Characterization of Translational-Control Ribonucleic Acid Isolated from Embryonic Chick Muscle†

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ABSTRACT: Myosin heavy chain (MHC) mRNP particles have been purified from 13-day chick embryonic skeletal muscle by a combination of sucrose density gradient centrifugation and metrizamide buoyant density centrifugation. Associated with the mRNPs are at least three distinct low molecular weight RNA molecules including translational-control RNA (tcRNA). This particular RNA contains 102 nucleotides and is uridine and guanine rich, and its nucleotide sequence has been determined. $tcRNA_{102}$ is capable of inhibiting the translation of the mRNAs with which it is associated upon preincubation in stoichiometric amounts. Under these conditions, endogenous reticulocyte mRNA is not inhibited. Under appropriate salt and temperature conditions, $tcRNA_{102}$ is capable of reassociating with myosin heavy chain (MHC) mRNA, thus altering the sedimentation characteristics of the mRNA. This suggests that the mRNA–$tcRNA_{102}$ interactions alter the secondary structure of the mRNA. In addition, $tcRNA_{102}$ does not associate with ribosomal RNA or globin mRNA, suggesting that some degree of specificity is involved with the RNA–RNA interactions.

Evidence for the control of gene expression at the translational level has continued to accumulate in recent years. Among the controls utilized by eukaryotic cells is the sequestering (in the cytoplasm) of mRNA into inactive particles (mRNPs) unassociated with ribosomes. We have previously reported that myosin heavy chain (MHC) mRNA can be isolated from such particles from both embryonic muscle and muscle cell cultures (Heywood & Kennedy, 1976; Bester et al., 1975; Havaranis & Heywood, 1981). In addition, we have suggested that a small RNA, designated translational-control RNA (tcRNA), is involved in maintaining the mRNA in an inactive state (Bester et al., 1975). Translational-control RNA has been demonstrated to stoichiometrically inhibit the translation of MHC in vitro and upon hybridization dramatically increase its RNase resistance (Heywood & Kennedy, 1976). Recently, a number of reports have shown small RNAs to be associated with stored mRNPs in a variety of cell systems (Northeman, et al., 1980; Vincent et al., 1980; O'Loughlin & Gross, 1981). The MHC mRNP has been purified and shown to contain, in addition to MHC mRNA, a number of low molecular weight RNAs (Havaranis & Heywood, 1981). In this report, we confirm the existance of tcRNA among these small RNAs and characterize tcRNA with regard to its nucleotide sequence and function in the repression of mRNA translation.

Experimental Procedures

Metrizamide Gradient Purification of MHC mRNPs. The isolation of chick muscle MHC mRNP by sucrose density gradient centrifugation and subsequent metrizamide buoyant density centrifugation has been previously described (Bester et al., 1980; Havaranis & Heywood, 1981).

Terminal 3′ End Labeling of tcRNA. MHC mRNP RNA isolated from the leg muscle of 10 dozen 13-day-old chick embryos was used for end labeling. Ethanol-precipitated RNA was resuspended in 10 μL of ligation buffer (0.05 M Hepes[1]–KOH, pH 7.5, 0.015 M $MgCl_2$, 0.0033 M dithiothreitol, 10% Me_2SO) heated to 65 °C for 5 min and then rapidly cooled. An additional 5 μL of ligation buffer containing 100 μg/mL ATP was added, and the total reaction volume of 15 μL was transferred to a siliconized Eppendorf microtube in which 40 pmol of [5′-^{32}P]-pCp (New England Nuclear) had been dried under vacuum according to the procedure of Donis-Keller et al. (1977). The equivalent of 10 units of T4 RNA ligase (P-L Biochemicals) was added, and the reaction was incubated at 4.0 °C for a minimum of 24 h at which time 5 μL of dye mix containing TBE (0.05 M Tris, 0.06 M boric acid, and 1 mM EDTA, pH 8.3), 0.05% bromophenol blue, 0.05% xylene cyanol, and 7 M urea was added.

Gel Electrophoresis of 3′-End-Labeled tcRNA. End-labeled tcRNA was electrophoresed on a 48-cm 10% polyacrylamide gel (made up in TBE buffer, pH 8.3, and 7 M urea). The running buffer was TBE, pH 8.3, and electrophoresis was carried out at room temperature at 800 V. tcRNA was excised from the gel and eluted by the addition of 3.0 mL of elution buffer (0.5 M sodium acetate, 0.010 M magnesium acetate, 0.0001 M EDTA, and 0.2% $NaDodSO_4$) and subsequently incubated at 37 °C for 24 h. At the end of the incubation period, the solution was passed through a sterile 5-cm^3 disposable hypodermic syringe containing a glass wool plug to remove gel fragments. Finally, the [^{32}P]-tcRNA was ethanol precipitated in the presence of unlabeled tRNA used as a carrier.

Sequencing tcRNA. Polyacrylamide gel purified, 3′-end-labeled tcRNA was pelleted at 10000 rpm for 1 h. The pellet was carefully resuspended in 50 μL of H_2O; 10-μL aliquots of [^{32}P]-tcRNA were used for each sequencing reaction. One microliter of 13× digestion buffer (0.43 M sodium citrate, pH 5.0, 0.022 M EDTA) was added to each 10-μL aliquot so that the final concentration was 0.033 M sodium citrate–0.017 M EDTA. The sequencing procedure used is a modification of the Donis-Keller et al. (1977) procedure. This buffering system was used for all the enzyme digestion reactions except the ribonuclease T1 reaction, which also contained 5 M urea. The units of enzyme used in each individual reactions were as follows: 0.026 unit of ribonuclease T1, 2.0 units of ribonuclease *Bacillus cereus*, 2.5 units of ribonuclease Phy M.,

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[1] Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; PBS, phosphate-buffered saline; IgG, immunoglobulin G.

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Table I: Oligo[d(A)]-cellulose Binding Properties of mRNP-Associated RNAs[a]

	RNA (nucleotides)					
	150		102		89	
experimental conditions	cpm bound	% bound	cpm bound	% bound	cpm bound	% bound
binding at 25 °C	158	<1	3840	20	240	1.2
heat denaturation at 65 °C followed by slow cooling to 25 °C	195	<1	12240	62	310	1.5

[a] ^{32}P-End-labeled RNAs were resuspended in 0.5 M NaCl–0.01 M Tris (pH 7.4) prior to column application. 20 000 cpm were applied in each case. 99% of bound counts were recovered from the oligo[d(A)]-cellulose by washing with 0.01 M Tris (pH 7.4) at 65 °C.

and 2.1 units of ribonuclease U2. Enzyme digestions were performed at 50 °C for 20 min. The reactions were terminated by the addition of 5 μL of urea–dye mix, followed by quick freezing on dry ice. All RNA sequencing enzymes were purchased from P-L Biochemicals. The enzymatically digested RNA samples were thawed and either loaded onto a 48-cm 20% polyacrylamide gel (capable of effectively resolving nucleotides 1–50) or loaded onto a 48-cm 12% polyacrylamide gel (capable of effectively resolving nucleotides 10–150); both gels contained TBE and 7 M urea. The 20% gel was electrophoresed until the bromophenol blue marker had migrated halfway down the gel. The 12% gel was electrophoresed until the same marker was 5 cm from the bottom of the gel. After electrophoresis, the sequences were visualized by autoradiography. In addition, either 3′-end-labeled tcRNA or tRNA was eluted from the 10% sizing gel and resuspended in 50 μL of 0.05 M Na_2CO_3–$NaHCO_3$, pH 9.3. Alkaline hydrolysis was performed under limiting conditions (incubation at 90 °C for 8 min).

Complex Formation between tcRNA and Other RNAs. [^{32}P]-tcRNA$_{102}$ was purified by acrylamide gel electrophoresis as described above and mixed with various mRNA species or rRNA in complex formation buffer (0.02 M Tris-HCl, pH 7.5, 0.05 M NaCl, 0.025 M $MgCl_2$). The final reaction volumes were 50 μL. The reactions were heated to 65 °C for 1 h and slowly cooled to room temperature. The reactions were diluted to 0.5 mL with 0.05 M Tris-HCl, pH 7.5–0.25% $NaDodSO_4$ and applied to an 11-mL 10–30% sucrose density gradient formed in the same buffer. All gradients were simultaneously centrifuged for 16 h (unless otherwise indicated) at 30 000 rpm in an SB283 rotor at 4 °C. The A_{260} profiles of the gradients were sequentially determined on a flow-through spectrophotometer while fractions were collected to determine the position of the [^{32}P]-tcRNA$_{102}$ or [^{3}H]-mRNA. Radioactivity was determined by scintillation counting in a DPM counter.

Inhibition of MHC mRNA Translation by tcRNA$_{102}$. MHC mRNA from MHC mRNPs was purified as previously described (Bester et al., 1980). A total of 5 μg of MHC mRNA was added to a reticulocyte cell free system either independently or after preincubation with polyacrylamide gel purified tcRNA$_{102}$. The preincubation was performed in 0.02 M Tris (pH 7.4), 0.1 M KCl, and 0.003 M magnesium acetate at 60 °C for 15 min, followed by slow cooling to room temperature. MHC mRNA alone was treated in the same fashion. The reticulocyte lysates (30-μL final reaction volume, 5 μCi of [^{35}S]methionine), supplemented with 2 μg of muscle tRNA, were incubated for 1 h at 30 °C. After incubation, immunoprecipitates with antibody specifically reactive with either fast MHC protein or slow MHC protein (gift of Dr. S. Lowey) were added to the various cell lysates and analyzed as previously described (Havaranis & Heywood, 1981).

Lupus Antibody Precipitation of MHC mRNPs. Muscle cell cultures were labeled by the addition of [^{3}H]uridine 10 h prior to harvesting. At the time of harvesting, the cells were approximately 30–40% fused. Cells were harvested as previously described (Havaranis & Heywood, 1981), and the postmitochondrial supernatants were assayed for lupus erythematosus antibody precipitable material. Buoyant density purified MHC mRNPs that had been either radioactively labeled in cell culture with [^{3}H]uridine or reductively labeled in vitro with [^{14}C]formaldehyde by the procedure of Jentoft & Dearborn (1979) were also tested with the antibody. The MHC mRNPs or muscle cell lysates were incubated for 3 h at 37 °C with 15 μL of either lupus erythematosus antibody Ro (reactive with cytoplasmic components) or lupus erythematosus antibody Sm (reactive with nuclear components) (gift of Dr. J. Steitz). At the end of the incubation period, 20 μg of protein A (Sigma) was added, and the reaction mixture was further incubated at 4.0 °C for 24 h. The immunoprecipitates were centrifuged and washed in PBS prior to determining the radioactivity.

Results

Isolation of tcRNA$_{102}$. The ability to obtain purified MHC mRNPs free of ribosomes has enabled us to characterize RNAs present in these particles. We have previously reported that a small RNA that binds oligo[d(A)]-cellulose is found associated with MHC mRNPs isolated from sucrose density gradients (Heywood & Kennedy, 1976; Kennedy et al., 1978). This RNA was found to inhibit the translation of MHC mRNA and also form complexes with the MHC mRNA, increasing its RNase resistance. These functions attributed to tcRNA were also shown to have a degree of specificity since tcRNA did not effect all mRNAs similarly. Therefore, it was of interest to determine if a similar RNA could be obtained and characterized from MHC mRNPs purified by metrizamide buoyant density centrifugation. After removal of poly(A$^+$) MHC mRNA by oligo(T)-cellulose chromatography, a reproducible pattern of RNAs is obtained when MHC mRNP associated RNA is 3′ end labeled and subsequently analyzed by electrophoresis on denaturing polyacrylamide gels (Figure 1). The major small RNAs on the gel consist of three species of approximately 150, 102, and 89 nucleotides. It should be noted that there is no detectable loss of any of the predominant small RNAs into the oligo[d(T)]-bound fraction (data not shown).

Since we have previously demonstrated that tcRNA binds oligo[d(A)]-cellulose (Heywood & Kennedy, 1976), an analysis of the three small RNAs was undertaken to independently determine their capacity to bind oligo[d(A)]-cellulose. As can be seen in Table I, the RNA of 102 nucleotides binds oligo[d(A)]-cellulose while neither the 150 nor 89 nucleotide species is found to bind to any significant extent. It should be noted that the binding of this RNA to oligo[d(A)]-cellulose is not maximally efficient under the conditions used. Only 20% of tcRNA$_{102}$ binds oligo[d(A)]-cellulose at 25 °C; however, if the sample is heated to 65 °C and applied

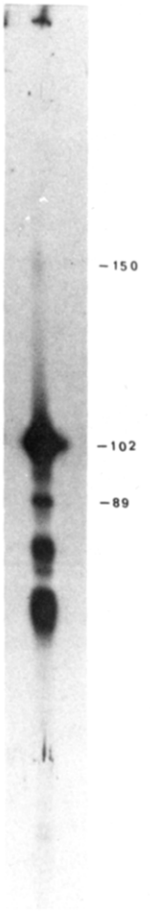


FIGURE 1: 3′ end labeling of oligo[d(T)]-unbound RNA from metrizamide-purified MHC mRNP particles. After oligo[d(T)]-cellulose chromatography, the unbound RNA, from metrizamide-purified MHC mRNPs, was 3′ end labeled and electrophoresed on a 10% polyacrylamide–urea gel as described under Experimental Procedures. The positions of RNA species of 150, 102, and 89 nucleotides in length are indicated.

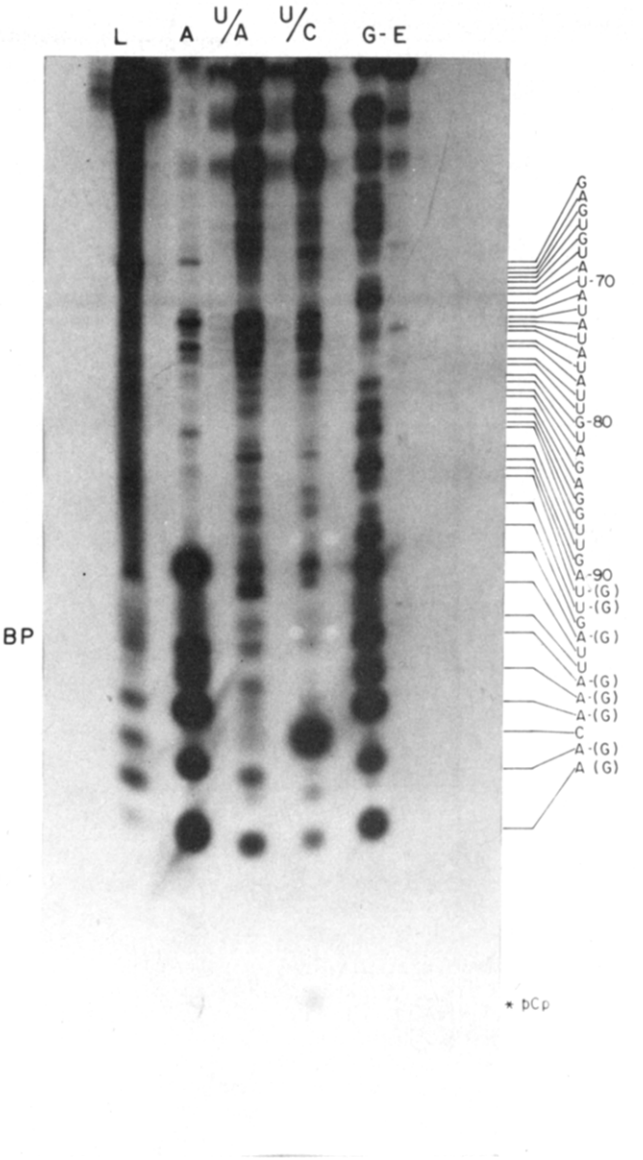


FIGURE 2: Enzymatic digestion of 3′ end labeled tcRNA$_{102}$. 3′ end labeled tcRNA, migrating at approximately 100 nucleotides and possessing inhibitory activity (Figure 3), was sequenced as described under Experimental Procedures. Enzyme-digested tcRNA$_{102}$ was electrophoresed on a 12% polyacrylamide–urea gel. L denotes end-labeled tRNA after limited alkaline hydrolysis. A contains tcRNA after limited digestion with ribonuclease U2, thus revealing adenine residues. U/A contains tcRNA$_{102}$ after limited digestion with ribonuclease *Phy. M*, thus revealing uridine and adenine residues. U/C contains tcRNA$_{102}$ after limited digestion with ribonuclease *B. cereus*, thus revealing uridine and cytidine residues. G contains tcRNA$_{102}$ after limited digestion with ribonuclease T1, thus revealing guanine residues. E contains undigested tcRNA. BP denotes the bromophenol blue dye.

to the column, 62% is bound. The bound material can be eluted with 0.01 M Tris-HCl (pH 7.4).

Nucleotide Sequence of tcRNA$_{102}$. After electrophoresis and autoradiography, the RNA species migrating at approximately 100 nucleotides was excised from the gel, and the RNA was eluted and subsequently digested with a series of RNases as described under Experimental Procedures. The partial digests were analyzed both on 12% and 20% polyacrylamide gels; an example is shown in Figure 2. The gel shown here is only one of the many gels run to make this sequence determination. Gels were run for varying lengths of time and at several voltage settings to bring about the separation of nucleotide residues in "hot" and "cold" regions. Regions where the sequence was either overexposed (hot) or underexposed (cold) were routinely observed, requiring multiple autoradiographic exposure times for all sequencing gels to accurately determine the nucleotide sequence (not shown). It is highly probable that these hot and cold spots result from the inherent structure of the molecule. For example, the region containing nucleotides 12–20 is considered a hot spot while that containing nucleotides 50–60 is considered a cold spot (as discussed below, these regions can be related to the secondary and tertiary structure of the molecule, which in turn is dictated by the primary sequence). The primary sequence and nucleotide composition of tcRNA$_{102}$ is shown in Table II. The molecule is 102 nucleotides in length, guanidine and uridine rich, and contains few cytidine residues.

From the sequencing ladders, the precise identity of a few bases, particularly near the 3′ end of the molecule, is unclear, although the choice of bases never exceeds two. This multiplicity of bases in similar positions may result from either a small degree of impurity or a variability in the sequence of tcRNA$_{102}$, suggesting it may arise from similar but different DNA sequences. Nevertheless, the purity of the tcRNA$_{102}$ eluted from the acrylamide gel allows for a sequence analysis identifying the molecule as a previously uncharacterized species of cytoplasmic RNA.

Interaction of tcRNA$_{102}$ with mRNA. We have previously reported that a low molecular weight RNA isolated with MHC mRNPs inhibits MHC synthesis. Therefore, we tested the

Table II: Sequence and Base Composition of $tcRNA_{102}$

Sequence

5'-UCGGUGAGACAGAAUGUGUUGCUGGUUGUUGAUUGUU-
U
10 20 30
GGGUUGUGCGUGUAGUUAAGUGUGAGAGUGUAUAUAUA-
U U
40 50 60 70
UAUUGUAGAGGUUGAGGGGUUGGGCGG-3'
UU A AAA AA
80 90 100

Base Composition

40% uridine	5% cytidine
32% guanine	6% unclear
17% adenine	

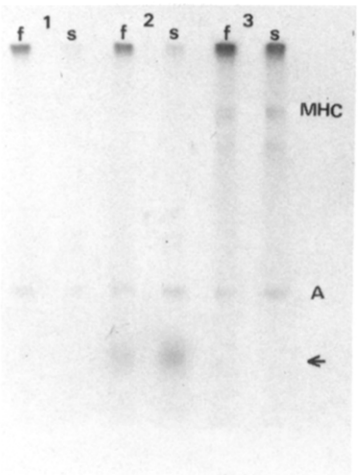


FIGURE 3: Inhibition of MHC mRNA translation by polyacrylamide gel purified $tcRNA_{102}$. After an incubation of the reticulocyte lysates without added mRNA (1), with 8 μg of MHC mRNA (3), and with 8 μg of MHC mRNA plus 0.12 μg of $tcRNA_{102}$ (2), MHC synthesis was analyzed with antibodies specific for fast MHC isoform (f) or slow MHC isoform (s). Precipitated protein products were analyzed on a 7.5–12% $NaDodSO_4$–polyacrylamide gradient gel and subsequently autoradiographed. MHC designates position of myosin heavy chain; A designates endogenous reticulocyte product; arrow shows ^{32}P radioactivity of $tcRNA_{102}$.

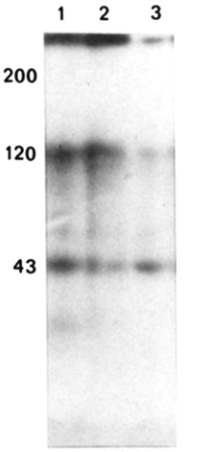


FIGURE 4: Reticulocyte cell free synthesized proteins. After removal of the MHC-immunoprecipitable material (Figure 3), the remaining supernatants were electrophoresed on a 7.5–12% polyacrylamide gradient gel followed by autoradiography. Lane 1 contains those proteins produced in the control lysates. Lane 2 contains those proteins produced by the lysates receiving $tcRNA_{102}$ preincubation with MHC mRNA. Lane 3 contains those proteins produced in lysates receiving MHC mRNA alone. Numbers denote the molecular weights × 10^3 of marker proteins.

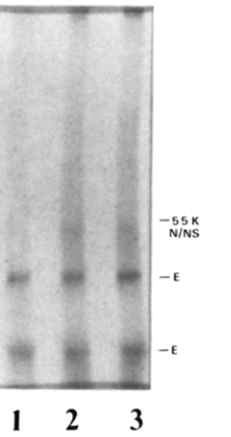


FIGURE 5: Inability of $tcRNA_{102}$ to inhibit VSV RNA translation. In vitro translation, acrylamide gel analysis, and autoradiography were as under Experimental Procedures and Figure 3: (lane 1) control with no mRNA plus 0.1 μg of $tcRNA_{102}$; (lane 2) 1 μg of VSV mRNA plus 0.1 μg of $tcRNA_{102}$; (lane 3) 1 μg of VSV mRNA plus 0.3 μg of $tcRNA_{102}$. E designates endogenous reticulocyte translation products.

ability of the 102-nucleotide oligo[d(A)]-binding $tcRNA_{102}$ to interfere with the translation of MHC mRNA. When stoichiometric amounts of MHC mRNA and $tcRNA_{102}$ are mixed, heated to 65 °C, and slow cooled to room temperature prior to addition to the cell-free system, MHC synthesis is completely inhibited (compare lanes 2f and 2s with lanes 3f and 3s, Figure 3). Endogenous protein synthesis in the reticulocyte lysate is not inhibited (Figure 4). In addition, VSV mRNA translation is also unaffected by the additioin of $tcRNA_{102}$ under these same conditions (Figure 5). We have previously reported that tcRNA does not inhibit globin synthesis in a wheat germ translation system (Kennedy et al., 1978). Similarly, we have found that the addition of $tcRNA_{102}$ has no effect on the total amount of radioactivity incorporated into protein in a reticulocyte lysate to which 1 μg of globin mRNA had been added. Autoradiographic analysis was not possible due to high levels of radioactive globin in the control (minus globin mRNA). In addition, when mRNA is isolated from muscle mRNPs of less than 40 S and incubated with the $tcRNA_{102}$ obtained from MHC mRNPs, the translation of these muscle mRNAs is also inhibited (to be published elsewhere). These results suggest that $tcRNA_{102}$ isolated from MHC mRNPs has a degree of specificity for the mRNAs with which it interacts; i.e., it inhibits the translation of those mRNAs found as mRNPs in muscle tissue but has no effect on the translation of the heterologous mRNAs so far tested. It should be noted that the importance of the sequence of addition of the RNAs to the translation system as well as the ability of tcRNA to selectively inhibit MHC mRNA translation in the presence of globin mRNA has been previously reported (Kennedy et al., 1978).

The interaction of $tcRNA_{102}$ with mRNA was also examined for its ability to form complexes as determined by sucrose density gradient centrifugation. Under the specified centrifugation conditions polyacrylamide gel purified [^{32}P]-$tcRNA_{102}$ is found to remain near the top of the gradient (Figure 6), while uncomplexed 26S MHC mRNA is found to sediment near the bottom. When both molecules are premixed as described above for the translation studies (Figure 7), essentially all of the 260-nm absorbance and approximately 70% of the [^{32}P]-$tcRNA_{102}$ sediments at the bottom of the gradient. This is a positive indication of complex formation between MHC mRNA and $tcRNA_{102}$. Due to the small amount of $tcRNA_{102}$ available, stoichiometry was not attempted in these experi-

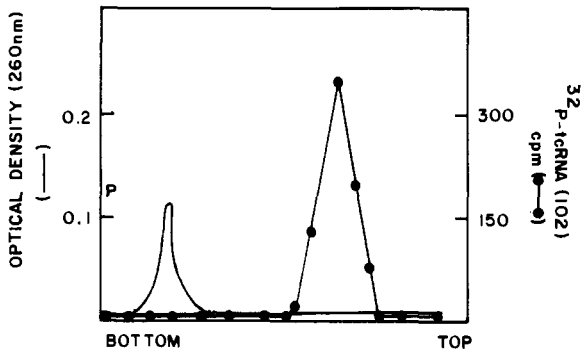


FIGURE 6: Sedimentation profile of end-labeled 102 nucleotide long tcRNA and MHC mRNA. The 3′-end-labeled 102 nucleotide long $tcRNA_{102}$ was excised and eluted from a 10% polyacrylamide–urea resolving gel. After ethanol precipitation, the RNA was centrifuged on a 10–30% sucrose density gradient at 30000 rpms for 16 h. Purified MHC mRNA was simultaneously centrifuged under the same conditions. The sedimentation profile was analyzed by recording the absorbance at 260 nm (—) and by collecting 25-drop fractions for scintillation counting (●). (●) Sedimentation profile of ^{32}P-end-labeled $tcRNA_{102}$; (—) optical density profile of MHC mRNA centrifuged under the same experimental conditions in the absence of $tcRNA_{102}$. The figure is a composite of two separate gradients.

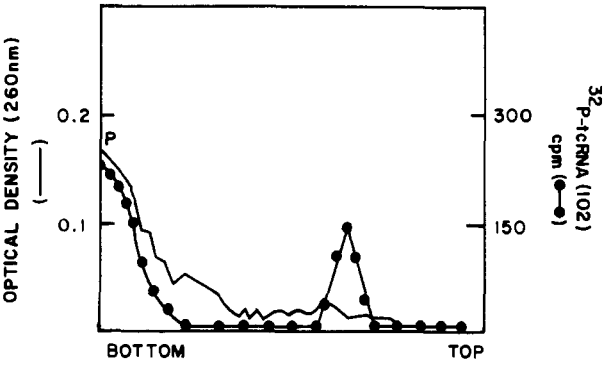


FIGURE 7: Sedimentation profile of end-labeled 102 nucleotide long tcRNA preincubated with MHC mRNA. Following preincubation of MHC mRNA and end-labeled 102 nucleotide long tcRNA under complex forming conditions as described under Experimental Procedures, the RNA was centrifuged on a 10–30% sucrose density gradient at 30000 rpms for 16 h. The sedimentation profile was analyzed by recording the absorbance at 260 nm (—) and by collecting 25-drop fractions for scintillation counting (●). P denotes pelleted fraction.

ments. However, not all of the $tcRNA_{102}$ sediments with the mRNA, suggesting that excess $tcRNA_{102}$ is present in the reaction mixture. In order to test the specificity of the RNA–RNA interaction, $tcRNA_{102}$ was similarly combined with poly(A^-) RNA that contained 18S rRNA and 28S rRNA in addition to tRNA (Figure 8). It can be seen that no shifts in the 260-nm absorbance or radioactivity occurs. This suggests that $tcRNA_{102}$ does not interact with these RNAs and that it complexes specifically with mRNAs.

Complex formation experiments were also conducted with a variety of other mRNAs, since we were not able to demonstrate the inhibition of translation of heterologous mRNAs by $tcRNA_{102}$. An example with 9S rabbit globin mRNA is shown in Figure 9. In this case, as well as others with TMV RNA and sea urchin poly(A^-) histone mRNA (results not shown), no complex formation could be demonstrated on the sucrose density gradients. Therefore, the negative assays demonstrating the inhibition of translation combined with the positive assays showing complex formation between $tcRNA_{102}$ and mRNA strongly suggest that a degree of specificity exists allowing $tcRNA_{102}$ to interact only with certain mRNA species.

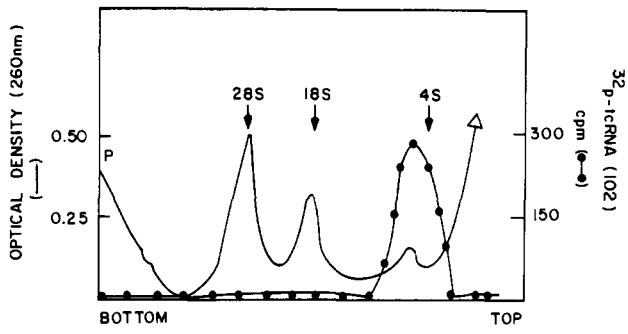


FIGURE 8: Sedimentation profile of end-labeled 102 nucleotide long tcRNA preincubated with ribosomal RNA. Following preincubation of ribosomal RNA with end-labeled $tcRNA_{102}$ under complex forming conditions, the RNA was centrifuged on a 10–30% sucrose density gradient at 30000 rpms for 16 h. The sedimentation profile was analyzed by recording the absorbance at 260 nm (—) and by collecting 25-drop fractions for scintillation counting (●). P denotes pelleted fraction.

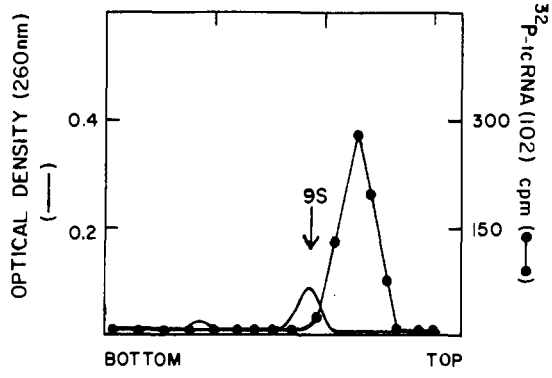


FIGURE 9: Sedimentation profile of end-labeled 102 nucleotide long tcRNA preincubated with globin mRNA. Following preincubation of globin mRNA and end-labeled 102 nucleotide long tcRNA under complex forming conditions the RNA was centrifuged on a 10–30% sucrose density gradient at 30000 rpms for 16 h. The sedimentation profile was analyzed by recording the absorbance at 260 nm (—) and by collecting 10-drop fractions for scintillation counting (●).

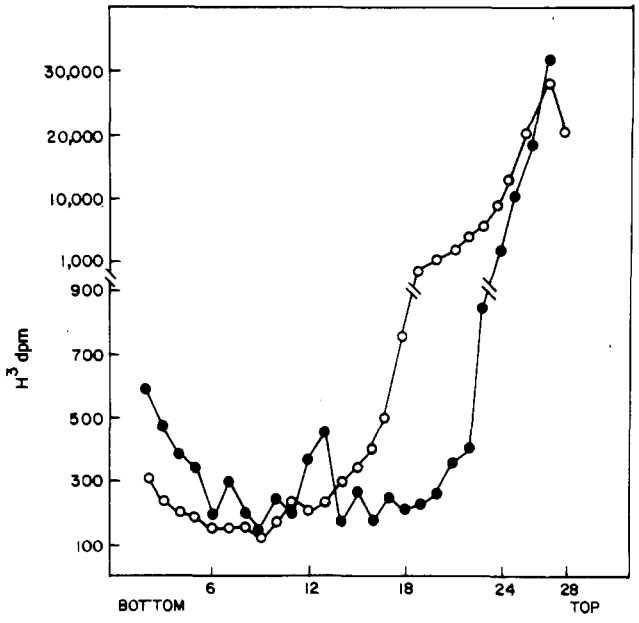


FIGURE 10: Sedimentation profile of [^{3}H]polyadenylic acid preincubated with $tcRNA_{102}$. [^{3}H]polyadenylic acid was centrifuged on a 10–30% sucrose density gradient at 30000 rpms for 16 h in the absence of $tcRNA_{102}$ (●) and in the presence of $tcRNA_{102}$ after preincubation (○). Radioactivity was monitored by collecting 25-drop fractions for scintillation counting.

Table III: Lupus Erythematosis Antibody Precipitation of MHC mRNP Particles[a]

	antibody precipitable radioactivity (dpm)/total radioactivity (dpm)	% total precipitated
IgG + [^{14}C]-MHC mRNPs	66/906	7.2
[^{14}C]-MHC mRNPs + Sm	82/942	8.7
[^{14}C]-MHC mRNPs + Ro	80/970	8.2
IgG + [^{3}H]-MHC mRNPs	210/5500	3.8
[^{3}H]-MHC mRNPs + Sm	184/5125	3.6
[^{3}H]-MHC mRNPs + Ro	305/5230	5.8

[a] Lupus erythematosis antibody precipitations were performed as described under Experimental Procedures. MHC mRNP particles were incubated with either Sm antibody or Ro antibody. After centrifugation, the pellets were resuspended, and radioactivity was determined by scintillation counting.

Furthermore, we have observed that this interaction is partially mediated by the hybridization of the mRNA poly(A) tail with $tcRNA_{102}$; supportive evidence is shown in Figure 10. An observed shift in the sedimentation pattern occurs when poly(A) is prehybridized to $tcRNA_{102}$ prior to centrifugation, under the same conditions employed above. Radioactivity associated with [^{32}P]-$tcRNA_{102}$ was found predominantly to reside in fractions 22–24 when $tcRNA_{102}$ alone was subjected to identical hybridization and centrifugation conditions.

Comparison of tcRNA to Lupus erythrematosus Antibody Precipitable RNAs. A number of both nuclear and cytoplasmic small RNAs associated with RNPs have been observed to react with lupus erythematosus antibodies (Lerner & Steitz, 1979; Lerner et al., 1981). It was therefore of interest to determine if the isolated MHC mRNP would form a precipitate with these antibodies. The mRNP proteins obtained from muscle tissue were radioactively labeled by reductive methylation while [^{3}H]uridine-labeled mRNPs were obtained from muscle cell cultures (Havaranis & Heywood, 1981). It can be seen in Table III that neither anti-Sm nor anti-R0 precipitates the MHC mRNP to a greater degree than the control IgG. We do not believe that the increase in ^{3}H-labeled MHC mRNP precipitable radioactivity with anti-R0 is significant since a similar result was not observed when ^{14}C-labeled MHC mRNP was used. While a [^{3}H]uridine-labeled muscle cell extract does contain material precipitable with anti-Ro, a further analysis of any RNAs precipitating with lupus erythematosus antibodies has not been undertaken since the Ro RNA nucleotide sequence has not been reported, although anti-Ro has been shown not to be species specific (Lerner & Steitz, 1981). Any relationship to $tcRNA_{102}$ would therefore be speculative.

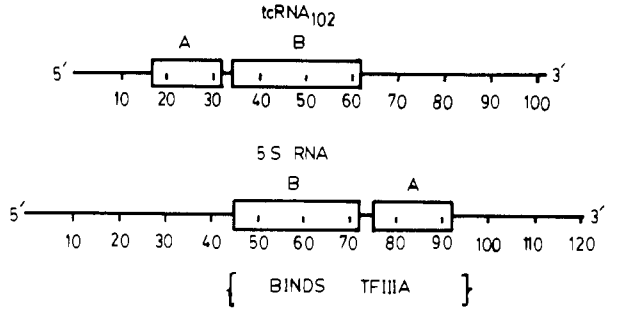
Discussion

mRNPs have been isolated from embryonic muscle tissue by a combination of sucrose density gradients and metrizamide buoyant density configuration (Bester et al., 1980; Havaranis & Heywood, 1981). MHC mRNPs have been obtained in which the only mRNA present is that coding for the MHC. Using antibodies specific for fast and slow MHC, it was determined that the mRNAs for both isoforms of MHC are present in these mRNPs. This is not an unexpected result since the mRNPs were prepared from a mixture of leg muscles. It is also possible than an embryonic isoform of MHC contains determinants that cross-react with the adult fast and slow antibodies. Nevertheless, the MHC mRNP contains only MHC mRNA(s). As in the case of erythroblasts (Vincent et al., 1980), liver (Northeman et al., 1980), and sea urchin (O'Loughlin & Gross, 1981), MHC mRNP contains a number of small RNAs. Among these is a 102-nucleotide RNA that weakly binds oligo[d(A)]-cellulose and specifically binds mRNA (but not all mRNAs) and inhibits their translation. The nucleotide subsequence of this RNA ($tcRNA_{102}$) has been determined and is shown in Table II. We have compared this sequence to known small RNA sequences using the SUMEX-AIM facility at Stanford University (Brutlag et al., 1982). It has no sequence homology to 4.5S RNA; however, it shows some interesting homologies to U1 snRNA (Table IV).

Table IV: Sequence Homology between $tcRNA_{102}$ and U1 snRNA

tcRNA	2	CGGUGAGACU GAAUG	16
U1 snRNA	115	CGG GAAACUCGACUG	129
tcRNA	9	ACUGAAUGUGUU GCU	23
U1 snRNA	32	AC GAAGGUGUUUUCU	46
tcRNA	32	AUU GUUGGGUUGUGCG	47
U1 snRNA	134	AUUUGU GG UAGUGCG	148
tcRNA	54	UAAUU GUGUGAGUG	67
U1 snRNA	132	UAAUUUGUGGUAGUG	146

Table V: $tcRNA_{102}$ Has Regions of Homology with the TFIIIA Binding Sequence Present in 5S RNA



The functional role of specific sequences present in snRNAs, as well as $tcRNA_{102}$, with respect to mRNA metabolism is not presently known. It may be that functional domains are held in common by these RNAs; this is yet to be determined. Although there are areas of homology between $tcRNA_{102}$ and snRNAs, these areas are not sequentially positioned, suggesting that $tcRNA_{102}$ is not a degradation product of a snRNA.

5S rRNA contains an internal sequence that has been shown to bind a protein (transcription factor III, TFIIIA) involved with the control of RNA polymerase III directed transcription of 5S rRNA genes (Engelke et al., 1980). Similar sequences have also been noted on tRNAs that are also RNA polymerase III products (Ciliberto et al., 1982). Similarly, as shown in Table V, $tcRNA_{102}$ also contains these homologous sequences, suggesting that $tcRNA_{102}$ may be an RNA polymerase III product.

During the sequencing of $tcRNA_{102}$, it was observed that very reproducible hot and cold spots were obtained on the sequencing ladders (Figure 2). These cold spots most likely represent areas relatively insensitive to nuclease digestion, while the hot spots correspond to regions readily accessible to enzymatic cleavage. For example, bases 41–64 form a cold spot on the sequencing ladder; these nucleotides have a potential of forming a double-stranded loop with only two mismatched base pairs if guanidine–uridine base pairing is allowed. It is likely that the hot and cold spots reflect the intramolecular folding of the molecule. The sequence of the small RNA migrating just ahead of $tcRNA_{102}$ [approximately 90 nu-

cleotides (Figure 1)] has been determined. It is both guanidine and uridine rich, does not contain the same hot and cold spots, and is 89 nucleotides in length (to be published elsewhere).

The fact that $tcRNA_{102}$ will form specific complexes with mRNA and can be isolated from mRNP particles from both primary tissue culture cells and leg muscle tissue strongly suggests that these RNAs are associated by hydrogen bonding in the cell. Nevertheless, it will be necessary to cross-link these RNAs in vivo and determine the precise regions containing complementary sequences between $tcRNA_{102}$ and the corresponding mRNAs before it can be definitively stated that $tcRNA_{102}$ is directly associated with mRNAs in vivo. Any interaction between mRNA and $tcRNA_{102}$ must account for significant alterations in the structure of the mRNA as demonstrated in this report by changes in sedimentation behavior and the increase in RNase insensitivity as reported previously (Heywood & Kennedy, 1976).

We propose that at least three regions of interaction exist between $tcRNA_{102}$ and MHC mRNA that may account for the observed dramatic alterations in sedimentation and RNAse sensitivity of complexed and uncomplexed message. It has been shown previously by Heywood et al. (1975) that the poly(A) tail on myosin mRNA is necessary for the inhibitory activity of tcRNA. The poly(A) tail of mRNA can form a rather strong bonding with $tcRNA_{102}$ by both Hoogstein and Watson–Crick base pairing (Bina et al., 1980), forming a triple-standard RNA. In addition, the 3′ end of $tcRNA_{102}$ has a complementary region to a sequence common to many eukaryotic mRNAs (AAUAAA) near the 3′ poly(A) tail. Base pairing between the first two codons for MHC (Met, Ser) with a region near the 5′ end of $tcRNA_{102}$ may also occur. Therefore, the tertiary structure of the mRNA may be altered by the interaction of $tcRNA_{102}$ with these regions of the mRNA, rendering the message translationally inactive. The fact that $tcRNA_{102}$ complexes with muscle mRNAs in such a way as to significantly alter the mRNAs sedimentation patterns suggests that large changes in structure are occurring. It is possible that under the conditions used in vitro, end to end or concatermeric complexes are occurring as well as those proposed above. Nevertheless, it is clear that nonspecific RNA–RNA aggregation is not occurring due to the fact that $tcRNA_{102}$ does not complex with all mRNAs or rRNA. Whether, in fact, this mode of interaction is correct will require sequence analysis of the regions of interaction between $tcRNA_{102}$ and MHC mRNA. In addition, until more sequence information is available concerning the mRNAs, we are unable to explain the specificity of interaction between $tcRNA_{102}$ and muscle mRNAs. Nevertheless, a function of $tcRNA_{102}$ appears to be involved with the cytoplasmic utilization of mRNA. It is also possible that $tcRNA_{102}$ and the other small RNAs isolated with MHC mRNP may be involved with nuclear packaging and transport of newly made MHC mRNA and serve secondarily as cytoplasmic repressors. Although we have stressed, in this report, the role of small RNAs in the translational repression of cytoplasmic mRNA, we do not exclude the possibility that mRNP proteins may also be involved in this function.

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