SEPARATION OF THE mRNAs CODING FOR α- AND β-TROPOMYOSIN

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1. Introduction

The tropomyosins are a family of related proteins which are widely distributed in nature [1–3]. In muscle tropomyosin performs a major structural and regulatory role. It is associated with actin in the thin filament and is essential in mediating the effect of Ca^{2+} on the actin–myosin interaction. Tropomyosins from different kinds of muscle share similar physical and biological properties but show chemical heterogeneity resulting from differences in primary structure [2,4,5]. Even within a single type of muscle distinct tropomyosins can be identified which have similar isoelectric points but differ in apparent molecular weight [6]. Skeletal muscle contains two types of tropomyosin, α-TM and β-TM, the proportions of which vary with fibre type [4,5]. The functional significance of these distinct tropomyosins is obscure since amino acid sequence analysis and peptide mapping studies indicate that α-TM and β-TM share a high degree of sequence homology [7,8]. In the experiments described here I show that these similar proteins are encoded by mRNA molecules which differ considerably in their physical properties.

2. Materials and methods

2.1. *Isolation and fractionation of RNA*

Total RNA was isolated from embryonic chicken tissue by disruption in 10 vol. 6 M guanidine–HCl, 0.2 M Na acetate pH 5.2 [9] followed by ethanol fractionation and phenol extraction as described previously [10]. Poly A-containing RNA was isolated by chromatography on oligo dT cellulose [11]. Poly A-containing RNA from 20-day embryo leg muscle was fractionated by preparative electrophoresis on a composite agarose–acrylamide gel [12] as described [13].

2.2. *Cell free translation*

mRNA activity was assayed using a micrococcal nuclease-treated, reticulocyte lysate, cell-free system [14]. ^{35}S-Labelled proteins synthesized in vitro were analyzed using one-dimensional SDS–polyacrylamide gel electrophoresis [15] or two-dimensional gels [16]. Immunoprecipitation of cell-free translation products was performed using a rat antiserum against adult chicken leg muscle tropomyosin and protein A–Sepharose as immunoadsorbent [17]. ^{35}S-Labelled proteins were detected by fluorography of the gels [18] and exposure to pre-fogged film at −80°C [19].

3. Results and discussion

RNA from embryonic chicken tissue was translated in a micrococcal nuclease-treated reticulocyte lysate cell free system [13] and proteins synthesized in vitro were examined using SDS–gel electrophoresis (fig.1). The period of incubation of chick embryos is 3 weeks with hatching occurring at day 20–21. During the third week of development there is a clear change in the mRNA population of leg muscle (fig.1A,a,b). The mRNAs coding for a small number of proteins such as myosin heavy chain (MHC), actin, creatine kinase (M-CK), glyceraldehyde-3-phosphate dehydrogenase (GPD) and myosin light chain (MLC) increase above the background of other mRNAs to reach apparent maximal levels just prior to hatching. These mRNA species almost certainly correspond to the limited number of mRNA sequences which become very abundant upon fusion of myoblasts during myogenesis in vitro [20].

Adult chicken leg muscle contains two types of tropomyosin molecules which differ in apparent molecular weight. By analogy with rat skeletal muscle tropomyosins the component of higher molecular

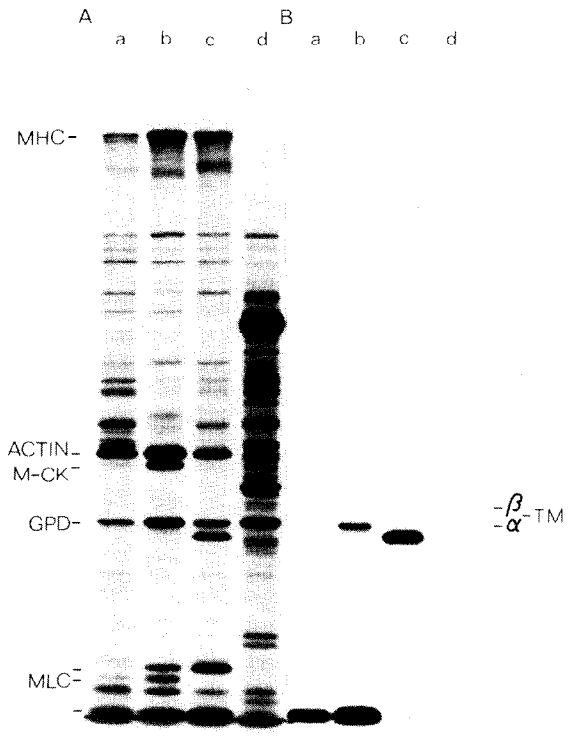

Fig.1. Cell-free translation of chick embryo RNA and immunoprecipitation with antitropomyosin antiserum. (A) Total cell-free translation products; (B) products immunoprecipitated with antitropomyosin antiserum. (a) 11-day embryo leg muscle, (b) 20-day embryo leg muscle, (c) embryonic heart, (d) embryonic liver. The positions of protein markers are indicated: MHC (myosin heavy chain), actin, M-CK (skeletal muscle creatine kinase), GPD (glyceraldehyde-3-phosphate dehydrogenase), MLC (myosin light chain), α-TM (α-tropomyosin), β-TM (β-tropomyosin).

weight is defined as β-TM since it is this component which is reduced in abundance in breast muscle, a fast twitch muscle (not shown). Similarly the component of lower molecular weight is defined as α-TM. These are presumed to be the same as α-TM and β-TM of skeletal muscle described by Cummins and Perry [4]. Antiserum against adult chicken leg muscle tropomyosin detects two bands in the 20-day embryo leg muscle pattern (fig.1B,b) which appear to be the products of translation of α-TM and β-TM mRNAs for the following reasons. These proteins co-migrate exactly with marker α-TM and β-TM. They are greatly reduced in abundance in the 11-day embryo leg muscle pattern indicating that the mRNAs coding for these proteins are induced at the same time as mRNAs coding for other major proteins of differentiated muscle. The mRNAs coding for these proteins are found only in leg muscle not in heart muscle (fig.1B,c) or in liver (fig.1B,d) consistent with the known tissue distribution of α-TM and β-TM [4,5]. When analysed using two-dimensional gel electrophoresis these two proteins are seen to possess nearly identical acidic isoelectric points characteristic of tropomyosins (not shown).

The antiserum also detects a single abundant protein in the heart muscle pattern of slightly lower molecular weight than α-TM (fig.1B,b,c). This component can be identified as cardiac tropomyosin (TMc) since this protein is known to cross react with antiserum against a mixture of α-TM and β-TM from skeletal muscle [21]. The incorporation of [^{35}S]-methionine into α-TM and β-TM, and TMc during synthesis in vitro is very different. This may reflect a real difference in mRNA abundance but could also be due to differences in methionine content of the three tropomyosins or in the relative efficiency of translation of their respective mRNAs.

The physical properties of mRNAs coding for α-TM and β-TM were compared by electrophoretic fractionation on a composite agarose–acrylamide gel [12,13]. The conditions of RNA electrophoresis were non-denaturing in order to facilitate the recovery of translatable activity. Poly A-containing RNA was isolated from 20-day embryo leg muscle RNA by chromatography on oligo dT cellulose [11]. Following electrophoresis the appropriate region of the gel was sliced, RNA electroeluted and translated in vitro (fig.2). Analysis of the products of translation of the fractionated mRNAs using one-dimensional SDS electrophoresis shows that they form a diagonal (fig.2A), indicating that in general the mobility of the mRNA is linearly related to the mobility of the protein for which it codes. The two most striking exceptions are the mRNAs coding for M-CK and for β-TM. This could be caused by a failure of either the mRNA or its protein product to migrate according to its true molecular weight. Indeed, both M-CK, α-TM and β-TM have been observed to migrate anomalously during SDS electrophoresis [4,5,22]. However, the intriguing result is the fact that the mRNAs coding for α-TM and β-TM can be separated almost completely from each other, (fig.2A) and this is confirmed by analysis of the protein products of the fractionated mRNAs using two-dimensional gels (fig.2B). This demonstrates conclusively that α-TM and β-TM are

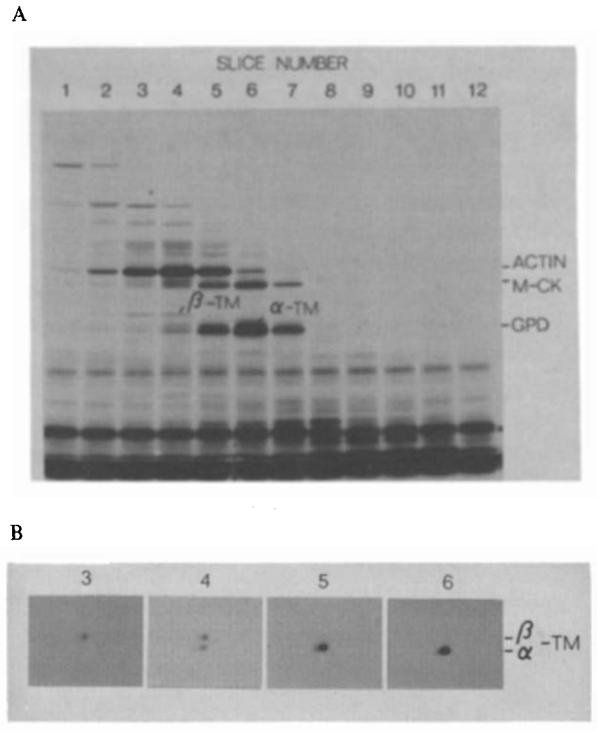

Fig.2. Cell-free translation of 20-day embryo leg muscle fractionated by preparative gel electrophoresis. (A) Translation products of RNA derived from the 18 S region of the preparative gel analyzed on an SDS–10% polyacrylamide gel. (B) Analysis of the translation products of RNA from gel slices 3, 4, 5 and 6 using two-dimensional gels. Only the area of the two-dimensional gel in the region of the tropomyosins is shown.

encoded by separate mRNA molecules. The difference in mobility of the two mRNAs suggests an apparent difference in size of at least 25%. Although the bulk of the protein chemical evidence indicates that α-TM and β-TM are very similar in length and in sequence [7,8] the exact nature of the differences between these two proteins is not well defined. It is therefore difficult to determine whether the difference in mobility of the two mRNAs is due to a significant difference in size such as exists between the mRNAs coding for α-actin and non-muscle actins [13] or to differences in secondary structure. Structural analysis of the mRNAs coding for α-TM and β-TM will be required to resolve the problem. Although this approach would require the construction of recombinant plasmids carrying sequences specific to α-TM and to β-TM, such plasmids could be identified using RNA-blotting techniques [23] which will take advantage of the known physical differences between the two mRNAs.

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