IN VITRO TRANSLATION OF RNA FROM EMBRYONIC AND FROM ADULT CHICKEN PECTORALIS MUSCLE PRODUCES DIFFERENT MYOSIN HEAVY CHAINS

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1. Introduction

A unique myosin heavy chain exists in embryonic fast muscle of the rat [1], rabbit [2,3] and chicken [4]. Other studies of embryonic rabbit fast myosin found the absence of a methylated sequence which is present in adult fast muscle myosin [5]. This methylation was shown to be a post-translational modification of the heavy chain [2,6]. Since differences between embryonic and adult myosin heavy chain are small [1,4,7], it is possible that the embryonic myosin heavy chain is not a unique gene product.

Here, we report for the first time results from RNA-translation experiments that argue strongly for the conclusion that the myosin heavy chains of adult and embryonic chicken breast muscle are produced from different genes or at least that distinct mRNAs exist for adult and embryonic myosin heavy chains from this muscle. The conclusion that embryonic myosin heavy chain is a unique gene product is based on the following results. RNA prepared from embryonic breast muscle or from cultures of that muscle, when translated in vitro, directs the synthesis of a myosin heavy chain whose peptide map is identical to that of myosin heavy chain isolated from embryo breast muscle. We also show that RNA isolated from adult breast muscle directs the synthesis of adult myosin heavy chain and that, by peptide map analysis, the adult and embryonic myosin heavy chains are distinct.

2. Materials and methods

Cell cultures were prepared from the pectoralis muscle of 12.5 day chick embryos as in [8,9]. Myosin was prepared from 7-day-old cultures which were striated and exhibited spontaneous contractions by the procedure in [10]. Myosins were also prepared from the pectoralis muscle of 15 day chick embryos and the pectoralis muscle and the anterior latissimus dorsi muscle of adult chickens by identical procedures. All chickens and eggs were of the white Leghorn variety. Myosin preparations were stored in 40 mM sodium pyrophosphate, 50% glycerol at −20°C.

Myosin heavy chain peptide maps were obtained by a modification of the procedure in [11]. Myosin (100 μg) was reduced with 1/4 vol. reducing buffer by boiling for 4 min and loaded into a 20 mm slot of a 10% SDS–polyacrylamide gel. Following electrophoresis for 4–5 h at 20 mA the gel was stained for 15 min and destained for 15 min. The myosin heavy chain band was cut out of the gel and cut into 4 mm lengths. Following equilibration with sample buffer (0.125 M Tris (pH 6.8), 1 mM EDTA, 1% SDS) for 30 min, these gel pieces were put into 4 mm slots of an 11.25% SDS–polyacrylamide gel and overlayed with sample buffer. Five concentrations of *Staphylococcus aureus* V8 protease (Miles) (1 μg/ml, 2.5 μg/ml, 5 μg/ml, 10 μg/ml, 25 μg/ml) were prepared in sample buffer containing 10% glycerol and bromophenol blue tracking dye and 10 μl protease solution was layered over the gel slices. Current (20 mA) was applied to the gel for 40 min followed by incubation at room temperature for 30 min. Electrophoresis was then carried out until the tracking dye was 1 cm from the bottom of the gel. A preliminary experiment was performed to compare the adult myosin preparation used in this report to a column purified preparation of adult myosin. The peptide maps of the 2 preparations were identical at all enzyme concentrations indicating there were no impurities present in the heavy chain regions of our myosin preparations.

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RNA was prepared from cell cultures and 15 day embryonic pectoralis by the guanidine–HCl method in [9]. Essentially the same procedure was used for RNA from the adult breast except that the initial guanidine extract was extensively deproteinated with chloroform prior to the addition of ethanol.

In vitro translation was performed in the reticulocyte lysate in [9] except that a nuclease-treated lysate [12] was used. Following translation, 0.5 M KCl was added to solubilize myosin and ribosomes were removed by centrifugation (105 000 × *g*, 1 h). Adult fast myosin (70 μg) was added to the supernatant as carrier and the myosin was precipitated in 9 vol. distilled water. Myosin was recovered by centrifugation and the specific activity of all samples was adjusted to 20 000 cpm/100 μg myosin with adult fast myosin. These samples were used for peptide map analysis as described and following electrophoresis the maps of the ^{35}S-labeled myosin heavy chain were visualized by fluorography [13,14].

3. Results and discussion

3.1. *Limited peptide map of embryonic fast, adult fast, and cell culture myosin heavy chains*

The heavy chains of embryonic fast, adult fast, cell culture and adult slow myosins were prepared on 10%

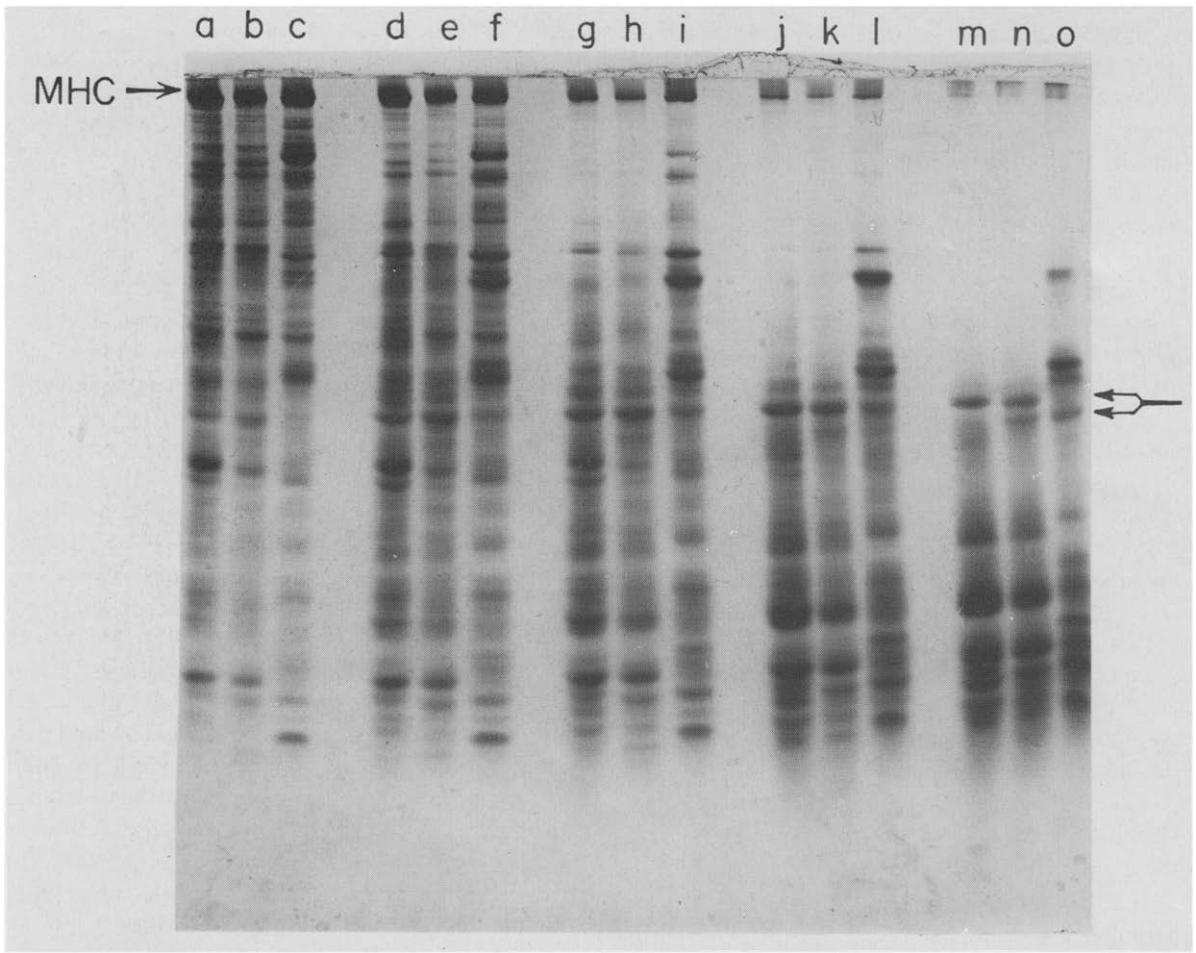

Fig.1. Peptide maps of adult pectoralis, 15 day embryonic pectoralis, and adult anterior latissimus dorsi (ALD) myosin heavy chains: lanes (a), (d), (g), (j) and (m) contain adult pectoralis myosin heavy chains; lanes (b), (e), (h), (k) and (n) contain 15 day embryonic pectoralis myosin heavy chain; lanes (c), (f), (i) and (o) contain adult ALD myosin heavy chain. Lanes (a)–(c) 10 ng *Staph. aureus* V8 protease, lanes (d)–(f) 25 ng *Staph. aureus* V8 protease, lanes (g)–(i) 50 ng *Staph. aureus* V8 protease, lanes (j)–(l) 100 ng *Staph. aureus* V8 protease, and lanes (m)–(o) 250 ng *Staph. aureus* V8 protease.

SDS–polyacrylamide gels. Each heavy chain was then reacted with 5 different concentrations of *Staph. aureus* V8 protease as in section 2. The advantage of this protocol is that it is not only possible to compare the end products of the digestion, but also to compare the intermediates generated by lower concentrations of the protease. Furthermore, the reproducibility of the pattern of peptides can readily be observed. Fig.1 compares the heavy chains of adult fast, 14.5 day embryonic fast and adult slow heavy chain. It is readily apparent that adult slow heavy chain is distinctly different from adult fast heavy chain at all enzyme concentrations (lanes a and c, d and f, g and i, j and l, m and o). The embryonic fast heavy chain is similar to adult fast but shows distinct differences. These differences between adult slow and fast and between embryonic and adult fast myosin have already been noted [1,4]. The presence of a doublet (indicated by the arrows) in the embryonic myosin heavy chain is not observed in the adult heavy chain (cf. lanes m and n). In addition, there are slight differences seen in the smallest peptides which accumulate with increasing enzyme concentration (cf. lanes j and k). Finally, the patterns of intermediate-sized polypeptides seen at the lower enzyme concentrations are different in embryonic and adult fast heavy chains (cf. lanes a and b, d and e, g and h). Fig.2 shows the peptide maps of adult fast, cell culture and adult slow

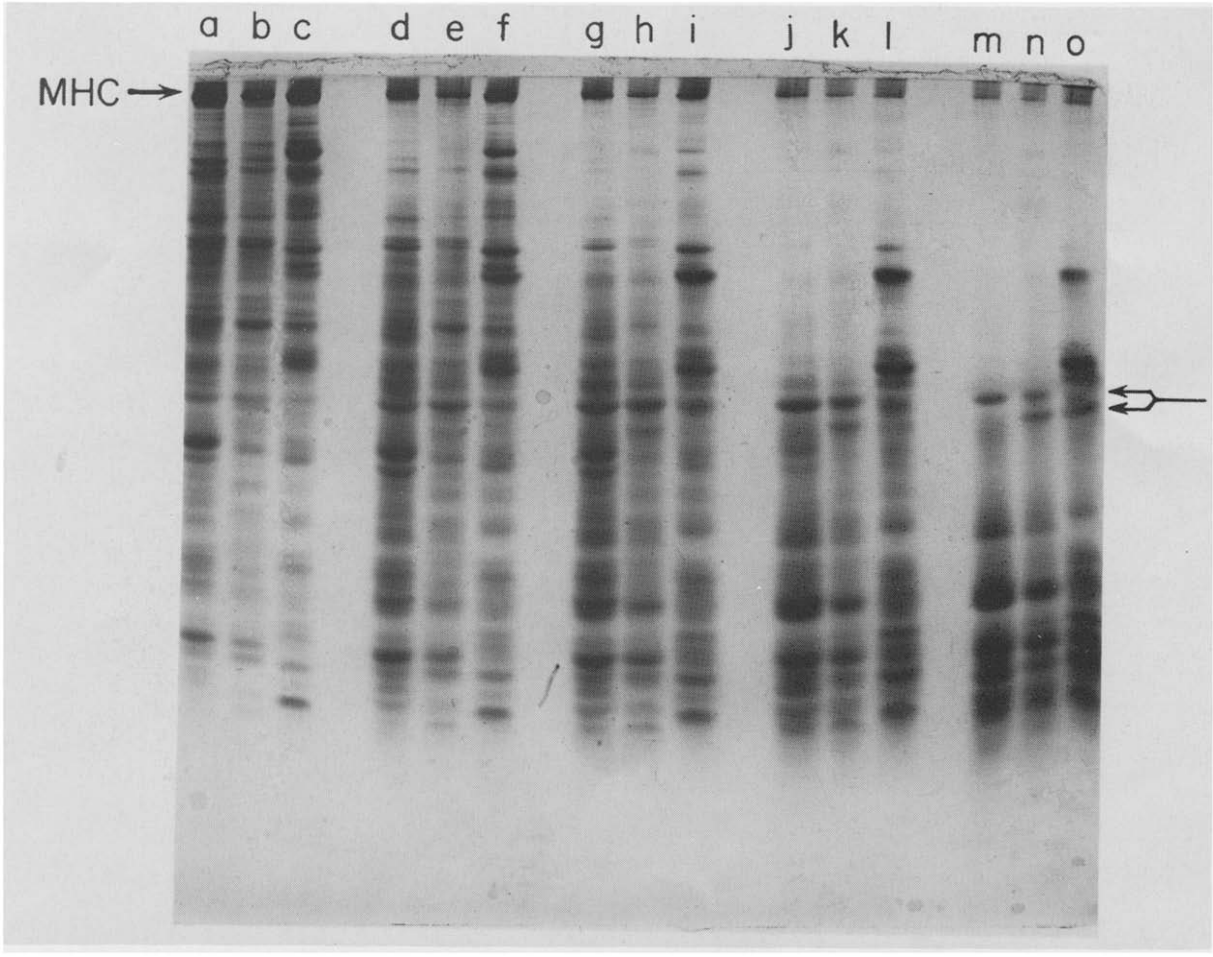

Fig.2. Peptide maps of adult pectoralis, 7 day myogenic cell culture, and adult anterior latissimus dorsi (ALD) myosin heavy chains: lanes (a), (d), (g), (j) and (m) contain adult pectoralis myosin heavy chains; lanes (b), (e), (h), (k) and (n) contain myosin heavy chain from 7-day-old myogenic cultures derived from the embryonic breast; lanes (c), (f), (i), (l) and (o) contain adult ALD myosin heavy chain. Lanes (a)–(c) 10 ng *Staph. aureus* V8 protease, lanes (d)–(f) 25 ng *Staph. aureus* V8 protease, lanes (g)–(i) 50 ng *Staph. aureus* V8 protease, lanes (j)–(l) 100 ng *Staph. aureus* V8 protease, lanes (m)–(o) 250 ng *Staph. aureus* V8 protease.

myosin heavy chains. The myosin heavy chain from cell cultures shows the same differences from adult fast heavy chain as was observed for embryonic myosin heavy chain. In particular the doublet seen in fig.1 is also seen in cell culture myosin heavy chain (cf. lanes m and n). All differences seen between embryonic fast heavy chain and adult fast heavy chain were also seen when comparing cell culture myosin heavy chain and adult fast myosin heavy chain.

3.2. *Embryonic fast myosin heavy chain is a unique gene product*

Since the peptide map of embryonic fast heavy chain is similar to that of adult fast heavy chain, it is possible that post-translational modifications in the primary structure of the heavy chain account for the observed differences in fig.1 and 2. In order to determine whether the embryonic fast myosin heavy chain is a unique gene product, RNA was prepared from embryonic breast, myogenic cell cultures, and adult breast and translated in the reticulocyte cell-free system as in section 2. As far as we are able to determine from the literature the reticulocyte lysate is not capable of processing translation products beyond the primary structure encoded by the specific mRNAs [16–19]. Translation of the RNAs from the different sources should therefore produce direct evidence that myosin heavy chain from embryonic sources is in fact encoded by a unique gene sequence.

The ^{35}S-labeled in vitro synthesized myosin was co-extracted with unlabeled carrier myosin as in section 2 and all 3 translation products were adjusted to

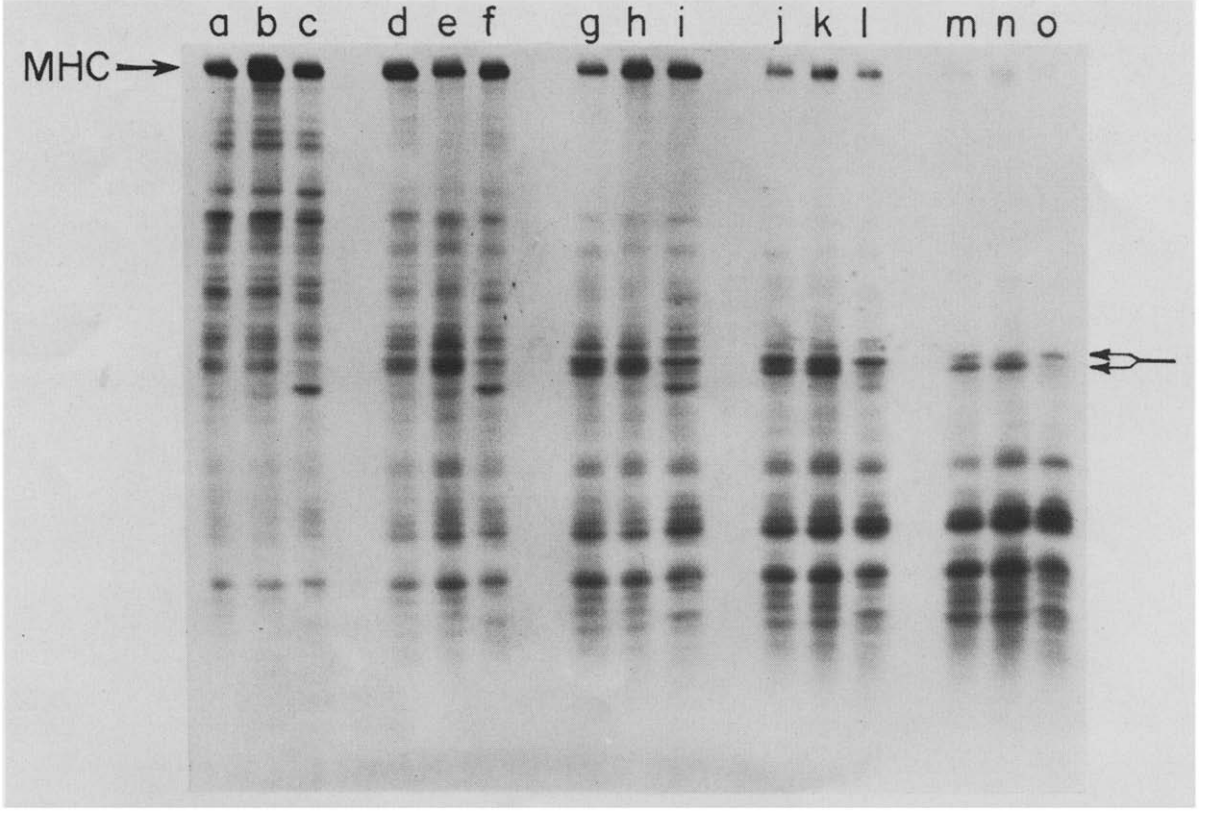

Fig.3. Fluorograph of peptide maps of ^{35}S-labeled myosin heavy chain synthesized in a reticulocyte lysate stimulated with RNA from myogenic cell cultures, 15 day embryonic pectoralis, and adult pectoralis: lanes (a), (d), (g), (j) and (m) contain myosin heavy chain synthesized in a reticulocyte lysate stimulated with RNA from 7-day-old myogenic cell cultures; lanes (b), (e), (h), (k) and (n) contain myosin heavy chain synthesized in a reticulocyte lysate stimulated with RNA from 15-day embryonic pectoralis; lanes (c), (f), (i), (l) and (o) contain myosin heavy chain synthesized in a reticulocyte lysate stimulated with RNA from adult pectoralis. Lanes (a)–(c) 10 ng *Staph. aureus* V8 protease, lanes (d)–(f) 25 ng *Staph. aureus* V8 protease, lanes (g)–(i) 50 ng *Staph. aureus* V8 protease, lanes (j)–(l) 100 ng *Staph. aureus* V8 protease, and lanes (m)–(o) 250 ng *Staph. aureus* V8 protease.

the same specific activity with adult myosin. Peptide maps of these myosin heavy chains were prepared as described and visualized by fluorography [13,14]. Fig.3 shows that the peptide maps of myosin heavy chains synthesized by the reticulocyte lysate coded by embryonic and cell culture RNAs are identical and clearly different from the peptide map of the heavy chain synthesized in the reticulocyte lysate coded by adult RNA. Specifically, the doublet seen in fig.1 and 2 can also be seen in the heavy chain maps made from embryonic and cell culture RNAs (fig.3). In addition, the pattern of intermediates is clearly different in the heavy chain synthesized from adult RNA (cf. a, b and c; d, e and f of fig.3). Therefore, the embryonic and the adult mRNAs are clearly different. The former codes for embryonic and the latter codes for adult-type myosin heavy chains. Whether these different RNAs originate from distinct genes [15] or result from different processing of the same gene transcript remains to be seen.

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