Expression of phosphoglycerate mutase mRNA in differentiating rat satellite cell cultures

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Poly(A)$^+$ mRNA was isolated from rat satellite cell cultures and analyzed by Northern blot analyses for mRNA content of phosphoglycerate mutase (PGAM) isozymes. In non-differentiating satellite cells only PGAM-B mRNA was detected, but when cells were differentiated into myotubes, which undergo spontaneous contraction, mRNA for PGAM-M muscle-specific isozyme was also detected. This finding is in perfect concordance with the transition of PGAM isozymes encountered in the same cell cultures, and strongly supports a transcriptional control of PGAM expression throughout myogenesis independently of nerve influence.

Phosphoglycerate mutase; Cell culture; Isozyme transition; mRNA

1. INTRODUCTION

The differentiation process of myoblasts into myotubes involves major changes in gene expression. Various proteins are induced during satellite cell differentiation, including components of the contractile apparatus and enzymes [1], and isozymic changes occur in a number of enzymes [2–4].

Phosphoglycerate mutase (PGAM; EC 5.4.2.1) is a convenient marker for the study of muscle cell differentiation. In mammals, PGAM is present in three isozymic forms, resulting from homo- and heterodimeric combination of two different subunits, types M (muscle) and B (brain) [4]. Both subunits, which are 80% homologous in their amino acid sequence [5,6] and possess similar immunological properties [7], are encoded by two different genes. The cDNA of both subunits has been cloned from human [5] and rat [8] skeletal muscle and human brain [6], and the human M gene recently isolated [9].

In early fetal life, type BB PGAM is the only form present in skeletal muscle. During development, an isozyme transition occurs, with type BB PGAM being replaced by type MM PGAM, through the MB hybrid isozyme [4]. Studies during rat, mouse and human muscle cell differentiation have demonstrated that PGAM isozyme begins to occur during the second half of pregnancy [10–12] and it has also been found that in human muscle cell cultures induction of type M PGAM subunit expression is influenced by de novo muscle cell innervation, although the factors involved in isozyme transition have not been clearly established [13,14].

The mechanism by which muscle-specific proteins are induced during muscle differentiation is largely unknown, although it appears to be regulated at the level of mRNA accumulation, which has been assumed to imply transcriptional control [3]. Thus, the present work was undertaken to study the timing of the changes in type M and B PGAM mRNA levels during differentiation in rat muscle cell cultures.

2. MATERIALS AND METHODS

2.1. *Cell cultures*

Rat satellite cells were prepared as previously reported [15,16]. Under experimental conditions, the maximum proliferation rate was observed on the 4th day after plating, as detected by [^{3}H]thymidine incorporation or cytophotometry [16]. To follow the differentiation process, the increase in creatine kinase activity was routinely recorded with a Merckotest kit (Merck) as described previously [16]. After the 6th day of culture, satellite cells fused into myotubes which spontaneously contracted on the 10th day.

Cell extracts were prepared on different days of culture and PGAM isozymes were analyzed by cellulose acetate electrophoresis as previously reported [17,18].

2.2. *mRNA isolation and Northern analyses*

Cells were lysed in buffer containing 4 M guanidinium isothiocyanate, 1 M β-mercaptoethanol and 0.5% Sarkosyl. Total RNA was isolated by ultracentrifugation on a CsCl cushion according to standard procedure [19]. Poly(A)$^+$ mRNA was purified by affinity chromatography on Hybound-Map (Amersham) according to the supplier's instructions. Northern blot of poly(A)$^+$ mRNA purified

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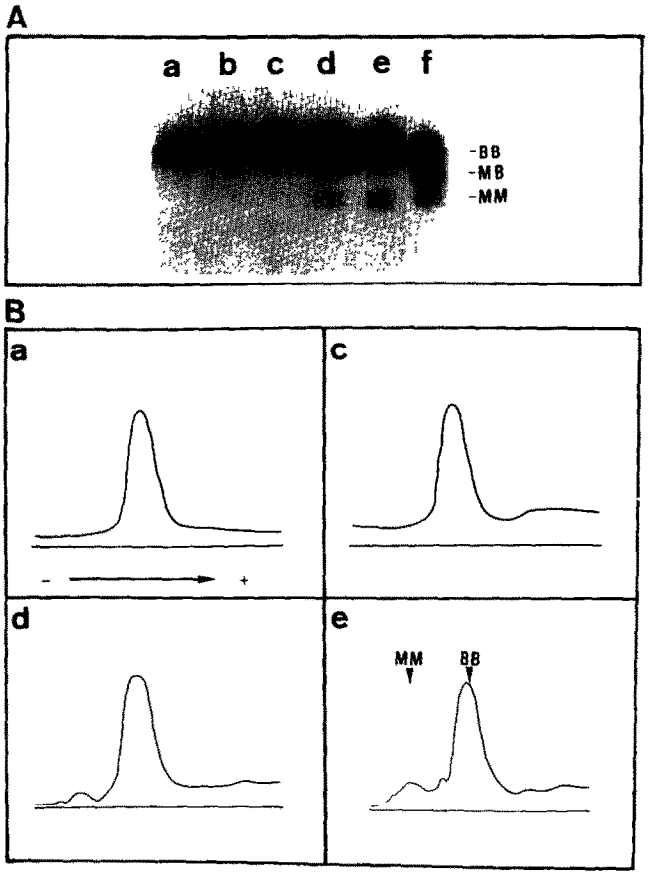

Fig. 1. Cellulose acetate electrophoresis of PGAM isozymes of cultured rat muscle cells. Electrophoregram (A) and electrophoretic traces (B) on the following indicated days of satellite cell culture: (a) 4 days, (b) 6 days, (c) 8 days, (d) 11 days, (e) 14 days; and (f) adult rat heart.

from satellite cells was performed as previously described [20] except that RNA was transferred onto nylon membranes (GeneScreen Plus, DuPont de Nemours, France) and hybridized using standard techniques [21]. The following cDNAs were used as probes: (i) the 820 bp rat PGAM-M cDNA [8] and (ii) the 400 bp long fragment, derived from *Bgl*II-*Eco*RI cleavage of rat cDNA, corresponding to the 5′ fragment of PGAM-B cDNA clone (unpublished). cDNAs were labelled by the random primed techniques. Filters were washed twice with 2 × SSC/0.1% SDS for 5 min at room temperature and twice with 2 × SSC/0.1% SDS for 30 min at 56°C, and exposed with Amersham Hyperfilm-MP films for 1–4 days.

3. RESULTS AND DISCUSSION

As shown in Fig. 1, before day 11 of differentiation only type BB PGAM is present in rat muscle cultured cells. From day 11 onwards type MM PGAM appears and increases progressively, although it represents a minor proportion (less than 15%) of total enzyme activity after 14 days of differentiation. Detachment of cells from the gelatinized culture substrate, induced by cell contraction, impeded further studies of isozyme transition.

Fig. 2 shows Northern experiments in cell cultures and adult rat muscle and brain tissues. Results of cell

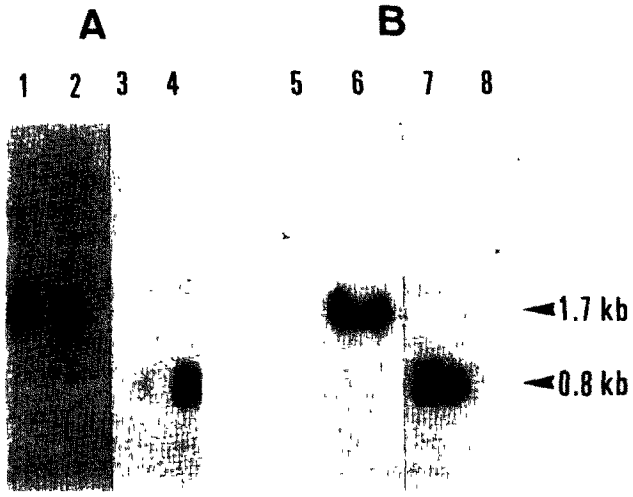

Fig. 2. Northern blot analysis of poly(A)$^+$ PGAM-RNA from rat muscle cell cultures (A) and adult rat tissues (B). (A) Cell cultures: B probe-hybridized RNA at 4 days (lane 1) and 11 days (lane 2) of culture; M probe-hybridized RNA at 4 days (lane 3) and 11 days (lane 4) of culture. (B) Adult rat tissues: B probe-hybridized RNA from skeletal muscle (lane 5) and brain (lane 6); M probe-hybridized RNA from skeletal muscle (lane 7) and brain (lane 8).

culture analysis are in agreement with the transition of the enzyme phenotype pattern. At day 4 of differentiation only type B PGAM mRNA is observed, whereas at day 11, when type MM and type BB PGAM are present, both B and M mRNA are detected (Fig. 2A). In adult rat brain and skeletal muscle, which possess only BB and MM PGAM isozymes respectively, only the corresponding type B and type M PGAM mRNA are observed (Fig. 2B).

These results strongly support a transcriptional control of PGAM throughout myogenesis, similar to that of creatine kinase [3]. They also clearly demonstrate that in rat expression of muscle-specific PGAM isozyme does not require cell innervation, although it has been shown that muscle-type PGAM transition is enhanced in innervated cultured human muscle fibres [13,14] and complete transition from BB- to MM-PGAM has not been observed in anneurally grown human myotube cultures [14]. Rat satellite cells undergo further differentiation in vitro than human cells. They contract spontaneously from the 10th day of culture onwards and fuse into myotubes in the absence of nerve. This could explain the earlier apparition of PGAM-MM isozyme in rat cells in comparison to human cells.

Our data also show that, in cultured rat muscle cells, detection of both PGAM-M mRNA and PGAM-M subunit correlates with the initiation of spontaneous cell contraction. Since it has been reported that electrical stimulation and tetrodotoxin paralysis do not affect PGAM isozyme transition in cultured rat muscle cells [22], involvement of other factors should be further investigated.

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