Most myosin heavy chain mRNA in L$_6$E$_9$ rat myotubes has a short poly(A) tail

(myoblast cell lines/translation *in vitro*/cDNA hybridization)

SUSAN BENOFF AND BERNARDO NADAL-GINARD

Department of Cell Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461

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ABSTRACT   The mRNA for rat muscle myosin heavy chain (MHC) was isolated from L$_6$E$_9$ myotubes by two rounds of sucrose density gradient centrifugation followed by fractionation on an agarose/polyacrylamide gel. The purity of the mRNA isolated was determined by translation *in vitro*, peptide analysis of the *in vitro* product and comparison with authentic MHC, analysis of the kinetics of hybridization with cDNA prepared with this RNA, and titration analysis of total cytoplasmic RNA from muscle and nonmuscle sources. By using the MHC cDNA as probe of myogenic differentiation, it was observed that the level of cytoplasmic MHC mRNA increased ≈200-fold as the dividing myoblast differentiated into the fused myotube. Titration analysis of RNAs fractionated by oligo(dT)-cellulose chromatography indicated that the majority of the increase occurred in that RNA population that failed to bind to an oligo(dT)-cellulose column.

Cultured rat myogenic cell lines (1) provide an *in vitro* model system for the study of myogenesis. The biochemical and morphological changes that occur during the differentiation of these cell lines parallels those occurring during myogenesis *in vivo*. Multinucleated fibers are formed by the fusion of single myoblasts. Concomitant with cell fusion, there begins the synthesis and accumulation of muscle-specific contractile proteins, such as the heavy and light chains of myosin, actin, tropomyosin, and the troponins.

We were interested in determining whether changes in myosin heavy chain (MHC; $M_r = 200,000$) accumulation during myogenic differentiation could be correlated with parallel changes in the level of cytoplasmic MHC mRNA. We had previously developed an *in vitro* translational system (2), which we employed to monitor for the presence of MHC mRNA. However, because it is possible that MHC mRNA can be sequestered in a manner that prevents its translation (3), it is necessary to directly measure the amount of MHC mRNA by hybridization with complementary DNA (cDNA) to pure MHC mRNA.

We report here on the isolation and purification of a mammalian MHC mRNA. The cDNA prepared with this mRNA as template has been used to investigate the controls on MHC accumulation during myogenesis. The data indicate that the level of cytoplasmic MHC mRNA increases ≈200-fold as the dividing myoblast differentiates into the fused myotube. A striking feature of this increase in MHC mRNA accumulation is its compartmentalization. The largest increase in cytoplasmic MHC mRNA occurs in that RNA population that fails to bind to oligo(dT)-cellulose.

MATERIALS AND METHODS

Cell Lines, Cell Culture, and Differentiation Conditions. L$_6$E$_9$ is a subclone of the L$_6$ rat myogenic cell line (1) isolated in our laboratory (4). Myoblast cultures were maintained in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 20% fetal calf serum (GIBCO). For induction of myogenic differentiation, exponentially growing cultures were transferred to Dulbecco's modified Eagle's medium supplemented with 10% horse serum (GIBCO) (4). All cultures were incubated at 37°C in a humidified atmosphere of 10% CO$_2$ in air.

Isolation of RNA. Total cytoplasmic RNA was isolated by using guanidine hydrochloride, following the procedure of Cox (5) with the following modification: cells were lysed with 0.5% Nonidet P-40 in a high-salt buffer (250 mM NaCl/5 mM MgCl$_2$/10 mM Tris·HCl, pH 7.4) to prevent the precipitation of polysomal MHC mRNA.

Oligo(dT)-cellulose (Collaborative Research, Waltham, MA) chromatography was performed according to the method of Aviv and Leder (6). All purified RNA was stored as ethanol precipitates at −60°C.

Cell-Free Protein Synthesis and Quantitation of the Amount of MHC Synthesized. RNA preparations were translated in a nuclease-treated rabbit reticulocyte cell-free amino acid incorporating system, and total protein and MHC syntheses were determined as described (2).

Sodium Dodecyl Sulfate (NaDodSO$_4$)/Polyacrylamide Gels. Electrophoresis of proteins in NaDodSO$_4$/polyacrylamide slab gels was performed as described by Laemmli (7). Fluorography was performed by following the procedures of Bonner and Laskey (8) and Laskey and Mills (9).

Peptide Analysis. Peptide mapping by limited chymotryptic proteolysis and electrophoresis was performed according to the procedure of Cleveland *et al.* (10) with modifications (2).

Fractionation of RNA on Polyacrylamide Gels. RNA was fractionated by electrophoresis on polyacrylamide gels in the REB buffer system of Sharp *et al.* (11). RNA (50–100 μg) applied to 8-cm disc gels (diameter 0.5 cm) consisting of 2.4% acrylamide, 0.32% agarose (Seakem; Marine Colloids, Rockland, ME), or 3.0% acrylamide, no agarose, and the following components: 0.5% N,N'-diallyltartardiamide (Eastman), 0.5 × REB, 2.5% (vol/vol) glycerol, 0.125% N,N,N',N'-tetramethylethylenediamine, 0.1% ammonium persulfate, 0.05% NaDodSO$_4$. The samples were electrophoresed at 7 mA per gel in a pH 7.8 electrode buffer (1 × REB/0.1% NaDodSO$_4$). The time the dye marker ran off the gels was noted and the samples were electrophoresed for 25% more time (approximately 5.5 hr total time elapsed). One-millimeter gel slices were depolymerized in 500

μl of 2% periodic acid/0.5% NaDodSO$_4$ for 2–6 hr at 37°C (12). RNA was recovered by Ca^{2+} precipitation according to the method of Dolja *et al.* (13) followed by ethanol precipitation.

Preparation of cDNA. MHC[^{3}H]cDNA was synthesized in a 100-μl reaction mixture containing: 100 mM Tris·HCl (pH 8.3), 60 mM NaCl, 6 mM Mg(C$_2$H$_3$O$_2$)$_2$, 10 mM dithiothreitol, 1.0 mM each dATP, dGTP, and dTTP, 0.6 μg of (dT)$_{10}$ (Collaborative Research), 40 μg of actinomycin D, 0.5–1.0 μg of RNA, 43.2 units of avian myeloblastosis virus RNA-dependent DNA polymerase (J. Beard through the Office of Program Resources and Logistics, National Cancer Institute), and 0.163 mM [^{3}H]dCTP (specific activity 22 Ci/mmol) (1 Ci = 3.7 × 10^{10} becquerels) as described (14).

cDNA·RNA Hybridization. Hybridizations were carried out in sealed microcapillaries as described (14). The amount of [^{3}H]cDNA protected by the test RNA was determined by digestion with 135 units of *Aspergillus oryzae* nuclease S1 (Miles). Radioactivity was determined in 10 ml of OCS scintillation cocktail (Amersham) with a 46% efficiency. Hybridization reaction mixtures not containing any RNA exhibited an average of 6% nuclease-resistant, acid-precipitable radioactivity (range 4–7%). As a standard in each analysis, the kinetics of rehybridization of mouse α + β globin mRNA to mouse α + β globin [^{3}H]cDNA (generous gifts of A. Skoultchi) were determined.

RESULTS

Isolation and Characterization of Rat MHC mRNA. The position of biologically active MHC mRNA in both total cytoplasmic RNA and poly(A)$^+$ RNA from well-fused L$_6$E$_9$ cells after centrifugation through NaDodSO$_4$-sucrose density gradients was monitored by separately translating a portion of the RNA in each fraction in a nuclease-treated reticulocyte lysate cell-free amino acid incorporating system (Fig. 1 A and B). This system had been optimized for both MHC synthesis and total protein synthesis (2). In both instances the MHC mRNA activity sedimented at approximately 26 S, in good agreement with the previously reported sedimentation value for other MHC mRNAs from other systems (15–17). MHC represented about 30–40% of the total protein synthesis directed by the RNA from two peak gradient fractions. Because the synthesis of cDNA from an RNA template is dependent on the presence of a poly(A) tail on the RNA, all further work was performed with poly(A)$^+$ RNA.
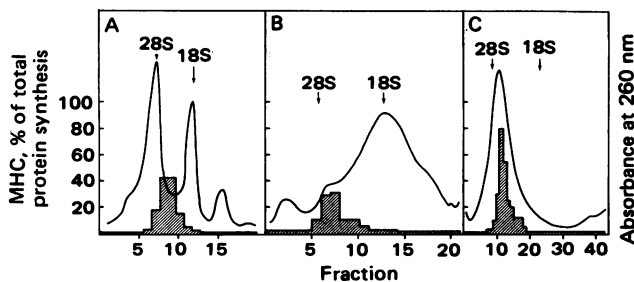
FIG. 1. Sucrose density gradient centrifugation of RNA extracted from well-fused L$_6$E$_9$ cells. RNA was isolated on day 6 after plating of cells in differentiation medium. RNA in ETS (10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.2% NaDodSO$_4$) was heated at 65°C for 5 min and quick cooled before application to 12-ml 15–30% sucrose density gradients in ETS. The gradients were centrifuged at 31,000 rpm for 15 hr at 27°C in a Spinco SW 41 rotor. The gradients were scanned at 260 nm and fractions were collected. The arrows in the figures represent the positions of 28S and 18S rRNA size markers. The step graph in the figure represents the amount of MHC synthesized as a percentage of the total proteins synthesized *in vitro* by an aliquot of the RNA in each gradient fraction. (*A*) Total cytoplasmic RNA; (*B*) poly(A)$^+$ RNA; (*C*) refractionation of MHC mRNA-enriched fractions from *B*.
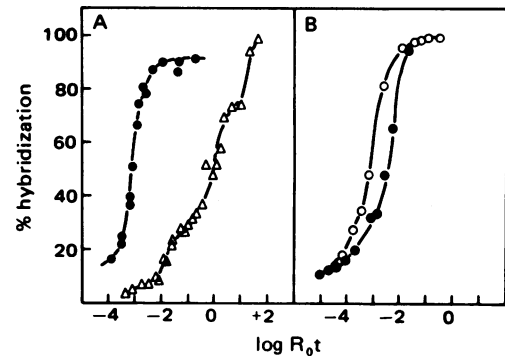
FIG. 2. Complexity analysis of RNA enriched in MHC mRNA. R$_0$t, product of RNA concentration (moles of nucleotide per ml) and incubation time (sec). (*A*) cDNA was prepared to the RNA fraction in Fig. 1*C*, which was shown to be enriched in MHC mRNA by translation *in vitro*. The cDNA was hybridized back to its template RNA and the kinetics of the reaction were compared with those of α + β globin mRNA to α + β globin cDNA. The complexity of the test sample was calculated as described (18). ●, α + β globin mRNA; △, MHC-mRNA-enriched RNA. (*B*) cDNA was prepared to the 30–32S RNA fraction obtained by electrophoretic fractionation of MHC-mRNA-enriched RNA (see Fig. 3) that directed the synthesis of MHC *in vitro*. ○, α + β-globin cDNA; ●, cDNA to 30–32S MHC mRNA.

The fractions from the poly(A)$^+$ RNA gradient that contained MHC mRNA activity were pooled and rerun on another NaDodSO$_4$-sucrose density gradient (Fig. 1*C*). Again the MHC mRNA activity sedimented at 26 S and was localized in two fractions. MHC now represented between 50 and 80% of the total protein synthesized by the RNA from these fractions. Nevertheless, when the cDNA made from these fractions was hybridized back to its RNA template (Fig. 2*A*), the RNA was more complex than expected on the basis of the translational analysis, displaying three abundance classes. The most abundant class had a calculated complexity of 5980 nucleotides. Because chicken MHC mRNA has been reported to be 6000 nucleotides long (16, 17, 19, 20), it appeared that MHC mRNA was the most abundant RNA in this population.

This MHC-mRNA-enriched RNA was further fractionated by agarose/acrylamide gel electrophoresis. An RNA fraction was obtained that migrated at 30–32 S and that upon translation *in vitro* yielded a single protein (Figs. 3 and 4*A*). This polypeptide displayed a physical characteristic of authentic MHC: i.e., precipitation at low ionic strength. Limited peptide analysis of this polypeptide by a modification of the procedure of
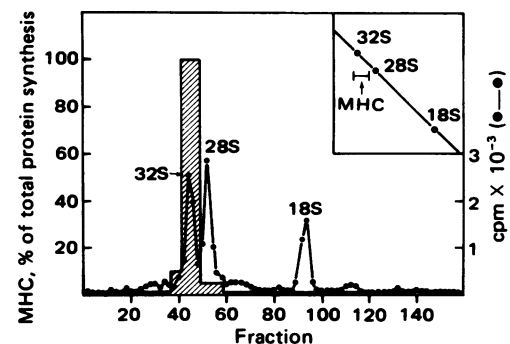
FIG. 3. Migration of MHC mRNA in agarose/polyacrylamide gels. Poly(A)$^+$ RNA (50 μg) from the MHC-mRNA-enriched fraction from the gradient depicted in Fig. 1*C* was electrophoresed on a 16-cm 0.32% agarose/2.4% polyacrylamide gel. The line graph in the figure depicts the migration of ^{3}H-labeled HeLa rRNA markers that were electrophoresed on a separate gel. The mobility of MHC mRNA was determined by translation *in vitro* of the fractionated RNA. The position of MHC mRNA is indicated by the step graph. (*Inset*) Molecular weight determination of MHC mRNA.
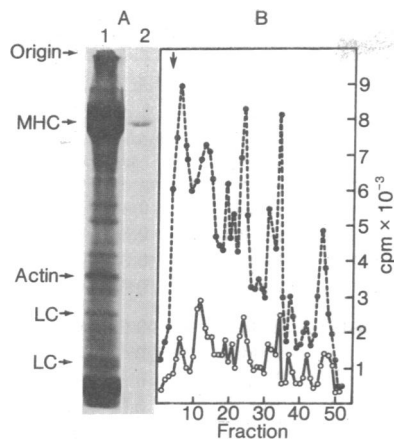
FIG. 4. Identification of the polypeptide whose synthesis is directed by 30–32S RNA as authentic MHC. (A) Polyacrylamide gel electrophoresis of the products of translation in vitro. LC, light chain. The single ^{35}S-labeled polypeptide (lane 2) whose synthesis is directed by the 30–32S RNA fraction isolated by agarose/acrylamide gel electrophoresis (see Fig. 3 and text) migrates slightly more rapidly than authentic [^{3}H]MHC (lane 1) isolated from cells labeled in vivo. (B) Peptide mapping of in vivo-synthesized MHC and polypeptide whose synthesis is directed by 30–32S RNA. Peptide mapping is limited chymotryptic digestion performed by a modification of the procedure of Cleveland et al. (10). In vivo [methyl-^{3}H]methionine-labeled MHC and the in vitro [^{35}S]methionine-labeled polypeptide in question were mixed and isolated by low salt precipitation in the presence of ATP followed by polyacrylamide gel electrophoresis. Digestion with 500 μg of chymotrypsin was for 1 hr at room temperature. Similar results were obtained when the in vivo- and in vitro-labeled polypeptides were analyzed on separate gels (data not shown). The arrow in the figure represents the position of the intact MHC. ●---●, In vivo-labeled [methyl-^{3}H]methionine MHC isolated from well-fused L₆E₉ cells; O—O, [^{35}S]methionine-labeled polypeptide isolated from an in vitro translational reaction mixture containing 0.5 μg of RNA from the 30–32S RNA fraction recovered after agarose/acrylamide gel electrophoresis.

Cleveland et al. (10) demonstrated complete homology of the resultant peptides with those of authentic MHC (Fig. 4B). No MHC mRNA activity was observed in any other gel fraction. Although chicken MHC mRNA sediments at 26 S on sucrose density gradients, the same RNA has previously been observed to migrate at 32 S on nondenaturing polyacrylamide gels (20–22). The 30–32S RNA was used to prepare cDNA, and the cDNA was hybridized back to its template (Fig. 2B). The kinetics of hybridization demonstrate that the RNA is a single species that is >90% pure. By comparison with an α and β globin mRNA standard, it was determined that this RNA was composed of 5360 nucleotides, a number that is quite compatible with the 6000 residues expected for MHC mRNA. Furthermore, this RNA is muscle-specific (Fig. 5). When the cDNA was hybridized with total cytoplasmic RNA from hepatoma cells, a low level of hybridization was observed only at high inputs of nonmuscle RNA. This low level of hybridization could be due to hybridization of the cDNA with mRNA sequences for nonmuscle myosin. We thus conclude that this 30–32S RNA fraction is MHC mRNA that is >90% pure.

To confirm the size of MHC mRNA present in total cytoplasmic RNA, total cytoplasmic RNA from well-fused L₆E₉ cells was fractionated on polyacrylamide gels that did or did not permit the entrance of 32S rRNA (Fig. 6 A and B). RNA isolated from defined gel fractions were hybridized with the MHC cDNA probe described above. Greater than 95% of the total hybridizable mRNA sequences migrated at 30–32 S. The small percentage of sequences migrating more rapidly was probably due to degradation of MHC mRNA during the iso-
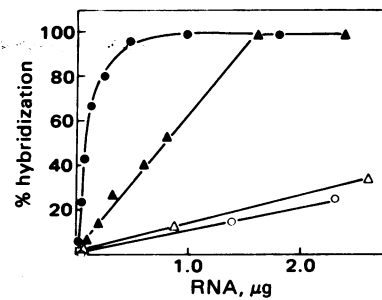

FIG. 5. Hybridization analysis of RNA from muscle and nonmuscle sources. Increasing amounts of RNA were hybridized with a constant amount of MHC cDNA in cDNA excess. The amount of double-stranded cDNA (cDNA in hybrid form with RNA) was determined by digestion of the reactions with S1 nuclease. Each reaction mixture contained 500–600 cpm of [^{3}H]cDNA. △, Total cytoplasmic RNA isolated from GH3 cultured rat cells; O, total cytoplasmic RNA isolated from HTC cultured hepatoma cells; ▲, total cytoplasmic RNA isolated from dividing L₆E₉ myoblasts; ●, total cytoplasmic RNA isolated from well-fused L₆E₉ myotubes.

lation of the RNA. These observations were confirmed by translation in vitro of the RNA isolated from the gel fractions. Only the 30–32 S RNA directed the synthesis of MHC. In addition, total cytoplasmic RNA that was fully denatured in formamide at 65°C (23) was fractionated by sucrose density gradient centrifugation and hybridized to the cDNA probe (Fig. 6C). The only significant hybridization occurred with RNA migrating at approximately 26 S. These studies demonstrate that all rat muscle-specific MHC mRNA migrates at 26 S on sucrose density gradients but migrates at 30–32 S on nondenaturing polyacrylamide gels.

MHC mRNA Is Induced during Myogenic Differentiation. In order to determine whether translational or pretranslational controls regulate MHC synthesis during myogenic differentiation, total cytoplasmic RNA from dividing myoblasts and from well-fused myotubes was analyzed by hybridization in solution to the MHC cDNA probe in cDNA excess (Table 2; Fig. 5). We observed that RNA from myotubes hybridized to the cDNA probe 83 times faster than RNA from dividing myoblasts. These results indicate that MHC mRNA accumulation
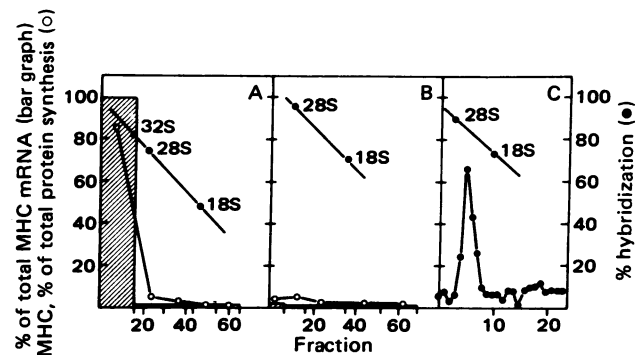

FIG. 6. Size determination of MHC mRNA from total cytoplasmic RNA from L₆E₉ myotubes. Total cytoplasmic RNA was fractionated on 0.32% agarose/2.4% acrylamide (A) or straight 3% acrylamide gels (B). Gels were cut into 1-mm slices with a Mickle gel slicer and the RNA was extracted. The RNA was pooled into discrete size fractions as shown in the figure. The position of MHC mRNA was determined by both hybridization with MHC cDNA (bar graph) and translation in vitro (O). (C) Total cytoplasmic RNA was denatured in 50% formamide at 65°C for 3 min, quick cooled, and layered over 3.8-ml 5–20% sucrose density gradients in ETS. The gradients were centrifuged at 58,000 rpm in a Spinco SW 60 rotor for 2.5 hr at 22°C and scanned at 260 nm, and fractions were collected. An aliquot from each fraction was immediately hybridized with 600 cpm of MHC [^{3}H]cDNA (●).

Table 1. Translation *in vitro* of total cytoplasmic RNA from L₆E₉ cells*

Source of RNA	Incorporation, cpm		MHC, % of total protein synthesis
	Total	MHC[†]	
Total RNA[‡]	106,455	15,968	15.0
Peak 1 (0.5 M NaCl wash)[§]	72,715	5,963	8.2
Peak 2 (0.1 M NaCl wash)[§]	73,055	10,593	14.5
Peak 3 (0.0 M NaCl wash)[§]	188,085	28,400	15.1

* RNA was isolated from well-fused L₆E₉ cells 6 days after the switch to differentiating medium. RNA was translated in the nuclease-treated reticulocyte cell-free system (2).

[†] It is apparent that the specific activity of MHC mRNA in each fraction (cpm incorporated into MHC per μg of RNA in the translation reaction) is different. The bulk of the RNA in total RNA and peak 1 represents nontranslatable rRNA sequences. We do not know at this time whether the lower specific activity of MHC mRNA in total RNA and peak 1 RNA as compared to that found in peak 2 and peak 3 RNA is due to true differences in translational efficiency or to the presence of high amounts of nontranslatable RNA.

[‡] Five micrograms of total RNA was used per 50-μl translational reaction.

[§] Oligo(dT)-cellulose chromatography was performed by the method of Aviv and Leder (6). After one round of oligo(dT)-cellulose chromatography, peaks 2 and 3 were combined and rechromatographed to remove any contaminating ribosomal RNA and poly(A)⁻ RNA. Peak 1 was also rechromatographed to be sure no sequences that could bind to oligo(dT)-cellulose were present. Ten micrograms of peak 1 RNA, 0.5 μg of peak 2 RNA, or 0.5 μg of peak 3 RNA was used per 50 μl translational reaction. Ribosomal and poly(A)⁻ RNA are found in peak 1. Poly(A)^{short} RNA is found in peak 2. Poly(A)^{long} RNA [poly(A)⁺ RNA] is found in peak 3 (6). We have determined that the mean length of the poly(A) tracts in peaks 1, 2, and 3 are 13 nucleotides, 31 nucleotides, and 230 nucleotides, respectively.

is induced during myogenic differentiation. However, there is a low but detectable level of MHC mRNA in dividing myoblasts. Although in our *in vitro* protein-synthesizing system we are able to efficiently translate MHC with mRNA from dividing myoblasts (ref. 2; unpublished data), *in vivo* most of this MHC mRNA is not located on polysomes and no MHC is accumulated by these cells (unpublished data). Therefore, these results suggest that controls at the level of cytoplasmic MHC mRNA accumulation as well as at the level of MHC mRNA translation regulate MHC synthesis in L₆E₉ cells.

The data presented here (Table 2, Fig. 5) indicate that MHC mRNA represents 0.4% of the total cytoplasmic RNA of the well-differentiated myotube. This corresponds to 47,500 molecules of MHC mRNA per myotube nucleus and is 5- to 10-fold higher than the estimates of MHC mRNA present in differentiated cultures of embryonic avian myotubes (21, 24, 25). This difference in cytoplasmic MHC mRNA levels could be species specific. It is, however, more likely due to the culture systems used. Primary cultures are contaminated with large amounts of fibroblasts, which do not synthesize MHC. Myotube preparations from our cell line are not contaminated with nonmuscle or undifferentiated cells (4), and therefore it is unlikely that we are overestimating MHC mRNA levels.

The Major Portion of Induced MHC mRNA Fails to Bind to Oligo(dT)-Cellulose. There have been conflicting reports as to whether or not MHC mRNA is polyadenylated (26, 27). For this reason MHC mRNA was initially isolated from poly(A)⁺ RNA. In the course of these experiments we observed significant translatable MHC mRNA activity in RNA isolated from well-fused L₆E₉ cells in both the poly(A)⁺ RNA fraction and the RNA that fails to bind to an oligo(dT)-cellulose column (Table 1). On the basis of the percentage of MHC mRNA in poly(A)⁺

Table 2. Compartmentalization of cytoplasmic MHC mRNA in fused myotubes and growing myoblasts

Source of RNA*	RNA fraction[†]	MHC mRNA, % of total cytoplasmic RNA[‡]	MHC mRNA molecules per cell[§]
Fused myotubes	Total	0.413	47,500
	Peak 1	0.369	42,440
	Peak 2	0.0100	1,150
	Peak 3	0.0395	4,540
Dividing myoblasts	Total	0.00678	290
	Peak 1	0.00102	40
	Peak 2	0.000797	30
	Peak 3	0.00439	190

* RNA was isolated from rapidly dividing myoblasts or from fused myotubes 6 days after transfer to differentiating medium.

[†] Total RNA was fractionated by two rounds of oligo(dT)-cellulose chromatography as described in the legend to Table 1.

[‡] The values given for the RNA fractions after oligo(dT)-cellulose chromatography have been normalized to reflect the percentage of total MHC mRNA present in a particular RNA fraction.

[§] Calculated by a modification of the formula of Ross *et al.* (18). The RNA content per 10⁶ cells was 37.95 and 14.19 μg in myotubes and myoblasts, respectively.

RNA (1.1%) and the percentage of total cytoplasmic RNA that binds to oligo(dT)-cellulose (4.2%), it was estimated that MHC mRNA represented only 0.0462% of the total cytoplasmic RNA from a well-fused L₆E₉ cell. However when the cDNA probe was hybridized with total cytoplasmic RNA, we observed that MHC mRNA represents 0.4% of the total cytoplasmic RNA of the well-differentiated myotube. This value is 10-fold higher than that obtained with poly(A⁺) RNA and suggested that a significant fraction of the total MHC sequence did not bind to oligo(dT)-cellulose.

In order to determine the distribution of MHC mRNA in well-fused L₆E₉ cells, the three RNA fractions from an oligo(dT)-cellulose column were each hybridized with MHC cDNA (Table 2). The data demonstrate that 89% of the total MHC mRNA sequences do not bind to oligo(dT)-cellulose. By nuclease digestion (28) we have determined that these MHC mRNA sequences contain poly(A) tracts of only 13 residues (data not shown). In contrast, only 16% of the MHC mRNA sequences present in dividing myoblasts fail to bind to oligo(dT)-cellulose and contain short poly(A) tracts. The majority of MHC mRNA sequences in dividing myoblasts contain a poly(A) tail of about 231 residues (data not shown). It appears, therefore, that the induction of MHC mRNA accumulation during myogenic differentiation is correlated with the accumulation of mRNA that fails to bind to oligo(dT)-cellulose. Similar results were obtained with RNA fractionated by poly(U)-Sepharose chromatography (data not shown).

Our data suggest that pretranslational controls are very important in the regulation of MHC synthesis during myogenic differentiation. Cytoplasmic MHC mRNA accumulation is induced 164-fold during the course of differentiation of the L₆E₉ myogenic cell line. More importantly, the most significant increase (1061-fold) in MHC mRNA sequences is observed in the fraction of the total cytoplasmic mRNA that contains only a short poly(A) tail and fails to bind to oligo(dT)-cellulose.

DISCUSSION

This paper presents the isolation and purification of a mammalian MHC mRNA. The purity and identity of the mRNA were established by several criteria. The mRNA displayed the migration characteristics attributed to MHC mRNA on sucrose density gradients and agarose/acrylamide gels. It directed the

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cell-free synthesis *in vitro* of a single protein with a peptide profile characteristic of authentic MHC. The extent and kinetics of hybridization of the mRNA to its cDNA indicates that the RNA is not contaminated with other sequences to any significant extent, and its complexity determined from the $R_0t_{1/2}$ corresponds to the expected complexity of MHC mRNA. Previous reports of MHC mRNA purification (16, 21, 27) show nucleotide complexities for the putative MHC mRNA that are too high and are incompatible with its gel migration characteristics, suggesting contamination with other muscle mRNAs. Recently Patrinou-Georgoulas and John (17) have isolated a MHC mRNA from embryonic chicken muscle with a nucleotide sequence complexity very similar to the rat MHC mRNA reported here.

Although controls at the level of translation cannot be excluded, it is clear from our results that pretranslational controls are important in the regulation of MHC synthesis and accumulation during myogenic differentiation. Cytoplasmic MHC mRNA levels increase ≈200-fold during the course of myogenic differentiation of L_6E_9 cells. Eighty-nine percent of this increase occurred in that RNA population that failed to bind to oligo(dT)-cellulose. In this fraction MHC mRNA concentration increased 1061-fold during the course of differentiation. This finding is in complete contrast to translational studies that reported that MHC mRNA has a long 3′ poly(A) tail (3,15–17, 19–22, 25, 27, 29–33). We have determined that the majority of MHC mRNA sequences in the well-fused myotube contain a poly(A) tract of about 13 residues, in contrast with the 230 residues of the average poly(A) tract found in the oligo(dT)-bound fraction. It is possible that different translational efficiencies of MHC mRNA in *in vitro* systems, depending on the length of the poly(A) as well as the stage of differentiation of the cells, could explain these conflicting results (2). In this regard, it should be noted that, on the basis of translational studies, a substantial fraction of the mRNA for actin (34, 35) and for myosin light chains (36) does not bind to oligo(dT)-cellulose. Together with our observations, these data might suggest that contractile protein synthesis is specifically directed by poly(A)⁻ or poly(A)short RNA populations. It has been reported that actin mRNA (34) and two of the early messages of adenovirus-2 (28) display a spectrum of poly(A) lengths, ranging from lengths that readily bind to oligo(dT)-cellulose down to lengths that fail to bind even to poly(U)-Sepharose (34). We suspect the same to be true of MHC mRNA. The reason for this large increase in MHC mRNA with a very short poly(A) tail during the course of myogenic differentiation of L_6E_9 cells is unknown. The half-life of MHC mRNA has never been measured, but it has been postulated that it is greatly increased during myogenic differentiation (15, 29). It is possible that all MHC mRNA initially contains a long poly(A) tail that is progressively shortened as the age of the MHC mRNA increases. This "aging" of poly(A) tails has previously been observed for globin mRNA (37, 38). Whatever the reason, we have discovered a specific increase in a differentiation-specific mRNA that fails to bind to oligo(dT)-cellulose or poly(U)-Sepharose. At this point we do not know whether the low levels of MHC mRNA detected in growing myoblasts are due to contamination with a small fraction of differentiated cells or whether the MHC mRNA is present in all cells. It is clear that a comprehensive study of the synthesis, processing, and stability of MHC mRNA in relation to the translational activity during the course of myogenic differentiation is required to resolve unequivocally the relationship of pretranslational and translational controls on MHC synthesis.

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