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VARIATIONS IN CONTRACTILE PROPERTIES OF RABBIT SINGLE MUSCLE FIBRES IN RELATION TO TROPONIN T ISOFORMS AND MYOSIN LIGHT CHAINS

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SUMMARY

1. The maximal velocity of shortening (V_{max}), tension–pCa relationships and the contractile and regulatory protein composition were determined in single, chemically skinned fibres from adult rabbit plantaris muscles.

2. Three groups of fibres were identified based on their protein compositions. One group had exclusively the slow-type myosin heavy chain (MHC) and myosin light chains (LC) and had low velocities. Another group of fibres had mixtures of fast-type and slow-type MHCs and LCs and had intermediate shortening velocities. The third group of fibres had fast-type myosin heavy and light chains and high velocities.

3. The low-velocity fibres had a mean velocity ($\pm$S.E.M.) of $0{\cdot}86 \pm 0{\cdot}03$ muscle lengths/s (ML/s) at 15 °C. The remaining fibres formed a continuum with respect to V_{max} from 1·37 to 3·94 ML/s. These results indicate that a much greater diversity exists among single fibres from adult mammalian skeletal muscle than previously recognized. The intermediate- and high-velocity fibres formed a continuum (from slow to fast) with respect to the amount of myosin light chain 3 (LC3). That is, V_{max} increased with the relative LC3 content in single fibres in the intermediate- and high-velocity groups in a quantitative, statistically significant manner.

4. Three isoforms of fast-type troponin T were identified among the intermediate- and high-velocity fibres. These fibres also contained fast-type troponin C and troponin I. As was the case with the relative LC3 content, these fibres also formed a continuum with respect to the relative proportions of the three isoforms of fast-type troponin T. It appears that different isoforms of troponin T are responsible for a slightly higher Ca^{2+} sensitivity of tension development in the high-velocity fibres compared to the intermediate fibres. The continuum in troponin T isoform composition paralleled an increase in V_{max} among these fibres.

5. The low-velocity fibres had the highest Ca^{2+} sensitivity of the three groups and had exclusively the slow-type isoforms of the regulatory proteins in the troponin complex.

6. The co-ordinated variations in troponin T and LC3 compositions among the

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intermediate- and high-velocity fibres are discussed as a possible means for the further differentiation of the contractile properties of the fibres in these two groups, beyond that provided by myosin heavy chain isoforms alone.

INTRODUCTION

Heterogeneity in the contractile and regulatory protein composition of vertebrate skeletal muscle has been extensively described (see recent reviews by Bandman, 1985; Obinata, 1985; Ohtsuki, Maruyama & Ebashi, 1986; Swynghedauw, 1986; Syrovy, 1987). We have previously shown that the myosin heavy chain (MHC) composition is strongly correlated with the maximal velocity of shortening (V_{max}) of individual fibres from adult rabbit soleus (slow-twitch) muscle (Reiser, Moss, Giulian & Greaser, 1985*a*) and from neonatal rabbit psoas (fast-twitch) muscle (Reiser, Moss, Giulian & Greaser, 1985*b*). Cardiac and skeletal muscle troponin C (TNC) isoforms are clearly associated with differences in the Ca^{2+} sensitivity of tension development in these two tissues as demonstrated by the effects of substituting the TnC of skinned fibres from skeletal muscle with cardiac TnC (Moss, Lauer, Giulian & Greaser, 1986) and vice versa (Babu, Scordilis, Sonnenblick & Gulati, 1987). Thus, the functional significance of different isoforms of several contractile and regulatory protein isoforms of striated muscle is beginning to be understood.

Isoforms of myosin light chains (LC) and the other components of the troponin complex, troponin T (TnT) and troponin I (TnI), as well as of tropomyosin (Tm), are also known to exist in striated muscle. For example, the myosin LCs of slow-twitch muscle are different from those of fast-twitch muscle (Sarkar, Sreter & Gergely, 1971; also see Bandman, 1985, and Syrovy, 1987, for reviews). The functional roles of the various LCs have never been clearly demonstrated, however. Similarly for the components of troponin, relatively little is known concerning the functional significance of the extensive heterogeneity of the troponin subunit isoforms, especially of TnT (Medford, Nguyen, Destree, Summers & Nadal-Ginard, 1984; Breitbart, Nguyen, Medford, Destree, Mahdavi & Nadal-Ginard, 1985). Some progress has recently been made in this area by Tobacman & Lee (1987) who demonstrated a difference in the Ca^{2+} sensitivity of myosin subfragment-1 Mg-ATPase activity in a reconstituted protein system from bovine heart, depending on which of two TnT isoforms was present.

The objective of the present study was to define further the functional roles of isoforms of troponin subunits and the myosin light chains. This was done by determining the maximal velocity of shortening and the Ca^{2+} sensitivity of tension development in single fibres from adult rabbit plantaris muscle. Subsequently, the contractile and regulatory protein compositions of these same fibres were determined using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). The rabbit plantaris muscle was chosen based on previous reports which demonstrated that this muscle, in several mammalian species, is composed of a mixture of histochemically defined fibre types (e.g. Ariano, Armstrong & Edgerton, 1973; Armstrong & Phelps, 1984).

METHODS

Plantaris muscles were removed from adult male White New Zealand rabbits and placed in cold relaxing solution (described below). Bundles (~1 mm thick) of fibres were dissected from a muscle, tied to glass capillary tubes at slightly stretched lengths and stored at −20 °C in 50% (v/v) glycerol-containing relaxing solution (described below) for 5–27 days. On the day of an experiment, a segment of a single, chemically skinned muscle fibre was isolated from a bundle and mounted in an experimental apparatus similar to one described previously (Moss, 1979) which was mounted on the stage of a Zeiss WL microscope. The fibre was attached on one end to a servo-controlled DC torque motor (model 300H, Cambridge Technology, Cambridge, MA, U.S.A.) and, on the other end, to an isometric tension transducer (model 403, Cambridge Technology) using connectors described by Moss (1979). The length of the fibre segment between the connectors (i.e. the portion of the fibre exposed to the solutions in the experimental apparatus) was determined by measuring the horizontal displacement of the microscope stage while viewing the segment from one end to the other. Fibre length (L_0) was set such that the resting sarcomere length was between 2·45 and 2·65 μm, by moving the torque motor which was mounted on a three-way positioner. Sarcomere length and fibre width were measured from Polaroid photographs obtained with a camera mounted on the microscope. The depth of the fibre was measured by noting the vertical displacement of the microscope stage while focusing on the top and bottom surfaces of the fibre. Fibre cross-sectional area (CSA) was calculated from the width and depth measurements and assuming an elliptical circumference (Gordon, Huxley & Julian, 1966). The reported values for fibre CSA and isometric tension normalized with respect to CSA have been corrected by a factor of 1·44 for the swelling that is known to occur (approximate 20% increases in fibre width and in depth) during skinning (Godt & Maughan, 1981).

Relaxing and activating solutions contained 7·0 mM-EGTA, 5·45 mM-total Mg, 1·0 mM-free Mg^{2+}, 4·39 mM-total ATP (i.e. 4·39 mM-Mg-ATP), 14·5 mM-creatine phosphate, 10 mM-caffeine, 20 mM-imidazole, at pH 7·00 and with sufficient KCl to achieve a final ionic strength of 180 mM. Creatine phosphokinase was not included in these solutions since previously it was shown that endogenous kinase activity is sufficient to buffer [ATP] in rabbit skinned muscle fibres (e.g. Julian, Moss & Waller, 1981). Relaxing solution had a pCa (i.e. $-\log[Ca^{2+}]$) of 9·0 and the maximally activating solution had a pCa 4·5. A solution with a pCa of 4·0 was also prepared. Submaximally activating solutions had pCas ranging from 4·8 to 7·4 and were prepared by mixing appropriate volumes of the solutions of pCa 4·5 and 9·0. The amount of each substance to be added when preparing the pCa 4·0, 4·5 and 9·0 solutions was determined by using the computer program of Fabiato & Fabiato (1979) along with the stability constants listed by Godt & Lindley (1982). The apparent stability constant used for Ca-EGTA ($2·27 \times 10^6$ M^{-1}) had been corrected for the effect of ionic strength (Martell & Smith, 1974) and to 15 °C (Fabiato & Fabiato, 1979). The relaxing solution in which the dissections and fibre isolations were performed and the glycerol-relaxing solution contained 2 mM-EGTA, 1 mM-$MgCl_2$, 4 mM-ATP, 10 mM-imidazole and 100 mM-KCl at pH 7·00.

The slack tast method (Edman, 1979) was used to measure V_{max} as described and illustrated previously (Reiser *et al.* 1985*a*). Briefly, a fibre was transferred from the relaxing solution to the maximally activating solution whereupon tension was generated under isometric conditions. During the plateau of the tension response, a controlled amount of slack (ΔL) was rapidly (< 1·0 ms) introduced into the fibre via the torque motor. At this instant, tension fell to the baseline. Since the fibre was still activated, shortening occurred to take up the slack. At the instant the slack was just taken up, tension began to redevelop. The time (Δt) between the introduction of slack and the beginning of tension redevelopment was measured from the screen of a digital storage oscilloscope (model 2090, Nicolet Instrument Corp., Madison, WI, U.S.A.) with a resolution of 500 ns/address. The beginning of tension redevelopment was determined as the first point in the record where tension consistently rose above the baseline. The point is always well defined due to the high signal/noise ratio in the records obtained from large fibres as used in the present study. During successive activations, ΔL was varied and Δt values were recorded. A straight line was fitted to a plot of ΔL *vs.* Δt using a least-squares regression and the slope of the line was taken as V_{max}. It was important to determine the reliability of the slack test method since the goal of the present study was to determine if differences in V_{max} between fibres are quantitatively correlated with differences in protein composition of the same fibres. Therefore, V_{max} was measured on two adjacent segments of twenty-two different fibres isolated from adult rabbit psoas (fast-twitch), soleus (slow-twitch) and

plantaris (mixed) muscles. As shown in Fig. 1, V_{max} of the first segment was plotted against V_{max} of the second segment for each fibre. A straight line was fitted to the data using a least-squares regression. The correlation coefficient obtained was 0·991, the slope was 0·998 and the intercept on the ordinate was −0·01 muscle length/s (ML/s). These results demonstrate that the slack test method is a highly reliable method for measuring V_{max} as required for the present study.

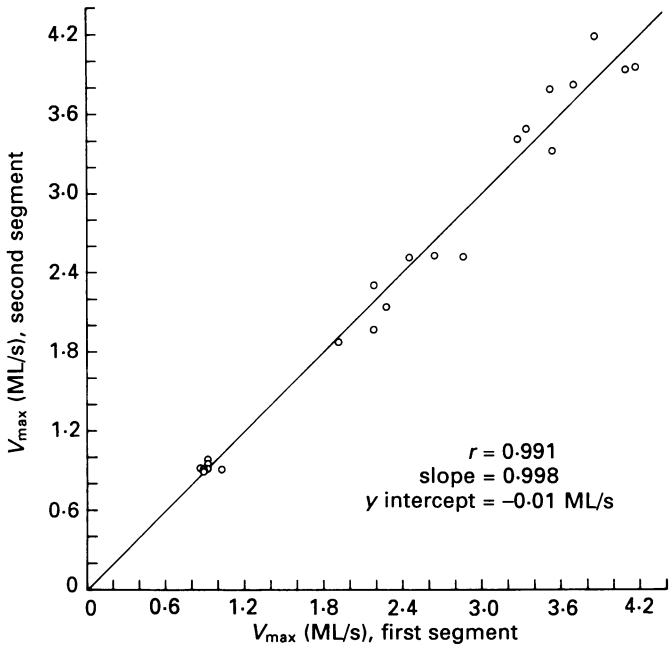

Fig. 1. Plot of the maximal velocity of shortening (V_{max}), in muscle lengths/s (ML/s), of one segment of a skinned fibre *v*. V_{max} of a second segment of the same fibre. Each symbol represents a single fibre from rabbit plantaris, soleus or psoas muscle. The linear regression and correlation coefficients determined from a least-squares fit were 0·998 and 0·991, respectively. The drawn line has a slope equal to 1·00.

To characterize the Ca^{2+} sensitivity of tension development in single fibres, the isometric tension generated at several randomized submaximal levels of activation was recorded. Every third activation on a given fibre was performed at pCa 4·5 and the tension obtained (P_0) was used to normalize the tension during each submaximal activation (P) before and after each maximal activation. If P_0 decreased by 10% or more of the P_0 at the beginning of tension measurements on any fibre, the measurements were stopped. Peak tension during each activation was determined by measuring the change in tension that occurred when sufficient slack was introduced into the fibre during the plateau of the tension response to drop the tension to the baseline. The change in tension that occurred in relaxing solution (i.e. resting tension) was subtracted from the peak tension generated in activating solution to obtain the active tension developed by the fibre. The fibres that were used to characterize the Ca^{2+} sensitivity of tension development were soaked in relaxing solution containing 0·5% (w/v) Brij 58 (polyoxyethylene 20 cetyl ether, Sigma Chemical Co.) for 30 min at room temperature prior to the tension measurements. This step was performed to solubilize further the sarcoplasmic reticulum which normally retains some functional activity even after prolonged glycerol treatment (Eastwood, Wood, Back & Sorenson, 1979).

Every activation of a fibre for either the V_{max} determinations or the tension measurements was immediately preceded by soaking the fibre in a solution with a reduced Ca^{2+}-buffering capacity.

This solution was prepared in the same way as the relaxing solution except that the [EGTA] was reduced to 0·5 mM and HDTA (1,6-diaminohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid; Fluka Chemical Corp.) was added to a concentration of 6·5 mM, thereby maintaining the ionic strength at 180 mM (a modification of the methods of Moisescu, 1976, and Moisescu & Thieleczek, 1978). Following a soak in this solution, the fibre generated tension more rapidly and maintained a more uniform sarcomere length during activation. If the average sarcomere length of a fibre changed by 10% or more between the resting and fully activated states at the beginning of the tension measurements, the fibre was discarded.

TABLE 1. Summary of V_{max} measurements on single fibres from adult rabbit plantaris muscles

Group	V_{max} (ML/s)	P_0/CSA (kN/m^2)	Resting tension (% P_0)	CSA ($\times 10^{-5}$ cm^2)
Low velocity, $n = 22$	0·86 ± 0·03	251 ± 16	2·1 ± 0·1	1·22 ± 0·07
Intermediate velocity, $n = 29$	2·27 ± 0·08	253 ± 7	0·8 ± 0·1	1·39 ± 0·08
High velocity, $n = 27$	3·38 ± 0·06	249 ± 10	0·8 ± 0·1	2·32 ± 0·15

All values are given as mean ± S.E.M. Abbreviations: V_{max}, maximal velocity of shortening in muscle lengths/s (ML/s); P_0/CSA, peak isometric tension developed during maximal calcium activation (P_0) normalized with respect to fibre cross-sectional area (CSA). CSA values have been corrected for the fibre swelling that occurs during skinning (see Methods).

SDS–PAGE was performed according to Giulian, Moss & Greaser (1983) with the following modifications: (1) the acrylamide concentrations were 3·5% (w/v) in the stacking gel and 9 and 12% in the myosin heavy chain and myosin light chain gels, respectively; (2) sample loads were kept low (approximately 0·5 nl of fibre volume for each gel lane) on the heavy chain gels to improve resolution of the closely spaced isoforms; (3) sample loads on the myosin light chain gels were approximately 1·5 nl of fibre volume in each lane; and (4) glycerol was added to the gel matrix to a final concentration of 10% (w/v) as described by Carraro & Catani (1983). The volume of each fibre was determined from the segment length and cross-sectional area measurements obtained while the fibre was mounted in the experimental apparatus. Following a gel run, the gels were silver stained according to the methods of Giulian *et al.* (1983). The fast-type and slow-type myosin heavy chains and light chains were identified on the gels on the bases of their known molecular weight (for the light chains) and their co-migration with the predominant heavy chain and light chain bands of the rabbit fast-twitch psoas and slow-twitch soleus muscles, respectively. The slow-type and fast-type troponin subunit bands were identified on the basis of known molecular weights. In addition, the migration pattern of purified troponin subunits from rabbit fast muscle (Fig. 4) facilitated the identification of fast-type troponin bands. The minor troponin T bands were identified on the basis of the molecular weights of α- and β-tropomyosin, as well as of troponin T, and the migration pattern of troponin T isoforms on gels in previous reports in the literature (e.g. Schachat, Bronson & McDonald, 1985). A soft laser scanning densitometer (model SL-504-XL, Bio-Med Instruments, Fullerton, CA, U.S.A.) was used to determine the relative proportions of myosin heavy and light chains in each fibre. The area of each protein band was determined from the output of the integrator of the densitometer.

The shortening velocity and tension measurements were made at 15 °C. Tests for statistical significance of differences between means were performed using the Student's t test.

RESULTS

The maximal shortening velocity (V_{max}) and the maximum tension generated per unit of fibre cross-sectional area (P_0/CSA) were measured on one set of seventy-eight fibres. The results are summarized in Table 1. Three groups of fibres, identified on the basis of differences in protein composition (explained in detail below), differed from each other with respect to V_{max}. The low-velocity fibres had a mean V_{max}

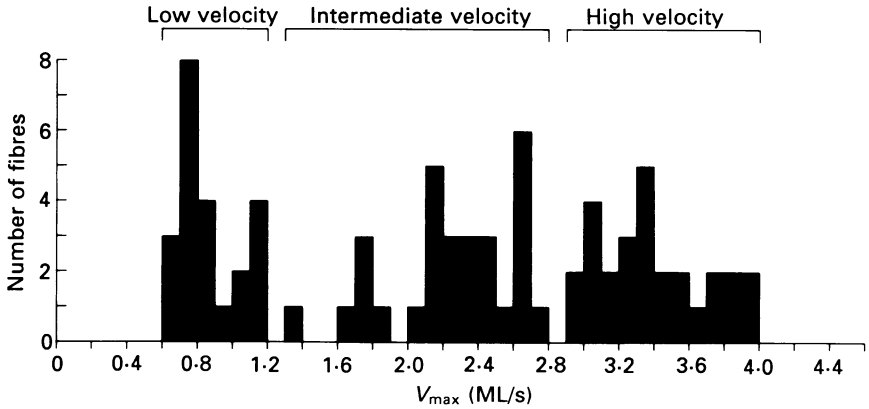

Fig. 2. Distribution of the maximal velocity of shortening (V_{max}) measurements obtained on seventy-eight fibres from rabbit plantaris muscles.

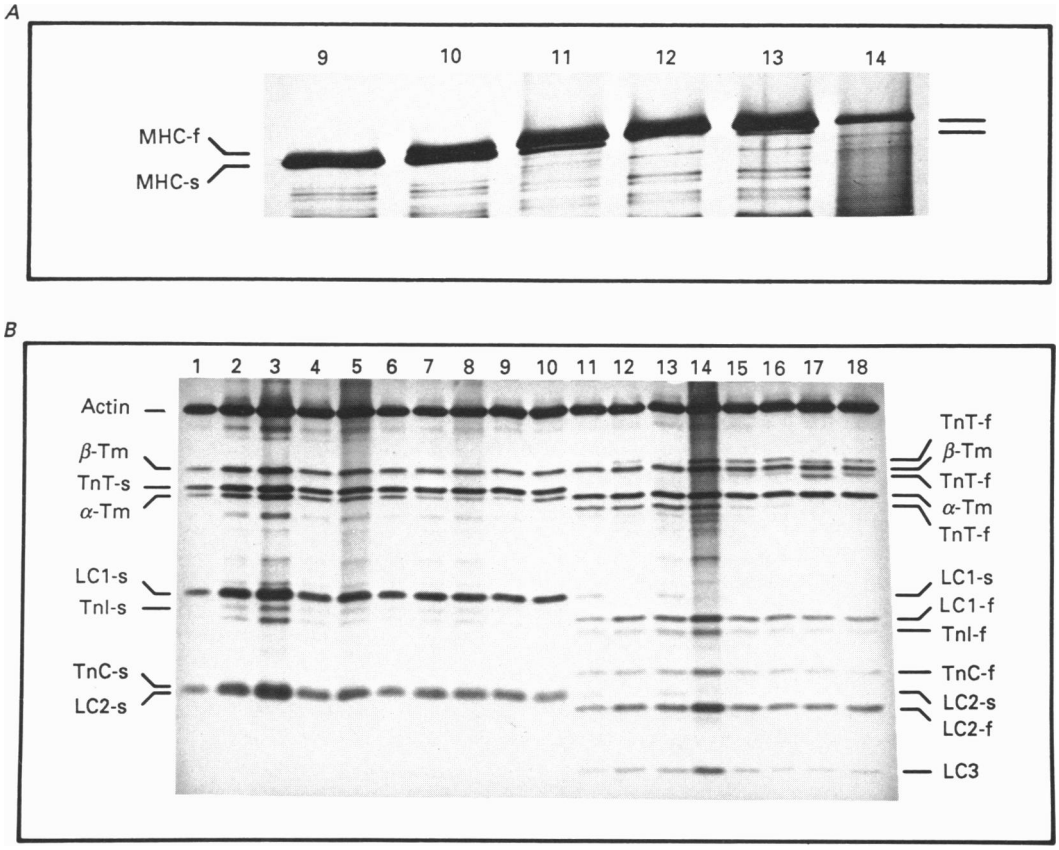

Fig. 3. The myosin heavy chain (MHC) region (*A*) and low molecular weight region (*B*) of two SDS gels. Each lane represents a single fibre. The fibres in lanes 9 to 14 in the gel in *A* are the same as those in lanes 9 to 14 in the gel in *B*. The abbreviations are the same as those used in the text. The shortening velocities in the fibres, given in muscle lengths/s and with the gel lane number in parentheses, are: 0·81 (1), 0·85 (2), 0·86 (3), 1·09 (4), 1·09 (5), 1·10 (6), 0·62 (7), 0·75 (8), 0·79 (9), 1·02 (10), 1·61 (11), 1·71 (12), 2·15 (13), 2·67 (14), 3·06 (15), 3·19 (16), 3·38 (17) and 3·61 (18).

(±S.E.M.) of 0·86±0·03 ML/s while the fibres in intermediate- and high-velocity groups formed a continuum with respect to V_{max} from 1·37 to 3·94 ML/s (Fig. 2). Despite an approximate fourfold difference in mean V_{max} between the low- and high-velocity fibres, P_0/CSA was invariant among the low-, intermediate- and high-velocity fibres. The approximate ranges in V_{max} among the low-, intermediate- and

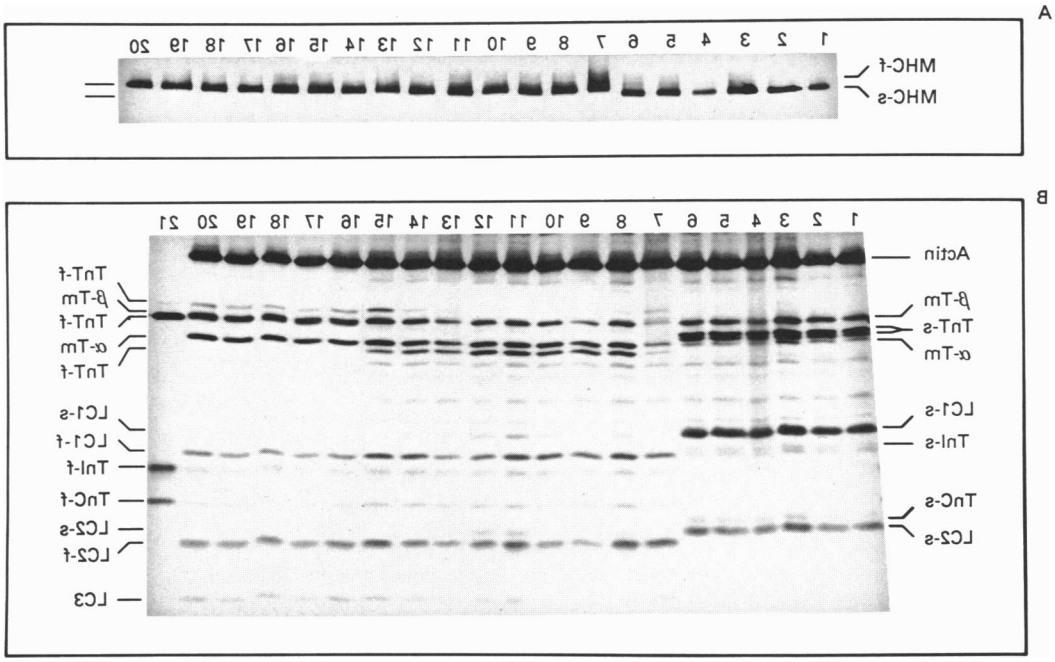

Fig. 4. The myosin heavy chain (MHC) region (*A*) and low molecular weight region (*B*) of two SDS gels. Lanes 1 to 20 contain samples from separate single fibres, with the same fibre in the same numbered lane of the gels in *A* and *B*. Lane 21 represents troponin T, troponin I and troponin C isolated from rabbit fast back muscle. The shortening velocities of the fibres, given in muscle lengths/s and with the gel lane number in parentheses, are: 0·83 (1), 1·09 (2), 1·11 (3), 1·14 (4), 1·16 (5), 1·19 (6), 2·46 (7), 2·60 (8), 2·62 (9), 2·64 (10), 2·68 (11), 2·70 (12), 2·94 (13), 2·96 (14), 3·01 (15), 3·21 (16), 3·31 (17), 3·70 (18), 3·87 (19) and 3·90 (20).

high-velocity fibres are indicated in Fig. 2. Virtually all of the fibres examined with SDS–PAGE in the low-velocity group had exclusively the slow-type MHC (MHC-s) and LCs (LC-s). However, one fibre (lane 10, Fig. 3) in this group (with a V_{max} of 1·02 ML/s) had a small portion of fast-type MHC (MHC-f) and exclusively slow-type LCs. All the fibres in the high-velocity group contained exclusively the fast-type MHC and LCs (Figs 3 and 4). The fibres in the intermediate-velocity group had mixtures of slow-type and fast-type MHCs and/or LCs. A detailed description of the relationships between MHC, LC and regulatory protein compositions and V_{max} of single fibres follows.

The relative amounts of fast-type MHC and LCs increased with V_{max} among the fibres at the low end of the intermediate-velocity range. However, in the majority of fibres in the intermediate group, only one MHC (i.e. fast-type) was observed, with variable amounts of fast-type LCs among individual fibres which varied in direct

proportion to V_{max}. The amount of LC3 of single fibres was normalized to the amount of LC1 (slow-type plus fast-type LC1 (LC1-s + LC1-f) whenever both were present in the same fibre), based on the results of laser densitometric scanning. The relationship between V_{max} *vs.* the LC3/(LC1-s + LC1-f) ratio for the intermediate- and high-velocity fibres represented in the gels shown in Fig. 4 is illustrated in Fig. 5. As the

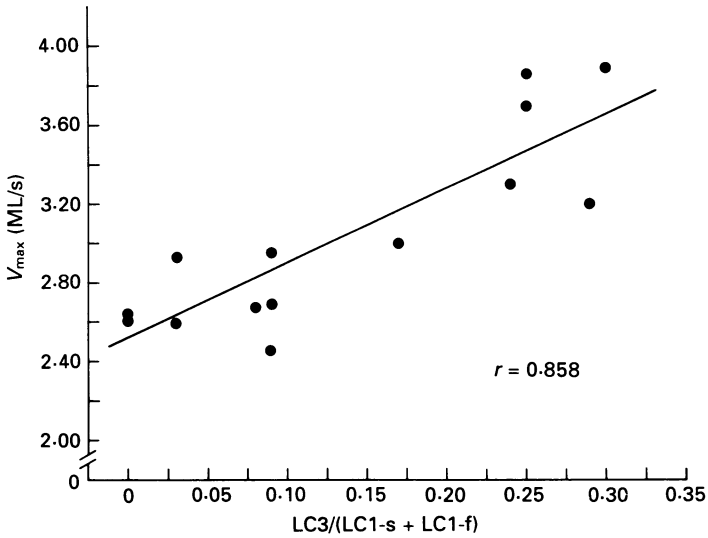

Fig. 5. Plot of the maximal velocity of shortening (V_{max}) *vs.* the LC3/(LC1-s + LC1-f) ratio of single fibres. Each symbol represents an individual fibre. The linear correlation coefficient determined from a least-squares fit between V_{max} and relative LC3 content is shown.

amount of LC3 increased among these fibres, so also did V_{max}. The linear correlation coefficient between V_{max} and relative amount of LC3 was 0·858 ($P < 0·001$). A 10% increase in LC3 was associated with an approximate 0·4 ML/s increase in V_{max}. Essentially the same results were obtained with the LC3/LC1-f ratio since, although a few fibres contained both LC1-s and LC1-f, the amount of LC1-s was always much less than LC1-f. From this result, it appears that the MHC composition of rabbit skeletal muscle fibres determines whether a fibre will have a high or low shortening velocity but, among the fast fibres, the LC composition may modulate the shortening velocity. Fibres with mixtures of fast-type and slow-type MHCs were also observed to have intermediate velocities. These fibres also had fast/slow LC ratios that were proportional to fast/slow MHC ratios (e.g. lanes 11 and 13, Fig. 3). This result from adult rabbit plantaris muscle (which is mixed with respect to fibre types) is in contrast to an earlier observation (Reiser *et al.* 1985*a*) that some fibres of the adult rabbit soleus muscle (almost exclusively slow-twitch fibres) contain mixtures of slow-type and fast-type MHCs and LCs but that the MHCs and LCs appear to vary independently of each other.

A relationship was also observed between V_{max} and the regulatory protein composition of the intermediate- and high-velocity fibres. Specifically, three forms of

troponin T (the troponin subunit that binds tropomyosin) were observed in these fibres (Figs 3 and 4). These fast-type isoforms of troponin T were clearly separated from slow troponin T on our gels. As the velocity of the intermediate and fast fibres increased, there was a gradual shift from one form of troponin T which migrated ahead of α-Tm to a mixture of two other forms of troponin T with greater apparent

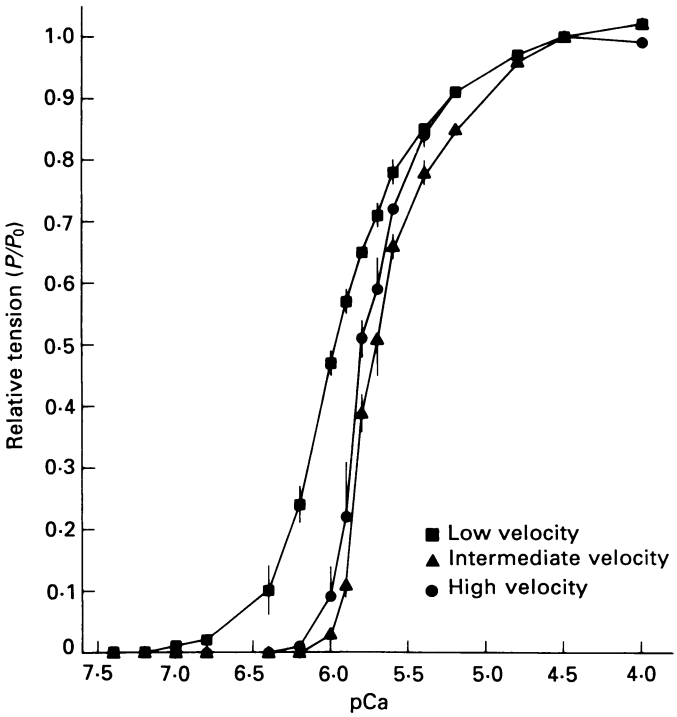

Fig. 6. Plots of relative tension (P/P_0) *vs.* pCa for fibres in the low- (●), intermediate- (▲) and high- (■) velocity groups. Each point represents the mean ±S.E.M. of measurements on four to six fibres. In some cases, the error bars are smaller than the symbol size.

molecular weights that migrated ahead and behind β-Tm. This pattern can be observed in the gels shown in Figs 3 and 4. The tropomyosin content of the fibres in the intermediate- and high-velocity groups was invariant; that is, all the fibres contained α-Tm and β-Tm in a nearly constant 1:1 ratio. Also, all of these fibres contained only the fast-type isoforms of TnC and TnI.

Since the regulatory proteins are believed to be involved in the calcium activation of striated muscle, we determined the Ca^{2+} sensitivity of tension generation on a separate set of fibres from the low-, intermediate- and high-velocity groups. Following the determination of the tension–pCa characteristics of one segment of a given fibre, V_{max} was measured on another segment of the same fibre. The results of the tension–pCa measurements are illustrated in Fig. 6. The low-velocity fibres had the greatest Ca^{2+} sensitivity of tension development as evidenced by (1) their relatively low threshold for Ca^{2+} activation (i.e. ~pCa 7·2 compared to pCa 6·2 and 6·4 for the intermediate- and high-velocity fibres, respectively) and (2) the shift of the

entire tension–pCa curve to higher pCas relative to the curves for the intermediate- and high-velocity fibres. Differences also existed, although relatively small, between the intermediate- and high-velocity fibres with the intermediate fibres having a slightly higher threshold for Ca^{2+} activation and a tension–pCa curve shifted to lower pCas by ~0·1 pCa unit, compared to the curve for the high-velocity fibres. A Hill transformation of the data represented in Fig. 6 (a method used to linearize the data

TABLE 2. Summary of V_{max} and tension–pCa relations of single fibres from adult rabbit plantaris muscles*

Group	V_{max} (ML/s)	P_0/CSA (kN/m²)	CSA ($\times 10^{-5}$ m²)	n_1	n_2	Threshold pCa	pCa_{50}
Low velocity, $n = 6$	0·75±0·07	227±19	1·22±0·13	1·24	2·25	7·2	5·95
Intermediate velocity, $n = 6$	2·04±0·20	213±16	1·32±0·11	1·40	4·65	6·2	5·80
High velocity, $n = 5$	3·26±0·12	220±9	2·17±0·22	1·66	5·10	6·4	5·70

* These fibres are not represented in Table 1. Values for V_{max}, P_0/CSA and CSA are expressed as mean ±S.E.M. Abbreviations: n_1 and n_2 are the Hill coefficients of the transformed, relation tension *vs.* pCa data shown in Fig. 6; pCa_{50} is the pCa at which tension is half of the maximum; all other abbreviations are the same as those in Table 1. CSA values have been corrected for the fibre swelling that occurs during skinning (see Methods).

for convenient quantitative comparisons; Moss, Swinford & Greaser, 1983) highlights differences between the fibres from the three groups (Table 2). The Hill coefficients (n) which were obtained from this transformation are interpreted as reflecting differences in the amount of molecular co-operativity along the thin filament during tension development. The greater coefficients for the high-velocity compared to the intermediate-velocity fibres indicate a slightly higher degree of apparent co-operativity in tension development in the high-velocity fibres.

DISCUSSION

The primary results of the present study are (1) there was a virtual continuum of maximum shortening velocities among individual rabbit plantaris fibres, (2) although V_{max} appeared to be primarily determined by the myosin heavy chain composition (in agreement with our previous results), variations in the LC3 content of single fibres from adult rabbit plantaris muscle were quantitatively correlated with V_{max}, suggesting a modulatory role for the alkali light chains of myosin, (3) differences in TnT isoform content among fibres containing fast-type myosin heavy chain paralleled differences in the relative LC3 content and (4) variations in the proportions of TnT isoforms were associated with differences in the Ca^{2+} sensitivity of tension development of fibres with intermediate and high shortening velocities.

Diversity in skeletal muscle function has traditionally been thought to result from the presence of varying proportions of two distinct types of fibres, slow-twitch and fast-twitch, between different muscles or from alterations in motoneurone firing rates. Subsets have been identified among fast-twitch fibres by a variety of means (e.g. histochemical reactions based on myosin ATPase activity) but little information is available concerning functional differences between fibres other than slow and fast rates of shortening which generally differ by three- or fourfold. The results of the

present study clearly demonstrate a much more elaborate diversity in physiological properties among single fibres of skeletal muscle than previously recognized.

Our previous studies have shown a strong correlation between V_{max} and the MHC composition of single cells from adult rabbit soleus muscles (Reiser *et al.* 1985*a*). The soleus muscle of most mammals consists of a majority of slow, type I fibres and a few fast, type II A fibres but does not contain type II B fibres (Barnard, Edgerton, Furukawa & Peter, 1971; Ariano *et al.* 1973; Armstrong & Phelps, 1984). We reported (Reiser *et al.* 1985*a*) that a majority of fibres in the adult soleus have exclusively slow MHC and low V_{max} values. A few fibres had significantly higher shortening velocities over a relatively wide range which were quantitatively correlated with mixtures of slow MHC and fast MHC. Type II A and type II B mammalian muscle fibres have different heavy chains, as clearly demonstrated by several laboratories. For example, Staron & Pette (1987) have shown that the MHCs of histochemically verified type II A and type II B fibres of normal rabbit anterior tibialis muscle can be separated on 5% SDS gels. Similar results were obtained on single fibres from rat hindlimb muscles by Danieli Betto, Zerbato & Betto (1986) using 6% SDS gels. Sweeney, Kushmerick, Mabuchi, Gergely & Sreter (1986) demonstrated, through peptide mapping, that the MHC of type II B fibres of normal rabbit anterior tibialis muscles is different from the MHC of transformed type II A fibres from anterior tibialis muscle that had been intermittently stimulated for 7 weeks. Differences were also detected by Mabuchi, Pinter, Mabuchi, Sreter & Gergely (1984) in the peptide maps of MHCs of types II A and II B fibres of rabbit skeletal muscle. Since the plantaris muscle of several mammalian species is composed of II A and II B (as well as I) fibres (Ariano *et al.* 1973; Armstrong & Phelps, 1984), it is likely that the fibres in the intermediate-velocity and high-velocity groups in the present study do not all have the same single type of MHC. Therefore, differences in V_{max} might also be correlated with variations in MHC composition (undetected with our gel system) among these fibres as well as with differences in LC3 content (Fig. 5). However, the present study is the first to demonstrate that differences in alkali light chain content are correlated with a physiological property at the single muscle fibre level.

The intermediate- and high-velocity fibres also formed a continuum with respect to V_{max} and TnT isoform composition. One possible mechanism to explain this relationship is that the genetic basis for the control of TnT isoform expression is shared by that for myosin light and/or heavy chain expression. It is logical that a selective advantage might be gained from a co-ordinated control of regulatory and contractile proteins. Related to this is the observation that the fibres in the high-velocity group have a slightly greater Ca^{2+} sensitivity of tension development. This suggests that, in an intact fibre, as intracellular $[Ca^{2+}]$ increases during the normal course of activation, the high V_{max} fibres will be activated more quickly than the intermediate V_{max} fibres, due to their greater Ca^{2+} sensitivity. This could provide a mechanism for a further differentiation of the contractile properties of these two groups of fibres thus providing a more elaborate array of responses for specific tasks during motor activity. Evidence for co-ordinated expression of myosin light chains and troponin subunits in avian skeletal muscle has also been reported (Mikawa, Takeda, Shimuzu & Kitaura, 1981).

Differences in troponin T isoform composition among mammalian skeletal muscle fibres have also been demonstrated by others (Moore & Schachat, 1985; Schachat *et al.* 1985; Briggs, Lin & Schachat, 1987; Moore, Briggs & Schachat, 1987). The differences that we report between the intermediate- and high-velocity fibres in the plantaris muscle with respect to troponin T content are similar to differences between types II A and II B plantaris fibres observed by Schachat and co-workers. However, it is difficult to make precise comparisons between their results and ours due to differences in the gel systems between these studies which result in differences in the migration rates of several protein isoforms. We observe approximately equal amounts of the two forms of tropomyosin (α and β) and exclusively the fast-type TnC and TnI in all the fibres in the intermediate- and high-velocity groups in the plantaris muscle. Therefore, we conclude that the differences in the Ca^{2+} sensitivity of tension development between these two groups of fibres is related to the differences in their TnT composition. Using a reconstituted protein system, Tobacman & Lee (1987) demonstrated that different isoforms of cardiac TnT yield different Ca^{2+} sensitivities of bovine cardiac myosin subfragment-1 Mg-ATPase activity. Risnik, Verin & Gusev (1985) reported that the two TnT isoforms of bovine cardiac muscle differ by the insertion of an additional peptide in a region near the N-terminus of the molecule in the higher molecular weight isoform. This is also the region of TnT in which interactions with the tropomyosin of its functional group (i.e. seven actins, 1 troponin complex and 1 tropomyosin molecule along the thin filament) and adjacent functional groups are likely to occur (Flicker, Phillips & Cohen, 1982; White, Cohen & Phillips, 1987) during activation. Our results show that a greater Ca^{2+} sensitivity of tension development is associated with the presence of higher molecular weight isoforms of TnT in single muscle fibres. A hypothetical mechanism to explain this observation is that a greater co-operative interaction might occur between the higher molecular weight TnT isoforms with tropomyosin of their own and the adjacent functional groups during activation, based on the presence of an extra peptide in the region of the N-terminus of these isoforms of TnT as reported for cardiac TnT (Risnik *et al.* 1985). This, in turn, could result in greater disinhibition of the thin filament at a given $[Ca^{2+}]$ compared to when lower molecular weight isoforms of troponin T are present and, therefore, to greater tension development resulting in a leftward shift of the tension–pCa relationship, as observed (Fig. 6). This is similar to a mechanism recently suggested by Schachat, Diamond & Brandt (1987).

In conclusion, variations in the myosin light chain composition and in troponin T isoform composition of single fibres from rabbit plantaris muscles are correlated with differences in the maximal velocity of shortening and the Ca^{2+}-sensitivity of tension development. It is suggested that the observed co-ordinated differences in alkali light chain and troponin T isoform contents among intermediate- and high-velocity fibres might provide a mechanism for a greater functional distinction between these two groups of fibres than would otherwise exist if only one (i.e. LC3 or troponin T) varied while the other remained constant.

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REFERENCES

ARIANO, M. A., ARMSTRONG, R. B. & EDGERTON, V. R. (1973). Hindlimb muscle fiber population of five mammals. *Journal of Histochemistry and Cytochemistry* **21**, 51–55.

ARMSTRONG, R. B. & PHELPS, R. O. (1984). Muscle fiber type composition of the rat hindlimb. *American Journal of Anatomy* **171**, 259–272.

BABU, A., SCORDILIS, S. P., SONNENBLICK, E. H. & GULATI, J. (1987). The control of myocardial contraction with skeletal fast muscle troponin C. *Journal of Biological Chemistry* **262**, 5815–5822.

BANDMAN, E. (1985). Myosin isozyme transitions in muscle development, maturation, and disease. *International Review of Cytology* **97**, 97–131.

BARNARD, R. J., EDGERTON, V. R., FURUKAWA, T. & PETER, J. B. (1971). Histochemical, biochemical, and contractile properties of red, white, and intermediate fibers. *American Journal of Physiology* **220**, 410–414.

BREITBART, R. E., NGUYEN, H. T., MEDFORD, R. M., DESTREE, A. T., MAHDAVI, V. & NADAL-GINARD, B. (1985). Intricate combinatorial patterns of exon splicing generate multiple regulated troponin T isoforms from a single gene. *Cell* **41**, 67–82.

BRIGGS, M. M., LIN, J. J.-C. & SCHACHAT, F. H. (1987). The extent of amino-terminal heterogeneity in rabbit fast skeletal muscle troponin T. *Journal of Muscle Research and Cell Motility* **8**, 1–12.

CARRARO, U. & CATANI, C. (1983). A sensitive SDS–PAGE method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. *Biochemical and Biophysical Research Communications* **116**, 793–802.

DANIELI BETTO, D., ZERBATO, E. & BETTO, R. (1986). Type 1A, 2A, and 2B myosin heavy chain electrophoretic analysis of rat muscle fibers. *Biochemical and Biophysical Research Communications* **138**, 981–987.

EASTWOOD, A. B., WOOD, D. S., BOCK, K. L. & SORENSON, M. M. (1979). Chemically skinned mammalian skeletal muscle. 1. The structure of skinned rabbit psoas. *Tissue and Cell* **11**, 553–566.

EDMAN, K. A. P. (1979). The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *Journal of Physiology* **291**, 143–159.

FABIATO, A. & FABIATO, F. (1979). Calculator programs for computing the composition of the solutions containing metals and ligands used for experiments in skinned muscle cells. *Journal de physiologie* **75**, 463–505.

FLICKER, P. F., PHILLIPS JR, G. N. & COHEN, C. (1982). Troponin and its interactions with tropomyosin: an electron microscope study. *Journal of Molecular Biology* **162**, 495–501.

GIULIAN, G. G., MOSS, R. L. & GREASER, M. (1983). Improved methodology for analysis and quantitation of proteins on one-dimensional silver-stained slab gels. *Analytical Biochemistry* **129**, 277–287.

GODT, R. E. & LINDLEY, B. D. (1982). Influence of temperature upon contractile activation and isometric force production in mechanically skinned muscle fibers of the frog. *Journal of General Physiology* **80**, 279–297.

GODT, R. E. & MAUGHAN, D. W. (1981). Influence of osmotic compression on calcium activation and tension in skinned muscle fibers of the rabbit. *Pflügers Archiv* **391**, 334–337.

GORDON, A. M., HUXLEY, A. F. & JULIAN, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *Journal of Physiology* **184**, 170–192.

JULIAN, F. J., MOSS, R. L. & WALLER, G. J. (1981). Mechanical properties and myosin light chain composition of skinned muscle fibres from adult and new-born rabbits. *Journal of Physiology* **311**, 201–218.

MABUCHI, K., PINTER, K., MABUCHI, Y., SRETER, F. A. & GERGELY, J. (1984). Characterization of rabbit masseter muscle fibers. *Muscle and Nerve* **7**, 431–438.

MARTELL, A. E. & SMITH, R. M. (1974). *Critical Stability Constants*: *Amino Acids*, vol. 1, pp. xi–xii. New York: Plenum.

MEDFORD, R. M., NGUYEN, H. T., DESTREE, A. T., SUMMERS, E. & NADAL-GINARD, B. (1984). A novel mechanism of alternative RNA splicing for the developmentally regulated generation of troponin T isoforms from a single gene. *Cell* **38**, 409–421.

MIKAWA, T., TAKEDA, S., SHIMUZU, T. & KITAURA, T. (1981). Gene expression of myofibrillar proteins in single muscle fibers of adult chicken: micro two dimensional gel electrophoretic analysis. *Journal of Biochemistry* **89**, 1951–1962.

MOISESCU, D. G. (1976). Kinetics of reaction in calcium-activated skinned muscle fibres. *Nature* **262**, 610–613.

MOISESCU, D. G. & THIELECZEK, R. (1978). Calcium and stronium concentration changes within skinned muscle preparations following a change in the external bathing solution. *Journal of Physiology* **275**, 241–262.

MOORE, G. E., BRIGGS, M. M. & SCHACHAT, F. H. (1987). Patterns of troponin T expression in mammalian fast, slow and promiscuous muscle fibres. *Journal of Muscle Research and Cell Motility* **8**, 13–22.

MOORE, G. E. & SCHACHAT, F. H. (1985). Molecular heterogeneity of histochemical fibre types: a comparison of fast fibres. *Journal of Muscle Research and Cell Motility* **6**, 513–524.

MOSS, R. L. (1979). Sarcomere length–tension relations of frog skinned muscle fibres during activation at short lengths. *Journal of Physiology* **292**, 177–192.

MOSS, R. L., LAUER, M. R., GIULIAN, G. G. & GREASER, M. L. (1986). Altered Ca^{2+} dependence of tension development in skinned skeletal muscle fibres following modification of troponin by partial substitution with cardiac troponin C. *Journal of Biological Chemistry* **261**, 6096–6099.

MOSS, R. L., SWINFORD, A. E. & GREASER, M. L. (1983). Alterations in the Ca^{2+}-sensitivity of tension development by single skeletal muscle fibers at stretched lengths. *Biophysical Journal* **43**, 115–119.

OBINATA, T. (1985). Changes in myofibrillar protein isoform expression during chicken skeletal muscle development. *Zoological Science* **2**, 833–847.

OHTSUKI, I., MARUYAMA, K. & EBASHI, S. (1986). Regulatory and cytoskeletal proteins of vertebrate skeletal muscle. *Advances in Protein Chemistry* **38**, 1–67.

REISER, P. J., MOSS, R. L., GIULIAN, G. G. & GREASER, M. L. (1985*a*). Shortening velocity in single fibers from adult rabbit soleus muscles is correlated with myosin heavy chain composition. *Journal of Biological Chemistry* **260**, 9077–9080.

REISER, P. J., MOSS, R. L., GIULIAN, G. G. & GREASER, M. L. (1985*b*). Shortening velocity and myosin heavy chains of developing rabbit muscle fibers. *Journal of Biological Chemistry* **260**, 14403–14405.

RISNIK, V. R., VERIN, A. D. & GUSEV, N. B. (1985). Comparison of the structure of two cardiac troponin T isoforms. *Biochemical Journal* **225**, 549–552.

SARKAR, S., SRETER, F. A. & GERGELY, J. (1971). Light chains of myosins from white, red, and cardiac muscles. *Proceedings of the National Academy of Science of the U.S.A.* **68**, 946–950.

SCHACHAT, F. H., BRONSON, D. D. & MCDONALD, O. B. (1985). Heterogeneity of contractile proteins: a continuum of troponin–tropomyosin expression in mammalian skeletal muscle. *Journal of Biological Chemistry* **260**, 1108–1113.

SCHACHAT, F. H., DIAMOND, M. S. & BRANDT, P. W. (1987). Effect of different troponin T–tropomyosin combinations on thin filament activation. *Journal of Molecular Biology* **198**, 551–554.

STARON, R. S. & PETTE, D. (1987). The multiplicity of combinations of myosin light chains and heavy chains in histochemically typed single fibres: rabbit anterior tibialis muscle. *Biochemical Journal* **243**, 695–699.

SWEENEY, H. L., KUSHMERICK, M. J., MABUCHI, K., GERGELY, J. & SRETER, F. A. (1986). Velocity of shortening and myosin isozymes in two types of rabbit fast-twitch muscle fibres. *American Journal of Physiology* **251**, C431–434.

SWYNGHEDAUW, B. (1986). Developmental and functional adaptation of contractile proteins in cardiac and skeletal muscles. *Physiological Reviews* **66**, 710–771.

SYROVY, I. (1987). Isoforms of contractile proteins. *Progress in Biophysics and Molecular Biology* **49**, 1–27.

TOBACMAN, L. S. & LEE, R. (1987). Isolation and functional comparison of bovine cardiac troponin T isoforms. *Journal of Biological Chemistry* **262**, 4059–4064.

WHITE, S. P., COHEN, C. & PHILLIPS JR, G. N. (1985). Structure of co-crystals of tropomyosin and troponin. *Nature* **325**, 826–828.