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A novel protein, p19/6.8, specific for cardiac and slow skeletal muscle

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Upon in vitro translation of mRNAs from slow (soleus) muscles of the mouse a hitherto undescribed translation product has been detected that was absent from fast sekeletal muscles and was termed p19/6.8 according to its position in 2-dimensional gels. mRNA for p19/6.8 was also found in the ventricle of the heart. p19/6.8 was not detectable by Coomassie blue staining but could be characterised by fractionation of in vivo labelled muscle tissue. It was found to sediment with the particulate fraction at $14000 \times g$. The expression of p19/6.8 mRNA appears to be down-regulated in muscles with phasic activity.

In vitro translation; Organ culture; Myotonia; (Mouse, Rat)

1. INTRODUCTION

In skeletal muscle, the diversity of fiber types is reflected by the pattern of polypeptide species of the muscle fibers. Many muscle proteins exist as slow and fast isoforms in different fibers (e.g. myosins, tropomyosins, troponins, Ca^{2+}-Mg^{2+}-ATPases), while others are found to be expressed exclusively in fast fibers like parvalbumin [1] or in slow fibers, like the type I proteins [2]. The differential expression of these proteins is thought to be controlled by the activity of the motor neuron supplying the muscle fiber [3].

Proteins that are normally only expressed in slow muscles can be induced in fast muscles by artificial chronic stimulation [4,5]. Some neuromuscular diseases are accompanied by the expression of slow muscle specific proteins [6] in muscles that are purely fast in the absence of disease. Analysis of mRNAs from myotonic muscle of the mouse mutant 'arrested development of righting response', ADR [7,8], led to the discovery of a translation product, termed p19/6.8 [9,10]. The distribution and some properties will be described in the present communication.

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2. MATERIALS AND METHODS

For the origin of the myotonic mouse mutant ADR see [7,8]. Non-affected littermates were used as wildtype controls (genotype: +/adr?). RNA was extracted from shock-frozen muscles by homogenization in 4 M guanidinium thiocyanate and pelleted through a CsCl cushion [11]. In vitro translation was done in a commercial (NEN, Dreieich) reticulocyte lysate according to the manufacturer's instructions with minor modifications.

Freshly dissected soleus muscle (+/adr?) was incubated for 6 h in 200 μl methionine free medium (DMEM) supplemented with 50 μM unlabelled methionine and 50 μCi [^{35}S]methionine in a 5% CO_2 atmosphere at 37°C. Proteins were extracted by grinding with mortar and pestle in 2% Triton X-100, 10 mM Tris-Cl, pH 7.6. The homogenates were sonicated and then shaken for 30 min. The supernatant after centrifugation at $14000 \times g$ was referred to as the soluble fraction, the remaining pellet, extracted with 8 M urea, was regarded as the insoluble fraction.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) was carried out as described by O'Farrell [12] in a mini-electrophoresis chamber (Renner, Darmstadt). After staining with Coomassie brilliant blue or silver [13] the gels were processed for fluorography by immersing in Amplify (Amersham).

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3. RESULTS

Polypeptide p19/6.8 was one of the major in vitro translation products synthesized from RNAs that were isolated from the adult soleus of mouse (fig.1b) or rat (fig.1e). In contrast, the p19/6.8 translation product was below the detection limit in soleus from 10-day-old mice (fig.1a) and fast

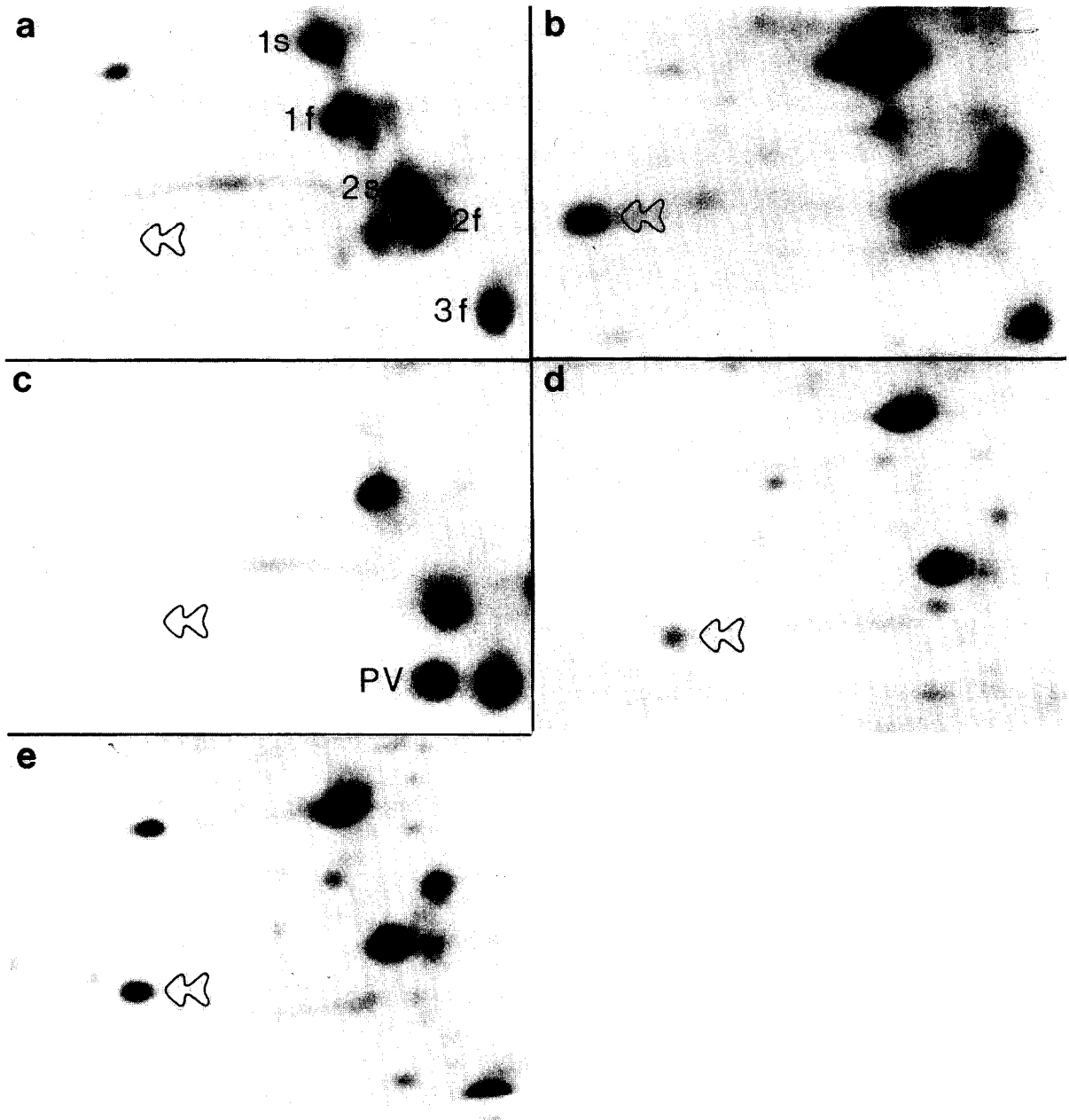

Fig.1. 2D-pattern (only part of the gels is shown) of in vitro translated mouse (a–d) and rat (e) muscle RNAs. RNAs were from (a) M. soleus, 10 day; (b) M. soleus, 80 day; (c) M. vastus, 120 day; (d) ventricle; (e) M. soleus. 1s, 1f, 2s, 2f, 3f, slow and fast isoforms of myosin light chains 1,2,3; PV, parvalbumin; arrow, p19/6.8.

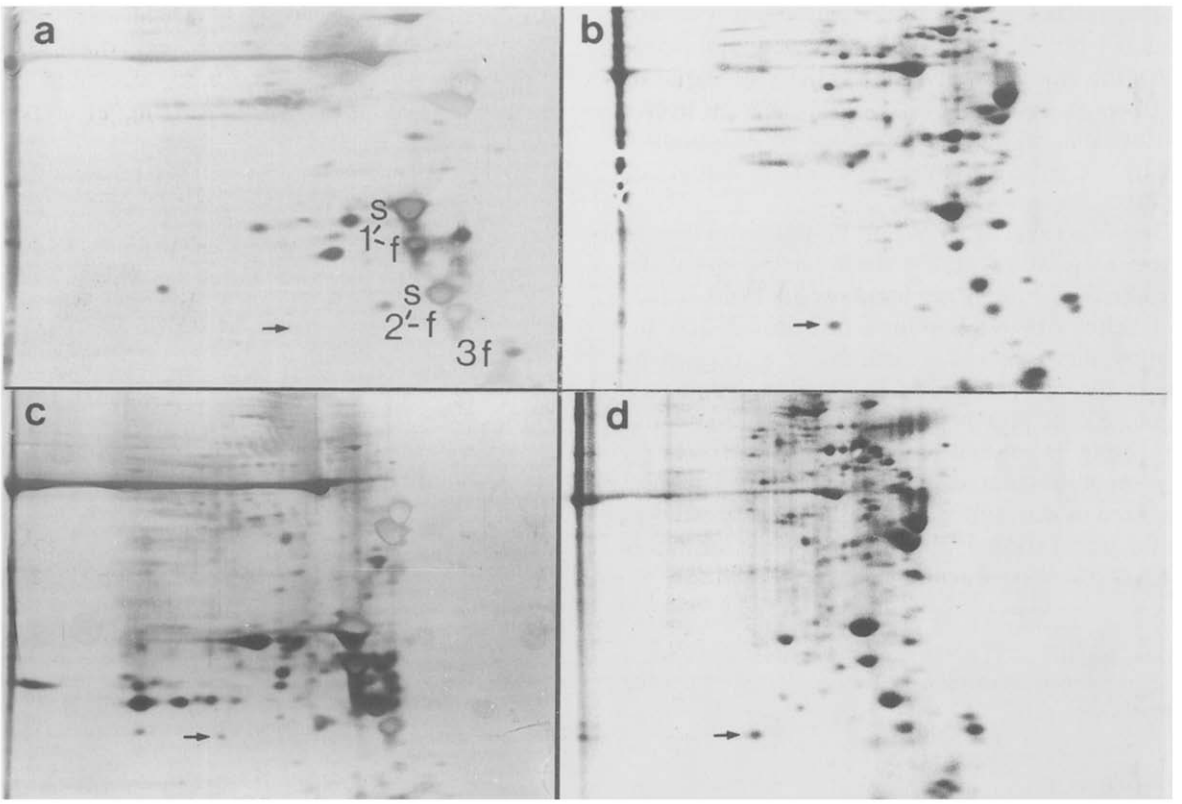

Fig.2. 2D-PAGE of proteins extracted from a mouse soleus muscle that was ^{35}S-labelled in organ culture. Fluorograph (b,d) and silver-stained gels (a,c) of the soluble (a,b) and the insoluble (c,d) fraction is shown. Abbreviations used were the same as in fig.1.

wildtype muscles of all ages (fig.1c). Furthermore, p19/6.8 mRNA was found in the ventricle of the heart (fig.1d).

Soluble proteins extracted from a slow muscle after [^{35}S]methionine labelling in vivo showed a similar pattern to major translation products, including p19/6.8 (fig.2b), however, p19/6.8 could not be detected on the same gel by silver staining (fig.2a).

Polypeptide p19/6.8 was found in the unsoluble fraction of silver-stained 2D-PAGE (fig.2c) but the specific activity (visually estimated by comparing p19/6.8 spots in silver-stained 2D-PAGE and fluorographs of the gels) was much lower. The identity of p19/6.8 in these 2D-PAGEs was confirmed by co-electrophoresis with in vitro synthesized ^{35}S-labelled polypeptides (not shown).

4. DISCUSSION

In skeletal muscle of the mouse, p19/6.8 is only found in slow soleus muscle. Since this muscle consists of 50% type I and 50% type II (oxidative) fibers, and the fast M. tibialis anterior of 50% type II (oxidative) and 50% type II (glycolytic) [14] fibers, the presence of p19/6.8 mRNA in the soleus and its absence in the tibialis anterior muscle suggests that in skeletal muscle p19/6.8 is expressed exclusively in type I fibers. In addition, in the rat soleus, which is composed of 70–90% type I fibers [15], the concentration of p19/6.8 mRNA is higher than in mouse soleus. p19/6.8 is also found in the ventricle of the heart, which shares several contractile proteins with slow skeletal muscles. For example, the slow myosin light chains are expressed both in slow skeletal muscle and in the ventricle [16].

In contrast, in the diseased myotonic muscle, which is not a slow muscle with respect to physiological, histochemical, or biochemical properties [14], considerable amounts of p19/6.8 mRNA are present and translated. In the same

muscle, mRNA coding for parvalbumin is down-regulated [9]. It is known, that parvalbumin concentration is regulated via the activity of the muscle fiber membrane [3,17–19], and it seems possible that p19/6.8 is regulated in an opposite way by the same stimulus, i.e. excessive activity of the myotonic muscle.

The function of p19/6.8 is unknown. It is neither a cytosolic protein nor a component of the myofibrils. On the other hand, newly synthesized, ^{35}S-labelled p19/6.8 is soluble in Triton X-100, indicating that it is either membrane associated or free in the cytoplasm (fig.2a,b). Subsequently, it might become attached to a subcellular structure, thus losing its solubility. In conclusion, p19/6.8 is a protein regulated by muscle activity and restricted to slow type I fibers. It is a useful marker for the maturation of slow muscles and for muscle diseases affecting fiber type differentiation.

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