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PROPERTIES OF THE MUSCLE PROTEINS – A COMPARATIVE APPROACH

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SUMMARY

The differences in performance that exist between skeletal muscles are in part determined by the presence of different forms of most of the contractile and regulatory proteins of the myofibril – isoforms. These isoforms have common properties but their amino acid sequences are not identical and they exhibit slight differences in biological activities, such as ATPase, affinity for calcium, etc., that are appropriate for the physiological properties of the muscle in which they are present.

With the exception of actin, all the major proteins present in the I and A filaments of skeletal muscle have been shown to exist in two or more isoforms. Whereas proteins such as troponin I and troponin C are present as a single isoform in each fibre type in normal muscle, others such as myosin and tropomyosin are present as two or more isoforms, usually in relative amounts characteristic for the fibre type. Type I and type II muscle fibres possess the capacity of synthesizing all the skeletal muscle isoforms of the myofibrillar proteins. The complement of isoforms present in a muscle fibre, however, depends on a number of factors such as the stage of development or regeneration, type of innervation, hormonal effects, etc. Complex mechanisms involving the coordinated control of gene expression must operate to ensure that the set of isoforms of the myofibrillar proteins present is characteristic for the cell type.

One of the striking features of muscle systems is the wide range in properties they exhibit. This adaptability of muscle to its physiological role is a consequence of the specialization that occurs by the normal process of development and the ability to adapt further to the functional demands when the normal developmental changes are assumed to be complete – a property often referred to as plasticity.

In all striated muscles the design of the I and A filament system is very similar and the whole contractile unit, the myofibril, exhibits relatively minor variations in structure in the different muscle types. A range in the biochemical properties of the myofibril is achieved by incorporating in the structure isoforms of the constituent protein components. These isoforms have common biological properties as might be expected from their similar secondary, tertiary and quaternary structures. They differ

Key words: Myofibrillar protein isoforms, muscle gene expression.

in amino acid sequence, but the regions of the molecules concerned in biological activity are usually strongly conserved. In most cases there is evidence that individual genes control the synthesis of each isoform whereas in some instances different pathways of processing the RNA produced by a single gene can give rise to the isoforms. The isoforms of a protein component of the myofibril usually exhibit slight differences in properties, such as ATPase activity, calcium-binding, etc., that are appropriate to the special properties of the muscle from which they are derived.

Isoforms have been found for all myofibrillar proteins that have been examined (Table 1).

Table 1. *Myofibrillar proteins that exist as isoforms*

A-filament	I-filament	Z-line
Myosin	Actin	α-Actinin
C protein	Tropomyosin Troponin C Troponin T Troponin I	

A FILAMENT PROTEINS

Myosin, the major protein of the A filament, has long been known to exist in forms of different ATPase activity in fast and slow adult skeletal, foetal skeletal, cardiac and smooth muscles. The molecule is hexameric consisting of four light chains, made up of two kinds, the P or regulatory light chains and the alkali light chains, and two heavy chains (Fig. 1).

The heavy chains extend along the length of the tail largely in α-helical conformation where they associate as a coiled coil. Each of the two heads contains part of the heavy chain organized with less secondary structure than in the tail, one alkali light chain and one P light chain. The simplest myosin molecule is composed of three different polypeptide chains with identical heads. If heterodimers with different heavy and light chains exist, then the number of different polypeptide chains in a

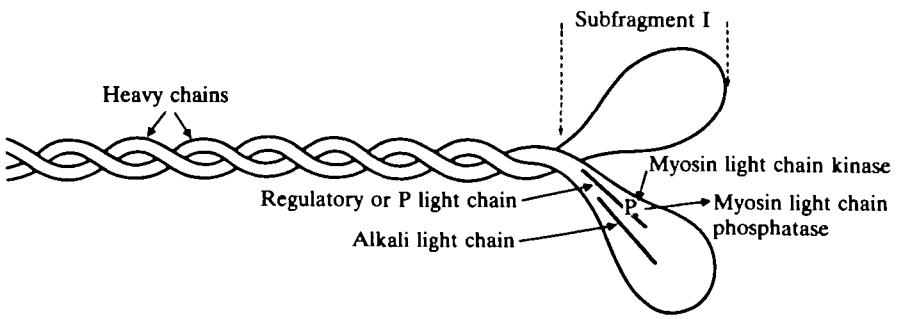

Fig. 1. Schematic representation of the myosin molecule.

myosin molecule could increase to a maximum of six. Genetic control of myosin synthesis is complex, for cloning studies suggest that there are at least 13 genes for the heavy chains in mammals (Leinwand, Fournier, Nadal-Ginard & Shows, 1983). Almost as many different forms of the light chains have been recognized but there is evidence (Robert *et al.* 1982; Nabeshima, Fujii, Kuriyama, Maramatsu & Ogata, 1982) that differences in the sequences of the alkali light chains may be a consequence of RNA processing.

The relationship of the different isoforms of myosin to muscle formation and development will be discussed in the next paper in this volume (Whalen, 1985). There is, however, another aspect of the properties of myosin which is of significance in relation to its function in different muscle types: that is its covalent modification by myosin light chain kinase involving phosphorylation of a single specific site on the P light chain (Fig. 1).

The role of this process is not understood in striated muscle although the enzyme is activated by calcium concentrations similar to those that activate the myofibrillar ATPase. Phosphorylation of the P light chain is presumed to modulate the contractile response. The extent of this covalent modification of the myosin that occurs on stimulation of muscle is in the order of fast skeletal > slow skeletal > cardiac muscle. The P light chain exists in two forms, P1 and P2, in both slow skeletal and cardiac muscle, but not in fast skeletal muscle (Westwood & Perry, 1982; Westwood, Hudlická & Perry, 1984). There is no information as to the physiological consequences of the presence of two P light chains in a given cell type but it could result in either two different species of myosin molecules or one species with a different P light chain on each head, i.e. non-identical heads. During development of the rabbit ventricle the amount of the P2 light chain slowly increases, reaching about 25 % of the total in the adult animals. The kinase levels in the striated muscles correlate well with the response that occurs on stimulation and there is evidence that the enzymes in fast skeletal, slow skeletal and cardiac muscles are different gene products. This is certainly the case in smooth muscle where the enzyme has a higher molecular weight and the activity per gram wet weight is similar to that in fast skeletal muscle. Its role in the former tissue also appears to be entirely different in that phosphorylation of the P light chain is essential for activation of the actomyosin ATPase of smooth muscle, which is not the case in striated muscle.

C protein

The C protein is a myofibrillar component of molecular weight about 140 000 Da that has been characterized and shown by Offer, Moos & Starr (1973) to be associated with the transverse stripes originally observed in the A band by Draper & Hodge (1950). Seven of the eleven stripes, stripes 5–11 (Fig. 2), consistently stain with antibody to C protein prepared from rabbit fast skeletal muscle (Starr & Offer, 1983). Depending on the conditions, stripes 3 and 4 (Fig. 2) may also stain with this antibody. Other proteins that are often isolated in association with myosin, the H and X proteins, have also been characterized, but their relationship to C protein and to the cross stripes of the A band is not clear. C protein isolated from slow skeletal muscle is

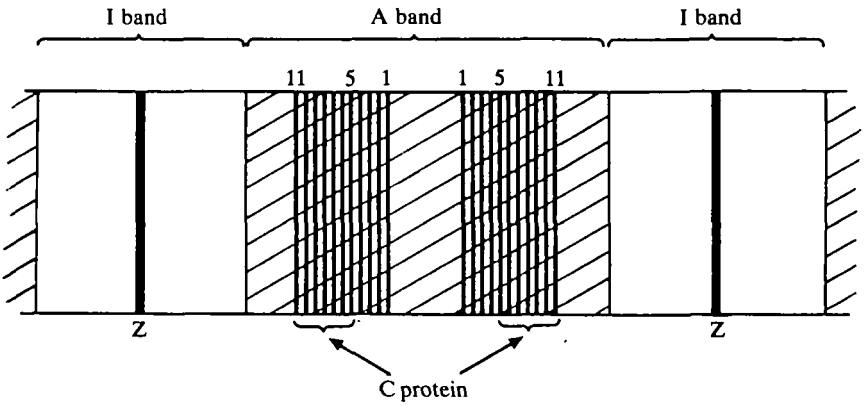

Fig. 2. Scheme illustrating the location of C protein and other A filament proteins in the skeletal myofibril. The cross-stripes staining for C and related proteins are numbered 1 to 11.

not identical with that present in fast muscle and shows many similarities with X protein. Because of its unusual distribution in type I and II fibres, Starr & Offer (1983) have reserved judgement on whether X protein is indeed the slow muscle isoforms of C protein, although the available evidence suggests that it is (Reinach *et al.* 1982; Dhoot, Hales, Grail & Perry, 1985).

The localization of the isoforms of C protein is somewhat different to that observed with other myofibrillar proteins. Type I cells and certain type II cells contain only slow and fast forms of C protein respectively. A number of cells that are apparently type II in that they stain with fast troponin I and are positive for myosin ATPase after preincubations at pH 9·4, stain for both fast and slow forms of C protein (Fig. 3). The relative amounts of the two isoforms of C protein in the latter cells vary as judged from the density of immunoperoxidase staining. As yet there has been no clear indication of a role for C protein although the evidence that it can be phosphorylated in cardiac muscle (Jeacocke & England, 1980; Hartzell & Titus, 1982) suggests that it may have a regulatory function, possibly at the cross-bridge level. The distribution of the isoforms is also suggestive of a function related to the activity pattern of the muscle. Nevertheless it is difficult to understand why some cells contain variable proportions of the two isoforms. In this respect it would be of interest to determine in cells containing both isoforms whether they are both present in the same myofibril and, if they are, whether each stripe contains a single isoform. If the latter is the case, then the varying staining intensities observed with the two antibodies may reflect the relative number of stripes containing each isoform.

I FILAMENT PROTEINS

Actin

Actin is the most strongly conserved of the muscle proteins. It is the only myofibrillar protein so far shown to be present in fast and slow skeletal muscles in

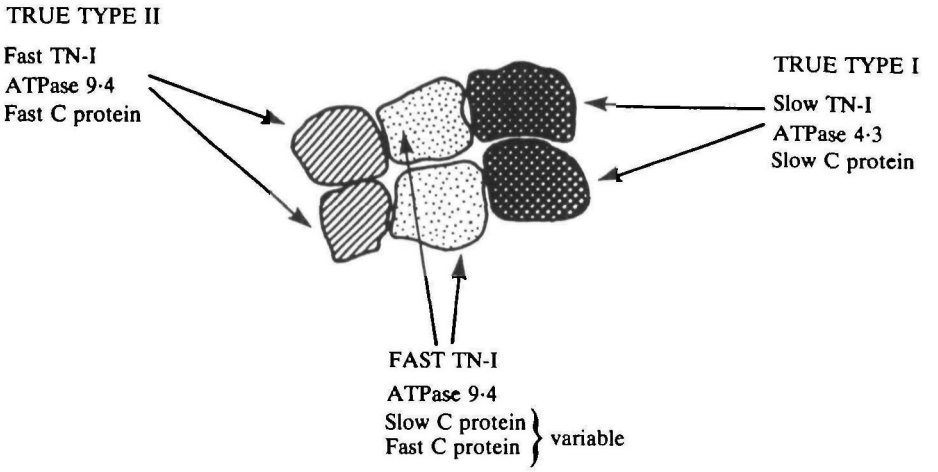

Fig. 3. Staining of rabbit skeletal muscle cells with the antibodies to the slow and fast isoforms of C protein. The diagram illustrates the three types of staining observed with the immunoperoxidase procedure, when skeletal muscle cells are treated with antibodies to the isoforms of troponin I (TN-I) and C protein and for myosin ATPase after preincubation at pH 9·4 and 4·3 (Dhoot, Hales, Grail, & Perry, 1985).

identical forms. The amino acid sequence of bovine cardiac actin differs from rabbit skeletal actin in only four out of 375 residues (Table 2). In different smooth muscles the actin sequences vary slightly, for example bovine stomach and aorta actins differ

Table 2. *Comparison of amino acid sequences of actin from various mammalian muscles*

Residue no.	Skeletal fast, slow Rabbit	Cardiac Cow	Smooth (Stomach) Cow	Smooth (Aorta) Cow
1	Asp		—	Glu
2	Glu	Asp		
3	Asp	Glu	Glu	Glu
4	Glu			Asp
5	Thr			Ser
17	Val		Cys	Cys
89	Thr	Ser	Ser	Ser
298	Met	Leu	Leu	Leu
357	Thr	Ser	Ser	Ser
374 (375)	Phe			
Number of residues changed		5	6	8

In the left-hand column the numbers of the variant residues are listed. The C-terminal residue is also listed as residue 374 to correspond with the original actin sequence (Lu & Elzinga, 1977), although there are probably 375 residues. By this convention, the serine residue after residue 234 is designated serine 234a (Vandekerckhove & Weber, 1979).

Unless otherwise indicated the residue is identical with that in rabbit skeletal muscle.

A horizontal line indicates a residue is absent (from Vandekerckhove & Weber, 1979).

from rabbit skeletal actin in six and eight residues respectively. With the exception of the replacement of valine 17 with cysteine in smooth muscle actin all the amino acid replacements are conservative and do not affect the charge at physiological pH values. Thus it would appear that the role of actin in the contractile system is very similar in all muscle types. Unlike the other components of the myofibril the isoforms of actin do not exhibit amino acid substitutions that would produce a significant difference in properties. In the I filament of skeletal muscle actin has to interact with a number of other proteins, the isoforms of which differ considerably in their amino acid sequences whereas the sequence of the actin is unchanged. It is probable that the relatively minor changes that exist between the actin isoforms are to accommodate interaction of actin with the proteins associated with it in the thin filament rather than to affect its role in the energy transduction process.

Tropomyosin

The filamentous nature of the tropomyosin molecule and its ability to form linear head-to-tail aggregates implies that it plays an important structural role in the myofibril. Nevertheless in striated muscle, there is clear evidence that tropomyosin has a regulatory role as it is essential for Ca^{2+}-sensitivity of the MgATPase of actomyosin in the presence of troponin. It is probable that this property is a special feature of tropomyosin adapted for the type of regulation characteristic of striated muscle. Tropomyosin is probably present in all tissues that possess actomyosin contractile systems even though troponin is restricted to striated muscle. Its function is presumably related to its ability to interact and form a linear aggregate with actin and thus possibly stabilize the structure of the filament. Although closely associated with one isoform of actin in skeletal muscle, different forms of tropomyosin are associated with type I and type II fibres.

The tropomyosin molecule is a dimer consisting of two subunits of molecular weight about 33 000 Da. Originally two forms of tropomyosin subunit were distinguished on the basis of their electrophoretic mobility. These were designated α and β subunits (Cummins & Perry, 1973) and have been shown to differ in 39 residues (Mak, Smillie & Stewart, 1980), only two of which lead to charge differences. The ratio of α and β subunits is constant for a given muscle and in general the α-tropomyosin content is higher in fast skeletal muscles. Application of high resolution, two-dimensional electrophoresis indicates that the tropomyosin isoform composition is rather more complex than was previously thought. In general, fast skeletal muscles contain α and β forms, whereas in slow skeletal muscles the α subunit is absent or partially replaced, depending on the species, by additional forms designated γ and δ (Heeley *et al.* 1983, Fig. 4). Thus several isoforms exist in a given cell type raising the question of whether tropomyosin can exist *in vivo* as a heterodimer as well as a homodimer. Little evidence of functional differences between the forms is available but it would appear that the tropomyosin variation is more related to its interaction with the components of the troponin complex which are known to exist in isoforms characteristic of the muscle type. There is some evidence, for example, that the binding to troponin T differs in α- and β-tropomyosins.

It is difficult to understand why several isoforms of tropomyosin are present in the same cell if there is a significant difference in their biological properties. The relative amounts of α and β subunits in a given fast muscle are remarkably constant despite the evidence that considerable variation can be observed in the $\alpha:\beta$ ratio in individual type II fibres of rabbit psoas muscle (Heeley *et al.* 1983). Tropomyosin from cardiac muscle of small animals at least, consists almost entirely of the α subunit.

In living muscle, α-tropomyosin is partially phosphorylated at serine 283. There is evidence for phosphorylation for the other isoforms, but usually less than is the case with the α subunit. The role of phosphorylation is not clear but higher levels are observed in foetal muscles. This suggests that tropomyosin phosphorylation may have a role in muscle growth and development rather than in the regulation of contraction.

Troponin complex

The components of the troponin complex exist in isoforms which in many species are present as a single form characteristic of the cell type. This applies particularly to troponin I and C, but troponin T in chickens has been reported to exist in several isoforms in fast skeletal muscle (Wilkinson, Moir & Waterfield, 1984).

Troponin C is unusual in that the cardiac and slow skeletal muscle isoforms have an identical sequence. This would suggest that the troponin complex exhibits similar Ca^{2+}-binding properties in the two types of muscle, but it should be pointed out that interaction with troponin I increases the binding constant of troponin C for Ca^{2+}. As the isoform of troponin I in slow skeletal muscle is not identical to that in cardiac muscle, they will presumably affect the Ca^{2+}-binding characteristics of troponin C differently. Thus although the troponin C molecules in slow skeletal and cardiac muscles are identical, they may have slightly different binding characteristics in the troponin complex *in situ*. Slow skeletal and cardiac muscle troponin C, however, possess only one Ca^{2+}-specific site, site II (Fig. 5). Site I in all forms of cardiac troponin C sequenced so far contains amino acid substitutions that render the site ineffective for calcium binding. As the Ca^{2+}-specific sites are considered to be sites that must be filled to trigger contraction, slow skeletal and cardiac muscles will be

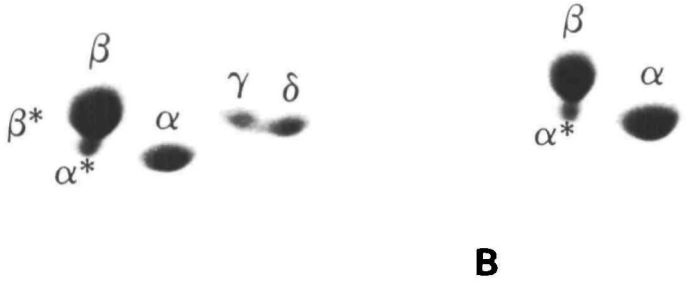

Fig. 4. Isoforms of tropomyosin in fast and slow skeletal muscles. Two-dimensional electropherograms of extracts of rabbit muscles. Asterisk indicates that the isoform is phosphorylated; (A) soleus muscle; (B) extensor digitorum longus muscle.

activated by the uptake of one Ca^{2+} per troponin molecule rather than two as is the case with fast skeletal muscle.

Each type of striated muscle cell (slow and fast skeletal and cardiac) contains a specific form of troponin I. Although considerable sequence differences involving up to 40 % of the residues exist between the three forms (Wilkinson & Grand, 1978), two regions are virtually identical in all three forms of the rabbit muscle protein (Fig. 6). These are regions that have been identified, by studies on partial digestion products, to be involved in interactions with troponin C and actin. They are also very close to serine or threonine residues that are sites or presumptive sites of phosphorylation in all three isoforms.

Cardiac troponin differs from the other isoforms in possessing an extra 26-residue sequence at the N-terminus. In this region serine 20 is readily phosphorylated by cAMP-dependent protein kinase and the phosphate at this site exchanges rapidly with the intracellular pool. In normally beating rabbit heart the site is about 30 % phosphorylated and on β-adrenergic stimulation this rises to approach 100 %. Phosphorylation of serine 20 of cardiac troponin I changes the Ca^{2+}-sensitivity of the actomyosin ATPase, i.e. a higher Ca^{2+} concentration is required for 50 % activation of the ATPase. The process is reversible for phosphorylation if troponin I returns to the normal value after β-adrenergic stimulation (Perry, 1983; England, 1983). This process represents a special adaptation for the regulation of contraction by the myocardial cell which is able to manipulate the Ca^{2+} flux to increase the contractile force developed by each cell.

Troponin T, like troponin I, also exists in forms that are specific for the muscle type but as yet there is little information about the differences in the biological properties of the three forms. In addition to the muscle type-specific isoforms of troponin T three isoforms are present in chicken fast skeletal muscle. One, of molecular weight 33 500 Da, is present in breast muscle whereas two forms, of molecular weight 30 500 and

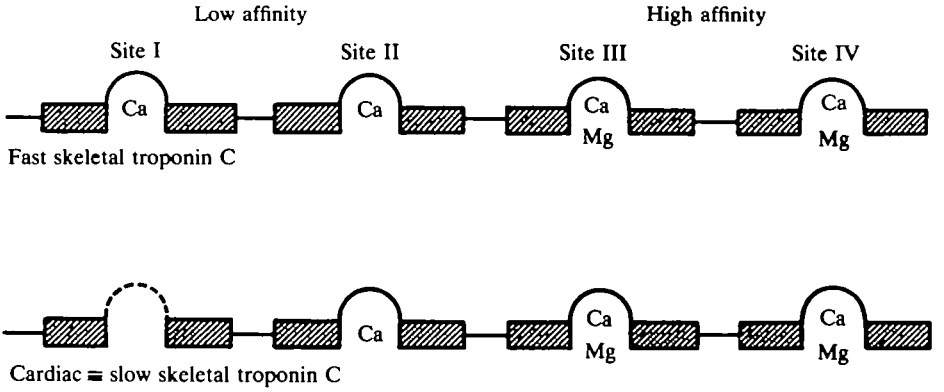

Fig. 5. Calcium-binding domains of troponin C from striated muscle. The shaded boxes and enclosing loop represent the calcium-binding domains, α-helix-calcium binding loop -α-helix.

29 800 Da, are found in the leg muscle. The sequence differences in these three forms of troponin T are confined to the N-terminal region and it has been proposed that all three proteins are derived from a single gene which gives rise to three different mRNA molecules by alternative pathways of splicing (Wilkinson *et al.* 1984).

FACTORS DETERMINING GENE EXPRESSION

From Table 3 it can be seen that in skeletal muscle at least 12 genes are concerned in the synthesis of the five proteins of which the I filament is composed. To produce an I filament corresponding to either a type I or type II fibre at least five of these genes must be expressed in a coordinated manner so that an appropriate set of isoforms is produced. The capacity of the skeletal muscle cell to express all the isoforms is indicated when the switch from the slow muscle to the fast muscle isoforms can be induced by replacing the slow with a fast nerve or by imposing direct stimulation of the appropriate frequency. This transformation has now been demonstrated with myosin (Buller, Mommaerts & Seraydarian, 1969; Barany & Close, 1971; Weeds, Trentham, Kean & Buller, 1974; Sreter, Gergely, Salmon & Romanul, 1973; Pette,

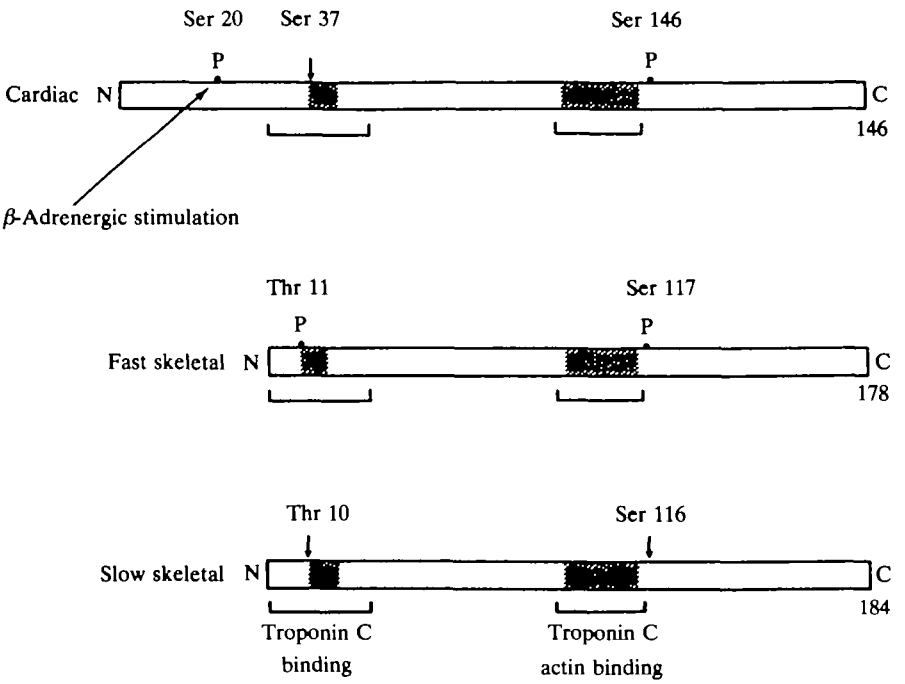

Fig. 6. Scheme illustrating the amino acid sequences of the isoforms of troponin I from striated muscle. Numbers indicate the positions of the amino acid residues. The regions on the isoforms involved in interaction with actin and troponin are indicated by brackets. The shaded areas are virtually identical in all three isoforms. P, residues known to be phosphorylated.

Muller, Leisner & Vrbová, 1976; Salmons & Sreter, 1976; Rubinstein *et al.* 1978), components of the troponin complex (Amphlett *et al.* 1975; Dhoot, Perry & Vrbová, 1981) and tropomyosin (Heeley *et al.* 1983). The tropomyosin transformation is of interest in that it is unlike that observed with myosin and troponin components where the isoforms are completely replaced. In rabbit tropomyosin the β subunit remains relatively constant in amount, the γ and δ subunits are replaced by the α subunit in the transformation of slow to fast muscle by cross innervation and *vice versa*. This suggests some kind of reciprocal mechanism controlling the expression of the genes for α and γ and δ tropomyosins.

The strict compartmentalization of the isoforms in type I and type II fibres seen in adult skeletal muscle requires innervation by the two types of nerve. In the absence of nerve the ground state of the gene expression in skeletal muscle produces the fast muscle isoforms of the troponin components and α and β tropomyosin, which are the forms characteristic of fast skeletal muscle. This is evident from studies on the distribution of the I filament protein isoforms in foetal muscle before nerve differentiation (Dhoot & Perry, 1980), in regenerating muscle (Dhoot & Perry, 1982) and after denervation (Heeley, Dhoot & Perry, 1985).

CONCLUSIONS

It is clear that the isoform composition of the myofibril is an important factor in determining its specialized function. Obvious examples are myosin, where there is a good correlation with the speed of contraction and the V_{max} of the enzyme (Barany, 1967), and troponin C, where only one Ca^{2+} site has to be filled to trigger contraction in slow skeletal and cardiac muscles compared to fast skeletal muscle. This may be related to the more restricted Ca^{2+}-sequestering systems in the former tissues compared to that in fast skeletal muscle. It is less easy to understand the significance of the isoform distribution of proteins such as tropomyosin and C protein which have a largely structural role; nevertheless the isoforms present are specific for the type of muscle cell. A further problem is to understand whether there is any functional significance in the presence of two or more different isoforms in a given cell type, as applies in the case of myosin and tropomyosin.

Table 3. *Isoforms of I-filament proteins in skeletal and cardiac muscles*

Protein	Skeletal		Cardiac
	Type I fibres (slow)	Type II fibres (fast)	
Actin	Skeletal	Skeletal	Cardiac
Troponin C	Slow (= cardiac)	Fast	Cardiac (=slow)
Troponin I	Slow	Slow	Cardiac
Troponin T	Slow	Fast*	Cardiac
Tropomyosin	$(\alpha),\beta,\gamma,\delta$	α,β	$\alpha(\beta)$
No. of genes	7–8	6	5–6

* There is evidence of several forms of troponin T in chicken fast skeletal muscle (see text).

The isoform composition of myofibrillar proteins is not changed rapidly but a reversible method of adapting the system to the activity pattern is covalent modification. Thus by the phosphorylation of proteins such as myosin and troponin I, processes that are marked in certain muscle types, the tissue is able to respond rapidly to the functional demands.

In skeletal muscle, only a fraction of the total number of genes that are available for the synthesis of the myofibrillar proteins needs to be activated to synthesize the proteins of a functional A or I filament system. Clearly a complex mechanism for control of gene expression that is related to the activity pattern of the muscle regulates the production of the appropriate complement of isoforms.

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