaptoethanol. Electrophoresis in the presence of sodium dodecyl sulfate was carried out using 5% polyacrylamide gels as previously described (25-27). After staining with Coomassie brilliant blue the gel was cut into 1-mm slices containing 1 mM Tris/acetate, pH 8.5, containing 1% sodium dodecyl sulfate and 1% β-mercaptoethanol. Electrophoresis was carried out using 2.5% gels containing 0.5% agarose cross-linked with 0.175% bisacrylamide. After the gels were prerun for 90 min at 4 mA/tube, 20 to 40 μg of RNA samples were applied to the gels with 10% sucrose and electrophoresis was carried out for 3 h at 4 mA/tube. The gels were then removed and fixed in a solution containing acetic acid/water/methanol (1/5/5) for 3 h. The gels were then scanned at 260 nm in a Gilford recording spectropho- tometer fitted with a linear transport device.

Sucrose Gradient Sedimentation of 120 S Particles – Due to the very large size of MHC mRNA (26, 27, 34, 35) the size of cytoplasmic nonpolysomal mRNP particles containing this mRNA is expected to be larger than the 40 S ribosomal subunit (1, 2). The postpolysomal pellet (Scheme 1) was, therefore, initially selected in order to look for cytoplasmic mRNP par- ticles containing MHC mRNA. In order to define more precisely the approximate size of the particles and to purify them fur- ther, the material present in the gradient fractions 18 to 25 (Fig. 1) of EDTA-treated postpolysomal particles (cf. "Experi- mental Procedures") was analyzed by centrifugation in a sec- ond 15 to 40% linear sucrose gradient made in 0.5 M KCl- containing buffer. Monosomes, 60 S and 40 S ribosomal sub- units, prepared from embryonic leg muscles, were used as markers. About 60 to 65% of the ultraviolet-absorbing mate- rial sedimented as a distinct peak (Fig. 2, indicated by the bar) which showed an A260/A280 ratio of about 1.2. The approximate S value of this peak calculated by the method of Martin and Ames (36), was about 120. Since the hydrodynamic properties of cytoplasmic mRNP particles are not precisely known, the S value should be considered only as approximate.

Sucrose Gradient Sedimentation of 120 S Particles – The RNA isolated from the peak fractions of 120 S particles (Fig. 2) was examined by agarose-polyacrylamide gel electrophoresis using chick embryonic muscle 18 S and 28 S rRNAs and MHC mRNA purified from chick embryonic myosin-synthesizing polysomes as markers. As shown in Panels A and B of Fig. 3, MHC mRNA migrated more slowly than the 28 S rRNA. This is in agreement with the known electrophoretic mobility and molecular weight of MHC mRNA previously reported in the literature (18, 25-27, 35). The RNA fraction isolated from the 120 S particles (Panel C) gave a densitometric peak with a mobility identical to that of purified MHC mRNA. Furthermore, the peaks of the gel runs of these two RNA samples were virtually identical (Fig. 3, B and C).

Detectable peak of other species of cellular RNAs was present in the RNA fraction isolated from the 120 S particles. The peaks in the gel scans of MHC mRNA (Fig. 3B) and the RNA from 120 S particles (Fig. 3C) represent about 85 to 90% of the total ultraviolet-absorbing material present in the samples. These results indicate that a large species of RNA, which is different from rRNAs and very similar to MHC mRNA in electrophoretic mobility, is the main component of the cyto- plasmic 120 S particles.

Characterization of in Vitro Translation Products Pro- grammed by RNA of 120 S Particles – In order to test if the RNA isolated from the 120 S particles directs the synthesis of myosin heavy chain, the products obtained from incubation of rabbit reticulocyte lysate programmed with this RNA and labeled with a mixture of "C-amino-acids were co-purified with carrier myosin (for details see Scheme 2). The recovery of CCl4COOH-insoluble protein-bound radioactivity at each step of the copurification scheme is shown in Table I. About 20% of the total nondialyzable counts was coprecipitated with carrier myosin at low ionic strength. When this material was further purified by chromatography on a column of DEAE-cellulose, two peaks in which myosin and nonmyosin chains are known
to be resolved (23, 25–27, 30, 34, 37, 38) were obtained (Fig. 4). About 40% of the total radioactivity applied to the column was eluted with the early protein peak. This presumably represents the globin and nonglobin polypeptides which were coded by the endogenous mRNAs present in the reticulocyte lysate and were co-precipitated with myosin at low ionic strength (23–27). In addition, this fraction may also include prematurely terminated chains as well as any nonmyosin polypeptides which may be synthesized in the reticulocyte lysate system programmed by the RNA fraction of the 120 S particles.

The identification of the in vitro products as myosin heavy chains is shown by the radioactivity profiles which indicated that about 55% of the total counts applied to the column were eluted with myosin heavy chains (Fig. 4, indicated by the bar). When these peak fractions were pooled, dialyzed against Buffer F (cf. "Experimental Procedures") and rechromatographed on a second DEAE-cellulose column, about 95% of the counts was recovered in the myosin peak (data not shown here). Thus, in terms of initial radioactivity about 10 to 11% was copurified with myosin on the DEAE-cellulose columns (Table I).

The characterization of the in vitro products as myosin heavy chains was further confirmed by the densitometric scanning and the radioactivity profile of the gel runs of the column purified material described above. About 70% of the counts applied to the gel comigrated with the densitometric peak which corresponded to the 200,000-dalton myosin heavy chain (Fig. 5). A major portion of the remaining 30% of the radioactivity was present at the top of the gel presumably representing some aggregated materials which did not enter the gel. No significant amount of radioactivity was observed in the light

![Figure 3](https://example.com/fig3.png)

**Fig. 3.** Densitometric scans of polyacrylamide gel runs of RNA samples. For details see "Experimental Procedures." Panel A: ---, 30 µg of 28 S chick embryonic muscle rRNA; --, 28 µg of 18 S chick embryonic muscle rRNA. Panel B: ---, 28 µg of purified MHC mRNA. Panel C: ---, 30 µg of RNA isolated from 120 S particles.

![Figure 4](https://example.com/fig4.png)

**Fig. 4.** DEAE-cellulose chromatography of in vitro products programmed by RNA isolated from 120 S particles. For details see "Experimental Procedures." The products co-precipitated with carrier myosin at low ionic strength and containing about 1.45 x 10⁵ cpm were dissolved in 1 ml of 0.03 M K₂HPO₄ (pH 7.5), 0.1 mM dithiothreitol, and 0.1 mM EDTA. The sample was then applied on a column (14 x 1 cm) of DEAE-cellulose previously equilibrated with the same buffer. After washing the column thoroughly with 30 ml of the column buffer the myosin was eluted with 0.35 M NaCl, 0.03 M K₂HPO₄ (pH 7.5), and 0.1 mM EDTA. After reading absorbance at 280 nm, 40-μl portions of the 2-ml fractions were analyzed for radioactivity. Fractions 19 to 22, indicated by the bar, were pooled and processed further for gel electrophoresis. The arrow indicates the position in the elution profile when the eluant was changed. The radioactivity is plotted as counts per min per 40-μl aliquot of the fractions.

<table>
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<tr>
<th>Purification step</th>
<th>RNA from 120 S particles</th>
<th>Purified MHC mRNA</th>
<th>Minus exogenous RNA</th>
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<td>Incubation mixtures after addition of carrier myosin and dialysis</td>
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<td>7.95 x 10⁶</td>
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<td>10.92</td>
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<td>Sodium dodecyl sulfate-polyacrylamide gel electrophoresis</td>
<td>3.2 x 10⁴</td>
<td>4.38</td>
<td>4.05 x 10⁴</td>
</tr>
</tbody>
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Table I

Recovery of CCl₄COOH-insoluble protein-bound radioactivity during co-purification of in vitro products with carrier myosin

For details see section on "Experimental Procedures." 

* The amounts of RNA used per incubation were: 10.5 µg of RNA from 120 S particles; 12 µg of purified MHC mRNA.

* Estimated from the ratio of radioactivity co-migrating with the 200,000-dalton myosin heavy chain band and the total radioactivity applied on the gel.
Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of in vitro products purified by DEAE-cellulose chromatography (Fig. 4). For details see "Experimental Procedures." Portions of the myosin sample containing about 900 to 1200 cpm (specific activity, 20,000 to 25,000 cpm/mg) were analyzed using 10.5 cm of 5% gels at 4 mA/gel for 2.5 h. After staining with Coomassie brilliant blue the gels were scanned at 595 nm, sliced, and processed for radioactivity as described in the text. The migration of the light chains present in the carrier myosin are indicated with arrows and marked as LC1 and LC2.

This indicates that the mRNAs coding for the myosin light chains were absent in the 120 S particle.

Since myosin and myosin-like proteins are currently believed to be present in a variety of non-muscle cells including erythrocytes (39), it was of interest to test whether or not the reticulocyte lysate synthesizes myosin heavy chain in the absence of exogenous mRNA. As shown in Table I, about 2.5% of the 14C-amino-acid counts incorporated in incubation mixtures, in which mRNAs were omitted, co-precipitated with carrier myosin. When this material was chromatographed on DEAE-cellulose, the radioactivity recovered in the ultraviolet-absorbing myosin peak decreased further to 0.082%. Due to this low level of incorporation subsequent analysis of this sample by gel electrophoresis could not be carried out. Experiments using [35S]methionine of high specific activity as the single radioactive amino acid and 19 unlabeled amino acids (cf. "Experimental Procedures") were then performed in order to estimate the endogenous synthesis of myosin heavy chain in the reticulocyte lysate. When a portion of the total CCl4COOH-insoluble proteins obtained from the lysate incubated alone was analyzed by sodium dodecyl sulfate-gel electrophoresis, a small radioactive peak indicated by Bar 1 and co-migrating with the myosin heavy chain marker was observed (Fig. 6). After co-precipitation at low ionic strength and chromatography on DEAE-cellulose, gel electrophoresis of the products gave a more distinct radioactive peak which co-migrated with the myosin heavy chain marker (Fig. 6, inset). The radioactivity recovered in this peak amounted to about 0.2% of the total counts incorporated in the reticulocyte lysate. The major fraction of the products, about 80 to 85%, was located in the large radioactive peak (indicated by Bar 6) which co-migrated with the band of rabbit hemoglobin.

In order to test the possibility that the low level of endogenous synthesis of myosin heavy chain, described above, is nonspecifically stimulated by exogenous mRNAs, we have studied the effect of two species of non-muscle mRNAs on myosin heavy chain synthesis in the reticulocyte lysate. When a sample of CCl4COOH-insoluble products programmed by heat-denatured 70 S AM virus RNA was subjected to electrophoresis, four additional components (indicated by Bars 2 to 5, Fig. 6) appeared in the gel run. The molecular weights of the labeled proteins present in bands 2 to 5 were calculated as: 75,000 to 80,000; 45,000 to 50,000; 28,000 to 32,000; and 18,000 to 20,000, respectively. These results are in good agreement with the published reports in literature on the electrophoretic behavior of AM virus RNA-directed products in reticulocyte...
lysate (40). This indicates that the radioactive components (indicated by Bars 2 to 5, Fig. 6) are polypeptides synthesized in the reticulocyte lysate in response to AM virus RNA. However, the addition of the AM virus RNA to the lysate did not cause any enhancement of amino acid incorporation into the myosin heavy chain peak, as shown by gel electrophoresis of either the total CCl₄-COOH-insoluble polypeptides (Bar 1 in Fig. 6) or the products copurified with carrier myosin by DEAE-cellulose chromatography (inset, Fig. 6). Similar results were also obtained when a sample of total calvaria mRNA of chicken embryos was used as the exogenous mRNA (results not shown in Fig. 6). These results indicate that the low level of endogenous myosin heavy chain synthesis in the rabbit reticulocyte lysate is not stimulated nonspecifically by exogenous mRNAs.

Comparison of in Vitro Translation of RNA of 120 S Particles and Purified MHC mRNA—In order to test whether MHC mRNA purified from embryonic leg muscle polysomes is also translated similarly in the rabbit reticulocyte lysate system, the experiments described above were repeated using a comparable amount of purified MHC mRNA. These results are also presented in Table I, indicating that the amounts of radioactivity recovered with carrier myosin during each step of the copurification scheme were very similar to those obtained with RNA from the 120 S particle. To compare the efficiency of translation of the two kinds of RNA, studies were carried out in which the amount of RNA used in the incubation was varied. The results (presented as Experiment 1 in Table II) show that at low concentrations of added mRNA the amount of radioactivity recovered in the myosin heavy chain band was a linear function of the mRNA concentration and that in this linear concentration range the RNA from 120 S particles and purified MHC mRNA were equally effective in directing the synthesis of the intact polypeptide chain in the rabbit reticulocyte lysate system. The degree of stimulation of myosin heavy chain synthesis, which was dependent on the concentration of mRNAs used, usually ranged from 15- to 40-fold. In contrast to these specific stimulating effects of the RNA of the 120 S particles and purified MHC mRNA, the low level of endogenous synthesis of myosin heavy chain in the lysate did not show any significant change in response to incubation with varying concentration of 70 S AM virus RNA (8 to 32 µg) and 5 µg of chicken embryonic calvaria mRNA (Experiment 2 in Table II).

**Protein Content of 120 S Particles**—When preparations of formaldehyde-fixed 120 S particles were centrifuged to equilibrium in preformed CsCl gradients, the absorbance profile gave a peak at a density of 1.415 g/cm³ (Panel A, Fig. 7). The protein content of the 120 S RNA was calculated from this value by the method of Spirin (1, 2) as 72.5%. The buoyant densities of 60 S and 40 S ribosomal subunits prepared from embryonic chicken muscles, on the other hand, usually ranged between 1.58 to 1.585 for 60 S and 1.52 to 1.53 g/cm³ for 40 S subunits, respectively (Panels B and C, Fig. 7). These values are in good agreement with those reported in the literature for eukaryotic ribosomal subunits (for a review, see Refs. 1 and 2). The shape of the absorbance profile and the absence of any peak in the density range of 1.5 to 1.6 g/cm³ indicate that the 120 S particle did not contain any detectable level of ribosomal subunits.

If the mRNA activity reported in the previous section was due to aggregates of cytoplasmic protein-free 26 S MHC mRNA species which may co-sediment with the 120 S particles, a considerable fraction of the ultraviolet-absorbing material would be expected to sediment at the bottom of the CsCl gradient, since protein-free RNA is pelleted in CsCl gradients. In order to test this possibility the bottom of the tubes, after the CsCl gradients were fractionated, was repeatedly rinsed with a small volume of distilled water using a rubber policeman to scratch the sides of the tubes. No detectable absorbance at 260 nm above the background level was found in the rinse solution. This indicates that protein-free MHC mRNA was absent in our preparations. The characteristic buoyant density of 120 S particles reported here is in good agreement with the published values of eukaryotic mRNPs in the literature.

**TABLE II**

| Experiment condition | % of total radioactivity incorporated in myosin heavy chain band
|----------------------|-------------------------------------------------------------------
| Experiment 1b:       |                                                                   |
| Lysate               | 0.12                                                              |
| + 4.5 µg MHC mRNA    | 2.28                                                              |
| + 9.0 µg MHC mRNA    | 4.42                                                              |
| + 18 µg MHC mRNA     | 5.65                                                              |
| + 5.0 µg RNA from 120 S particles | 2.05               |
| + 10.0 µg RNA from 120 S particles | 4.29               |
| + 16.5 µg RNA from 120 S particles | 5.38               |
| Experiment 2c:       |                                                                   |
| Lysate               | 0.26                                                              |
| + 8.0 µg AM virus RNA| 0.19                                                              |
| + 16.0 µg AM virus RNA| 0.21                                                             |
| + 32.0 µg AM virus RNA| 0.23                                                             |
| + 5.0 µg calvaria mRNA| 0.25                                                            |

* For details see legends to Table I and Fig. 5.

* Assays were carried out with 15 µCi of [3H]-amino-acid mixture per incubation. Control incubation of lysate without exogenous mRNA gave 7.48 x 10⁴ cpm of total CCl₄-COOH-insoluble protein-bound counts.

* Assays were carried out with 15 µCi of [35S]methionine (325 Ci/ mmol) per incubation. Control incubation of lysate without exogenous mRNA gave 15 x 10⁴ cpm of total CCl₄-COOH insoluble protein bound counts.

![Fig. 7. Determination of buoyant densities of 120 S particles and chick embryonic muscle ribosomal subunits. For details see "Experimental Procedures." Centrifugation was done at 35,000 rpm for 40 h in a Beckman Spinco SW 60.1 rotor at 20° using preformed CsCl gradients of densities 1.2 to 1.6 g/cm³ for 120 S particles (Panel A) and 1.4 to 1.8 g/cm³ for 60 S and 40 S ribosomal subunits (Panels B and C). The absorbance plotted is calculated from the ISCO ultraviolet recorder as previously described (11).](http://www.fc-rc.org/.../guest_on_September_22,2017)
unequal size, one containing MHC mRNA (i.e. 120 S particle).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Protein Moieties of 120 S Particles — The electrophoretograms of samples of alkylated proteins of 120 S particles and embryonic muscle ribosomal subunits are shown in Fig. 8. The typical ribosomal proteins in the molecular weight range of 15,000 to 30,000 (Gels A and B, Fig. 8) were absent in the 120 S particles (Gel C, Fig. 8). At least seven distinct polypeptides ranging from 44,000 to 100,000 daltons, which appeared to be nonribosomal proteins, were consistently observed in the electrophoretogram of the 120 S particle (Gel C, Fig. 8). The most prominent bands of these proteins, as judged by staining intensity, corresponded to molecular weights of 44,000, 59,000, 53,000, 51,000, 81,000, 83,000, 86,000, and 98,000. The 44,000-dalton band appeared to be the major component of this group of polypeptides. A number of additional minor bands, which were not well resolved and which represent polypeptides in the 85,000 to 100,000 range, were also observed in the 120 S particle.

Electrophoretic Comparison of Proteins of 120 S and Subribosomal mRNP Particles — In order to test whether cytoplasmic mRNP particles containing mRNAs coding for different myofibrillar proteins are associated with the same or different sets of proteins, we have compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis the proteins of mRNP particles containing mRNAs coding for two different proteins of unequal size, one containing MHC mRNA (i.e. 120 S particle) and the other containing actin mRNA (for details see also "Experimental Procedures", Scheme 1, and Ref. 11). Fig. 9A shows the electrophoretograms of subribosomal actin mRNP (Gel 1), myosin heavy chain mRNP (Gel 2) and a mixed sample of actin and myosin heavy chain mRNPs (Gel 3). It appears that the three faster components in the molecular weight range of 44,000 to 53,000 (see Fig. 8 for assignment of molecular weights) are present in both classes of mRNP particles. MHC mRNP, however, contains three distinct and a number of minor protein bands in the 81,000- to 85,000-dalton range (see also Fig. 8). Although actin mRNP particles upon electrophoresis consistently showed bands of some proteins of similar mobilities in this region of the gels, the relative amounts of these high molecular weight components were always found to be lower than those present in MHC mRNP (Fig. 9, Panel A, Gels 1 and 2). When samples of these mRNPs were run together, the three faster components migrated together (Fig. 9, Panel A, Gel 3). These features remained unaltered even when the time of electrophoresis was increased to 5 h (Panel B), or a combination of decreased sample load and longer run (6.5 h) was used to facilitate resolution of the bands (Panel C). These results indicate that except for some minor differences with respect to some of the polypeptide components, the sodium dodecyl sulfate-gel electrophoretic patterns of the protein moieties of different cytoplasmic mRNP particles of embryonic muscles were very similar.

**DISCUSSION**

The detection and isolation of a nonpolysomal cytoplasmic MHC mRNP particle reported here has been feasible due to two properties of this particle: (a) the known resistance of mRNP particles to EDTA treatment (1, 2) which allows the

**Fig. 8 (left).** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of 120 S particles and chick embryonic muscle ribosomal subunits. For details see "Experimental Procedures." Electrophoresis of alkylated samples of 120 S particles and ribosomal subunits was carried out using 10-cm long 10% gels at 4 mA/gel for 4 h. The gels were stained with Coomassie brilliant blue. The amounts of sample applied to the gels are: A, 0.3 A_260 unit of 60 S ribosomal subunit; B, 0.4 A_260 unit of 49 S ribosomal subunit; C, 0.1 A_260 unit of 120 S particle; D, a mixture of the following markers: 12 μg of conalbumin (molecular weight, 78 x 10^3); 8 μg of bovine serum albumin (molecular weight, 68 x 10^3); 6 μg of chicken actin (molecular weight, 42 x 10^3); and 8 μg of chicken tropomyosin (molecular weight 34 x 10^3). The bands corresponding to these four protein markers are shown in the electrophoretogram with arrows (a to d) in decreasing order of molecular weight.

**Fig. 9 (right).** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of 120 S particle and subribosomal mRNP particles containing actin mRNA. For details see "Experimental Procedures" and the legend to Fig. 8. The following amounts of samples were used: Panel A, 0.08 A_260 unit of subribosomal mRNP (Gel 1); 0.1 A_260 unit of 120 S particle (Gel 2); and a mixture of 0.04 A_260 unit of subribosomal mRNP and 0.06 A_260 unit of 120 S particle (Gel 3); Panel B, a mixture of 0.04 A_260 unit of subribosomal mRNP and 0.06 A_260 unit of 120 S particle; Panel C, 0.03 A_260 unit of subribosomal mRNP and 0.04 A_260 unit of 120 S particle. Conditions for electrophoresis were the same as described for Fig. 8 except that the time of electrophoresis was increased to 5 h for the gel run in Panel B and to 6.5 h in Panel C.
shown in Tables I and II, the products programmed by the MHC mRNA. As nonpolysomal RNA was also biologically active, we have compared its in vitro translation with that of MHC mRNA. As shown in Tables I and II, the products programmed by the RNA of 120 S particles in the rabbit reticulocyte lysate system could be co-purified with carrier myosin and very similar results were also obtained with MHC mRNA. Moreover, the efficiency of translation of these two RNA species into myosin heavy chains was identical (Table II). It should be noted that about 4 to 5% of the total radioactivity incorporated in the reticulocyte lysate system programmed by these two RNA species is identical (Table II). It should be noted that about 4 to 5% of the total radioactivity incorporated in the reticulocyte lysate system programmed by these two RNA species is identical (Table II). It should be noted that about 4 to 5% of the total radioactivity incorporated in the reticulocyte lysate system programmed by these two RNA species is identical (Table II).

The results presented in Fig. 3 show that the electrophoretic profile of the RNA isolated from the 120 S particle was strikingly similar to that of purified MHC mRNA isolated from chick embryonic muscle polysomes. In order to show that this nonpolysomal RNA was also biologically active, we have compared its in vitro translation with that of MHC mRNA. As shown in Tables I and II, the products programmed by the RNA of 120 S particles in the rabbit reticulocyte lysate system could be co-purified with carrier myosin and very similar results were also obtained with MHC mRNA. Moreover, the efficiency of translation of these two RNA species into myosin heavy chains was identical (Table II). It should be noted that about 4 to 5% of the total radioactivity incorporated in the reticulocyte lysate system programmed by these two RNA species is identical (Table II).

The protein content of the 120 S particle calculated from its buoyant density amounts to about 73%, which is much higher than typical ribosomal particles. Moreover, analysis of the protein moieties by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicates a limited number of distinct protein species which do not appear to be the typical ribosomal proteins. This shows that the 120 S particles are cytoplasmic entities distinct from ribosomes and initiation complex. It is of interest that two muscle-specific mRNPs of unequal sizes, viz., the above-mentioned 52,000- and 78,000-dalton proteins previously reported in polysomal mRNP particles, is associated with the poly(A) segment located at the 3'-end of eukaryotic mRNAs (43-45). Among the seven distinct proteins present in 120 S particles (Figs. 8 and 9) are two components of molecular weights 53,000 and 81,000, which presumably correspond to the above-mentioned 52,000- and 78,000-dalton proteins previously reported in polysomal mRNP particles. This is supported by our recent observation (46) that polysomal mRNP particles isolated from chick embryonic muscles also contain two main protein components of molecular weights 53,000 and 81,000. However, other proteins with molecular weights below 53,000 and above 81,000, which are present in 120 S particles, are absent in these polysomal mRNP particles.

In a recent report seven distinct protein components in the 34,000- to 90,000-dalton range have been described in gel runs of a mixture of subribosomal cytoplasmic mRNP particles isolated from Ehrlich ascites tumor cells (10). The electrophoretic pattern of the protein moieties of 120 S particles reported here is very similar to that of the cytoplasmic mRNP particles of ascites cells (10), except that a distinct band of 44,000 daltons is consistently observed in our preparation in place of the above-mentioned 34,000 molecular weight component. The 34,000-dalton component, according to Barriex et al. (10), presumably represents the protein associated with the 5 S RNA-protein particle present in their preparations. The electrophoretic pattern of the protein components of 120 S and subribosomal mRNP particles of embryonic muscles supports the view that proteins associated with eukaryotic mRNP are much more complex with respect to both size and number than previously thought (for a review, see also Ref. 10). The variation in the intensity and number of protein bands in the high molecular weight range (81,000 to 86,000 daltons) observed in subribosomal and MHC mRNP (Fig. 9) may be due to subpopulations of cytoplasmic mRNP containing additional or different proteins. This possibility seems quite likely for embryonic muscle which is known to contain a very heterogeneous distribution of mRNAs coding for a large number of muscle-specific proteins (11, 30, 34, 37).

The physiological function of the cytoplasmic mRNP parti-
icles is not clear at the present time. Our preliminary estimate indicates that about 25 to 30% of the total MHC mRNA of 14-day-old chick leg muscles are present as 120 S particles. One possibility is that these particles may participate in posttranscriptional controls of gene expression during the growth and terminal differentiation of the muscle cells as suggested by several workers (9, 11, 16-18, 21, 22, 24). We are currently investigating this possibility and have made an interesting observation that the 120 S particle is able to program the in vitro synthesis of myosin heavy chain in rabbit reticulocyte lysate as effectively as protein-free MHC mRNA (47). This suggests that the binding of specific proteins to the MHC mRNA per se does not exert a stringent negative control on the in vitro translation of the mRNA. The proteins, which are present in cytoplasmic mRNPs but seem to be absent in polysomal mRNPs (46), may participate in the in vivo regulation of the entry of the muscle-specific mRNAs from the cytoplasmic pool to the polysomes.

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