Myosin heavy-chain mRNA is present in both myofibrillar and subsarcolemmal regions of muscle fibres

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Hybridization *in situ* with riboprobes to the myosin heavy-chain slow isoform showed that, in the rat soleus muscle, the myosin heavy-chain mRNA was distributed throughout the myofibres. There was greater density of autoradiographic grains in the subsarcolemmal regions of the fibres, but there was also a considerable number of grains in the core myofibrillar region of the fibres. Microdensitometry showed that the grain density in the myofibrillar region was approximately half that in the subsarcolemmal rim; this would correspond to some 70% of the mRNA being present in the myofibrillar region. The results are consistent with the hypothesis that myosin is synthesized on polyribosomes present in the intermyofibrillar cytoplasm.

INTRODUCTION

Studies of *Xenopus* oocytes and mammalian cell lines in culture have provided evidence for the spatial localization of certain mRNAs (Jeffery, 1984; Sundell & Singer, 1990; Hesketh & Pryme, 1991). The sorting of mRNAs such that not only are those for membrane and secreted proteins directed to the endoplasmic reticulum, but also those for the bulk of cellular proteins are translated in different subpopulations of polyribosomes, could provide a mechanism whereby proteins are synthesized close to their site of function. Such a mechanism could be of particular importance in skeletal muscle, where the synthesis and turnover of myofibrillar proteins presents extreme logistical problems in synthesizing large quantities of actin and myosin which are required in precise locations within a highly organized framework (Millward, 1980). Although the turnover of myofibrillar proteins is asynchronous (Zak *et al.*, 1979), the replacement of these components appears to take place without loss of sarcomere integrity and, at least in the case of myosin, the protein is incorporated throughout the length and cross-section of the A-band (Franchi *et al.*, 1990). If myofibrillar proteins are synthesized close to their site of assembly, then one would expect the mRNAs for proteins such as myosin to be directed to the intermyofibrillar cytoplasm and to be translated in the polyribosomes that various data suggest are present in these regions of the myofibres, around the myosin-containing A-bands (Clavert *et al.*, 1949; Larson *et al.*, 1971; Horne & Hesketh, 1990a). The distribution of myosin heavy-chain mRNA has therefore been investigated in mature skeletal muscle using hybridization *in situ*.

MATERIALS AND METHODS

Cryostat sections (10 μm thick) were cut from the soleus muscle of three male rats of 30 days of age. After fixation in 4% paraformaldehyde the sections were dehydrated in ethanol and stored at -80°C . After rehydration they were subsequently treated with proteinase K (20 $\mu\text{g}/\text{ml}$), refixed and acetylated before hybridization with either a sense or an antisense ^{35}S -labelled riboprobe prepared from a 68 bp fragment of a slow myosin heavy-chain cDNA probe ligated into a Bluescribe vector. The probe (MHC5r3), from Professor G. Goldspink, Royal Veterinary College, Royal College Street, London N.W.1, U.K., was originally from Dr. K. Schwartz, Unite de Recherches sur le Metabolism du Coeur, U127 INSERM, Paris, France, and it comprised 19 nucleotides of the coding region and the remainder from untranslated sequences. Approx. 2×10^5 c.p.m. of labelled

RNA was added to each slide. After washing to remove non-specifically bound probe, the slides were dehydrated and dipped in autoradiographic emulsion (LM1; Amersham International) and exposed for 14 days. Autoradiographic grain densities were quantified by microdensitometry (Bitensky, 1980) by using a Vickers M85 microdensitometer and an aperture of 5 μm diameter; measurements were made at random points in both the myofibrillar region (five measurements/fibre) and subsarcolemmal area (five measurements/fibre on transverse sections; ten on longitudinal sections) of 20 fibres cut in longitudinal section and 40 cut transversely. Values for each fibre were corrected for blank absorbance measured on a neighbouring 'fast' fibre, which showed little or no labelling.

RESULTS AND DISCUSSION

As shown in Fig. 1, hybridization *in situ* using the antisense probes for the gene for the myosin heavy-chain slow isoform showed that, in the soleus muscle, which contains predominantly slow fibres, autoradiographic grains were present in the majority of fibres. Importantly, in those fibres which were labelled, the myosin heavy-chain mRNA was detected throughout the myofibres. It was evident from both transverse and longitudinal sections that, although the myosin heavy-chain mRNA was present in greater amounts in the subsarcolemmal region underlying the plasma membrane, significant amount of the mRNA were also present in the core of the fibres, presumably in the intermyofibrillar cytoplasm. Microdensitometry of the longitudinal sections showed that the absorbance due to the presence of autoradiographic grains was 6.0 ± 0.4 (mean $\pm$ S.E.M.) in the subsarcolemmal region and 3.9 ± 0.4 in the core myofibrillar region; measurements on transverse sections gave values of 5.6 ± 0.3 and 2.5 ± 0.2 respectively for the corresponding regions. The mean myofibrillar/subsarcolemmal grain density ratio was 0.63 ± 0.04 in longitudinal sections and 0.44 ± 0.02 in transverse sections. Thus the concentration of myosin heavy-chain mRNA in the myofibrillar region appears to be approximately half of that in the subsarcolemmal cytoplasm. In view of the fact that the volume of the myofibrillar 'compartment' is considerably bigger than that of the rim of subsarcolemmal cytoplasm, these relative concentrations, taken together with simple geometric considerations, indicate that, at least in the soleus muscle of rapidly growing rats, the majority of the myosin heavy-chain mRNA is present in the myofibrillar region. Assuming that the subsarcolemmal rim is 1.5 μm wide in a fibre of diameter 45 μm (cf. Horne & Hesketh, 1990b), then a relative grain density ratio

Fig. 1. Hybridization *in situ*, illustrating the distribution of myosin heavy-chain mRNA in rat soleus muscle

The dark autoradiographic grains reflect the mRNA distribution in (a) longitudinal sections and (b) transverse sections of soleus muscle hybridized with an antisense riboprobe to the myosin heavy-chain slow-isoform gene. There was very little non-specific hybridization, as demonstrated (c) by using a sense riboprobe generated from the same template. The bar represents 32 μm .

of 0.5 suggests that some 70% of the myosin mRNA is present in the intermyofibrillar cytoplasm.

The high proportion of myosin heavy-chain mRNA found in the myofibrillar region of the fibres probably reflects the relatively high rates of protein synthesis both in young rats of 30 days of

age (Lewis *et al.*, 1984) and in slow muscles such as the soleus. The distribution of myosin mRNA may be different in muscles from adult rats, in which the rate of protein synthesis is considerably reduced, and in predominantly fast-type muscles, which exhibit a lower protein-synthesis rate. It is not possible using techniques of hybridization *in situ* to distinguish between mRNAs which are being translated and those that are in some untranslated form. However, since it has been reported that some 70% of muscle ribosomes are present in the intermyofibrillar cytoplasm (Horne & Hesketh, 1990a,b), it would seem likely that the myosin heavy-chain mRNA detected in the myofibrillar region is being actively translated on polyribosomes present in this region and associated with the myofibrils.

The results are thus compatible with, but do not prove, the hypothesis that such intermyofibrillar polyribosomes are involved in the synthesis of sarcomeric proteins such as myosin. This hypothesis requires that a mechanism exists to direct mRNAs to the intermyofibrillar cytoplasm; although there is at present no evidence for such a mechanism, there is accumulating evidence that, in other cells, mRNAs are indeed directed to different cellular domains, and in part this involves interactions with the cytoskeleton [see Hesketh & Pryme (1991) for a review]. Such specific location of mRNAs is not dependent on the presence of nascent polypeptide chains, indicating that the information required for direction of the mRNA is present in the messenger molecule itself (Yisraeli & Melton, 1988; Sundell & Singer, 1990). During muscle growth induced by stretching, there is an increase in protein synthesis at the distal end of the muscle fibres (Williams & Goldspink, 1971) and this may be associated with a concentration of myosin mRNA at the myotendinous junction (Dix & Eisenberg, 1990). Thus it appears both that the synthesis of myosin in skeletal muscle is spatially organized and that any mechanisms proposed to direct mRNAs to specific locations in the muscle fibre must be able to adapt to different physiological needs. Since both muscle ribosome distribution and protein-synthesis rates alter with age and during hypertrophy (Lewis *et al.*, 1984; Horne & Hesketh, 1990a,b), future work should examine whether the distribution of mRNA is also different in muscles synthesizing protein at rates different from that of the rapidly growing soleus muscle.

We thank Professor G. Goldspink for kindly providing the myosin heavy-chain gene probe and Ms. Philippa Whitelaw for her assistance in the initial stages of this work.

REFERENCES

- Bitensky, L. (1980) Ciba Found. Symp. **73**, 181–202
- Clavert, J., Mandel, P. & Jacob, M. (1949) C.R. Séances Soc. Bio. Ses Fil. **143**, 539–540
- Dix, D. J. & Eisenberg, B. R. (1990) J. Cell Biol. **111**, 1885–1894
- Franchi, L. L., Murdoch, A., Brown, W. E., Mayne, C. N., Elliot, L. & Salmons, S. (1990) J. Muscle Res. Cell Motil. **11**, 227–239
- Hesketh, J. E. & Pryme, I. F. (1991) Biochem. J. **277**, 1–10
- Horne, Z. & Hesketh, J. E. (1990a) Biochem. J. **268**, 231–236
- Horne, Z. & Hesketh, J. E. (1990b) Biochem. J. **272**, 831–833
- Jeffery, W. R. (1984) Dev. Biol. **103**, 482–492
- Larson, P. F., Hudgeson, P. & Walton, J. N. (1971) Nature (London) **222**, 1168–1169
- Lewis, S. E. M., Kelly, F. J. & Goldspink, D. F. (1984) Biochem. J. **217**, 517–526
- Millward, D. J. (1980) Comp. Biochem. **19B**, 153–232
- Sundell, C. L. & Singer, R. H. (1990) J. Cell Biol. **111**, 2397–2403
- Williams, P. E. & Goldspink, G. (1971) J. Cell Sci. **9**, 751–767
- Yisraeli, J. K. & Melton, D. A. (1988) Nature (London) **336**, 592–595
- Zak, R., Martin, A. F. & Blough, R. (1979) Physiol. Rev. **59**, 407–447