Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 3087–3091, May 1982
Biochemistry

Quantitation of muscle-specific mRNAs by using cDNA probes during chicken embryonic muscle development *in ovo*

(myogenesis/abundant-class muscle-specific cDNA probe/subcellular distribution of mRNAs/mRNA sequence complexity)

CHANDRIKA R. SAIDAPET*, H. N. MUNRO†, K. VALGEIRSDÓTTIR†, AND SATYAPRIYA SARKAR*‡

*Department of Muscle Research, Boston Biomedical Research Institute, Boston, Massachusetts 02114; †Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts and U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts 02111; and ‡Department of Neurology, Harvard Medical School, Boston, Massachusetts 02114

Contributed by H. N. Munro, January 18, 1982

ABSTRACT **The emergence of abundant-class mRNAs specific for contractile muscle proteins and their distribution between polysomal and free mRNP fractions were studied in skeletal muscle excised from chicken embryos during the transition from myoblasts (day 9) to myotubes (day 18). Muscle-specific cDNA was selectively prepared by hybridizing cDNA to template RNA (polysomal poly(A)$^+$mRNA) from day-14 embryos followed by isolation of the abundant class, which represents ≈20% of total mRNA. The specificity of the cDNA probe for this class was confirmed by the differential degree of hybridization to cytoplasmic RNA from cultured myotube and myoblast cells and by its inability to hybridize with mRNA from nonmuscle cells such as liver. Except for muscle from day-9 embryos, the concentrations of the abundant-class muscle-specific mRNAs were higher in polysomes than in free mRNP fractions. Furthermore, the levels of these mRNAs in polysomes increased 12-fold from day 9 (myoblast) to day 14 (intermediate) with a further 3.6-fold increase from day 14 to day 18 (myotube). In contrast to this 45-fold net increase in the polysomal level of these mRNAs from day 9 to day 18, the levels in the free mRNP fraction showed only a 3-fold decrease during this period. Because the amount of mRNA lost from the mRNP fraction is much less than the net increase in the polysome fraction, mRNP does not serve as a reservoir of untranslated muscle-specific mRNA for transfer to polysomes. Consequently, the emergence of muscle-specific polysomal mRNA for contractile proteins during myogenesis *in ovo* appears to be regulated primarily by transcriptional control.**

In embryonic muscle, the mononucleated myoblast cells replicate for two or three cycles to become postmitotic mononucleated myoblasts, which then fuse and form multinucleated myotubes. After fusion there is a several-hundred-fold increase in synthesis of muscle-specific contractile (myofibrillar) proteins. At issue is whether this change in pattern of protein synthesis during myogenesis is regulated at the transcriptional or translational level. Much of the evidence relates to muscle that differentiates in tissue culture. *In vitro* translation shows that the mRNAs coding for the myofibrillar proteins in cultures of quail breast muscle are present in negligible amounts in the dividing myoblasts and accumulate coordinately after fusion, suggesting transcriptional regulation (1). A similar conclusion was based on studies of the abundance of mRNA for myosin heavy chain in myoblasts and myotubes in chicken embryonic muscle culture that use either translation of mRNA (2) or *in situ* hybridization with a cDNA probe (3). Other studies show that the transition from the myoblast to myotube stage in primary cultures of chicken embryonic muscle (4) and rat skeletal muscle (5) is associated with the appearance of a new abundant-class population of mRNA consisting of at least six different mRNA sequences—the increased synthesis of myofibrillar proteins at or after fusion being coincident with the appearance of this new class of mRNAs (4). On the other hand, several reports reviewed elsewhere (6) suggest that myogenesis in cultured cells is regulated by translational control of dormant message. Thus, translational control is suggested by (*i*) the presence of myosin heavy chain mRNA as a stored translationally repressed free mRNP particle prior to fusion and the subsequent transfer of this mRNA to the polysomes at or after fusion (7, 8), (*ii*) the ability of cultured rat muscle cells treated with actinomycin D to synthesize muscle-specific proteins (9, 10), and (*iii*) differences in the stability of mRNAs at myoblast and myotube stages (11, 12).

Apart from the contradictory nature of this evidence, studies on cultured muscle cells may not reflect *in vivo* myogenesis (13), which is influenced by physiological factors such as innervation, activity, and hormones absent from muscle cell cultures. Therefore, we have used abundant-class muscle-specific cDNA probes to quantitate muscle-specific mRNAs and their distribution patterns between polysomal and free mRNP fractions during chicken skeletal muscle development *in ovo*. There was an extensive increase in muscle-specific mRNAs in polysomes concomitant with development into myotubes, whereas during differentiation the small amount of muscle-specific mRNAs observed in the free mRNP fraction showed only a slight decrease that was insufficient to account for this accumulation of polysomal muscle-specific mRNAs.

MATERIALS AND METHODS

Leg and breast muscle excised at different stages of chicken embryogenesis was homogenized as described (14) with 10 mM vanadyl ribonucleoside complex (15) to inhibit RNase activity, and the postmitochondrial supernatant was separated into polysomal and postpolysomal fractions (14, 16). Poly(A)$^+$RNA was isolated by oligo(dT)-cellulose chromatography of the RNA extracted from each fraction (17). The RNA samples were analyzed electrophoretically in 1% agarose slab gels containing 2.2 M formaldehyde (18). As described (16, 19), mRNAs were translated in micrococcal nuclease-treated reticulocyte lysate (20) and specific translation products (19) were separated by $NaDodSO_4$/polyacrylamide gel electrophoresis with 7–15% gradient slab gels, followed by identification of the labeled proteins by fluorography (21).

Reaction mixtures (50-μl volume) for the synthesis of cDNA (22) contained 40 mM Tris·HCl (pH 8.3), 5 mM dithiothreitol, 6 mM $MgCl_2$, 100 mM KCl, actinomycin D at 50 μg/ml, dTTP, dGTP, and dATP at 50 μM each, 50 μM [^{3}H]dCTP (specific activity, 15–30 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels; New England Nuclear) or 50 μM [α-^{32}P]dCTP (specific activity, 400 Ci/mmol; New England Nuclear), polysomal poly(A)$^+$RNA at

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

40 μg/ml, oligo(dT)$_{12-18}$ at 80 μg/ml, and AMV reverse transcriptase at 80 units/ml (gift of J. W. Beard, Life Sciences, St. Petersburg, FL). After incubation at 42°C for 30 min, 5-μl samples were treated with Cl_3CCOOH at 4°C and the radioactivity of the insoluble material was measured by liquid scintillation counting (23). After extraction with phenol, the extract was passed through a Sephadex G-50 column. The cDNA eluted in the void volume was precipitated with ethanol by using *Escherichia coli* tRNA as carrier and the cDNA had a specific activity of $\approx 10^7$ cpm/μg. When sized by alkaline agarose slab gel electrophoresis followed by autoradiography that uses a *Hin*dIII digest of λ phage DNA as markers (New England BioLabs), the cDNA was in the range of 500 to 750 nucleotides.

Abundant-class muscle-specific cDNA (4, 5) was isolated by using a large batch of cDNA made against polysomal poly(A)$^+$RNA from day-14 chicken embryos that was back-hybridized to its template RNA to a R_0t value of 1.0. The reaction mixture was then digested with S_1 nuclease (Miles) in the presence of denatured *E. coli* DNA (50 μg/ml) for 60 min at 37°C, boiled for 5 min in 0.5 M NaOH, and then neutralized with glacial acetic acid. When passed through a Sephadex G-50/Chelex 100 column (24), ≈10–12% of the cDNA eluted in the void volume. This was rehybridized to day-14 polysomal poly(A)$^+$RNA to a R_0t value of 1.0 and the procedure was repeated. After the second recycling, recovery was ≈60–70%. This cDNA was isolated by ethanol precipitation for hybridization studies.

RNA·cDNA hybridizations in the presence of excess RNA were performed at 44°C in siliconized capillaries in 10-μl reaction mixtures that contained 20 mM 1,4-piperazinediethanesulfonic acid (pH 6.8), 50% formamide, 1 M NaCl, 2 mM EDTA, 0.2% $NaDodSO_4$, 2,000 cpm of total cDNA or 1,000 cpm of abundant-class cDNA, and amounts of RNA as indicated in the figure legends. The completed reactions were then treated with S_1 nuclease (25) for estimation of the percentage of cDNA in duplex form. The hybridization data were analyzed by using the computer program of Pearson *et al.* (26). Hybridization of a mixture of α- and β-globin mRNAs with the homologous cDNA under identical conditions was performed as a kinetic standard. Samples of the total and poly(A)$^+$RNA used in this study were hybridized with poly([^{3}H]U) to measure their poly(A) content (27).

Myoblast and myotube cells that were harvested after 24 hr and 5 days, respectively, from cultures of 10-day chicken embryonic breast muscle were kindly donated by Howard Holtzer (University of Pennsylvania).

RESULTS AND DISCUSSION

To study the appearance of muscle-specific mRNAs during myogenesis in the chicken *in ovo*, three representative stages of muscle differentiation were chosen—namely, day 9 (early), day 14 (intermediate), and day 18 (late), representing replicative myoblasts, postmitotic myoblasts, and fused myotubes, respectively (28, 29). First, products of *in vitro* translation directed by total muscle RNA at these stages of development were analyzed by fluorography by using samples of purified chicken myofibrillar proteins as markers (Fig. 1). The translation products of 14-day and 18-day chicken muscle mRNAs contained major bands, some corresponding to muscle-specific marker proteins (Fig. 1 *B* and *C*). In contrast, the translation products of mRNA from 9-day chicken embryos gave a different band pattern with a major protein at M_r 42,000 and a number of minor bands in the M_r 16,000–200,000 range (Fig. 1*A*). The difference in abundance of muscle-specific translation products in 9-day muscle preparations could be due to low levels of muscle-specific mRNAs or to poor translatability.

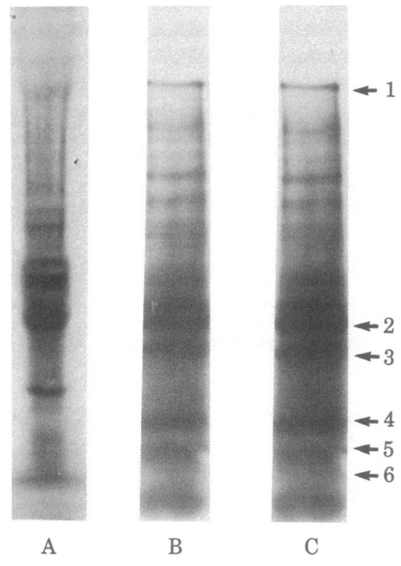

FIG. 1. Fluorography of [^{35}S]methionine-labeled cell-free products synthesized in a mRNA-dependent rabbit reticulocyte lysate in response to 20 μg of total RNA extracted from 9-, 14-, and 18-day chicken embryonic skeletal muscle and resolved on 7–15% gradient $NaDodSO_4$/polyacrylamide slab gels. Lane A, 9-day RNA; lane B, 14-day RNA; lane C, 18-day RNA. Arrows indicate the positions of nonradioactive myofibrillar proteins that were run as markers on the same gel and stained with Coomassie blue: 1, myosin heavy chain (M_r 200,000); 2, actin (M_r 42,000); 3, tropomyosin (M_r 35,000); 4, myosin light chain LC_1 (M_r 24,000); 5, myosin light chain LC_2 (M_r 19,000); 6, myosin light chain LC_3 (M_r 16,000).

Quantitation of mRNA as a function of development was carried out by using abundant-class muscle-specific cDNA probes because RNA·cDNA hybridization provides more precise estimates than those based on *in vitro* translation. Because 14-day chicken muscle mRNA is essentially similar in muscle-specific messages to mRNA from 18-day muscle (Fig. 1), polysomal poly(A)$^+$RNA of 14-day chicken embryonic muscle was selected for preparation of the cDNA probe as this was easier to excise and fractionate. To correct the R_0t values for interpretation of the hybridization data, the amount of RNA binding to poly([^{3}H]U) was determined and expressed as the percentage of poly(A)$^+$RNA that can be accounted for on the basis that 100 of the 2,000 nucleotides (5%) in the average chicken poly(A)$^+$RNA are contributed by the polyadenylylated sequence (30–32). Approximately 82–84% of the polysomal poly(A)$^+$RNA isolated from 9-, 14-, and 18-day muscle and liver could be accounted for as RNA with poly(A) sequences of this mean size (data not shown). The poly(A)$^+$RNA content of cytoplasmic nonpolysomal RNA and total RNA from cultured myoblasts and myotubes gave values that ranged, as expected, from 1 to 3%.

When cDNA prepared from 14-day chicken polysomal poly(A)$^+$RNA was back-hybridized to its template RNA, ≈80% of the cDNA formed hybrids at saturation (Fig. 2). Hybridization occurred over a range of R_0t values of almost five orders of magnitude, indicating considerable heterogeneity of the mRNA population. Least squares fit of the cDNA hybridization data to the standard pseudo-first-order hybridization rate equation is consistent with three components or concentration classes of RNAs—as shown by the transitions in the curve (Fig. 2) that is a computer fit (26) to the actual data points—based on the assumption that the data can be represented by the sum of a small number of ideal first-order reactions. The sequence complexities of the three classes of mRNAs indicated by the transitions in Fig. 2 are analyzed further in Table 1. This shows

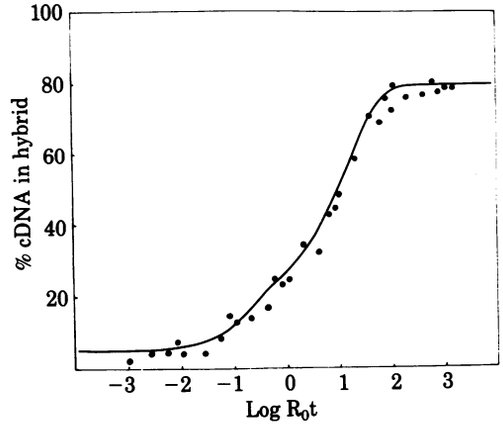

FIG. 2. Hybridization of 14-day polysomal poly(A)$^+$RNA to its homologous cDNA transcript. The concentration of mRNA used in the hybridization reaction varied from 4 to 200 μg/ml. The percentage of cDNA resistant to nuclease digestion in controls lacking the mRNA (1–3%) was subtracted from all values. The solid line is the computer-calculated pseudo-first-order curve (26) that gives the best least squares fit to the data. The parameters from which this curve is derived are listed in Table 1.

that, at day 14, polysomal poly(A)$^+$RNA in chicken muscle is comprised of ≈12,000 unique average-sized RNA species. Approximately 23% of the cDNA is complementary to a small group of 32 abundant RNA species present at about 400 copies per cell. Another 35% of the cDNA is complementary to a larger group of 1,500 RNA species present at an intermediate concentration of 12 copies per cell; the remainder of the cDNA (42%) is complementary to a large class containing 10,000 different RNA species averaging 2 copies per cell. These results agree reasonably well with reported values for the three classes of poly(A)$^+$RNA populations in myoblast and myotube stages of cultured chicken muscle cells (4) and in total RNA from 9-day embryonic chicken muscle (33).

When cDNA prepared against day-14 polysomal poly(A)$^+$RNA of chicken muscle was hybridized to chicken liver polysomal poly(A)$^+$RNA the sequences that appeared to be common to liver and muscle did not fall in this abundant frequency class (Fig. 3*A*). Therefore, the muscle-specific abundant-class cDNA was isolated by hybridizing cDNA to day-14 polysomal poly(A)$^+$RNA to a low R_0t value of 1.0, followed by treatment with S_1 nuclease. This isolated abundant-class cDNA did not hybridize to liver poly(A)$^+$RNA to a significant extent at saturation (Fig. 3*A*), indicating that it was indeed muscle-specific. The specificity of the abundant-class cDNA was further checked by its hybridization to total cytoplasmic RNA isolated from chicken muscle cells cultured for 24 hr (myoblast) and for 5 days (myotube). Even when corrected for poly(A)$^+$RNA content, <20% of the myoblast RNA hybridized to the abundant-class muscle-specific cDNA even at high R_0t values (Fig. 3*B*), whereas hybridization with myotube RNA progressed to ≈75–80% [corrected for poly(A)$^+$RNA content] and was thus similar to that obtained with the template polysomal poly(A)$^+$RNA (Fig. 2). Polyacrylamide gel electrophoresis of the myoblast and myotube RNA samples showed that their rRNA contents were similar (results not shown) and hybridization to poly([^{3}H]U) indicated the same proportion of the poly(A)$^+$RNA in these two RNA samples. Consequently, the difference in hybridization of the two RNA samples with the cDNA probe (Fig. 3*B*) reflects a change in the contents of the abundant-class mRNAs at these two stages in culture.

The abundant-class muscle-specific cDNA probe was then used to study the distribution patterns of mRNA sequences in the polysomal and free mRNP fractions of 9-, 14-, and 18-day chicken embryonic muscle. The hybridization reactions were driven to saturation with RNA samples from both polysomal and mRNP fractions (Fig. 4). The concentration of abundant-class muscle-specific mRNA sequences was higher in the polysomes than in the mRNP fractions on day 14 and day 18, whereas on day 9 the proportion of muscle-specific mRNA sequences was higher in the mRNP fraction than in the polysomes (Fig. 4 and Table 2). The progressive increase in the concentration of muscle-specific mRNAs in polysomes coincident with development represented a 12-fold increment between days 9 and 14, followed by a further 3.6-fold increase from day 14 to day 18. In contrast, there was a progressive decrease in the concentration of muscle-specific mRNAs in the mRNP fractions from day 9 through day 18. This decrease was only 3-fold, with the R_0t values ranging from 0.40 at day 9 to 1.12 at day 18.

These studies represent an *in vivo* attempt to dissect the relative contributions of translational and transcriptional controls during muscle myogenesis. Though they do not agree with all observations made on developing muscle cells in tissue culture, it should be stressed that myogenesis in cultured muscle cells may not mimic that in intact muscle—primarily because of the heterogeneity of the cell populations *in vivo* and also because of hormonal and other physiological factors in the intact organism. This has been emphasized by a recent report showing that the protein and mRNA patterns in cultured cardiocytes differ from those of embryonic heart (13).

Because the observed developmental changes in the mRNA

Table 1. Complexity analysis of poly(A)$^+$RNA of 14-day chicken embryonic muscle

Abundance class	Fraction of hybridizable cDNA	Apparent kinetic constant,* k_{obs}	$R_0t_{1/2}$ Observed†	$R_0t_{1/2}$ If pure‡	Base sequence complexity,§ $N_t \times 10^{-6}$	mRNA species,¶ no.	Copies per cell,‖ no.
Abundant	0.17	4.6	0.15	0.02	0.07	32	396
Intermediate	0.25	0.16	4.47	0.94	3.05	1,525	12
Rare	0.30	0.03	24.75	6.25	20.31	10,155	2

* k_{obs} is the rate constant of the reaction and is equal to ln $2/R_0t_{1/2}$ for each component.

† Observed $R_0t_{1/2}$ is the $R_0t_{1/2}$ value at which half-maximal hybridization is reached for each component.

‡ $R_0t_{1/2}$, if pure, means the half-maximal R_0t value corrected for the percentage of cDNA reacting in each component and multiplied by the fraction of RNA that is polyadenylylated (0.83).

§ The base sequence complexity (N_t) in nucleotides is given by [$R_0t_{1/2}$ (if pure) for each component]/($R_0t_{1/2}$ for α- and β-globin mRNA) × 1,300, in which 1,300 is the combined nucleotide lengths of chicken α- and β-globin mRNA. Under the hybridization conditions used, $R_0t_{1/2}$ for globin is 4×10^{-4} (mol/liter)sec.

¶ The number of RNA species is calculated assuming an average size of 2,000 nucleotides (30, 32) for chicken poly(A)$^+$RNA.

‖ The number of copies of each sequence per cell (33) is given by {[poly(A)$^+$RNA content/cell (μg)]$(6 \times 10^{23})\alpha$}/$(330 \times 10^6)N_t$, in which α is the fraction of hybridizable cDNA.

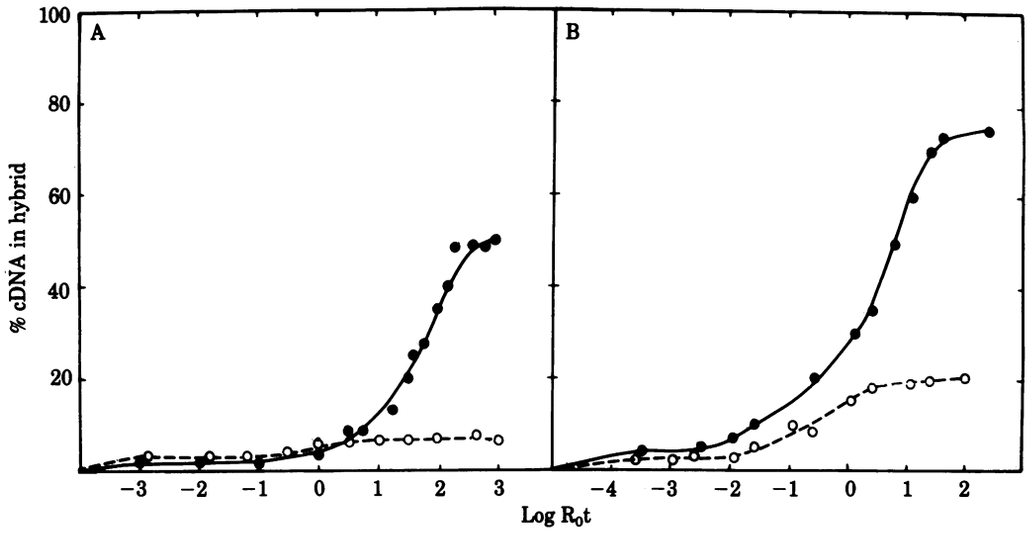

FIG. 3. (*A*) Hybridization of cDNA enriched for abundant-class muscle-specific sequences (○) and unfractionated cDNA made against 14-day polysomal poly(A)$^+$RNA (●) to liver polysomal poly(A)$^+$RNA. The concentration of RNA used in the hybridization reaction varied from 3 to 400 μg/ml. The R_0t values have been corrected to account for the fact that 84% of the liver polysomal poly(A)$^+$RNA was polyadenylylated. (*B*) Hybridization of cDNA enriched for abundant-class muscle-specific sequences to cytoplasmic RNA extracted from cultured myoblast (○) and myotube (●) cells. The concentration of RNA used in the hybridization reaction varied from 100 μg/ml to 2 mg/ml. The curves have been adjusted on the *x* axis to account for the fact that 2.28% of myoblast RNA and 2.46% of the myotube RNA were polyadenylylated.

content of the two cytoplasmic fractions were not strictly reciprocal, our results suggest that the bulk of the muscle-specific mRNAs do not remain dormant in a large untranslated pool in the form of free mRNP particles. Therefore, it is concluded that myogenesis *in ovo* is primarily regulated by transcriptional control. The possibility of some translational control involving certain individual muscle-specific mRNAs as an additional subtle regulatory mechanism during myogenesis has been suggested in the literature and is not excluded by our data. Thus, it has been proposed (7, 8) that myosin heavy chain mRNA is transcribed and stored as a translationally repressed mRNP prior to fusion, at which time it is transferred to the polysomes. Other workers (11, 12) have reported that the stability of cellular mRNAs, including a 26S putative myosin heavy chain mRNA, is considerably increased during terminal differentiation in culture. Recent work from this laboratory (34) has shown that the mRNA for myosin light chain LC_3 is present in 18- and 19-day chicken embryonic muscle in considerable amounts, whereas the LC_3 protein is barely detectable at this stage of development, suggesting a major role for translational control involving LC_3 mRNA. If the level of some of the myofibrillar proteins is regulated by a combination of both transcriptional and translational controls, the situation is reminiscent of that observed during the transition from a pluripotent mouse embryonic carcinoma cell line to a committed mouse teratocarcinoma myoblast cell and finally to the fused myotube (30). The levels of both polysomal mRNAs and nuclear RNAs containing mRNA sequences during these transitions have been shown to be con-

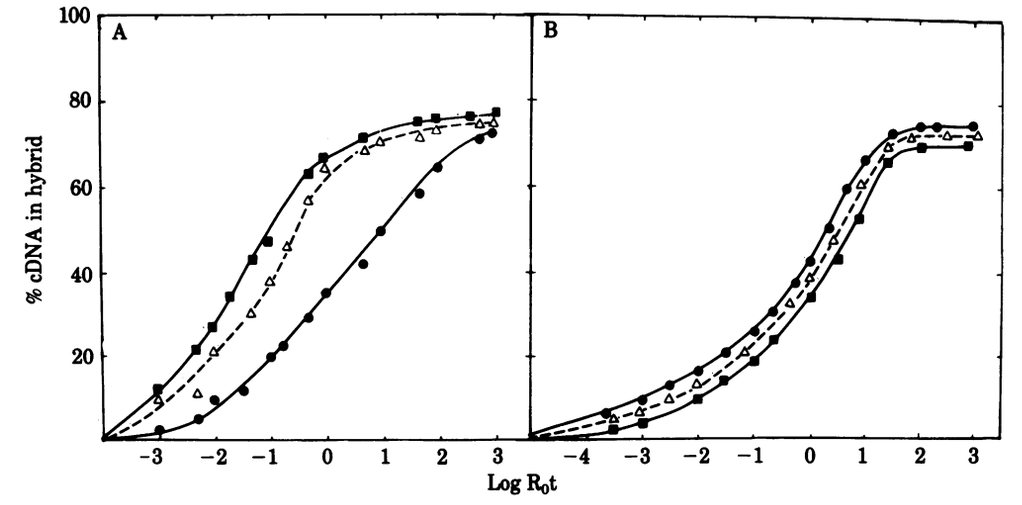

FIG. 4. (*A*) Hybridization of cDNA enriched for abundant-class muscle-specific sequences to chicken embryonic skeletal muscle polysomal poly(A)$^+$RNA. ●, Nine-day; △, 14-day; ■, 18-day polysomal poly(A)$^+$RNA. The concentration of RNA used in the hybridization reaction varied from 2 to 200 μg/ml. The R_0t values have been corrected to account for the fact that ≈83% of the various RNA samples were polyadenylylated. (*B*) Hybridization of cDNA enriched for abundant-class muscle-specific sequences to cytoplasmic nonpolysomal mRNP RNA from chicken embryonic skeletal muscle. ●, Nine-day; △, 14-day; ■, 18-day cytoplasmic mRNP RNA. The concentration of RNA used in the hybridization reaction varied from 200 μg/ml to 3 mg/ml. The curves have been adjusted on the *x* axis to account for the fact that 3.34% of the 9-day RNA, 2.18% of the 14-day RNA, and 0.89% of the 18-day RNA were polyadenylylated.

Table 2. Analysis of hybridization data involving abundant-class cDNA and various subcellular RNAs

Stage of development	Polysomal fraction		Cytoplasmic nonpolysomal fraction	
	$R_0t_{1/2}$*	Relative amounts†	$R_0t_{1/2}$*	Relative amounts†
Early (day 9)	1.000	0.08	0.40	0.2
Intermediate (day 14)	0.080	(1.0)	0.56	0.14
Late (day 18)	0.022	3.6	1.12	0.07

* Data taken from Fig. 4.

† Calculated by normalization of the various $R_0t_{1/2}$ values with respect to day-14 polysomal $R_0t_{1/2}$ of 0.08 as 1.0.

trolled transcriptionally, whereas the quantitative differences in their concentrations appear to be modulated posttranscriptionally (30). An answer to the question of whether or not the transcription and translation of mRNAs coding for individual myofibrillar proteins are coordinately regulated will require further work with specific cloned cDNA probes.

We thank Drs. D. Blumberg, E. Fodor, H. Drabkin, and R. Yanofsky for valuable discussions during the course of these studies. We also thank Drs. W. R. Crain and E. P. Morris for help in the computer analysis of the hybridization data. This investigation was supported by grants from the National Institutes of Health (AM 13238) and the Muscular Dystrophy Association of America and by the U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University.

1. Devlin, R. B. & Emerson, C. P. (1978) *Dev. Biol.* **69**, 202–216.
2. Strohman, R. C., Moss, P. S., Micou-Eastwood, J., Spector, D., Przybala, A. & Paterson, B. (1977) *Cell* **10**, 265–273.
3. John, H. A., Georgoulas, M. P. & Jones, K. W. (1977) *Cell* **12**, 501–508.
4. Paterson, B. M. & Bishop, J. O. (1977) *Cell* **12**, 751–765.
5. Zevin-Sonkin, D. & Yaffe, D. (1980) *Dev. Biol.* **74**, 326–334.
6. Heywood, S. M. & Kennedy, D. S. (1976) *Prog. Nucleic Acid Res. Mol. Biol.* **19**, 477–484.
7. Robbins, J. & Heywood, S. M. (1978) *Eur. J. Biochem.* **82**, 601–608.
8. Dym, H. P., Kennedy, D. S. & Heywood, S. M. (1979) *Differentiation* **12**, 145–155.
9. Dym, H., Turner, D. C., Eppenberger, H. M. & Yaffe, D. (1978) *Exp. Cell Res.* **113**, 15–21.
10. Yaffe, D. & Dym, H. (1972) *Cold Spring Harbor Symp. Quant. Biol.* **37**, 543–548.
11. Buckingham, M. E., Caput, D., Cohen, A., Whalen, R. G. & Gros, F. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1466–1470.
12. Buckingham, M. E., Cohen, A. & Gros, F. (1976) *J. Mol. Biol.* **103**, 611–626.
13. Brenton, R., Fiszman, M. & Gros, F. (1980) *Biochem. Biophys. Res. Commun.* **95**, 281–288.
14. Jain, S. K. & Sarkar, S. (1979) *Biochemistry* **18**, 745–753.
15. Berger, S. L. & Birkenmeier, C. S. (1979) *Biochemistry* **18**, 5143–5149.
16. Sarkar, S., Mukherjee, A. K. & Guha, C. (1981) *J. Biol. Chem.* **256**, 5077–5086.
17. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
18. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. (1977) *Biochemistry* **16**, 4743–4751.
19. Mukherjee, A. K., Guha, C. & Sarkar, S. (1981) *FEBS Lett.* **127**, 133–138.
20. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256.
21. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
22. Buell, G. N., Wickens, M. P., Payvar, F. & Schimke, R. T. (1978) *J. Biol. Chem.* **253**, 2471–2482.
23. Roy, R. K., Lau, A. S., Munro, H. N., Baliga, B. S. & Sarkar, S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1751–1755.
24. Hastie, N. D. & Bishop, J. O. (1976) *Cell* **9**, 761–774.
25. McKnight, S. & Schimke, R. T. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4327–4331.
26. Pearson, W. R., Davidson, E. H. & Britten, R. J. (1977) *Nucleic Acids Res.* **4**, 1727–1737.
27. Bergmann, I. E. & Brawerman, G. (1980) *J. Mol. Biol.* **139**, 439–454.
28. Herrmann, H., Heywood, S. M. & Marchok, A. C. (1970) *Curr. Top. Dev. Biol.* **21**, 181–234.
29. Marchok, A. C. & Herrmann, H. (1967) *Dev. Biol.* **15**, 129–155.
30. Affara, N. A., Robert, B., Jacquet, M., Buckingham, M. E. & Gros, F. (1980) *J. Mol. Biol.* **140**, 441–458.
31. Jain, S. K., Roy, R. K., Pluskal, M. G. & Sarkar, S. (1980) in *Biomolecular Structure, Conformation, Function and Evolution*, ed. Srinivasan, R. (Pergamon, Oxford), Vol. 2, pp. 461–472.
32. Monahan, J. J., Harris, S. E. & O'Malley, B. W. (1976) *J. Biol. Chem.* **251**, 3738–3748.
33. Ordahl, C. P. & Caplan, A. I. (1978) *J. Biol. Chem.* **253**, 7683–7691.
34. Roy, R. K. & Sarkar, S. (1981) *J. Cell Biol.* **91**, 370 (abstr.).