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Factors determining the subunit composition of tropomyosin in mammalian skeletal muscle

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1. Adult rat fast-twitch skeletal muscle such as extensor digitorum longus contains α- and β-tropomyosin subunits, as is the case in the corresponding muscles of rabbit. Adult rat soleus muscle contains β-, γ- and δ-tropomyosins, but no significant amounts of α-tropomyosin. 2. Evidence for the presence of phosphorylated forms of at least three of the four tropomyosin subunit isoforms was obtained, particularly in developing muscle. 3. Immediately after birth α- and β-tropomyosins were the major components of skeletal muscle, in both fast-twitch and slow-twitch muscles. 4. Differentiation into slow-twitch skeletal muscles was accompanied by a fall in the amount of α-tropomyosin subunit and its replacement with γ- and δ-subunits. 5. After denervation and during regeneration after injury, the tropomyosin composition of slow-twitch skeletal muscle changed to that associated with fast-twitch muscle. 6. Thyroidectomy slowed down the changes in tropomyosin composition resulting from the denervation of soleus muscle. 7. The results suggest that the 'ground state' of tropomyosin-gene expression in the skeletal muscle gives rise to α- and β-tropomyosin subunits. Innervation by a 'slow-twitch' nerve is essential for the expression of the genes controlling γ- and δ-subunits. There appears to be reciprocal relationship between expression of the gene controlling the synthesis of α-tropomyosin and those controlling the synthesis of γ- and δ-tropomyosin subunits.

Each of the myofibrillar proteins exists in a number of different forms, isotypes or isoforms of similar biological function that possess different amino acid sequences. The small differences in properties of the isoforms of each myofibrillar protein are in part responsible for the differences in physiological properties that exist between muscle types. Cells of slow-twitch and fast-twitch skeletal muscle and of ventricle and atrium of the heart contain isoforms of the myofibrillar proteins that are characteristic of the cell type (Perry *et al.*, 1984). As the skeletal-muscle cell is capable of synthesizing the fast-twitch-muscle and slow-twitch-muscle isoforms of each of the myofibrillar proteins, very precise regulation of the genes (or in some cases RNA-processing systems) controlling the synthesis of the different isoforms must exist in differentiated muscle. Some myofibrillar proteins, for example the components of the troponin complex or actin, exist as a single isoform in a given cell type, whereas others, such as myosin and tropomyosin, exist in several isoforms, the complement of which is characteristic of the muscle fibre type (Perry *et al.*, 1984).

The earlier view that in striated muscle tropomyosin existed as two subunits, designated α and β, and that different cell types merely contained different proportions of the α- and β-subunits (Cummins & Perry, 1973, 1974) is clearly oversimplified. Evidence has been produced for the existence of additional isoforms, particularly in slow-twitch skeletal muscles, which have been designated γ and δ forms (Heeley *et al.*, 1983). Indeed, there are suggestions that even the α and β forms of tropomyosin may be muscle-type-specific (Billeter *et al.*, 1981; Kardami *et al.*, 1983). Although it is recognized that the complement of tropomyosin subunits is characteristic of the muscle type, apart from some studies on the effect

Abbreviation used: EDL, extensor digitorum longus.

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of cross-innervation on the tropomyosin composition (Roy *et al.*, 1979; Dhoot *et al.*, 1981; Heeley *et al.*, 1983) and on development (Amphlett *et al.*, 1976; Montarras *et al.*, 1982; Matsuda *et al.*, 1983) there is relatively little information about the factors that affect tropomyosin-gene expression in mammalian skeletal muscle. The present investigation was undertaken to throw more light on this process. By study of a number of conditions that change the skeletal-muscle cell phenotype it has been demonstrated that the genes coding for the γ- and δ-tropomyosin subunits appear to be expressed together and that there is a reciprocal relationship between their expression and that of the gene controlling synthesis of the α-tropomyosin subunit. Also, as has been demonstrated with the troponin components (Dhoot & Perry, 1980, 1981, 1983) and with myosin (Rubinstein & Kelly, 1978), the 'ground state' of tropomyosin-gene expression, which occurs in the absence of nerve or before its differentiation into fast-twitch and slow-twitch types, gives rise to the α- and β-subunits characteristic of fast-twitch skeletal muscle.

This present study and our earlier investigations on the troponin components (Dhoot & Perry, 1980, 1981, 1982, 1983) form part of a general investigation into the factors that regulate the genetic control of the synthesis of a multiprotein structure in muscle, the I-filament.

Materials and methods

Animals

Normal and thyroidectomized rats were of the Wistar strain purchased from Charles River, Margate, Kent, U.K. Thyroidectomy was carried out at 12 weeks.

Denervation

Leg muscles were denervated at the ages stated in the text by cutting the sciatic nerve in the proximal part of the thigh under diethyl ether anaesthesia. The nerves were then reflected to prevent re-innervation of the lower leg muscles. The leg muscles prepared specifically for this study were denervated at 16 and 40 weeks and examined 12 weeks later in each case. Other samples examined were muscles from previous studies on the effect of thyroidectomy (Dhoot & Perry, 1981) and denervation for 12 and 24 weeks (Dhoot & Perry, 1982) on the distribution of the fast-twitch-muscle and slow-twitch-muscle isotypes of troponin I. Animals were denervated 3–4 days after removal of the thyroid at 12 weeks, and muscles were examined 12 weeks later. Muscles from the earlier studies had been stored at -70°C before electrophoretic analysis.

Regeneration

Leg muscles of rats which were at least 14 weeks old were injured under ether anaesthesia by the application of solid CO_2 to the exposed surfaces of muscles for periods of up to 1 min (Price *et al.*, 1964). The animals were killed 7–12 days after injury, and the areas of necrosis were confirmed by staining frozen sections with haematoxylin and Van Geisen stain. Regenerating cells were separated from the original uninjured muscle by dissection and then prepared for electrophoretic analysis.

Immunohistochemistry

Immunoperoxidase staining of sections was carried out as described by Dhoot *et al.* (1978).

Electrophoresis of muscle samples

Muscles were homogenized in 10 vol. (w/v) of 9 M-urea and the solution then made 15 mM with respect to 2-mercaptoethanol and 2% (v/v) with respect to the detergent Nonidet P40. The homogenate (5–8 μl) was immediately subjected to isoelectric focusing and two-dimensional electrophoresis as described by O'Farrell (1975), with the IsoDalt apparatus (Anderson & Anderson, 1978*a*,*b*). The range for the isoelectric-focusing dimension was pH 4–6, with 2% Ampholines (LKB Instruments, Croydon, U.K.), and the samples were focused for a total of 10000 V·h. The first-dimension gels were then applied to the second-dimension gel, which consisted of a linear gradient of 10–20% (w/v) polyacrylamide.

Staining

Two-dimensional electrophoretograms were stained overnight, in multiples of ten, in a shaking bath containing 0.15% Coomassie Brilliant Blue R-250 in 50% (v/v) ethanol/10% (v/v) acetic acid and destained in 10% (v/v) ethanol/5% (v/v) acetic acid. Destained gels were photographed with a 535 nm interference filter and Ilford technical Orthofilm.

Densitometry

When the different electrophoretic forms of tropomyosin were adequately separated, the relative amounts of the tropomyosin subunits and also the proportion of phosphorylated and dephosphorylated derivatives of these subunits were estimated by densitometric scanning as described previously (Heeley *et al.*, 1982).

Results

High-resolution two-dimensional electrophoresis of whole extracts of fast-twitch and slow-twitch muscles of adult rats gave rise to a number

of spots in the region of the gel corresponding to the M_r and isoelectric point of tropomyosin (Figs. 1 and 2). The patterns of spots were similar to those obtained with the same muscles from other species, especially the rabbit. From densitometric analysis it was estimated that the EDL muscle of the rat contained α- and β-tropomyosin subunits in the ratio of 60:40. A somewhat similar ratio for these two subunits was found in rabbit EDL muscle (Heeley *et al.*, 1983). In those electrophoretograms in which the resolution was especially good, it was noted that each subunit and its phosphorylated derivative, identified by experiments in which intact muscles were incubated with [^{32}P]P_i migrat-

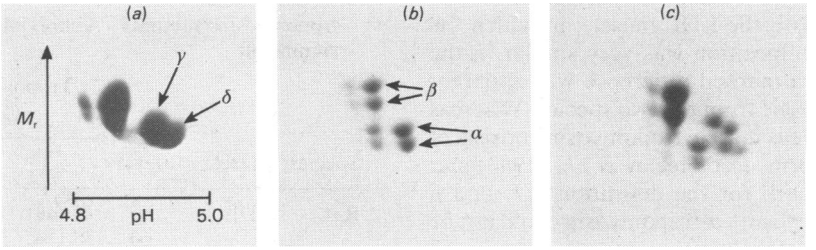

Fig. 1. *Subunits of tropomyosin in rat and rabbit skeletal muscles*
Two-dimensional electrophoresis was carried out on extracts of whole muscle (see the Materials and methods section). The region of electrophoretograms illustrated corresponds to M_r values of 30000–35000 and pI in the range 4.8–4.9. (*a*) Rat soleus muscle (1 year old); (*b*) rat EDL muscle (1 year old); (*c*) combined rat and rabbit soleus muscles. Arrows in (*a*) indicate the γ and δ forms that have not been resolved as separate spots.

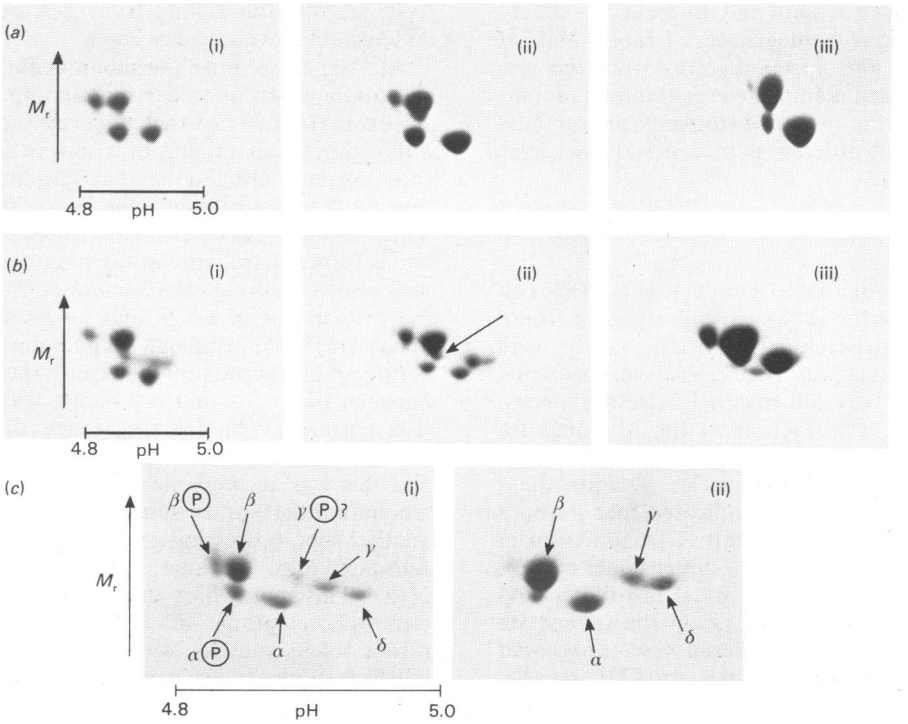

Fig. 2. *Changes in tropomyosin subunit composition during development of skeletal muscle*
Electrophoretograms were prepared as indicated in Fig. 1 legend. (*a*) Rat EDL muscle: (i) 5 days; (ii) 19 days; (iii) 60 days. (*b*) Rat soleus muscle: (i) 5 days; (ii) 19 days; (iii) 60 days. Arrow indicates phosphorylated form of either γ- or δ-subunits. (*c*) Rabbit soleus muscle: (i) 2 days before birth; (ii) 1 year old. Arrows indicate in *c*(ii) the α-, β-, γ- and δ-subunits of tropomyosin, and in *c*(i) the phosphorylated (Ⓟ) and the dephosphorylated derivatives of these subunits are indicated.

ed as a double spot (Fig. 1*b*). This observation, to which reference has been made previously (Heeley *et al.*, 1982, 1983), was not considered to be an artifact, for proteins migrating in neighbouring regions of the electrophoretograms, e.g. the myosin light chains appeared as single spots. The extent of phosphorylation of the β-subunit was always less than that of the α-subunit. This was especially apparent in adult muscles, in which the β-subunit was almost completely dephosphorylated.

In contrast with the EDL muscle, in which the tropomyosin composition was very similar in the rat and rabbit, a marked difference was apparent in the soleus muscle from the two species. Whereas in the rabbit soleus muscle tropomyosin consists of α, β, γ and δ forms [see Heeley *et al.*, (1983) and Figs. 1 and 2(*c*)(ii) for the definition of γ and δ forms of tropomyosin], α-tropomyosin could not be detected in electrophoretograms of extracts of adult rat soleus muscle. In this muscle only the β, γ and δ forms could be detected, and frequently the last two subunits merged into an asymmetric spot (Fig. 1*a*). As was the case with β-tropomyosin from EDL muscle (Fig. 1*b*), the phosphorylated and dephosphorylated forms of β-tropomyosin of rat soleus muscle migrated as two spots of slightly different isoelectric point and different M_r. On co-electrophoresis of homogenates of rabbit and rat soleus muscle the β-tropomyosins from the two species possessed identical electrophoretic mobilities, whereas the γ- and δ-tropomyosin subunits exhibited slight differences in size and isoelectric point (Fig. 1*c*).

Changes in tropomyosin composition during development

Earlier investigations (Amphlett *et al.*, 1976) on the changes in the subunit composition of tropomyosin during development in the rabbit were limited by the relatively poor resolution of subunits obtained on one-dimensional electrophoresis, coupled with the fact that the tropomyosin analysed was isolated from the whole foetal carcass containing a mixture of muscles. Despite these shortcomings, the studies indicated that β-tropomyosin was the major subunit of tropomyosin in foetal rabbit muscle. On two-dimensional electrophoresis of extracts of individual fast-twitch and slow-twitch muscles of the rat in the immediate postnatal period, marked differences were observed between the two muscle types. In EDL muscle, postnatal development merely involved a change in the ratio of α- to β-subunits. At 5 days after birth the β-subunit represented 55% of the total tropomyosin, this falling to the normal adult value of approx. 40% at 60 days (Table 1). During the same period no γ- or δ-subunit forms could be detected in EDL muscle and the extent of phosphorylation of the α-subunit fell from 48% to 14%. At all ages β-tropomyosin was phosphorylated to a lesser degree, this falling from 37% at day 5 to undetectable levels at day 28.

Table 1. *Relative proportions of tropomyosin subunits in rat and rabbit skeletal muscles at different ages*

The relative amounts of tropomyosin isoforms and their phosphorylated derivatives were determined by densitometry as described in the Materials and methods section. Each value is the average of at least three determinations on a minimum of four separate samples: no determination in each group deviated by more than ±5% from the mean, and most were usually within ±2% of this value. Values in parentheses are percentage phosphorylation of tropomyosin subunits. Abbreviations: N.S., not significant.

Species	Muscle	Age (days)	Tropomyosin subunits (% of total) α	β	γ+δ
Rat	EDL	5	45 (48)	55 (37)	0
		60	58 (14)	42 (N.S.)	0
	Soleus	5	40	55	5
		60	N.S.	60	40
Rabbit	Soleus	365	25	46	29

At 5 days after birth the subunit composition of the tropomyosin of soleus muscle appeared very similar to that of a fast-twitch muscle such as EDL, although a small amount of a spot in addition to those corresponding to the α- and β-subunits could also be detected at this stage [Fig. 2(*b*)(i)]. This additional spot was considered to consist of the γ and δ forms of tropomyosin, which had not been well resolved during electrophoresis. At this stage the proportions of α-, β- and γ+δ-subunit were about 40:55:5, although this value must be considered as approximate because the resolution between the α-, γ- and δ-subunits was not good [Fig. 2(*b*)(i)]. With increasing age there was a gradual decline in the amount of α-tropomyosin, and this was accompanied by an increase in the amounts of the γ- and δ-subunits. At the same time, another spot, possessing a very similar mobility to β-tropomyosin, became more apparent [Fig. 2*b*(ii)]. This spot, which could also be detected on electrophoretograms of homogenates of foetal rabbit soleus muscle, was presumed from ^{32}P-labelling studies to correspond to the phosphorylated derivative of either the γ or the δ form of tropomyosin [Fig. 2*c*(i)].

Regeneration

The results described suggest that the 'ground state' of tropomyosin-gene expression is similar to that in fast-twitch skeletal muscle and that the

tropomyosin subunits characteristic of slow-twitch muscle appear in parallel with the development of innervation by 'slow-twitch' nerve. In this respect the findings resemble those of an earlier investigation on changes in the isoforms of the components of the troponin complex during development (Dhoot & Perry, 1980). To investigate whether this general observation also relates to regenerating muscle, the pattern of tropomyosin-gene expression in muscle recovery after injury was studied.

The area of injury by the solid-CO_2 burn was identified by microscopic study of muscle sections. Deeper regions of the muscles contained cells of normal diameter, which had escaped injury. Neither these nor regions of necrosis were used for analysis. Regeneration was confined to regions on the surface of the muscle that were characterized by the presence of small circular cells. Electrophoretic analysis was therefore restricted to small pieces of muscle about 2–3 mm thick and 6–7 mm long. No significant changes were apparent in the subunit composition of the tropomyosin present in these regions removed from EDL muscle during regeneration (Fig. 3*b*). In all cases the α-/β-subunit remained at the normal adult value of 60:40. In regenerating soleus muscle the tropomyosin subunit composition was very similar to that of a fast-twitch muscle, although an additional spot characteristic of γ- or δ-subunits or an unresolved mixture of these isoforms could be detected on the electrophoretograms (Fig. 3*a*). The significance of the presence of the latter subunits is not clear, and it is possible that it was due to a small degree of contamination by uninjured tissues of the section used for analysis. The presence of α-tropomyosin confirms that the muscle was regenerating, for this subunit is absent from adult rat soleus muscle.

Denervation

The effect of denervation on the tropomyosin subunit composition of EDL and soleus muscles was very similar to that observed after injury, although, unlike in the latter condition, the muscle cells were clearly not regenerating. The subunit composition of tropomyosin in EDL muscle did not change significantly after 12 or 24 weeks of denervation. The age at which denervation was carried out also did not significantly affect the results obtained (Table 2). Denervation of soleus muscle led to a decrease in the amounts of the γ- and δ-subunits and an increase in the α-subunit, but the pattern of change depended on the age at which denervation was performed. Soleus muscles that had been denervated at 12 weeks for periods of 3 and 6 months both contained α- and β-subunits but no γ- and δ-subunits [e.g. Fig. 4(*b*)(iii)]. On the other hand, 3 months after denervation of soleus muscles in some older animals, e.g. denervated at 16 and 40 weeks, a small amount of unresolved γ- and δ-tropomyosin remained [Fig. 4(*b*)(ii)], sug-

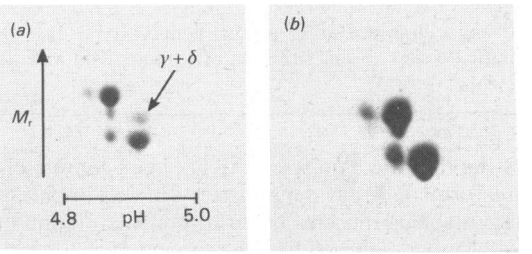

Fig. 3. *Tropomyosin subunits in regenerating rat soleus and EDL muscles*
Electrophoretograms were prepared as indicated in Fig. 1 legend. The muscles were obtained from a 56-week-old rat, 12 days after injury. (*a*) Soleus muscle; (*b*) EDL muscle. The spot indicated by the arrow in (*a*) probably represents unresolved γ- and δ-tropomyosins.

Table 2. *Effect of denervation on the subunit composition of tropomyosin in rat skeletal muscle*
Relative proportions of tropomyosin isoforms were determined as indicated for Table 1. Results are average of at least three determinations on each sample.

Age when denervated (weeks)	Time denervated (weeks)	Age at examination (weeks)	No. of muscles	Proportion of tropomyosin subunits (%) EDL α	EDL β	EDL γ+δ	Soleus α	Soleus β	Soleus γ+δ	Cells in soleus staining for fast-twitch troponin I (% of total)
Control*	–	12	3	59	41	0	0	60	40	2
12*	12	24	3	59	41	0	61	39	0	75
12*	24	36	1	61	39	0	63	38	0	92
16	12	28	3	55	45	0	Incomplete change			–
40	12	52	3	59	41	0	Incomplete change			–

* Muscles from a previous study (Dhoot & Perry, 1982), which had been stored at −70°C before analysis.

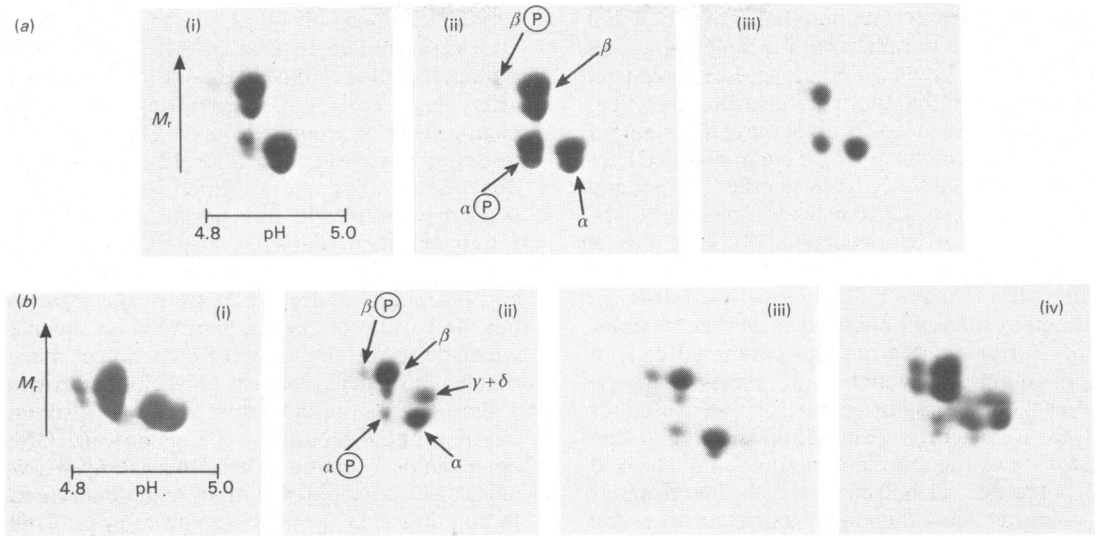

Fig. 4. *Effect of denervation on the tropomyosin subunits of rat skeletal muscle*
Electrophoretograms were prepared as indicated in Fig. 1 legend. (*a*) EDL muscle denervated at 12 weeks of age: (i) control; (ii) 3 months after denervation; (iii) 6 months after denervation. (*b*) Soleus muscle: (i) control; (ii) denervated at 40 weeks, examined at 52 weeks; (iii) denervated at 12 weeks, examined at 36 weeks; (iv) thyroidectomized at 12 weeks, denervated 3 days later and examined at 24 weeks. Arrows in (*a*)(ii) and (*b*)(ii) indicate the various subunits of tropomyosin and the phosphorylated derivatives (Ⓟ) of α- and β-tropomyosin.

gesting that these muscles had not been completely transformed. From densitometry it was apparent that in soleus muscles the β-tropomyosin content had also been altered by the removal of the nerve. In such cases the α-/β-subunit ratio approximated to 60:40, indicating that the synthesis of β-tropomyosin had been slightly suppressed in the denervated soleus muscle. The failure of Roy *et al.* (1980) to demonstrate changes in the tropomyosin of rabbit skeletal muscle after denervation can be explained by their use of one-dimensional electrophoresis to determine the subunit composition. As this procedure does not resolve α-tropomyosin from the γ- and δ-subunits, changes in relative proportions of these isoforms would not be detected (Heeley *et al.*, 1983). With the use of two-dimensional electrophoresis, Carraro *et al.* (1981) also report an increase in the α-/β-subunit ratio of tropomyosin in rat diaphragm after long-term denervation.

These results suggest that the innervation with a 'slow-twitch' nerve was a major factor in stimulating expression of the genes controlling the synthesis of tropomyosin subunits in slow-twitch muscle. Other factors must also be involved, however. When the denervation was carried out in thyroidectomized animals, i.e. in the absence of thyroid hormone, only a small amount of α-tropomyosin could be detected in soleus muscle after 12 weeks of denervation, and γ- and δ-tropomyosin were present in significant proportions [Fig. 4(*b*)(iv)]. In soleus muscles of age-matched animals denervated for a similar period with the thyroid intact ($n = 3$) virtually all the γ- and δ-subunits disappeared and the tropomyosin subunit composition corresponded to that of a fast-twitch muscle.

Phosphorylation of tropomyosin

Previous studies in this laboratory (Heeley *et al.*, 1982) and by others (Montarras *et al.*, 1981; Dabrowska *et al.*, 1983) have indicated that in circumstances in which active myofibrillogenesis was occurring, for example during early development, the proportion of tropomyosin in the phosphorylated form was much higher than in normal adult muscle. The developmental studies reported in the present paper confirm that this is the case with both α- and β-tropomyosin in EDL and soleus muscles, which are more highly phosphorylated immediately after birth than in the adult muscle. Invariably, α-tropomyosin was more highly phosphorylated than was the β-subunit. Phosphorylation was found not to be restricted to α- and β-tropomyosins, for phosphorylated forms of γ- and δ-subunits could be detected in developing slow-twitch muscle [Figs. 2(*b*)(ii) and 2(*c*)(i)]. The association of increased phosphorylation with active muscle growth was not observed in all studies, however, for little significant increase in phosphorylation could be observed in the tropo-

myosin subunits present in regenerating muscle, where myofibrillogenesis would be expected to be very active. Although in some cases increases in the phosphorylation of α-tropomyosin were detected, no consistent trend in the extent of phosphorylation could be observed after denervation [compare Figs. 4(*a*)(ii) and 4(*b*)(ii)] or after cross-innervation (Heeley *et al.*, 1983), situations in which marked changes in tropomyosin-gene expression were occurring.

Discussion

This study provides further evidence for the presence in slow-twitch muscle of additional tropomyosin subunits, the so-called γ and δ forms that are normally not found in fast-twitch skeletal muscle. The latter subunits were first shown to be present in the rabbit soleus muscle (Heeley *et al.*, 1983), but their presence also in the rat suggests that they are a general feature of slow-twitch skeletal-muscle fibres. It would seem likely that the spot in addition to those due to α- and β-subunits observed by other investigators on electrophoretograms of human (Billeter *et al.*, 1981), rabbit (Salviati *et al.*, 1982) and cat muscle (Steinbach *et al.*, 1980) represented unresolved γ and δ components. It was reported previously (Heeley *et al.*, 1983) that the α- and β-subunits from both fast-twitch and slow-twitch skeletal muscles of the rabbit migrated as a doublet under conditions of high resolution. Similar observations have now been made with rat muscle, and in some experiments the spots were clearly separated (see Fig. 1*b*). We do not believe this phenomenon is an artifact of electrophoresis, for it appeared to be a specific property of the tropomyosin subunits and their phosphorylated derivatives (Fig. 1*b*). The components in each doublet differed only in their M_r, and the appearance cannot be ascribed to a charge modification such as might occur if the protein underwent partial covalent modification during electrophoresis. At the moment we can offer no explanation for these observations.

Phosphorylated derivatives of the α- and β-subunits were persistently observed, and evidence was also obtained for phosphorylation of the γ- and δ-subunits, particularly in the developing soleus muscle of the rat and rabbit. α-Tropomyosin was usually more highly phosphorylated even under circumstances when it was not the major subunit present. This finding may be a consequence of the higher rate of phosphorylation of α-tropomyosin as has been reported with tropomyosin kinase prepared from chicken embryo (Montgomery & Mak, 1984). The extent of phosphorylation of all subunits was higher in developing muscle, but increased extents of phosphorylation were not always associated with active myofibrillogenesis, for they were not observed in regenerating muscle and only occasionally in denervated muscle, particularly in rats denervated at younger ages. In all subunits phosphorylation slightly increased the apparent M_r as determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

This was a general observation when the resolution obtained on electrophoresis was good [Fig. 2(*c*)(i)]. The increase was greater than would be expected from the addition of one phosphate group to the subunit molecule. It implies that phosphorylation of tropomyosin may produce a conformational change that persists even in the presence of sodium dodecyl sulphate and hence causes changed electrophoretic mobility. Similar changes in mobility on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis after phosphorylation have been observed with the regulatory subunit of type II cyclic AMP-dependent protein kinase (Hofmann *et al.*, 1975; Zoller *et al.*, 1979), glycogen synthetase kinase (Ahmad *et al.*, 1982), the 21000Da protein coded for by Harvey or Kirsten mouse sarcoma virus (Shih *et al.*, 1979), the avian β-adrenergic receptor (Stadel *et al.*, 1983) and phospholamban (Wegner & Jones, 1984). When 3.5M-urea was included in the sodium dodecyl sulphate used in the two-dimensional electrophoresis, the phosphorylated form migrated slightly faster than the dephosphorylated form of tropomyosin, rather than more slowly, as was the case in the absence of urea (D. H. Heeley & S. V. Perry, unpublished work).

The present studies indicate that the 'ground state' of tropomyosin-gene expression in rat skeletal muscle is that giving rise to the α- and β-subunits, i.e. those present in fast-twitch muscle. This conclusion is supported by the studies on skeletal muscle after denervation and injury and during the early stages of regeneration and development. The ability to express γ and δ forms of tropomyosin was restricted to certain cells, and particularly occurred in adult muscle when the fibre was innervated with a 'slow-twitch' nerve. During periods of transformation in rat skeletal muscle, changes in the subunit composition of tropomyosin were confined mainly to the α, γ and δ isoforms, although a minor alteration also occurred in the proportion of β-tropomyosin in the soleus muscle after denervation (Table 2). Similar changes occur after cross-innervation in the rabbit (Heeley *et al.*, 1983), although the proportion of total tropomyosin represented by the β-subunit remained constant during the transformation. These results suggest a reciprocal relationship between the gene controlling the expression of α-tropomyosin and those coding for the γ and δ forms of tropomyosin. It is noteworthy that in the

developmental studies by Montarras *et al.* (1982) a similar relationship was also observed for the fast-twitch and slow-twitch α-subunits of chicken muscle tropomyosin.

The changes in the tropomyosin isoforms resemble those observed in the isoforms of troponin I in muscles subjected to conditions similar to those described in this paper (Dhoot & Perry, 1982). Thus when the muscle undergoes transformation, under influence of nerve, development or hormone action, the genes controlling the isoforms of tropomyosin and the components of the troponin complex are under some kind of co-ordinated control. Actin, the other component of the I-filament, is not changed, for it is identical in both type I and type II fibres.

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