Low M_r RNAs of chicken muscle cells and their interaction with messenger and ribosomal RNAs

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Chicken muscle *Cytoplasmic RNA* *Low M_r RNA* *mRNA regulation*

1. INTRODUCTION

Control of gene expression at the post-transcriptional level has generated considerable attention [1]. Whether regulation of mRNA translation in eukaryotic cells is a significant factor in this process, has been tested by different systems [1,2].

We have revealed a low-M_r 4.4 S RNA in a ribonucleoprotein fraction capable of interfering with protein synthesis [3]. This low-M_r RNA inhibited translation of both capped and uncapped mRNAs and appeared to act at a stage early in the translation process [3]. This study was designed to determine whether this inhibitory RNA acts by interacting with complementary sequence in mRNA. The data obtained indicate that a low-M_r RNA comigrating with bulk tRNA hybridizes to both polysomal and free cytoplasmic mRNAs.

2. MATERIALS AND METHODS

2.1. *Growth and labeling of muscle cells*

Primary cultures of muscle cells were prepared from leg muscle of 10-day-old chick embryos [4]. Cells were plated at 5×10^5/plate in a 25 cm^2 flask (Corning), precoated with gelatin; 24 h after plating, RNA of these cells was labeled for 16 h with [5,6-^{3}H]uridine.

2.2. *Subcellular fractionation and isolation of RNA*

The cells were lysed by the method in [5]. Following the removal of nuclei and mitochondria by centrifugation at 20 000 × *g* for 20 min, the post-polysomal supernatant was obtained by centrifugation at 100 000 × *g* for 1 h. The post-polysomal supernatant was further centrifuged in a 75 Ti rotor at 40 000 rev./min for 16 h to pellet post-polysomal ribonucleoprotein complexes [3]. To isolate RNA, the pellet was suspended in 10 mM sodium-acetate, 100 mM NaCl, 1% SDS (pH 5.0) buffer to give 5 A_{260} unit/ml and extracted with a mixture of phenol:chloroform (1:1) as in [3].

2.3. *Polyacrylamide gel electrophoresis for separation of low-M_r RNA*

Low-M_r RNAs were separated by electrophoresis on a 10–15% exponential gradient slab gel 9 cm long and 0.75 cm thick using a modification of the method in [6].

2.4. *Hybridization of low-M_r RNAs to RNAs covalently linked to diazobenzyloxymethyl(DBM) cellulose*

Various species of RNA (5 mg) were covalently linked to DBM-cellulose paper prepared as in [7,8]. In all cases > 75% of the RNA applied was covalently bound. Following pre-hybridization of the DBM–RNA filters, 10^6–10^7 cpm ^{3}H-labeled RNAs suspended in 50 μl solution were layered on the filters. Pre-hybridization and hybridization were carried out by minor modification of the methods in [6,8]. Filters were incubated at 43°C for 68 h and subsequently washed with the same hybridization solution. Washing of filters was accomplished by shaking at 43°C until the radioactivity present in the washings dropped to background levels. The hybridized RNA was eluted at 65°C with a solution containing 90% deionized formamide,

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1 mM EDTA and 0.1% SDS. The eluted RNA was precipitated by adjusting the solution to 0.2 M potassium-acetate (pH 5.5) and adding 2.5 vol. ethanol.

3. RESULTS

3.1. *Low-M_r cytoplasmic RNAs of muscle cells*

As a first step to examining the ability of low-M_r cytoplasmic RNAs to hybridize to mRNAs the low-M_r RNAs were separated by polyacrylamide gel electrophoresis. These RNAs were isolated from the non-polysomal cytoplasmic fraction [3,9]. The electropherogram (fig.1) indicates that in addition to the major species tRNA, 5 S and 5.8 S rRNA, at least 8 low-M_r RNA species were present in the post-polysomal cytoplasmic fraction. The 5 S and 5.8 S rRNAs in this fraction are probably derived from the 40 S and 60 S ribosomal subunits.

3.2. *Hybridization of low-M_r RNAs to cytoplasmic RNAs*

To determine whether low-M_r RNAs interact directly with various species of cytoplasmic RNA, labeled RNAs were hybridized to DBM-cellulose-bound RNA. Hybridization analyses presented in the autofluorograms (fig.2A) show that no cytoplasmic low-M_r RNA hybridized to filters loaded with 5 S and 5.8 S rRNA (slot g), poly(A) (slot h), or with control filter (slot i). In contrast low-M_r RNA, species B, C and D hybridized to filters carrying 5–10 S mRNAs (slot b), 10–20 S mRNAs (slot c), 18 S chick muscle rRNA (slot d), 28 S chick muscle rRNA (slot e) and 16 S and 23 S *E. coli* rRNA (slot f). Several of the low-M_r RNA species hybridized to 28 S, 18 S eukaryotic mRNAs and 16 S, 23 S prokaryotic rRNAs. However, a strongly hybridized 4 S species did not hybridize to 16 S and 23 S *E. coli* rRNA but hybridized to mRNAs and eukaryotic 28 S and 18 S rRNAs. These observations suggest a degree of specificity of this reaction. Furthermore, the inability of poly(A) to hybridize with low-M_r RNAs implies that the observed hybridization to mRNAs was not mediated via the poly(A) track. The ability of both eukaryotic and prokaryotic rRNAs and of eukaryotic mRNAs to hybridize with the same species of low-M_r cytoplasmic RNAs suggests some common features among these various RNAs [10,11]. Also possible is that low-M_r RNAs hybridize to different complementary regions within the various RNAs used.

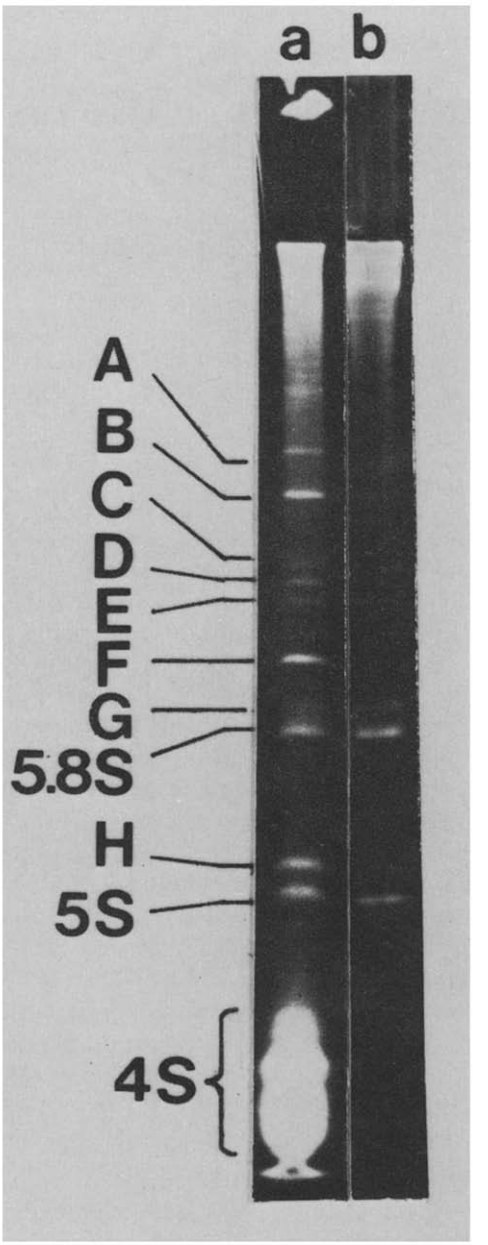

Fig.1. Analysis of low-M_r RNA by polyacrylamide gel electrophoresis: ethidium bromide stained RNA bands were photographed under UV light; (a) RNA from ≥ 10 S pellet of post-polyribosomal fraction (15 μg); (b) polysomal RNA (15 μg).

Hybridization of 4 S RNA to the 18 S and 28 S

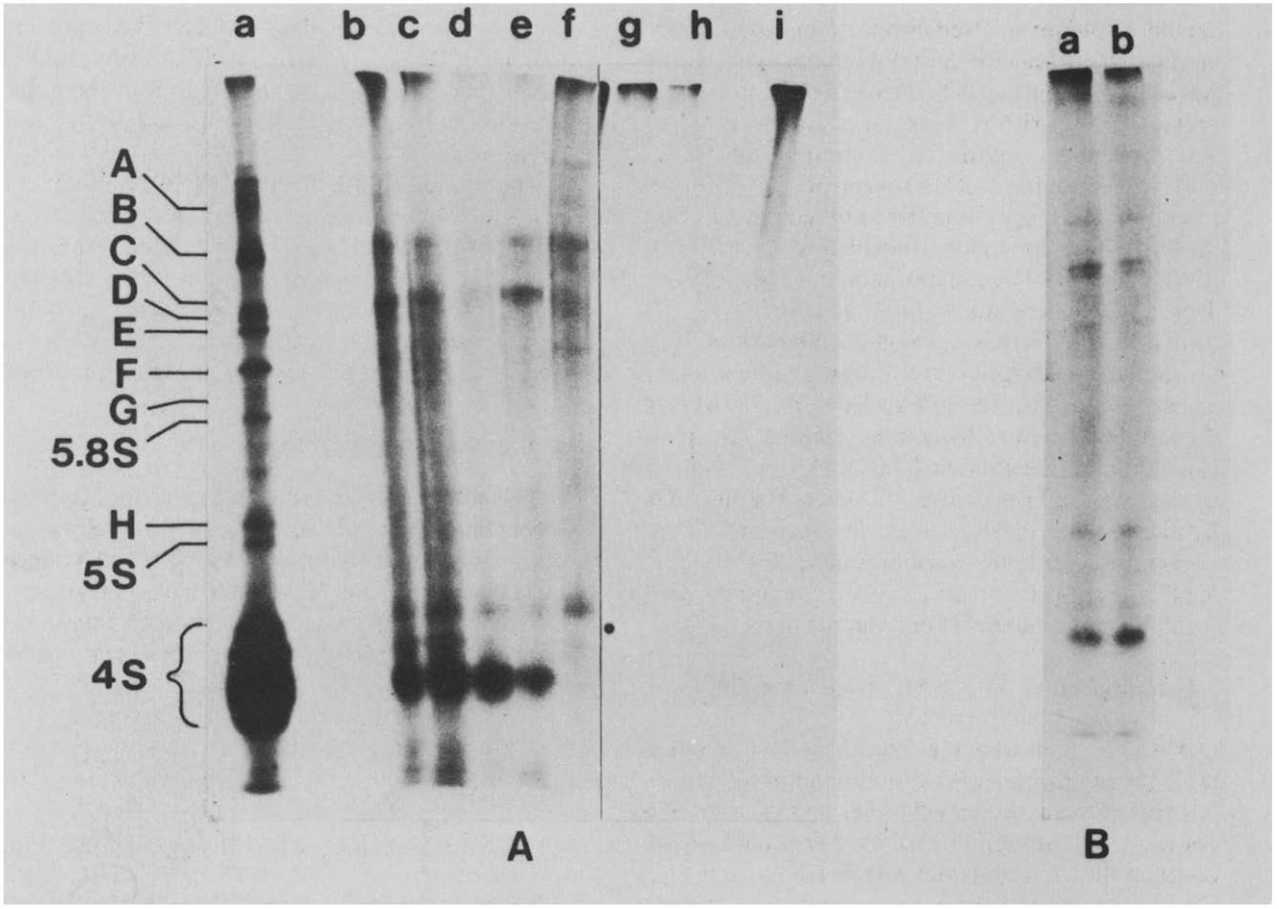

Fig.2. Analysis of low-M_r RNAs hybridized to muscle cytoplasmic mRNA and rRNAs: The ^{3}H-labeled low-M_r RNAs hybridized to different populations of RNAs covalently attached to DBM paper were eluted. The eluted RNAs were electrophoresed in 10–15% polyacrylamide gel as in [6]. Autofluorograph was developed after exposure of dried gels with Kodak X-Omat R film. (A) Hybridization of 1.5×10^7 cpm low-M_r cytoplasmic RNA: (a) RNA from $\geq$ 10 S post-polysomal pellet before hybridization, 250 000 cpm; RNA samples eluted from filters containing; (b) 5–10 S polysomal mRNA, 30 000 cpm; (c) 10–20 S polysomal mRNA, 42 000 cpm; (d) chick myoblast 18 S rRNA, 25 000 cpm; (e) chick myoblast 28 S rRNA, 22 000 cpm; (f) *E. coli* 16 S + 23 S rRNA, 7000 cpm; (g) 5 S + 5.8 S chick myoblast RNA, 2700 cpm; (h) poly(A), 2000 cpm; (i) minus RNA, 2500 cpm; autofluorographs were developed after 7 days exposure. (B) Hybridization of low-M_r cytoplasmic RNA to polysomal and free mRNA: 3×10^6 cpm ^{3}H-labeled post-polysomal cytoplasmic RNA was used for hybridization to DBM-RNA filters. Eluted RNA samples from filters containing: (a) polysomal mRNA, 6000 cpm; (b) post-polysomal (free) mRNA, 4000 cpm. Autofluorographs were developed after 15 days exposure. Polysomal, post-polysomal mRNA was isolated as in [9], 5–10 S and 10–20 S mRNA was isolated by fractionation of total polysomal mRNA in a 5–20% sucrose gradient in 10 mM sodium-acetate, 100 mM NaCl (pH 5.0) buffer [3,9]. rRNAs (18 S, 28 S, 5 S, 5.8 S) were obtained by centrifugation of the flow-through RNA from oligo(dT)-cellulose column in a similar gradient [3,9].

rRNA fraction may result from the presence of non-poly(A)-containing mRNAs contaminating the RNA preparation. This possibility seems unlikely, however, in view of the methods used to purify rRNA. rRNAs were isolated by oligo(dT)-cellulose chromatography following removal of the poly(A)-containing mRNAs from total polysomal RNA. The flow-through RNA from an oligo(dT)-

cellulose column was separated by sucrose density gradient centrifugation and only the peak fractions containing 18 S and 28 S rRNAs [9] were used to prepare DBM–rRNA filters. It is doubtful, therefore, whether significant amounts of non-poly(A)-containing mRNAs were present in these fractions. Conversely, it may be argued that hybridization of low-M_r cytoplasmic RNAs to mRNAs resulted from rRNA contamination of the mRNAs. This possibility is unlikely for 2 reasons:

(i) The 5–10 S mRNA fraction which lacks 18 S and 28 S rRNA hybridized to the same low-M_r RNAs (slot b); in contrast, low-M_r RNAs did not hybridize to 5 S and 5.8 S rRNA;

(ii) The poly(A)-containing mRNAs were isolated by oligo(dT)-cellulose chromatography and the poly(A) mRNAs were further purified by sucrose gradient centrifugation; RNA sedimenting in a broad 10–20 S region of the gradient was used for our studies.

3.3. *Hybridization of low-*M_r *cytoplasmic RNAs to polysomal and free mRNA*

A 4.5 S cytoplasmic low-M_r RNA was obtained in [12] by melting poly(A)-containing mRNAs from the non-polysomal (free) cytoplasmic fraction, but not from the polysomal mRNA. Whether low-M_r cytoplasmic RNAs interact with mRNAs from the free cytoplasmic fraction in a manner different from that observed with polysomal mRNAs was, therefore, examined. The low-M_r cytoplasmic RNAs which hybridized to polysomal and free mRNAs are illustrated in the autofluorogram (fig. 2B). These results indicate that both polysomal and free mRNAs bind the same low-M_r RNAs (slot a,b). However, in vivo the proteins associated with low-M_r cytoplasmic RNAs may play a role in regulating their interaction with polysomal and free mRNAs.

4. DISCUSSION

An RNA similar in size to the major 4 S cytoplasmic low-M_r RNA species which hybridizes to mRNA and rRNA was observed to inhibit protein syntehsis by interfering with an early event in translation [3]. Whether this RNA inhibits protein synthesis by interacting with mRNA and/or rRNA is as yet unresolved. Low-M_r RNA of similar size and hybridization properties have been reported in the cytoplasm and nucleus of CHO cells [12]. The low-M_r RNAs may be associated with the free mRNP complexes [13,14] and play a role in regulating the entry of the free mRNP complexes into polysomes.

The precise nature of this 4 S RNA and its relation to tRNA is unclear. That a 4 S RNA different from tRNAs was able to inhibit mRNA translation [3,14–16] argues against this possibility. Further characterization of this 4 S RNA with complementary base sequence to mRNAs and rRNAs is necessary for complete resolution of this question.

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