Correlation between the protein and mRNA levels for myosin light chains and tropomyosin subunits during chick fast muscle development in vivo

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Using myosin light chains and tropomyosin subunits as representative myofibrillar proteins, we have characterized their isoprotein forms and also correlated them with the accumulation of the corresponding mRNAs during development of a fast muscle in chicken, viz, pectoralis. Both slow and fast myosin light chain isoforms, except fast myosin light chain LC_3, and the two subunits of tropomyosin are present in early embryonic muscle. During development, the slow myosin light chains and β-tropomyosin appear in reduced amounts in pectoralis muscle and finally they disappear in adult muscle. Translation studies with total cellular RNA from developing muscle indicates that while the protein levels of the above isoforms, in general, correlate with the accumulation of corresponding mRNAs, for LC_3, additional post-transcriptional control appears to modulate the expression of this isoprotein skeletal muscle development in vivo.

Muscle development *Myosin light chain* *Tropomyosin subunit* *Translational control*

1. INTRODUCTION

Myofibrillar proteins are present in several isoforms in various striated muscles and these are known to be coded by distinct but closely related structural genes (review [1,2]). The adult skeletal muscles contain two main types of fibers, fast and slow, each of which are characterized by the presence of specific sets of myofibrillar protein phenotypes [3,4]. The myofibrillar protein isoforms are altered during processes such as muscle development and transformation of adult skeletal muscle fibers. However, the nature of the myofibrillar protein isoforms in embryonic skeletal muscle and the molecular mechanisms underlying the transition in these isoforms during muscle development are not clearly understood. For example, both fast and slow myosin light chains are present in embryonic fast and slow muscle [5,6] whereas the adult muscle contains only the light chains characteristic of the adult phenotype. Using tropomyosin (Tm) subunits and myosin light chains as representative proteins of the thin and thick filaments, we have reported that the appearance of the fast myosin light chain LC_3 is developmentally regulated and furthermore, the isoformic changes of myofibrillar proteins during skeletal muscle development reflect a model of differential gene expression [1]. Here we attempt to correlate the levels of myosin light chain isoforms and Tm subunits with the accumulation of their corresponding mRNAs, during development of a fast muscle, viz. chicken pectoralis. Our results in-

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Abbreviations: 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; LC_1F, LC_2F, LC_3, fast skeletal muscle myosin light chains, numbered according to increasing mobility in gels; SDS, sodium dodecyl sulfate; Tm, tropomyosin; LC_1S, LC_2S, slow skeletal muscle myosin light chains, numbered according to increasing mobility in gels

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dicate that except for myosin light chain LC_3, the contents of the above-mentioned myofibrillar protein isoforms are in general correlated with the level of the corresponding mRNAs. However, for LC_3 the light chain mRNA and protein levels show a non-coordinate relationship in late embryonic and adult muscle. The implication of these results in relation to cytoplasmic regulation of LC_3 mRNA during fast muscle development in vivo is discussed.

2. MATERIALS AND METHODS

Myofibrils were isolated from 10-, 14- and 19-day embryonic and from adult chicken pectoralis muscles [7]. For isolation of total cellular RNA, muscles were homogenized in 5 vol. buffer (0.1 M NaCl, 25 mM Tris–HCl (pH 9.0), 1 mM EDTA, 1% SDS, 0.5 mg heparin/ml) containing an equal volume of a mixture of phenol:chloroform:isoamylalcohol (50:50:1, by vol.). Total RNA was subsequently precipitated from the extract and processed as in [8]. Polysomes from embryonic pectoralis muscle were prepared according to [9] and those from adult muscle were made as in [10]. Cell-free protein synthesis was done at 30°C for 60 min in 25 μl reticulocyte lysate assay system [11] and contained the following: 0.1 M potassium acetate, 1 mM (for RNA) or 2.5 mM (for polysome) magnesium acetate, 10 mM creatine phosphate, 50 μg creatine phosphokinase/ml, 30 μM 19 unlabeled amino acids except methionine, 10 μCi [^{35}S]methionine (1000–1270 Ci/mmol; New England Nuclear), 50% (v/v) reticulocyte lysate and nonsaturating levels of either total RNA (5 μg) or polysomes (0.2 A_{260} unit). The translation products were analyzed by 2D-PAGE [13] together with adult chicken skeletal myofibrils used as marker. Following staining and destaining [14], gels containing cell-free translation products were dried and exposed to Kodak XAR-5 films for autoradiograms. Radioactivity associated with specific myofibrillar proteins in the 2D-gels were determined by scintillation counting [5].

Cell-free synthesis of fast myosin light chains was also confirmed by immunoprecipitation [15] of the translation products with antibodies to LC_3 (gift of Dr S. Lowey, Brandeis University.) The immunoprecipitates were analyzed by SDS–PAGE and autoradiography [13].

3. RESULTS AND DISCUSSION

The Coomassie blue-stained 2D-gel patterns of myosin light chains and Tm subunits in chicken pectoralis myofibrils during development are shown in fig. 1. Both slow and fast myosin light chains, except LC_3, are present in significant amounts in 10-day embryonic muscle (A). Thereafter, the slow myosin light chains are reduced in the myofibrils and by day 19 in ovo, they appear in insignificant quantities in the myofibrils (C), while LC_3 increases in considerable amounts in myofibrils at this stage. With respect to Tm, the gel patterns confirm [1] that β-Tm is the major subunit present in early embryonic pectoralis, while in adult muscle it is replaced by α-Tm (A–D). Muscle lysate from developing pectoralis gave identical patterns of the myofibrillar isoproteins (not shown), indicating that the protein patterns of the isolated myofibrils represent those present in vivo. At the level of in vitro translation products directed with total cellular RNA from developing pectoralis muscle, in general there is good correspondence between the in vivo levels of the myosin light chain isoforms and the accumulation of the corresponding mRNAs (cf. fig. 2 A–D *vs* fig. 1 A–D). The LC_3 light chain seems to be an exception to this correlation. Whereas LC_3 is not detectable either at the protein level or at the level of in vitro translation products with total RNA from early embryonic viz., 10-day muscle, the RNA from 19-day embryonic and adult muscle produces more LC_3 in vitro than that present in vivo (cf. fig. 2C, D *vs* fig. 1C, D): Interestingly, in adult pectoralis muscle show myosin light chain mRNAs are clearly detectable at a low level although, in vivo, the two proteins are apparently not present (cf. fig. 2D *vs* fig. 1D). The synthesis of LC_3 was verified by immunoprecipitation of the in vitro translation products with anti-LC_3, followed by SDS–PAGE of the immunoprecipitate. The immunoprecipitate (section 2) showed substantial synthesis of LC_3 with RNA from 19-day embryonic and adult pectoralis muscles (not shown). With respect to Tm subunits the total RNA directed cell-free translation products showed variable synthesis of the two Tm subunits, suggesting that the corresponding mRNAs may not be efficiently translated in nuclease-treated reticulocyte lysate.

The lack of correlation between the in vivo level

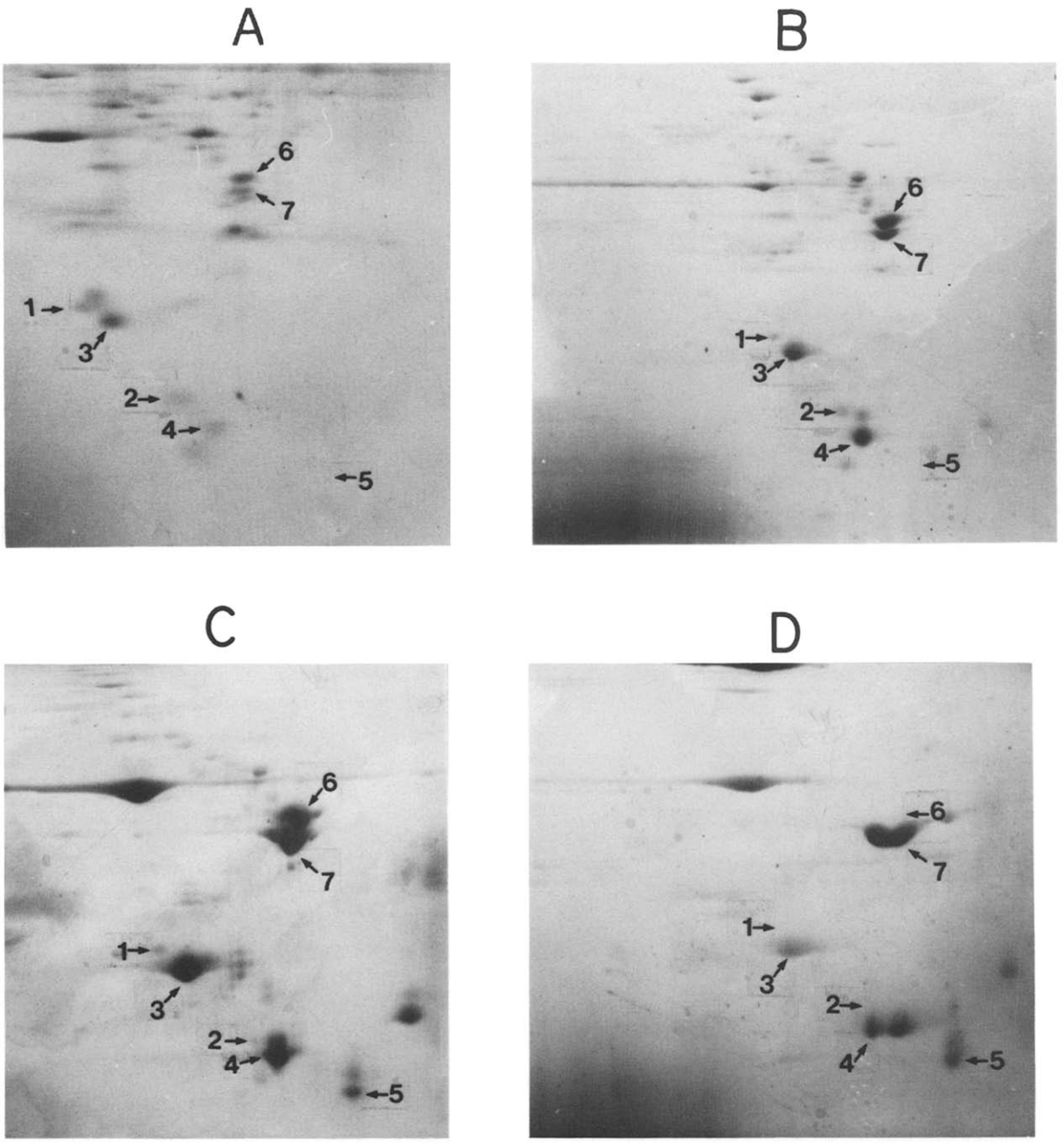

Fig. 1. Two-dimensional gel electrophoresis of myofibrils from chicken pectoralis muscles (details in section 2): (A) 10-day embryonic; (B) 14-day embryonic; (C) 19-day embryonic; (D) adult. Area of gels containing proteins of interest only are shown: (1) LC_1S; (2) LC_2S; (3) LC_1F; (4) LC_2F; (5) LC_3; (6) β-Tm; (7) α-Tm.

of myosin light chain LC_3, and the corresponding in vitro translation product led us to examine the level of LC_3 mRNA associated with polysomes in vivo. Polysomes isolated from developing pectoralis muscle were translated in vitro, and the translation products were analyzed. It was observed that the gel patterns showed good correspondence to the in vivo pattern of myosin light

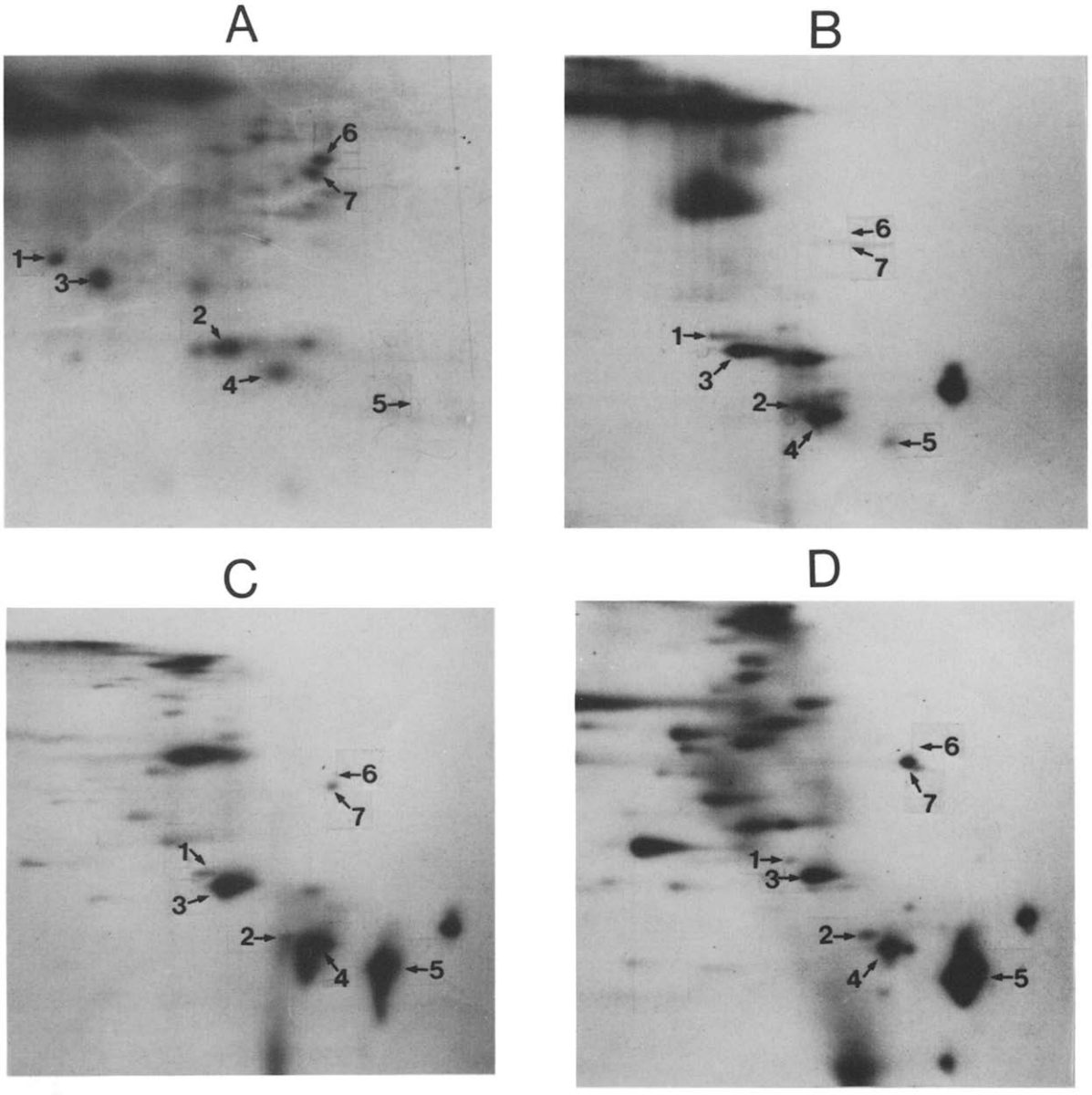

Fig. 2. Two-dimensional gel electrophoresis of translation products synthesized in cell-free system with RNA from chicken pectoralis muscles (details in section 2). Aliquots of reaction mixture containing 6×10^5 cpm, were mixed with carrier chicken skeletal myofibrils and analyzed by 2D-PAGE: (A) 10-day embryonic; (B) 14-day embryonic; (C) 19-day embryonic; (D) adult. Numbering of protein spots is according to fig. 1.

chain isoforms including LC_3 and Tm subunits present in embryonic and adult muscle (cf. fig. 3 *vs* fig. 1).

The relative amounts of myosin light chain isoforms synthesized in vitro in incubations directed by polysomes as well as total RNA isolated from the corresponding muscles, were quantitated. Several interesting features emerge from these results (table 1). At the polysomal level LC_1F, a major myosin light chain in embryonic muscle, is synthesized as a predominant isoform throughout embryonic development, being

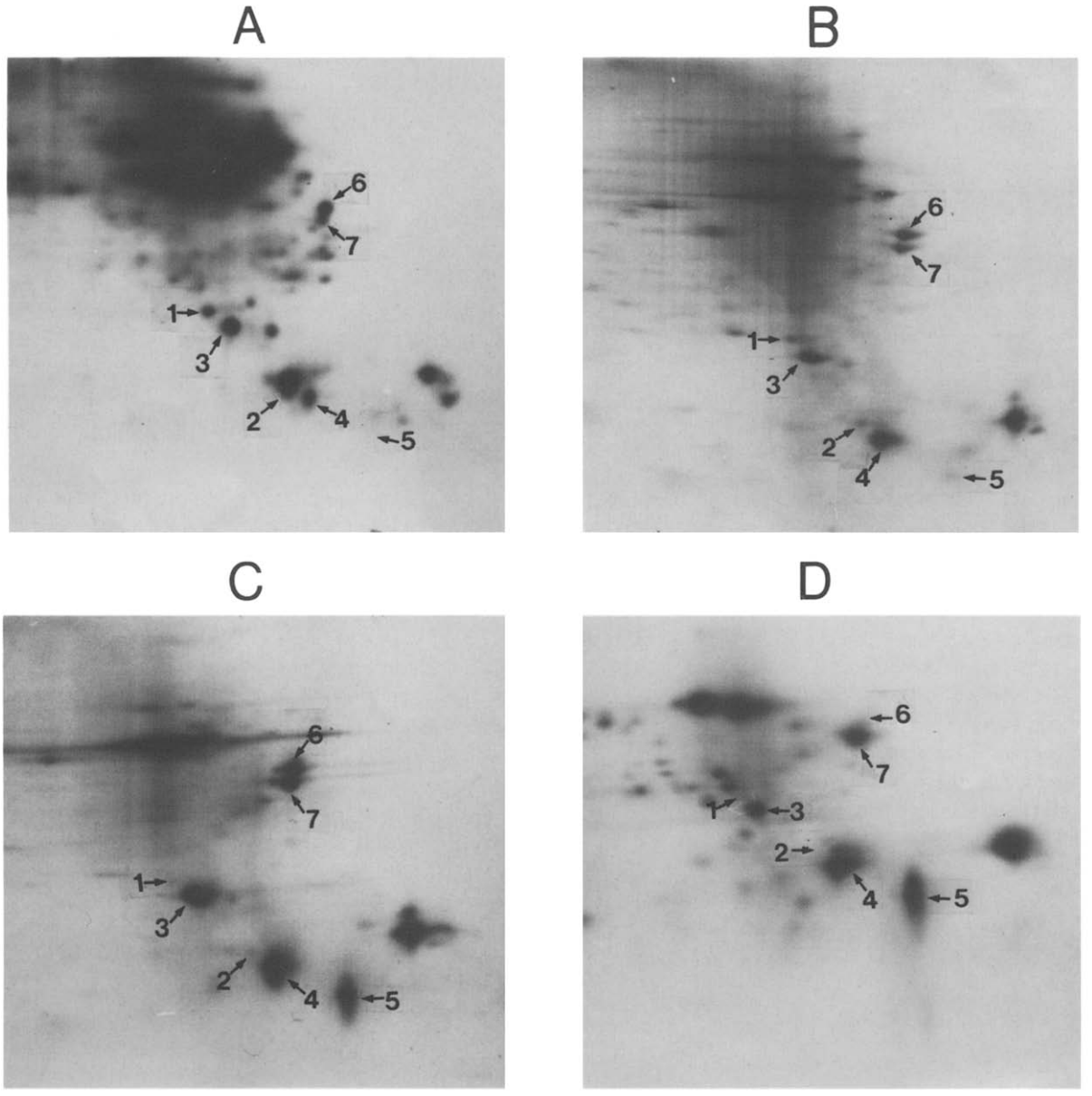

Fig. 3. Two-dimensional gel electrophoresis of translation products synthesized in cell-free system with polysomes from chicken pectoralis muscles (details in section 2). Aliquots and the reaction mixture containing 6×10^5 cpm, were mixed with carrier chicken skeletal myofibrils and analyzed by 2D-PAGE: (A) 10-day embryonic; (B) 14-day embryonic; (C) 19-day embryonic; (D) adult. Numbering of protein spots is according to fig. 1.

30–40% of total light chains synthesized. The polysome-directed synthesis of LC_2F, the other major light chain of embryonic myosin, increases from day-14 and in adult muscle amounts to 45% of the total light chains synthesized. A comparison of myosin light chain isoforms synthesized in vitro by polysomes as well as by total RNA isolated from the corresponding muscles indicates that in 10-day and 14-day embryonic muscles, there is no significant difference between the level of accumulation and subsequent translation of mRNAs of each of the isoforms. Thereafter, however, the

Table 1

Quantification of myosin light chains synthesized in vitro by total cellular RNA and polysomes from chick pectoralis muscles during development

Light chains	Pectoralis 10-day		Pectoralis 14-day		Pectoralis 19-day		Pectoralis Adult	
	RNA	Polysomes	RNA	Polysomes	RNA	Polysomes	RNA	Polysomes
LC_1S	13.2	14.4	9.7	9.9	5.2	1.9	2.0	–
LC_1F	32.4	31.0	33.5	35.2	25.1	25.1	14.0	26.5
LC_2S	36.1	35.6	12.3	9.4	4.7	1.8	3.5	–
LC_2F	18.3	19.0	37.0	39.5	24.1	35.2	12.5	45.2
LC_3	–	–	7.5	6.0	40.9	21.1	68.0	28.3

Results represent an average of 4 experiments with RNA or polysomes isolated from two different muscle samples. Data are expressed as percentage of each light chain present in total radioactivity associated with all myosin light chains. The number of methionine residues/mol light chain is the same for all myosin light chains [5]. Details in section 2

total RNA-directed cell-free synthesis of LC_3 exceeds its synthesis by polysomes from the corresponding muscle. Thus, in 19-day embryonic and in adult muscles the RNA-directed synthesis of LC_3 amounts to 40.9% and 68%, respectively. The corresponding values of LC_3 in polysome-directed translation products are 21.1% and 28.3%, respectively. Both slow myosin light chains, particularly LC_2S, is synthesized in appreciable amounts by polysomes from 10-day embryonic muscles. Thereafter, their synthesis is drastically reduced with development. Surprisingly, the mRNAs for slow myosin light chains are found at a low level in adult pectoralis muscle. The same pool of muscle, however, failed to show any slow myosin light chain in gel analysis, thus eliminating the possibility of contamination of the pectoralis by slow fibers [16].

In view of these results, the possibility that LC_3 mRNA is translated in vitro with relatively higher efficiency was also considered. Total RNA isolated from polysomes of 14-day embryonic and adult pectoralis muscle was translated in vitro, and the translation products were analyzed. The same pattern of synthesis of myosin light chains, including LC_3, as that obtained with the corresponding polysomes was obtained (not shown). Thus, the accumulation of LC_3 mRNA in fast muscle during development (40.9% in 19-day *vs* 68% in adults) seems to occur in a non-coordinate manner with respect to the corresponding protein levels. This implies that a substantial fraction of the mRNA remains untranslated.

Our results indicate that the appearance or disappearance of the myosin light chain and Tm isoproteins during avian fast muscle development are, in general, correlated with the levels of corresponding mRNAs, suggesting that their in vivo level is regulated primarily by transcriptional control. However, for LC_3, the light chain characteristic of the fast muscle, the mRNA may be regulated by additional cytoplasmic controls during the late embryonic and adult stages.

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