CHARACTERISTICS OF RIBOSOMES OF SKELETAL MUSCLE TISSUE

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Characteristics of the ribosomes of skeletal muscle tissue were investigated by electron-microscopic and chemical analysis of fractions of RNP particles obtained from the tissues of the hind limb muscles of rats and the human rectus abdominis muscle. The fraction of RNP particles was shown to contain functionally active mono- and polyribosomes. Two RNA fractions were distinguished: RNA_I and RNA_{II}. The first, RNA_I, is an assortment of ribosomal RNAs with sedimentation coefficients of 26-28S, 16-18S, and 4-5S; while RNA_{II} does not contain ribosomal RNAs and is similar in its nucleotide composition to rat DNA. The structural organization of ribosomes in the cytoplasm of the muscle fibers corresponds to that observed in the fraction of RNP particles. Polysomes are found in areas of physiological regeneration of myofibrils and they consist of combinations of five or more monoribosomes, arranged like beads. Ribosome-like particles were seen to escape through the nuclear membrane; in conjunction with the results of chemical analysis this indicates the periodic passage of RNA from the nucleus into the cytoplasm of the muscle fiber.

KEY WORDS: ribosomes; RNA; skeletal muscles.

The study of plastic processes taking place in skeletal muscle tissue during normal activity is fundamental to the understanding of the principles of its development and aging, its physiological regeneration, and adaptation to functional loads. This group of problems is connected with investigation of the mechanism of protein synthesis in muscle fibers. Accordingly the characteristics of the ribosomes of normal skeletal muscle tissue is of great interest.

EXPERIMENTAL METHOD

The test object consisted of the hind limb muscles of rats and the human rectus abdominis muscle. The rat muscles were homogenized in 3 volumes of 0.03 M Tris-HCl buffer, pH 7.7, containing 0.25 M KCl, 0.01 M $MgCl_2$, 0.006 M β-mercaptoethanol, and 0.11 M sucrose (buffer A). The homogenate was centrifuged at 30,000g for 30 min. The resulting supernatant was treated with Triton X-100 in a final concentration of 1% and centrifuged at 105,000g for 2 h through a layer of 1 M sucrose solution in buffer A. The residues were suspended in 0.05 M Tris-HCl buffer, pH 7.6, containing 0.05 M KCl and 0.001 M $MgCl_2$ and were used for analysis by centrifugation in a sucrose gradient, for determination of functional activity as described previously [2, 3], and for differential RNA extraction [7]. Pieces of RNP particles for electron-microscopic investigation were fixed for 1 h in 2% OsO_4 solution by Millonig's method, dehydrated in alcohols, and embedded in methacrylate or Araldite. Ultrathin sections, stained with uranyl acetate and lead salts, were studied in the IEM 7A electron microscope.

EXPERIMENTAL RESULTS

Analysis by centrifugation in a sucrose gradient showed the presence of polyribosomes, monoribosomes, and ribosomal subunits among the RNP particles isolated. Preincubation of the preparations with pancreatic RNase led to degradation of the polysomes and displacement of UV-absorbing material into the monomer region. Degradation of the polysomes also was observed when RNA synthesis was blocked by actinomycin D (Fig. 1). Electron-microscopically the monoribosomes appeared as dense irregularly spherical particles measuring

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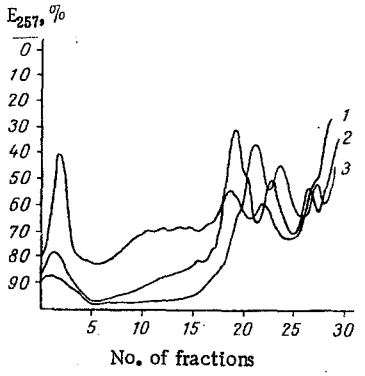

Fig. 1

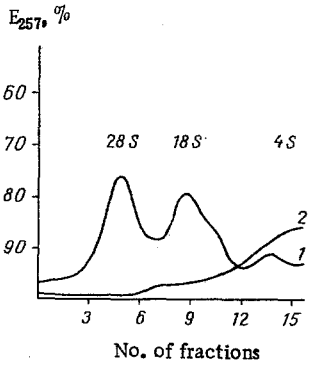

Fig. 2

Fig. 1. Sedimentation analysis of RNP particles in a sucrose gradient (40-10%): 1) initial preparation of RNP particles; 2) RNP particles after preincubation with RNase (10 μg/ml); 3) RNP particles obtained after injection of actinomycin D into animals (0.5 mg per animal).

Fig. 2. Sedimentation analysis of RNAs extracted from muscle RNP particles in a sucrose gradient (20-5%): 1) RNA_I; 2) RNA_{II}.

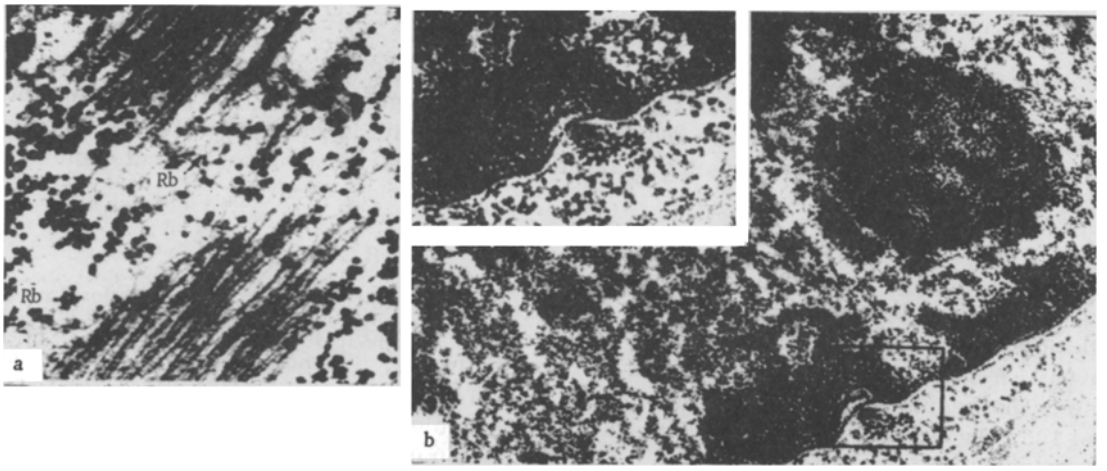

Fig. 3. Electron microscopy of ribosomes in muscle tissue: a) collection of mono- and polyribosomes (Rb) and glycogen in zone of physiological regeneration and of myofibrils (60,000 ×); b) escape of ribosome-like particles from nucleus into cytoplasm of muscle fiber (inset, 48,000 ×).

150-200 Å. The polyribosomes were aggregates consisting of five or more ribosomes arranged in a chain. Preparations of muscle RNP particles could incorporate leucine-C^{14} into the acid-insoluble material in a cell-free medium, evidence of their functional activity. Two RNA fractions were isolated by extraction with phenol at different pH values [7]: RNA_I and RNA_{II}.

The fraction RNA_I consisted of an assortment of ribosomal RNAs with sedimentation coefficients of 26-28S, 16-18S, and 4-5S (Fig. 2), and RNA_{II} did not contain ribosomal RNAs, but consisted of heterogeneous material located mainly in the low sucrose concentration zone. The preparations also differed in their nucleotide composition, as determined by a direct spectrophotometric method at different wavelengths [1]. The nucleotide composition of RNA_{II} is close to the nucleotide composition of rat DNA [4]. It can be postulated from these results that the RNA_{II} preparation contained messenger RNA. The structural organization of ribosomes in the cytoplasm of muscle fibers corresponded to that observed in the fraction of RNP particles. The polyribosomes consisted of groups of five or more monoribosomes, arranged like beads. They were detected in areas of physiological regeneration of the myofibrils and were structurally connected with fibrillary material which evidently

was related to muscle proteins in process of synthesis (Fig. 3a). The largest polyribosomes are considered [5, 6] to participate in the synthesis of myosin. In some cases ribosome-like particles were seen to escape through the nuclear membrane (Fig. 3b).

The experiments thus showed that during normal activity the cytoplasm of the skeletal-muscle fiber contains functionally active mono- and polyribosomes. Ribosomes participate in intracellular self-renewal processes and, in particular, renewal of the contractile system of the muscle fiber.

It can be concluded from these results that RNA passes periodically from the nucleus into the cytoplasm of the muscle fiber, possibly in connection with resumption of the cycle of intracellular self-renewal.

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CALCIUM TRANSPORT AND ATPase ACTIVITY OF THE SARCOPLASMIC RETICULUM OF NORMAL AND DENERVATED RABBIT MUSCLES

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The properties of the calcium pump of the sarcoplasmic reticulum (SR) from normal and denervated rabbit muscles were studied. The kinetics of transport of Ca^{++} ions in SR from denervated muscles obeys the Michaelis–Menten law. After denervation the rate of fast outflow of Ca^{++} from the vesicles is increased, leading to a decrease in the efficiency of transport and an increase in the activity of "basal" ATPase. Meanwhile the rate of Ca^{++} accumulation and the activity of transport Ca-ATPase are increased by 1.5 times. The kinetic properties of the reticulum from denervated muscles correspond to the pattern of the contraction–relaxation cycle in those muscles.

KEY WORDS: denervation; Ca^{++} transport; transport Ca-ATPase; sarcoplasmic reticulum.

Denervation leads to considerable changes in metabolism and, consequently, to morphological and physiological changes in muscle tissue. After denervation of fast muscles hypertrophy of the sarcoplasmic reticulum (SR), linked with an increase in the synthesis of membrane protein [11], and changes in the phospholipid composition of the membranes [5, 9] are observed. The effect of denervation on the Ca^{++} transport system in SR has received little study.

The object of this investigation was to study the properties of the calcium pump of SR fragments isolated from rabbit skeletal muscles after denervation.

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