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Cytochrome *c* protein-synthesis rates and mRNA contents during atrophy and recovery in skeletal muscle

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It is known that immobilization of the rat hindlimb by plaster casting leads to muscle atrophy and loss of muscle protein. In the present study, immobilization of the rat hindlimb for 6 h resulted in a significant 27% decrease in the absolute rate of cytochrome *c* synthesis in the red quadriceps muscle, without any change in the relative amount of cytochrome *c* mRNA. Cytochrome *c* mRNA in normal red quadriceps muscle was observed to be of four different lengths (1400, 1050, 650 and 580 bases). After 7 days of immobilization, the absolute rate of cytochrome *c* synthesis remained depressed and cytochrome *c* mRNA decreased by 40%; each of the cytochrome *c* mRNAs decreased, with a preferential disappearance of the 1050- and 1400-base lengths. Immobilization was ended on day 7, and the atrophied muscle was allowed to recover. At day 4 of recovery, the absolute rate of cytochrome *c* synthesis was 92% higher and the amount of cytochrome *c* mRNA had returned to control values. The abundances of the 1050- and 1400-base cytochrome *c* mRNAs had increased more than the shorter cytochrome *c* mRNAs, so that they were higher than control values. It appears that acute decreases in contractile activity of the red quadriceps muscle alter cytochrome *c* synthesis rates via translational or post-translational mechanisms, whereas chronic periods of modified contractile activity alter its synthesis rate via pre-translational mechanisms.

INTRODUCTION

Cytochrome *c* functions as one of the proteins in the chain of electron carriers in the mitochondrial inner membrane and transfers electrons from substrate molecules to oxygen (DePierre & Ernster, 1977). A positive feedback loop appears to exist between cytochrome *c* concentration and the amount of energy transformation in skeletal muscle. Limb immobilization, which decreases contractile activity and ATP formation, and the regrowth of atrophied muscle after ending immobilization, are models to down-regulate and to up-regulate, respectively, cytochrome *c* content in adult skeletal muscle (Booth, 1977, 1978). One approach to understanding cytochrome *c* gene expression in these models would be to correlate its synthesis rates with the amounts of its mRNA. Others have employed such an experimental design. For example, Everett *et al.* (1984) observed that the relative synthesis rates of α-myosin heavy chain and β-myosin heavy chain reflected the relative abundances of the α- and β-myosin heavy-chain mRNAs in the hearts of hypo- and hyper-thyroid rabbits. They interpreted these data to mean that thyroid-hormone regulation of cardiac myosin synthesis occurs pre-translationally.

We previously reported (Watson *et al.*, 1984) that the rate of α-actin synthesis decreased by 65% while the α-actin mRNA content was unchanged in skeletal muscle of adult rats during the first 6 h of hindlimb immobilization. This suggested that the fall in α-actin synthesis occurred independently of its mRNA content. At present it is unknown how the amount of mRNA coding for the mitochondrial protein cytochrome *c* in skeletal muscle responds during and after immobilization.

The aim of the present study was to determine whether cytochrome *c* mRNA content changed in parallel with the rate of cytochrome *c* synthesis, under conditions which cause rapid changes in cytochrome *c* content in adult-rat skeletal muscle.

MATERIALS AND METHODS

Animals

Female Sprague–Dawley (Charles River Breeding Laboratories) rats weighing 200–300 g were used. Animals were housed in temperature-controlled quarters (21 °C) with a 12 h-light/12 h-dark cycle and were provided with water and lab chow *ad libitum*. Immobilization of both hindlimbs was accomplished under diethyl ether anaesthesia as described by Booth (1977). Owing to the size of the muscle, RNA concentration (Fleck & Munro, 1962) and cytochrome *c* protein concentrations (Williams & Thorp, 1969) were measured on one set of rats, leucyl-tRNA and cytochrome *c* specific radioactivities were obtained with a second set of rats, and RNA extraction and cytochrome *c* mRNA determinations were made with a third set of rats.

Infusion protocol

At 2 days before the infusion, a chronic Silastic catheter was placed into the external jugular vein as previously described (Booth & Seider, 1979). Just before infusion animals were anaesthetized with ether and connected via the chronic catheter to an infusion swivel and pump. Within 10 min of recovery from ether, rats were awake and were able to move freely through 360° via the swivel in the infusion chamber. Continuous infusion for 5 h with L-[^{3}H]leucine (1 mCi/h) in Krebs–Ringer lactate solution containing 5% glucose (2 ml/h) began shortly after rats were placed into the infusion chambers. During the final 10 min of the 5 h infusion,

animals were anaesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt.), plaster casts were removed (if present), and while the infusion continued the quadriceps muscle was trimmed of superficial white muscle fibres *in situ*, and the remaining red quadriceps muscle was excised, and frozen quickly with Wollenberger tongs, which had been cooled to the temperature of liquid N_2. Muscles were stored at −80 °C until assayed. Muscles were powdered with a mortar and pestle at the temperature of liquid N_2 before use. The red quadriceps muscle excised first was used for assay of the specific radioactivity of leucyl-tRNA and the red quadriceps muscle removed next was used for purification of cytochrome *c*.

Specific radioactivity of leucyl-tRNA

tRNA was extracted from the powdered red quadriceps muscle by the method of Allen *et al.* (1969), as modified by Everett *et al.* (1981). The final RNA precipitate from the RNA extraction was dissolved in 0.5 ml of 25 mM-$NaHCO_3$ (pH 10.5) and leucyl-tRNA was deacylated by incubating the solution at 37 °C for 90 min. Analysis of the specific radioactivity of leucine freed from tRNA was performed by application of the ultramicro-method described by Airhart *et al.* (1979).

Cytochrome *c* isolation, purification and hydrolysis

Cytochrome *c* was extracted as described by Margoliash & Walasek (1967), with the modification that centrifugation at 28000 *g* for 15 min was substituted for filtration to remove protein precipitates (Booth & Holloszy, 1977). Purity of cytochrome *c* extracted from each muscle was verified by electrophoresis of a small portion of the sample on a denaturing SDS/urea/polyacrylamide gel for low-M_r proteins. Bromophenol Blue or silver was used to stain for proteins on gels. Purified cytochrome *c* (Sigma Chemical Co.) was used as a standard. If the samples exhibited a contaminating protein after purification and electrophoresis, they underwent a second $(NH_4)_2SO_4$ precipitation, ion-exchange chromatography and electrophoresis, and thereafter showed only a single band corresponding to the M_r of purified cytochrome *c* (results not shown).

The purified cytochrome *c* obtained from muscle was hydrolysed in 6 M-HCl *in vacuo* at 110 °C overnight, and the hydrolysate was evaporated to dryness in a Savant Speedvac apparatus. The specific radioactivity of leucine in the hydrolysed cytochrome *c* was determined by the ultramicro-method of Airhart *et al.* (1979).

Protein-synthesis calculations

The rate of cytochrome *c* synthesis was estimated by using the following equation (Garlick *et al.*, 1973):

$$\frac{S_B}{S_i} = \frac{\lambda_i}{\lambda_i - k_s} \times \frac{(1 - e^{-k_s t})}{(1 - e^{-\lambda_i t})} - \frac{k_s}{(\lambda_i - k_s)}$$

where S_B is the specific radioactivity of leucine in cytochrome *c*, S_i is the specific radioactivity of leucine on tRNA, λ_i is the first-order rate constant for the rise in the specific radioactivity of leucine in plasma (53 day^{-1}; results not shown), k_s is the fractional rate of cytochrome *c* synthesis, and *t* is the infusion time of L-[^{3}H]leucine (0.208 day). Waterlow & Stephen (1968) and Garlick *et al.* (1973) indicate that accurate determination of the value of λ_i is unnecessary, since quite large variations in this value result in small variations in the value of k_s. Rannels *et al.* (1982) claim that precedence exists in which the specific radioactivity of tRNA is less than intracellular or extracellular values, so that usage of the latter would underestimate the actual (tRNA-based) rate of synthesis. As it was not our purpose to test this claim, we employed tRNA specific radioactivity.

Extraction of total RNA from red quadriceps muscle

RNA was extracted from powdered red quadriceps muscle by the LiCl/urea method (procedure C) of Auffray & Rougeon (1979). Purity of the RNA preparations was checked by the A_{260}/A_{280} ratio (Maniatis *et al.*, 1982). No contamination was observed in any of the preparations (results not shown).

Preparation of the cytochrome *c* mRNA probe

pRC4, containing a 0.96-kilobase insert of the rat somatic cytochrome *c* gene, was kindly supplied by Dr. Ray Wu (see Scarpulla *et al.*, 1981). The coding strand of the genomic cytochrome *c* insert of pRC4 was subcloned into bacteriophage M13mp18. A partially double-stranded ^{32}P-labelled genomic probe specific for cytochrome *c* mRNA was obtained as described by Hu & Messing (1982). In brief, a 17-mer hybridization primer (Bethesda Research Laboratories) was annealed near the 3′ end of the genomic cytochrome *c* insert. Synthesis of a radiolabelled second strand, which occurred away from the insert, was initiated by adding 50 μM each of dGTP, dCTP and TTP, 25μCi of [α-^{32}P]dATP (Amersham) and 1–2 units (1 unit = 10 nmol/30 min) of the Klenow fragment of DNA polymerase. The reaction was allowed to proceed for 90 min at 20 °C and terminated by the addition of 10 M-ammonium acetate to 2.5 M, 10 μg of yeast tRNA and 2 vol. of ethanol. After precipitation at −70 °C for 15 min, the radiolabelled probe was centrifuged, and the pellet dried and dissolved in 250 μl of water. A 1 μl sample was counted for radioactivity to determine the specific radioactivity (routinely 1×10^7–5×10^7 c.p.m./μg of DNA).

Preparation for dot hybridization

Nitrocellulose filters were prepared as described by Watson *et al.* (1984). Extracted total RNA was denatured by heating to 65 °C for 15 min in 2.2 M-formaldehyde/6 × SSC, pH 7 (1 × SSC = 0.15 M-NaCl/0.015 M-sodium citrate), followed by quick cooling on ice. Known quantities of the denaturated RNA samples were dotted on 0.45 μm (pore size) nitrocellulose paper (Schleicher & Schuell) using a Schleicher & Schuell minifold apparatus. The nitrocellulose paper was then baked at 80 °C *in vacuo* for 90 min.

Pre-hybridization and hybridization of cytochrome *c* mRNA

The baked nitrocellulose filter was pre-hybridized in a heat-sealed plastic bag at 43 °C for 1–2 h in a 10 ml solution containing 5 × Denhardt solution (Denhardt, 1966), 5 × SSC, 50% (v/v) formamide, 50 mM-sodium phosphate buffer, pH 6.8, 1% glycine, 250 μg of sonicated denatured salmon sperm DNA/ml and 0.1% SDS. The pre-hybridization solution was removed and replaced by 10 ml of a hybridization solution containing 1 × Denhardt solution, 5 × SSC, 50% formamide,

20 mM-phosphate buffer, pH 6.8, 100 μg of sonicated denatured salmon sperm DNA/ml, 0.1% SDS and 1×10^7–5×10^7 c.p.m./μg (100 ng) of the ^{32}P-labelled M13 probe. The bag was resealed and placed into a gyrorotary water bath at 43 °C for 96 h.

After hybridization the nitrocellulose filter was washed four times at room temperature (15 min each) with 2 × SSCP (1 × SSCP = 120 mM-NaCl/15 mM-sodium citrate/13 mM-potassium phosphate/1 mM-EDTA, pH 7.2) and 0.1% Sarkosyl. The nitrocellulose filter was washed four times (30 min each) at 50 °C with 0.2 × SSCP/0.1% Sarkosyl. After the filter was air-dried, it was exposed at −80 °C to Kodax X-RMAT film in the presence of an intensifying screen for 24–48 h. A LKB laser-beam densitometer was used to estimate the intensity of the hybridization product on the film. A correlation of 0.99 with direct radioactivity counting of the hybridization product was obtained for densitometry.

For Northern-blot analysis, total RNA was denatured as described by Maniatis *et al.* (1982) and electrophoresed in a 2% agarose gel containing 2.2 M-formaldehyde. It was transferred to a nitrocellulose filter overnight. The Northern blot was hybridized to the cytochrome *c* insert of ^{32}P-labelled M13mp18 under the same conditions as described for the dot-blots (Fig. 3*a*).

Data analysis

$P < 0.05$ was set as being statistically significant when Student's *t* test or ANOVA was used. The amounts of hybridizable cytochrome *c* mRNA were expressed in scanner units or in c.p.m. and plotted against RNA concentration. The slope and co-ordinates of each line were then determined by least-squares linear regression, with correlation coefficients greater than 0.97. A comparison of these slopes provides an estimate of the changes in the amounts of cytochrome *c* mRNA (Dobner *et al.*, 1981; Landefeld *et al.*, 1985).

RESULTS AND DISCUSSION

Cytochrome *c* content in the red quadriceps muscle decreased by approx. 40% and muscle size decreased by 22% during the 7-day period of hindlimb immobilization (Table 1). Thus cytochrome *c* concentration decreased (Table 1), forcing the expression of cytochrome *c* synthesis rates in two different manners. Cytochrome *c* synthesis is expressed as a fraction of existing cytochrome *c* that is synthesized and as an absolute rate per g of tissue.

The fractional synthesis rate of mixed proteins in skeletal muscle is decreased by 35% throughout a 7-day period of limb immobilization (Tucker *et al.* 1981), but the fractional synthesis rate of cytochrome *c* exhibits a different pattern. After an initial 33% decline up to 6 h of immobilization, there is no difference in the fractional synthesis rate of cytochrome *c* between controls and 7 days of immobilization (Table 2). Owing to the fall in cytochrome *c* concentration, absolute rates of cyto-

Table 1. Cytochrome *c* concentration and content, and RNA concentration in the red quadriceps muscle of control rats, of rats during hindlimb immobilization, and of rats recovering from the 7-day period of immobilization

Values are means ± S.E.M.: *$P < 0.05$ for difference from control by Student's *t* test. The number of observations/group is four. For further details see the text. N.D., not determined.

Parameter	Control	Immobilized for		Immobilized for 7 days plus recovery for:		
		6 h	7 days	6 h	2 days	4 days
Muscle wt. (g)	2.93 ± 0.03	N.D.	2.29 ± 0.10*	2.28 ± 0.07*	2.36 ± 0.10*	2.13 ± 0.15*
Cytochrome *c*						
Concn. (nmol/g wet wt. of muscle)	9.0 ± 0.2	N.D.	6.8 ± 0.4*	7.0 ± 0.5*	7.6 ± 0.6	8.5 ± 0.1
Content (nmol/muscle)	26.3 ± 0.7	N.D.	15.7 ± 1.5*	15.9 ± 0.8*	18.0 ± 1.9*	18.1 ± 1.0*
RNA						
Concn. (mg/g of muscle)	1.1 ± 0.26	N.D.	1.1 ± 0.05	1.1 ± 0.16	1.2 ± 0.22	1.0 ± 0.01

Table 2. Specific radioactivity of leucyl-tRNA and cytochrome *c* synthesis rate in the red quadriceps muscle of control rats, of rats during hindlimb immobilization, and of rats recovering from the 7-day period of immobilization

Values are means ± S.E.M., for the numbers of rats in parentheses: *$P < 0.05$ for the difference from the control group (Student's *t* test). The estimate of the amount of cytochrome *c* synthesized per g of muscle was obtained by the multiplication of k_s by the average cytochrome *c* concentration in the red quadriceps muscle of a separate group of rats, which prevents expression of data with S.E.M.

Parameter	Control (13)	Immobilized for:		Immobilized for 7 days plus recovery for:		
		6 h (6)	7 days (5)	6 h (3)	2 days (4)	4 days (3)
Specific radioactivity of leucyl-tRNA (d.p.m./pmol)	14 ± 1	14 ± 1	12 ± 2	16 ± 2	10 ± 3	17 ± 3
Cytochrome *c* synthesis rate						
k_s (%/day)	4.5 ± 0.3	3.0 ± 0.3*	4.4 ± 0.4	4.9 ± 0.5	3.6 ± 0.3	8.3 ± 0.4*
(nmol/day per g)	0.37	0.27	0.30	0.34	0.27	0.71

chrome *c* synthesis per g of atrophied muscle remained depressed at day 7 of limb immobilization.

By using modifications of procedures described by Laurent *et al.* (1978) and by Ashford & Pain (1986), estimations of the relative contributions of synthesis and degradation rates to the loss of cytochrome *c* can be made. From the content and absolute synthesis rate at 6 h and 7 days of immobilization, we estimated that most (70%) of the decrease in cytochrome *c* content was due to increased degradation and that a decline in cytochrome *c* synthesis played a minor role (30%).

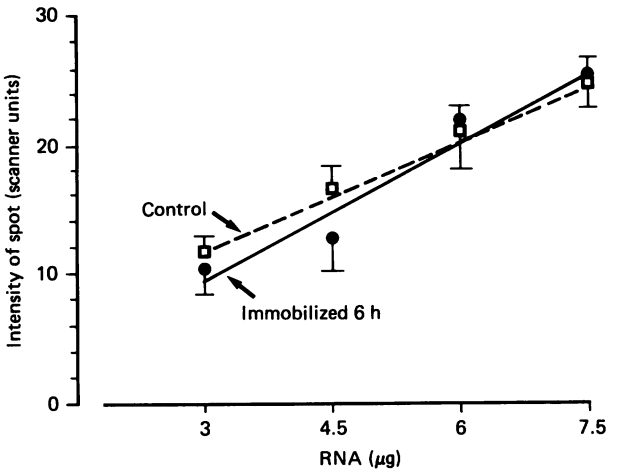

Fig. 1. Dot-blot hybridization analysis of relative cytochrome *c* mRNA amounts in red quadriceps muscle after 6 h of immobilization

The indicated amounts of total RNA, prepared by procedure C of Auffray & Rougeon (1979) from the red quadriceps muscle of either control rats (□) or rats having both hindlimbs immobilized for 6 h (●), were fixed to nitrocellulose paper and hybridized for 96 h at 43 °C to a genomic cytochrome *c* insert in M13mp18, which had a ^{32}P-labelled second strand generated from a hybridization probe primer annealed to the 3′ end of the genomic cytochrome *c* insert. Autoradiography was then performed. A densitometer was used to measure the intensity of the resultant spots (see the Materials and methods section). Values are means ± S.E.M; $n = 4$. Student's *t* test was used to test for statistical difference. Similar results were obtained in two additional experiments (results not shown).

Absolute and fractional rates of cytochrome *c* synthesis did not exceed control values during the first 2 days of recovery from the prior immobilization (Table 2). However, both rates of cytochrome *c* synthesis doubled by day 4 of recovery (Table 2). From day-4 recovery data we predict that most of the increase in cytochrome *c* protein during recovery will be due to an increase in its synthesis rate.

The findings discussed above and shown in Fig. 1 indicate that translational or post-translational mechanisms are likely to be responsible for the acute changes in cytochrome *c* synthesis when contractile activity is decreased. The observed 27% decrease in the absolute rate of cytochrome *c* synthesis in the red quadriceps muscle which occurred during the first 6 h of hindlimb immobilization (Table 3) was not associated with any change in amount of cytochrome *c* mRNA (Fig. 1). This finding parallels previous work on α-actin synthesis and its mRNA by Watson *et al.* (1984), which strengthens the possibility that the acute fall in mixed protein synthesis during immobilization, as reported by Tucker *et al.* (1981), occurs by a translational mechanism. Since we analysed mature protein, it is not possible at this time to differentiate between translational or post-translational mechanisms as being rate-limiting for the decrease in cytochrome *c* synthesis during the first few hours of limb immobilization.

Nevertheless, after more chronic periods of altered contractile activity, pre-translational mechanisms may be the controlling event in the synthesis of cytochrome *c*. For instance, after 7 days of hindlimb immobilization, the absolute rate of cytochrome *c* synthesis was 81% of control and cytochrome *c* mRNA was 60% of control values (Fig. 2, Table 2). After the 7-day period of limb immobilization was ended, cytochrome *c* synthesis rates did not exceed control values for the first 2 days, after which it increased more than 2-fold over the following 2 days (days 3 and 4 of recovery). One possible explanation for these observations is that the translational control of cytochrome *c* synthesis is limited by its mRNA concentration and that the doubling of cytochrome *c* synthesis can only occur when the amount of cytochrome *c* mRNA increases (Table 3, Fig. 2).

In the present study, cytochrome *c* protein contents are in non-steady-state conditions. Under these circumstances, we observed that there were changes in the relative proportion of each cytochrome *c* mRNA species

Table 3. Summary of changes in cytochrome *c* synthesis rate and in cytochrome *c* mRNAs in fast-twitch red skeletal muscle when contractile activity is altered

Cytochrome *c* synthesis rates were expressed as nmol/day per g.

Hindlimb immobilized for	Cytochrome *c* synthesis rate (% of control)	Total cytochrome *c* mRNA (% of control)	Relative profile of cytochrome *c* mRNAs to one another	
			1400 bases	1050 bases
6 h	73	100	No change	No change
7 days	81	60	↓	↓
7 days followed by:				
6 h recovery	92	68	↓	↓
2 days recovery	73	76	No change	No change
4 days recovery	192	126	↑	↑

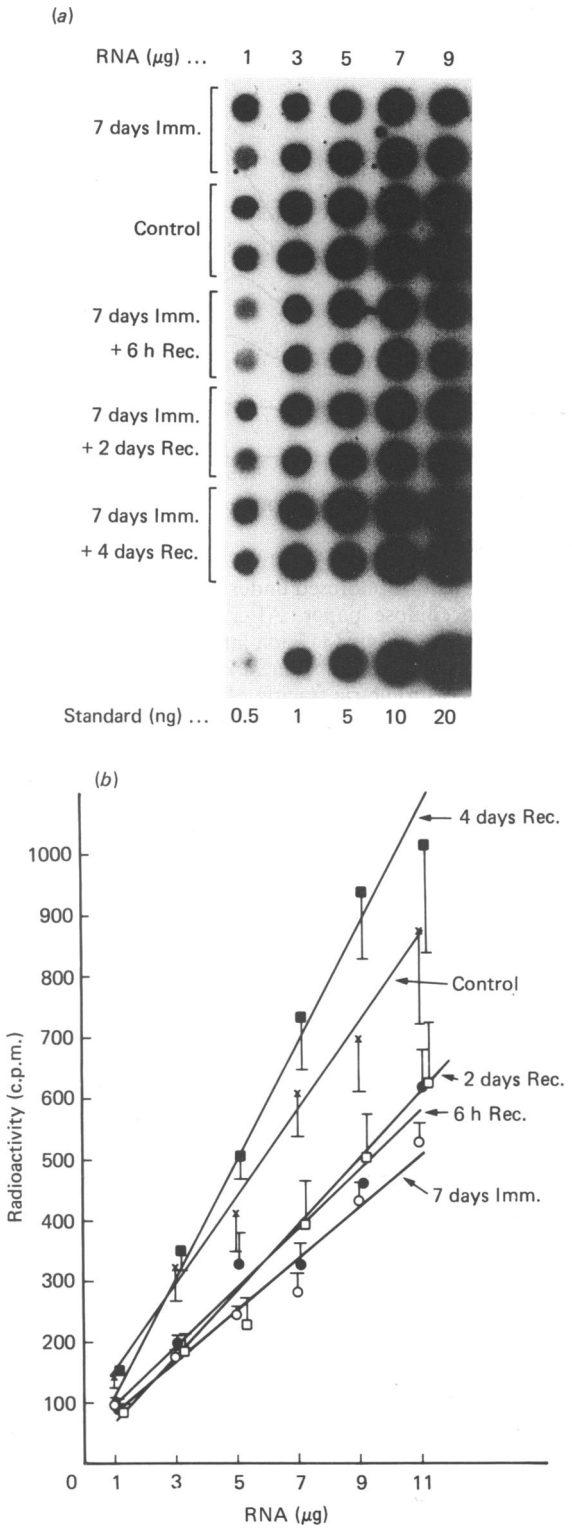

Fig. 2. Dot-blot hybridization analysis of relative cytochrome *c* mRNA amounts in the red quadriceps muscle of the following groups of rats: control and 7-day-immobilized limbs at 0 h, 6 h, 2 days or 4 days of recovery

(*a*) The amounts of total RNA dotted are given in the top row; see Fig. 1 legend for hybridization details. Each row of dots represents data from individual rats. The bottom row of dots was DNA complementary to the M13mp18 probe (M13mp19 with the genomic cytochrome *c* insert reversed) used to determine the relative cytochrome *c* mRNA concentrations in each of the samples. Hybridization of total RNA to a ^{32}P-labelled wild-type M13mp18 resulted in no detectable hybrid (results not shown). Abbreviations: Imm., immobilized; Rec., recovery. (*b*) Results from liquid-scintillation spectrophotometry of data shown in (*a*) plus data from an additional two rats/group. Hindlimb immobilization for 7 days significantly decreased cytochrome *c* mRNA, by 40% from control values. Neither 6 h nor 2 days recovery after a previous 7-day immobilization significantly altered cytochrome *c* mRNA from values observed during day 7 of immobilization. However, 4 days after the end of 7 days of immobilization, cytochrome *c* mRNA was significantly higher than after the 7-day immobilization, but not significantly higher than control. ANOVA with a Tukey's multiple-range test was used to test for significant differences.

(Fig. 3). The preferential loss of the 1400- and 1050-base cytochrome *c* mRNAs was noted in the red quadriceps muscle after 7 days of decreased contractile activity (Fig. 3). The disappearance of the 1400-base cytochrome *c* mRNA during day 7 of hindlimb immobilization is remarkable in that the absolute rate of cytochrome *c* synthesis was not further decreased below normal at this time (see Table 3).

On the other hand, during recovery from immobilization, the greater increase in both the 1400- and 1050-base cytochrome *c* mRNAs at day 4 was associated with a large increase in the synthesis rate of cytochrome *c* protein (Fig. 3, Table 3). Whether preferential expression of specific cytochrome *c* mRNA species correlates with changes in the synthesis rate of cytochrome *c* during non-steady-state conditions should be studied in future.

Previously, at least three different cytochrome *c* mRNAs (1400, 1100 and 700 bases) were detected from polyadenylated mRNA in four tissues (heart, liver, kidney and testis) of the rat (Scarpulla & Wu, 1983). Densitometry of the autoradiograph from the electrophoresed RNA showed that approximately equimolar amounts of each mRNA species were present, and this was interpreted as co-ordinate regulation of the steady-state amount of each mRNA species.

Scarpulla & Wu (1983) suggested that the size differences among the three cytochrome *c* mRNAs could be accounted for by length heterogeneity in their 3′ non-coding regions. They noted that there are five potential termination sites (565, 580, 840, 975 and 1190 bases) for the 3′ ends of cytochrome *c* mRNA molecules, as determined by the technique of S1 mapping. In addition to the three cytochrome *c* mRNA species found by Scarpulla & Wu (1983), we observed a fourth species of cytochrome *c* mRNA (580 bases) obtained by Northern hybridization analysis of total RNA from adult fast-twitch skeletal muscle (Fig. 3). The fourth cytochrome *c* mRNA molecule could be the product of the shortest 3′-end termination site identified by Scarpulla & Wu (1983).

There appear to be a variety of ways to produce mRNAs in skeletal muscle. For example, a single cytochrome *c* protein appears to be synthesized from multiple-length cytochrome *c* mRNAs produced by a single gene. On the other hand, a number of distinct isoforms exist for almost every contractile protein in muscle, and these are produced by a number of mechanisms. For instance, the existence of multigene

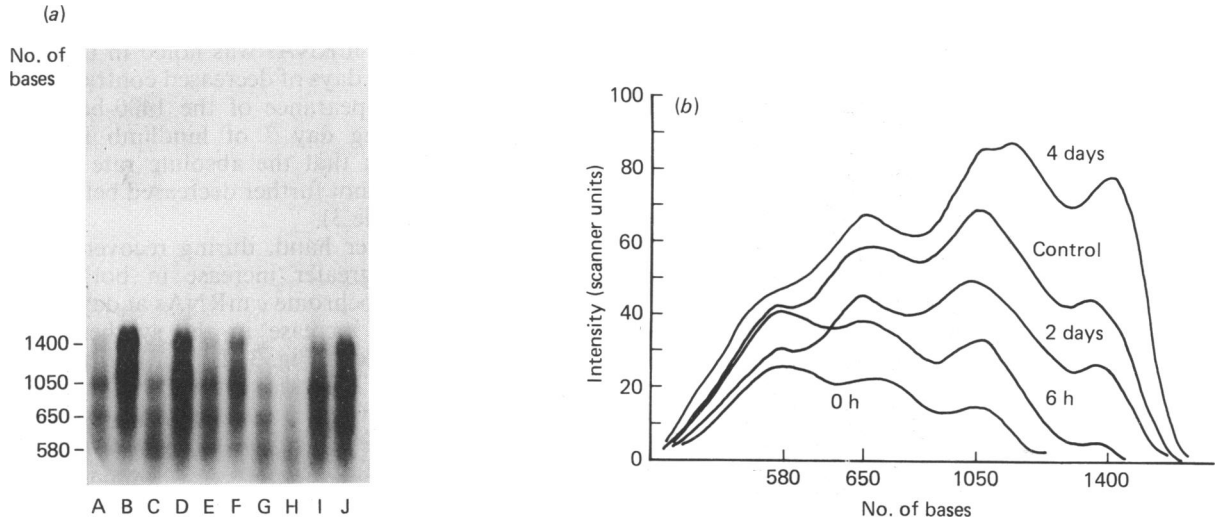

Fig. 3. Northern-blot analysis of cytochrome *c* mRNA from the red quadriceps muscle

(*a*) Autoradiograph: 15 μg of total RNA from five experimental groups of rats was electrophoresed under denaturing conditions on 2.0% agarose gel containing 2.2 M-formaldehyde and blotted on to nitrocellulose paper. After transfer of RNA to nitrocellulose, RNA was hybridized to a ^{32}P-labelled genomic cytochrome *c* insert in M13mp18. Each lane represents a sample from an individual rat. Recovery groups are from a prior 7-day period of limb immobilization. Lanes: I, J, control; G, H, 0 h recovery; A, C, 6 h recovery; E, F, 2 days recovery; B, D, 4 days recovery. (*b*) Densitometric tracing of data in (*a*).

families in which specific contractile protein isoforms are generated from distinct genes has been demonstrated (Buckingham, 1985). It has also been noted that a single gene can give rise to several distinct contractile protein isoforms via differential mRNA splicing of single gene products to produce more than one mRNA species (Medford *et al.*, 1984; Cooper & Ordahl, 1985; Hastings *et al.*, 1985).

The present studies show that cytochrome *c* gene expression is likely to be regulated by a number of processes. The loss of cytochrome *c* protein early in muscle atrophy may occur by a translational or post-translational modification of protein synthesis and by increased cytochrome *c* degradation. However, protein synthesis probably plays the predominant role in the regrowth of the cytochrome *c* poll after ending limb immobilization. Pre-translational controls also appear to play an important role in this process. Many mechanisms appear to underlie the adaptation of cytochrome *c* content to alterations in contractile activity.

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