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Purification of Myosin Translational Control RNA and Its Interaction with Myosin Messenger RNA†

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ABSTRACT: Myosin messenger ribonucleoprotein-translational control ribonucleic acid (mRNP-tcRNA) from myosin mRNPs found in embryonic chick muscle has been further purified by Dowex chromatography and, from a number of controls, it is suggested that this small RNA is not an artifact produced through the degradation of RNA during its isolation. This highly purified myosin mRNP-tcRNA is shown to have a molecular weight of 10 000 on formamide-acrylamide gels, and reacts stoichiometrically (on a 1:1 mole ratio) with myosin mRNA. The stoichiometric interaction between myosin mRNA and myosin mRNP-tcRNA is demonstrated by its ability to increase the nuclease resistance of the messenger, as well as inhibit its translation in a cell-free amino acid incorporating system.

The existence of messenger RNA that is not associated with ribosomes in eukaryotic cells is well documented (Brawerman, 1974; Gander et al., 1973; Williamson, 1973). In embryonic muscle both myosin mRNA (Buckingham et al., 1974; Heywood et al., 1975b) and actin mRNA (Bag and Sarkar, 1975) have been shown to exist as ribosomal free mRNP[1] particles in early stages of development. Although it has not been demonstrated that these stored messengers are precursors to polysomal messengers, it is clear that in the case of myosin mRNA, the stored and polysomal forms are identical as determined by hybridization experiments using myosin cDNA (Robbins and Heywood, 1976).

We have suggested that in muscle cells the mechanism by which certain mRNAs are maintained in an inactive form in the cytoplasm is by complexing with a small oligo(U)-containing RNA termed translational control RNA (tcRNA) (Bester et al., 1975). This RNA molecule is characterized by (1) its localization in mRNPs, (2) its oligo(U) region capable of forming hybrids with poly(A), (3) its ability to inhibit the translation of certain mRNAs (those with which it is isolated), and (4) its capability to change the structure of those mRNAs thereby increasing their nuclease resistance. Utilizing these properties of the molecule we have been able to isolate myosin tcRNA from myosin mRNPs and purify it to the extent that it appears as a single band on formamide-acrylamide gel electrophoresis. This highly purified myosin tcRNA was subsequently tested for its ability in one case to hybridize with myosin mRNA and in the other case to interfere with the in

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[1] Abbreviations used are: mRNP, messenger ribonucleoprotein; poly(A), poly(adenylic acid); DEAE, diethylaminoethyl; EDTA, (ethylenedinitrilo)tetraacetic acid; Hn, heterogeneous nuclear; ATP, GTP, adenosine and guanosine triphosphates.

vitro translation of myosin mRNA. The former results in an increase in ribonuclease resistance and the latter inactivates the messenger for translation. The completeness of these reactions involving the interaction of myosin tcRNA with myosin mRNA occurs when the molecules are present in a 1:1 mole ratio.

Materials and Methods

The preparation of rabbit reticulocyte lysate was as previously described (Evans and Lingrel, 1969). Cell-free synthesis was performed in 0.07 ml of reticulocyte lysate containing 50 μM hemin, 4 μg of muscle IF3, 4 μg of muscle tRNA, and ATP and GTP energy supply as described (Bester et al., 1975), 0.2 nmol each of 20 amino acids containing 10 μCi of [^{35}S]methionine (New England Nuclear). Both Mg^{2+} and K^+ concentrations had been optimized for the synthesis of myosin at 0.003 and 0.1 M, respectively (Heywood et al., 1974). When added to the reaction mixtures, mRNA was premixed with tcRNA in the ratios indicated in the different sets of experiments. These RNAs were premixed for 5 min at 5 °C in 0.005 ml of 0.15 M KCl, 0.005 M $MgCl_2$, and 0.02 M Tris-HCl (pH 7.4). Incubation of the reaction mixtures for protein synthesis was 45 min at 30 °C.

After incubation, the synthesis of myosin was determined as previously described (Heywood et al., 1975a). Briefly, 0.1 mg of carrier myosin was added and the myosin subsequently purified by ionic precipitation and DEAE-cellulose chromatography. Finally, the radioactivity co-electrophoresing with myosin on sodium dodecyl sulfate acrylamide gels was determined. For typical analysis see Figure 4 in Heywood et al., 1975a.

Muscle initiation factor 3 (IF3) was prepared from native 40S ribosomal subunits from 14-day embryonic chick leg muscle by a modification of the procedure of Freienstein and Blobel (1975). This procedure will be described in detail elsewhere.

Myosin mRNA (26 S) was prepared from the 70–120S myosin mRNPs found in 13-day embryonic chick leg muscle (Heywood et al., 1975b). After dialysis or chromatography on Sephadex G-50 (Superfine) (Pharmacia Fine Chemical) to remove the tcRNA, the myosin mRNA was purified as previously described (Heywood et al., 1975a) except that the oligo(dT)-cellulose columns were run at 60 °C to eliminate contaminating ribosomal RNA. Normally, 350 embryos yielded approximately 120 μg of 26S myosin mRNA and 0.5 μg of myosin tcRNA (see below).

Myosin tcRNA was prepared from the myosin mRNPs obtained from 13-day embryonic chick leg muscle. After isolation by sucrose density gradient centrifugation and pelleting of the myosin mRNPs (Heywood et al., 1975b), the entire pellet containing monomeric ribosomes was either directly used to obtain tcRNA or the mRNPs were separated from contaminating ribosomes by chromatography on poly(U)-Sepharose (Kish and Pederson, 1975). In the latter case, all the tcRNA could be recovered from the mRNPs after two passes through the poly(U)-Sepharose (tcRNA could not be obtained from ribosomes when subjected to identical conditions as were used to isolate tcRNA from the purified mRNPs).

The mRNP fraction was dialyzed against 10 volumes of 0.05 M potassium phosphate (pH 6.8) and 0.005 M EDTA for 4 h and the dialysate subsequently chromatographed on DEAE-cellulose as previously described (Heywood et al., 1974). Prior to dialysis, the dialysis tubing (0.6-cm diameter) was boiled in 20 mM EDTA for 1 h and washed extensively with H_2O. Only batches of dialysis tubing that yielded active

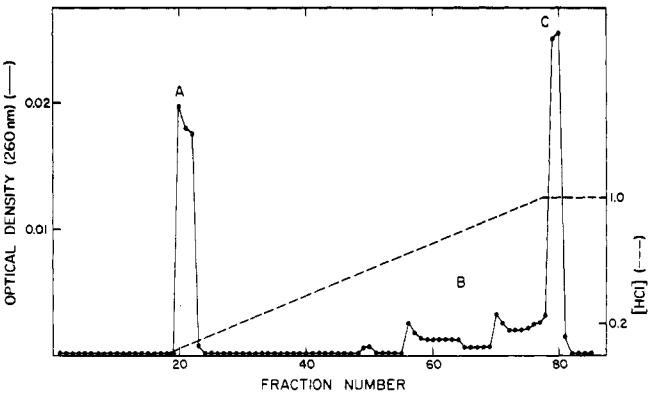

FIGURE 1: Dowex chromatography of myosin mRNP-tcRNA. The tcRNA was obtained from 13-day embryonic chick muscle myosin mRNPs, chromatographed on DEAE-cellulose and the alcohol precipitate was resuspended in distilled H_2O neutralized to pH 7.0 with 1.0 M NH_4OH. The Dowex column was also equilibrated with neutralized H_2O. The tcRNA was applied to the column, washed with 20 ml of H_2O and subsequently eluted with a 40-ml gradient of 0.1–1.0 N HCl. Fractions in peaks A, B, C were pooled and precipitated in 3 volumes of ethanol with 0.24 M ammonium acetate as indicated under Methods.

tcRNA preparations (oligo(U) containing) were used. Alternatively, the mRNP fraction can be resuspended in the above buffer and chromatographed on a 0.5 × 60 cm Sephadex G-50 (Superfine) column using the phosphate-EDTA buffer. The myosin mRNA fraction elutes in the excluded volume, while the tcRNA fraction elutes from 10–20 ml of the included volume. Due to greater recovery and ease of handling the dialysis method was normally preferred—although the subsequent purification of myosin tcRNA and its activity were equivalent using either procedure. After myosin tcRNA was eluted from the DEAE-cellulose by 1 M NaCl (Heywood et al., 1974) it was precipitated in 2 volumes of ethanol with 0.24 M ammonium acetate for 2 days at −20 °C. The RNA precipitate was subsequently resuspended in 0.1 ml of H_2O neutralized with NH_4OH and applied to a 0.5 × 4 cm Dowex column (Bio-Rad AG1-X2, 200–400 mesh) previously equilibrated with neutralized H_2O. After washing the column with 20 ml of H_2O, the RNA was eluted using a 40-ml gradient of 0.01–1.0 N HCl as shown in Figure 1. Fractions of 0.5 ml were collected. The chromatography was performed at 2 °C and was completed within 20 min. Very little, if any, acid hydrolysis occurs under these conditions (Bock, 1967). In addition, we have determined that globin mRNA maintains its characteristic sedimentation value of 9S after similar treatment. The fractions to be tested for mRNP-tcRNA activity (A, 20–22; B, 56–71; C, 79–80 in Figure 1) were precipitated in 2 volumes of ethanol with 0.24 M ammonium acetate for 2 days at −20 °C.

Hybridization conditions for either poly([^{3}H]A)-tcRNA hybrid formation or [^{3}H]uridine labeled myosin mRNA-myosin tcRNA hybrid formation were as previously described (Bester et al., 1975). In brief, unless otherwise specified, 10 μg of RNA was hybridized to either poly([^{3}H]A) or to tcRNA as indicated in the table and figure legends. Hybridizations were performed at 27 °C for 1 h in 0.1 M NaCl, 0.05 M Tris-HCl (pH 7.2), 0.002 M EDTA. The 0.05-ml reaction mixture was then made 0.3 M NaCl and 10 units each of T_1 and T_2 RNase were added. After 30 min, the nuclease resistant hybrids were analyzed by Sephadex G-25 chromatography (Bester et al., 1975; Heywood et al., 1975a).

Acrylamide gel analysis of myosin tcRNA was performed in 98–99% formamide using 0.01% sodium dodecyl sulfate on

TABLE I: Occurrence of Oligo(U)-Rich Regions in Myosin mRNPs.

RNA	% RNase Resistant[a]
1 μg of mRNP-tcRNA	4.1
10 μg of T_1 RNase fragments myosin mRNA[b]	0.0
10 μg alkaline hydrolyzed myosin mRNA[c]	0.0
10 μg ribosomal RNA	0.1

[a] 30 000 cpm of poly([^{3}H]A) added per assay. Hybridization and digestion by T_1 and T_2 RNase as described under Materials and Methods. Reactions were started by addition of poly([^{3}H]A) at 60 °C followed by cooling and 1 h at 25 °C. After RNase digestion the reaction mixtures were made 5% Cl_3CCOOH at 5 °C with 50 μg of carrier yeast RNA and collected by Millipore filters. [b] T_1 RNase fragments produced by 30 min nuclease reaction with 10 units of T_1 RNase at 0 °C and subsequent phenol extraction to remove nuclease (Dasgupta et al., 1975) fragments sedimented at less than 4S on sucrose density gradient centrifugation. [c] Limited alkaline hydrolysis (2-3S fragments on sucrose density gradient) produced by 0.3 M KOH for 15 min at 5 °C.

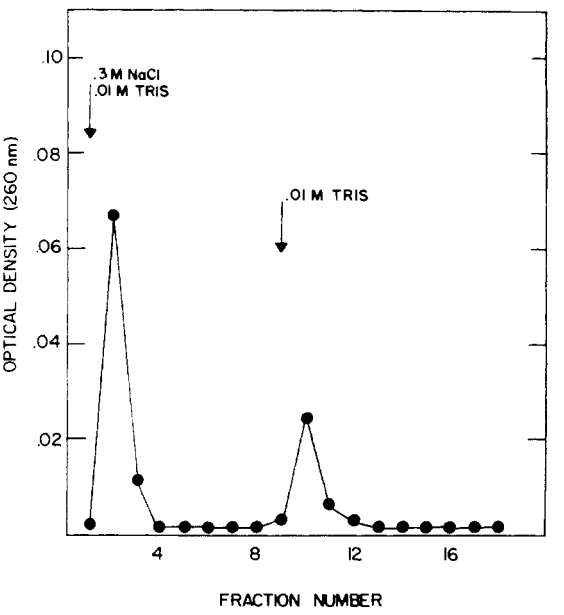

FIGURE 2: Oligo(dA)-cellulose chromatography of myosin tcRNA. The tcRNA, previously purified on DEAE-cellulose, was chromatographed on a 0.5 × 3 cm oligo(dA)-cellulose column at 27 °C with the buffers indicated. The pH of the buffers was 7.5; 0.5-ml fractions were collected.

6-cm, 12% acrylamide gels as described by Duesberg and Vogt (1973). The gels were run at 1 mA/tube until the tracer dye was 4-5 cm through the gel. The gels were stained using 0.2% methylene blue in 1 M acetic acid, scanned at 750 nm, and the radioactive gels sliced and counted in a liquid scintillation counter. Standards for molecular-weight determination were 5S rRNA, 4S tRNA, and oligo(U)$_{15}$ (4800 mol wt) (obtained from Collaborative Research, Cambridge, Mass.).

Oligo(dT)-cellulose and oligo(dA)-cellulose were obtained from Collaborative Research, Cambridge, Mass., while poly(U)-Sepharose was obtained from Pharmacia. In all cases, determination of radioactivity was as previously described (Rourke and Heywood, 1972) using a liquid scintillation counter.

Results

We have previously demonstrated that mRNP-tcRNA contains an oligo(U)-rich region capable of forming nuclease resistant hybrids with poly([^{3}H]A), while tRNA does not form hybrids (Bester et al., 1974). In order to assure that the tcRNA obtained from the mRNP fraction was not an artifact produced during its preparation the following experiments were performed: (1) 3000 cpm of [^{3}H]myosin mRNA was added to the myosin mRNP fraction prior to dialysis. Under the conditions used for the isolation of myosin tcRNA no radioactive material was found in the dialysate, suggesting that the tcRNA was not a degradation product of myosin mRNA produced during this procedure. (2) In addition, as shown in Table I, neither T_1 RNase fragments of myosin mRNA nor fragments produced by limited alkaline hydrolysis show regions of the myosin mRNA capable of forming stable hybrids with poly([^{3}H]A). In order to ascertain that the added poly([^{3}H]A) could compete for any such regions with the poly(A) derived from the messenger itself, the hybridization mixtures were heated to 60 °C for 10 min, the poly([^{3}H]A) was added, and the reactions were slowly cooled to 25 °C (10 min) and allowed to remain at 25 °C for 1 h before analysis. (3) rRNA will not form stable hybrids with poly([^{3}H]A) under the conditions at which tcRNA does (Table I). In addition, as stated in the Methods, no tcRNA can be recovered from ribosomes after poly(U)-Sepharose chromatography to remove mRNPs. These experiments suggest that the tcRNA obtained from either mRNPs or mRNPs contaminated with ribosomes is not a degradation product of these larger RNAs produced during preparation of the tcRNA.

Translational control RNA obtained from mRNPs has been shown to contain an oligo(U)-rich area by its ability to hybridize to poly(A). Therefore, the possibility exists that myosin tcRNA could be obtained by passing the ethanol-precipitated tcRNA obtained after dialysis and DEAE-cellulose chromatography through oligo(dA)-cellulose. As can be seen in Figure 2, a major portion of this material is not bound to the column at high-salt concentrations; however, material is bound that can be eluted at low-salt concentration. When these two fractions from the oligo(dA)-cellulose column were chromatographed on the Dowex column (Figure 1) the material not binding oligo(dA)-cellulose eluted in fraction A and B, while the oligo(U)-rich RNA binding to the oligo(dA)-cellulose at high-salt concentrations eluted in fractions B and C on the Dowex column. These results suggest that the mRNP-tcRNA (oligo(U)rich) will be found in those latter fractions eluting from the Dowex column. It should be noted that the tcRNA must be removed from the poly(A) containing mRNA prior to its isolation by oligo(dA)-cellulose chromatography.

In order to determine which fraction (A, B. or C; Figure 1) from the Dowex column is myosin mRNP-tcRNA, a number of tests were applied. mRNP-tcRNA is characterized by its ability (1) to form RNase resistant hybrids with poly([^{3}H]A), (2) to significantly increase the RNase resistance of the mRNA with which it is found in the mRNPs, and (3) to inhibit the translation of this mRNA without significantly affecting the translation of other messengers (Heywood et al., 1975a). Fraction B from the Dowex column was not present in enough quantity to recover reproducibly; therefore, it was pooled from repeated preparations and tested only twice in the latter assay (inhibition of myosin mRNA translation). As shown in Table II the poly([^{3}H]A) was completely hydrolyzed by T_1 and T_2 RNase, and, while fraction A (Figure 1) RNA gives negligible protection to poly([^{3}H]A) from RNase digestion, fraction C RNA contained the oligo(U)-rich RNA as measured by its

TABLE II: Protection of Poly([^{3}H]A) from T_1 and T_2 RNase Digestion Fractions of tcRNA Prepared from Myosin mRNPs.[a]

tcRNA Fraction	% RNase Resistant
A	0.18
C	3.47
-	0.00

[a] 30 000 dpm of poly([^{3}H]A) per assay. Hybrids were detected by Superfine G-25 (Sephadex) chromatography (Bester et al., 1975).

TABLE III: Protection of Myosin mRNA from T_1 and T_2 RNase Digestion by tcRNA.[a]

Myosin mRNP-tcRNA Fraction	% RNase Resistance
-	6.0
A	5.6
C	17.1
A + C	9.8

[a] 4500 cpm of [^{3}H]uridine labeled myosin mRNA per assay; 10 μg of myosin mRNA and 0.05 μg of tcRNA prepared by Dowex chromatography were used in each hybridization assay as indicated. Nuclease resistant fragments of RNA were determined by Sephadex chromatography (Heywood et al., 1975a).

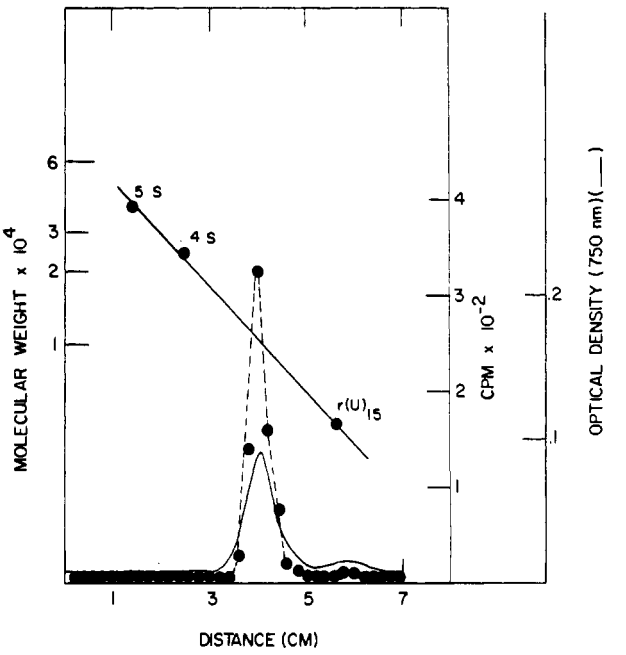

FIGURE 3: Acrylamide gel analysis of myosin mRNP-tcRNA (fraction C, Figure 1) using 98% formamide. Electrophoresis, staining, and counting of gel slices are as described under Methods. 5S rRNA, 4S tRNA, and oligo(rU)$_{15}$ (4800 mol wt) markers were run on a parallel gel. All of the radioactivity applied to the gel is accounted for within the radioactive profile shown.

ability to protect poly([^{3}H]A) from T_1 and T_2 RNase. These results agree with the fact that the tcRNA obtained by chromatography on oligo(dA)-cellulose (Figure 2) elutes as fraction C on the Dowex column (Figure 1).

Myosin mRNA has been shown to be 5–8% T_1 and T_2 RNase resistant in 0.3 M NaCl (Heywood et al., 1975a). As shown in Table III, the addition of fraction A (Figure 1) from the Dowex column did not increase the resistance of myosin mRNA to RNase hydrolysis. However, when fraction C is hybridized to [^{3}H]myosin mRNA there is a threefold increase in nuclease resistance, further suggesting that this fraction is the oligo(U)-containing myosin tcRNA. It was observed that when equal amounts of fractions A and C are added to [^{3}H] myosin mRNA an intermediate value of RNase resistance occurs (Table III). This may suggest that the two fractions contain molecules that compete for binding sites on the myosin mRNA. If fraction A contains polysomal tcRNA (Bester et al., 1975) by virtue of initiation complexes (80S) cosedimenting with myosin mRNPs, it is possible that these tcRNAs may compete for the second binding site, not utilizing the oligo(U) region of mRNP-tcRNA on the messenger and thereby causing an intermediate value for RNase resistance. Such a result would be predicted from our model (Bester et al., 1975) of tcRNA-mRNA interaction.

The experiments shown in Table III were done at a 1:1 mole ratio of tcRNA to myosin in mRNA. The molecular weight of myosin mRNA is assumed to be 2×10^6. This is a reasonable assumption for a mRNA coding for a protein of 200 000 molecular weight that would contain approximately 6000 coding nucleotides, a poly(A) tail, and nonreading portions. In order to determine the molecular weight of myosin mRNP-tcRNA, five muscle cell cultures (8×10^6 cells each) were labeled with ^{32}P for 4 h before extensive cell fusion was allowed to occur and the labeled myosin mRNPs added to those obtained from 100 13-day embryos. The myosin mRNP-tcRNA obtained from fraction C (Figure 1) was subsequently analyzed by formamide-acrylamide gel electrophoresis using 5S rRNA, 4S tRNA, and oligo(U)$_{15}$ (Collaborative Research) as markers. After staining the gels with methylene blue and rapid destaining, the gels were scanned for the position of both markers and tcRNA and the radioactivity was determined after slicing the tcRNA containing gel. As shown in Figure 3, myosin mRNP-tcRNA has a molecular weight of 10 000. This molecular weight was used for determining the stoichiometric relationship between the interaction of myosin mRNA and myosin mRNP-tcRNA.

Figure 4 illustrates the stoichiometric relationship of myosin mRNA-tcRNA with myosin mRNA as measured by the increase in RNase resistance after hybridization. As can be seen, increasing amounts of tcRNA increase the RNase resistance until a 1:1 mole ratio of myosin mRNP-tcRNA and myosin mRNA is reached. Additional amounts of tcRNA over this value do not result in a further increase in RNase resistance. These results suggest that one molecule of tcRNA (10 000 mol wt) can induce considerable structural changes in a messenger of approximately 2×10^6 molecular weight. Such a change can be brought about by bringing two distal portions of the mRNA into close proximetry of each other and in so doing allowing additional areas of the mRNA to form double-stranded regions that would otherwise not be stable. Further studies are under way to examine the manner by which tcRNA induces these structural changes in mRNA.

Myosin mRNP-tcRNA has been shown to inhibit the translation of myosin mRNA. When 20 μg of myosin mRNA is added to the reticulocyte cell-free system a substantial amount of radioactivity is incorporated in myosin as determined by a three-step copurification and sodium dodecyl sulfate-acrylamide gel electrophoresis (Table IV). When IF-3, isolated from native muscle 40S ribosomal subunits, is added to the incubation mixture, a five to sixfold increase in radioactivity incorporated into myosin is found. This is in agreement with our previous results (Rourke and Heywood, 1972)

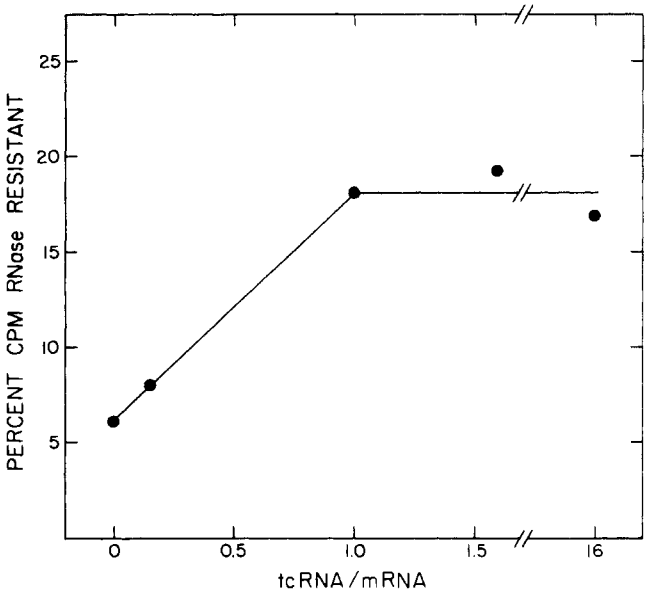

FIGURE 4: Stoichiometric relationship between myosin tcRNA and myosin mRNA in the increase in resistance to T_1 and T_2 RNase digestion of [^{3}H]myosin mRNA. Each reaction contained 2500 cpm of [^{3}H]myosin in mRNA (obtained by in vivo labeling of embryos). Hybridization reactions were performed and nuclease digests (T_1 and T_2 RNase) were analyzed by Sephadex chromatography as previously described (Heywood et al., 1975a). The ratio shown on the abscissa is the mole ratio.

TABLE IV: Effect of IF3 and Myosin mRNP-tcRNA Fractions on Myosin Synthesis

Myosin mRNA	IF3	tcRNA	cpm (Myosin)	Total cpm
−	+	−	150	92 400
+	−	−	3 800	90 170
+	+	−	17 100	93 140
+	+	(A)[a]	19 450	82 320
+	+	(B)[b]	14 700	88 420
+	+	(C)[c]	1 400	89 600
+	+	(C)[d]	16 800	91 430

[a] IF3 prepared from 1 M KCl wash of muscle 40S RSU, 20S protein complex chromatographed on DEAE-cellulose (4) μg/reaction mixture). [b] Myosin synthesis determined after $(NH_4)_2SO_4$ fractionation, ionic precipitation, DEAE-cellulose chromatography, and acrylamide gel electrophoresis (Heywood et al., 1975a). [c] 20 μg of myosin mRNA and 0.1 μg of tcRNA were used where indicated. [d] Alkaline hydrolyzed tcRNA (RNase treated tcRNA gave similar results as previously reported (Heywood et al., 1975a).

suggesting a role of these factors in the translational control of muscle proteins. When the three fractions from the Dowex column (Figure 1) of the myosin mRNP-tcRNA were premixed with myosin mRNA prior to its addition to the cell-free system, only fraction C resulted in a substantial inhibition of myosin synthesis. Only a 3% decrease in total radioactivity (indicative of globin synthesis, see Heywood et al., 1975a) occurs when fraction C is added with myosin mRNA suggesting that the inhibition is specific (Table IV). The addition of fraction A actually causes a small increase in the radioactivity incorporated into myosin, while fraction B is slightly inhibitory. These results further indicate that myosin mRNP-tcRNA is found in fraction C from the Dowex column. We have previously reported the RNase sensitivity of the active fraction (mRNP-tcRNA) (Heywood et al., 1974, 1975a). As shown in Table IV, the active fraction eluting from the Dowex

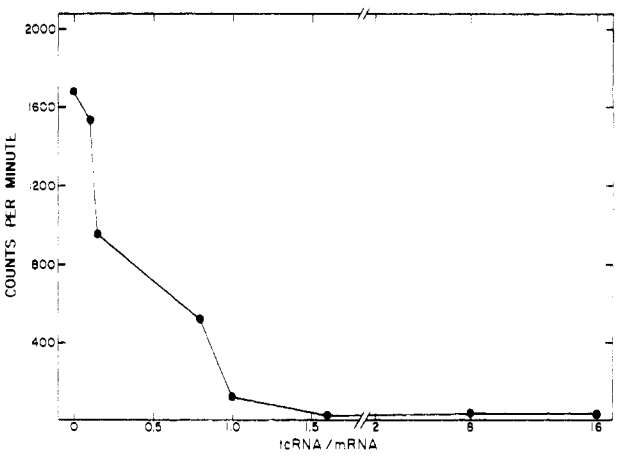

FIGURE 5: Stoichiometric relationship between myosin tcRNA and myosin mRNA as measured by the inhibition of myosin mRNA translation. Cell-free synthesis and determination of radioactivity incorporated into myosin are described under Methods and by Heywood et al., 1975a. The ratio shown on the abscissa is the mole ratio.

column is also sensitive to alkaline hydrolysis. This further indicates that the active material is an RNA and is not similar to the material found in ribosomal washes by Berns et al., 1975.

The experiments reported in Table IV utilized the premixing of stoichiometric amounts of myosin mRNP-tcRNA and myosin mRNA (a 1:1 mole ratio assuming 2×10^6 and 10 000 for the mol wt of myosin mRNA and myosin mRNP-tcRNA, respectively). As shown in Figure 5, when increasing amounts of myosin mRNP-tcRNA are premixed with myosin mRNA before addition to the incubation mixture, myosin synthesis is increasingly inhibited until virtually all translation of myosin mRNA is stopped when a 1:1 mole ratio is achieved. These results agree with those presented in Figure 4 and indicate that the interaction of the messenger and tcRNA occur stoichiometrically and not catalytically.

Results previously reported, using the less purified mRNP-tcRNA preparations (Bester et al., 1975; Heywood et al., 1975a), have suggested that a degree of specificity exists in the interaction of messenger with tcRNA. We have repeated these experiments using the highly purified myosin mRNP-tcRNA obtained from the Dowex-fractionation procedure. When poly(A)-containing mRNA, isolated from mRNPs sedimenting at less than 30S from 14-day embryonic chick muscle (Heywood et al., 1974; Bester et al., 1975), is premixed with myosin mRNP-tcRNA and the products of the cell-free system subsequently analyzed on acrylamide gels, the mRNP-tcRNA had little effect on the translation of these messengers (Figure 6). It should be pointed out that this group of messengers undoubtedly contains a large number of different mRNAs and there may indeed be a number of these that do interact with myosin mRNP-tcRNA but are not detectable by the methods used here or previously reported (Bester et al., 1975).

Discussion

An RNA molecule has been isolated from myosin mRNPs found in embryonic chick muscle. We have previously suggested that this molecule may specifically interact with mRNA by virtue of both an oligo(U)-rich region and a region as yet undefined thereby altering the structure of the messenger and in so doing rendering it inaccessible for translation (Bester et al., 1975). Although evidence is not available, as yet, it is

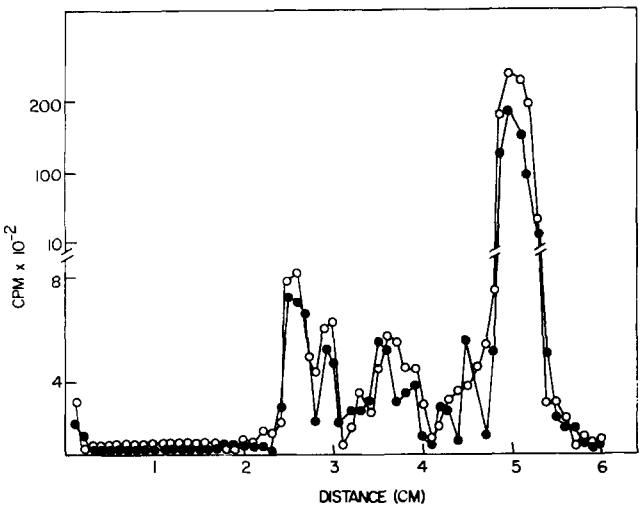

FIGURE 6: Myosin tcRNA does not inhibit the translation of poly(A)-containing mRNA obtained from less than 30S mRNPs obtained from muscle. The isolation of small mRNPs, in vitro synthesis, and electrophoretic analysis of radioactivity incorporated into proteins were as previously described (Heywood et al., 1975a). (O—O) 10 μ of poly(A)-containing mRNA; (●—●) 10 μg of poly(A)-containing mRNA premixed with 1 μg of myosin tcRNA.

possible that the original transcript that contains the poly(A)-containing mRNA also has within it the sequence of nucleotides constituting the tcRNA for the messenger. Such a possibility becomes more convincing from the finding by Molloy et al. (1972) that HnRNA contains an oligo(U) region distal from the poly(A)-containing mRNA. If this is the case, a direct link between transcription and translational control would be likely by having a messenger and a control RNA as part of the original transcript.

mRNP-tcRNA has a number of characteristics that may be utilized to aid in its identification and purification. It contains an oligo(U)-rich region that hybridizes to poly([^{3}H]A), thereby giving a rapid and simple procedure for initial identification of the molecule. The oligo(U)-rich property of mRNP-tcRNA may also be used to isolate the molecule, once poly(A) containing mRNA is removed, by oligo(dA)-cellulose chromatography. In addition, mRNP-tcRNA has the ability to alter the structure of mRNA thereby making it more nuclease resistant; finally, it does not allow the messenger to be translated in cell-free amino acid incorporating systems.

We have isolated myosin mRNPs by virtue of their high-sedimentation rate on sucrose density gradients. The mRNP-tcRNA obtained from these mRNPs has been purified by dialysis on Sephadex (Pharmacia) chromatography, DEAE-cellulose, Dowex chromatography, and ethanol precipitation until a molecule showing a single band of formamide gels has been obtained. This myosin mRNP-tcRNA interacts on a 1:1 mole ratio with myosin mRNA resulting in its greatest resistance to T_1 and T_2 nuclease and its complete inhibition of translation. In addition, the amount of recoverable tcRNA from myosin mRNPs suggests that this stoichiometry exists in the tissue itself.

From these results, and the number of controls performed which were concerned with the possible degradation of RNA during preparation, we suggest that tcRNA is not an artifact produced during isolation but that mRNP-tcRNA is indeed a bona fide entity that may control the utilization of messengers in embryonic muscle cells. The stoichiometric relationship between the mRNA and the tcRNA as demonstrated in this report lends further support to our suggested model for tcRNA activity (Bester et al., 1975). However, further experiments are necessary to establish that the interaction between mRNA and tcRNA observed in vitro reflects their physiological role in the translation of mRNA.

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