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THE 60S RIBOSOMAL SUBUNIT IS ALTERED IN THE SKELETAL MUSCLE OF DYSTROPHIC HAMSTERS

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SUMMARY: Polysomes from the skeletal muscle of normal and dystrophic hamsters were dissociated into ribosomal subunits by treatment with puromycin and the subunits from both strains were reassociated in all possible combinations. When their protein synthesis activity was assayed in a poly(U)-directed cell-free system at a low magnesium concentration, the reassociated ribosomes from dystrophic hamsters were less active than the ribosomes from control animals. The ribosomal defect is a property of the 60S subunit and is due to a ribosomal component rather than to abnormal binding of a non-ribosomal protein.

The biochemical basis for human Duchenne muscular dystrophy is still unidentified, in spite of intensive investigations (reviewed in 1). A variety of animal models have been investigated to give further insight into the disease and, among them, the Syrian hamster suffering from hereditary myopathy has proved to be a valuable model (2). Protein synthesis is known to be impaired in dystrophic hamsters (3-7). We reported in a previous study that polysomes from the skeletal muscle of dystrophic hamsters that had been purified by washing with a moderate ionic strength buffer differ from normal ones: when assayed in a cell-free system, they are less active than normal ones at low magnesium concentrations but more active at high magnesium concentrations (8). The aim of the present study is to identify the ribosomal subunit which is defective in dystrophic hamsters. To this end, ribosomes

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Abbreviations: Hepes, N-2-hydroxyethylpiperazine - N'-2-ethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid.

Definition: A_{260} unit, the quantity of material in 1 ml of a solution having an absorbance of 1 at 260 nm when measured in a 1-cm pathlength-cell.

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from the skeletal muscle of healthy and dystrophic hamsters were dissociated into their subunits and reconstituted to give hybrid ribosomes.

MATERIAL AND METHODS

Animals. Male Syrian hamsters with a genetic myopathy (CHF 14.6) were obtained from Canadian Hybrid Farm, Nova Scotia, and unrelated healthy Syrian hamsters (Lakeview strain) were obtained from a commercial breeding farm (Canadian Breeding Farm and Laboratories, Saint-Constant, Quebec). Fed animals were killed by decapitation at 30 days of age.

Preparation of ribosomal subunits. Polysomes were obtained from the skeletal muscle (hindleg) of normal and dystrophic hamsters by differential centrifugation and purification through a 50% (w/v) sucrose cushion in the preparation buffer I (50 mM Hepes, 250 mM potassium acetate, 15 mM magnesium acetate, 0.5 mM EDTA, 5 mM dithiothreitol, 0.1 mM PMSF, pH 7.8) as described previously (8), or in the preparation buffer II (same as buffer I except that the concentration in potassium acetate has been raised to 500 mM). Ribosomal subunits were obtained by treatment with puromycin, according to a procedure adapted from that of Blobel and Sabatini (9): polysomes were incubated with 0.5 mM puromycin and 0.1 mM GTP for 30 min at 37°C in the dissociation buffer (50 mM Hepes, 250 mM potassium acetate, 1 mM magnesium acetate, 5 mM dithiothreitol, pH 7.8). Subunits were separated by centrifugation through a linear 10 - 30% (w/v) sucrose density gradient in the dissociation buffer in which the concentration in magnesium acetate had been raised to 3 mM. Centrifugation was for 90 min at 45 000 rev./min in a Beckman SW 50.1 rotor. It was carried out at 25°C to avoid dimerization of subunits. The fractions containing the subunits were pooled, their concentration in magnesium acetate was raised to 20 mM and the subunits were pelleted by centrifugation at 4°C for 4 h at 65 000 rev./min in the 70.1 Ti rotor of a Beckman ultracentrifuge. The pellet was resuspended in the conservation buffer (the same as the dissociation buffer except that potassium and magnesium acetate are 100 and 4 mM, respectively) and stored in aliquots at -70°C. The purity of the subunits was estimated by centrifugation through a sucrose gradient. The conditions of centrifugation were as described above for the fractionation of subunits.

Poly(U)-directed polyphenylalanine synthesis. The protein synthesis activity of ribosomal subunits was assayed in 100 µl of a mixture containing: 2 mM ATP; 0.5 mM GTP; 8 mM creatine phosphate; 4 µg creatine phosphokinase; 50 mM Hepes; 170 mM potassium acetate; 7 mM (suboptimal) magnesium acetate; 5 mM dithiothreitol; 15 µg wheat germ tRNA; 100 µg poly(U); 0.01 mM [^{3}H] phenylalanine (New England Nuclear, 5 Ci/mmol); an optimized amount of the high-speed supernatant of a wheat germ extract as a source of soluble factors, about 400 µg protein; 0.1 A_{260} unit of 40S and 0.2 A_{260} unit of 60S; the final pH was 7.8. The high-speed supernatant of the wheat germ extract was prepared as described previously (8). The incorporation was for 60 min at 30°C. The reaction was stopped by the addition of 5% trichloroacetic acid and the samples were processed and counted as described (8). The efficiency of counting was 18%.

RESULTS AND DISCUSSION

An example of the fractionation through a sucrose gradient of ribosomal subunits from the skeletal muscle of dystrophic hamsters is

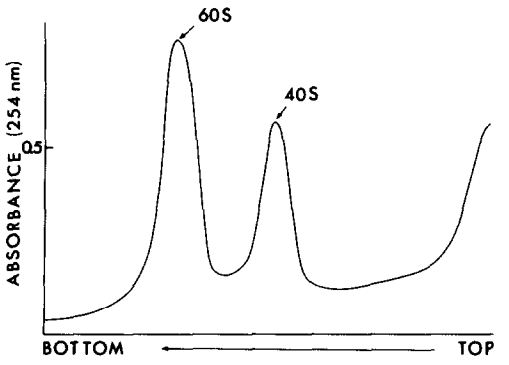

Figure 1. Fractionation of ribosomal subunits from the skeletal muscle of dystrophic hamsters. About 2 A_{260} units of dissociated ribosomes in 0.2 ml of the dissociation buffer were applied to a 5 ml linear 10 - 30% (w/v) sucrose gradient. Centrifugation was at 25°C for 90 min at 45 000 rev./min in a Beckman SW-50.1 rotor.

given in Fig. 1. Polysomes were obtained after centrifugation through a buffer of moderate ionic strength (preparation buffer I). Fig. 1 shows that the polysomes are completely dissociated into undegraded ribosomal subunits after incubation in the presence of puromycin. An identical pattern of fractionation was obtained with polysomes from dystrophic hamsters that had been washed through a buffer of high ionic strength (preparation buffer II) or with polysomes from control hamsters. The purity of the isolated ribosomal subunits was estimated by sucrose gradient analysis. Only one peak was observed with the 40S subunit while about 95% of the 60S subunit (as assessed by planimetry) sedimented as a single peak, the remaining 5% being contaminant 40S subunit (data not shown).

Subunits prepared from normal and dystrophic hamsters were recombined and the protein synthesis ability of homologous and hybrid ribosomes was assayed in a poly(U)-directed cell-free system at a low magnesium concentration (7 mM). The data of Table 1 show that reassociated homologous dystrophic ribosomes are less active than normal ones. This difference was observed with ribosomes obtained from polysomes purified by centrifugation through a buffer of either moderate or high ionic strength. In a study with ribosomes washed with a moderate

TABLE 1

Poly(U)-directed incorporation of phenylalanine at a low magnesium concentration with reassociated ribosomal subunits from the skeletal muscle of control and dystrophic hamsters.

Origin of the subunits 40S	60S	Type of purification	Incorporation of [^{3}H] phenylalanine (cpm)
A. Homologous association			
N	N	moderately-washed	147 126 ± 20 133
D	D	subunits	76 907 ± 11 165
N	N	highly-washed	137 015 ± 21 924
D	D	subunits	67 588 ± 10 273
B. Heterologous association			
N	D	moderately-washed	84 849 ± 12 573
D	N	subunits	156 233 ± 22 092
N	D	highly-washed	69 952 ± 10 045
D	N	subunits	126 544 ± 18 843

N is for normal control hamsters and D for dystrophic hamsters; the 40S and 60S ribosomal subunits were reassociated in a mass ratio of 1 to 2 and their protein synthesis activity was assayed at a 7 mM concentration in magnesium acetate. Moderately-washed subunits and highly-washed subunits originated from polysomes that had been spun down through 250 mM and 500 mM potassium acetate, respectively, during preparation. Results are corrected for the incorporation in the absence of ribosomes which is less than 2% of the incorporation in the presence of ribosomes. Experimental data are the means ± standard deviation of five independent determinations.

ionic strength buffer, we previously reported that there is a ribosomal defect in the skeletal muscle of dystrophic hamsters (8). The present results demonstrate that this defect is caused by a ribosomal component and cannot be ascribed to the binding of an additional non-ribosomal protein. Indeed, an extraneous protein would have been stripped off when the polysomes were washed with the high ionic strength preparation buffer (10,11).

Furthermore, the assays with hybrid ribosomes allow identification of the altered subunit in dystrophic hamsters. As shown in Table 1, hybrid ribosomes, with the 40S subunit originating from the dystrophic animals, incorporated phenylalanine as well as homologous ribosomes from control hamsters, indicating that the 40S subunit of dystrophic hamsters is not responsible for the ribosomal defect. In contrast, hybrid ribosomes with the 60S subunit originating from dystrophic hamsters were less active than the reassociated control ribosomes and their

activity was equal to that of homologous dystrophic ribosomes. This shows that the ribosomal defect in dystrophic hamsters is a property of the 60S subunit.

The results in the present study thus unambiguously demonstrate the occurrence of a ribosomal defect in dystrophic hamsters. A ribosomal defect has also been observed in other animal models of muscular dystrophy such as the chicken (12) and the mouse (13) as well as in humans suffering from Duchenne muscular dystrophy (14-15). This indicates that a ribosomal defect might play an important part in the development of the disease. A membrane defect is generally considered to play a key role in the pathogenesis of muscular dystrophies (reviewed in 1, 16) and we propose that there might be a relationship between this membrane defect and the ribosomal defect that we have observed. Polysomes are bound to the endoplasmic reticulum through their 60S ribosomal subunit (17) and these bound-polysomes synthesize several membrane proteins (reviewed in 18). We suggest that the defect in the 60S subunit in dystrophic hamsters might specifically affect the interaction of polysomes with the endoplasmic reticulum and thus perturb the synthesis of several membrane proteins. This is presently under investigation.

Ribosomes from the skeletal muscle of dystrophic hamsters were found previously to be less active than normal ones at low magnesium concentrations but more active at high magnesium concentrations (8). The same type of response to changes in magnesium concentration has also been observed with ribosomes from diabetic rats (19). Our recent finding that the 60S subunit is altered in ribosomes from dystrophic hamsters further reinforces this similarity, since it is also the 60S subunit that was found to be altered in ribosomes from diabetic rats (20). Moreover, the rate of protein synthesis in the skeletal muscle from dystrophic hamsters exhibits a decreased responsiveness to insulin

(5). It will be interesting to further investigate the similarities in the ribosomal defect of dystrophic and diabetic animals.

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