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Myosin light chain gene expression in developing and denervated fetal muscle in the mouse

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Summary

We have investigated the accumulation of mRNA transcripts of the atrial (or embryonic) myosin light chain $MLC1_A$ ($MLC1_{emb}$), and the two adult fast muscle myosin light chains ($MLC1_F$ and $MLC3_F$) during fetal skeletal muscle development in the mouse. In 15-day fetal muscle, $MLC1_A$ is the predominant mRNA detectable, by 18 days $MLC1_F$ has become the major transcript and $MLC3_F$ mRNA is detectable for the first time. By 12 days after birth, $MLC1_A$ transcripts are undetectable and $MLC1_F$ and $MLC3_F$ are similar in abundance. In fetuses treated with β-bungarotoxin and which therefore develop in the absence of functional nerve, $MLC1_A$ and $MLC1_F$ undergo normal transitions but $MLC3_F$ mRNA accumulation is significantly retarded. This demonstrates that these myosin light chain mRNAs accumulate with differing kinetics, and that $MLC3_F$ mRNA accumulation is nerve-dependent during fetal development. The results are discussed in terms of secondary muscle fibre formation, and in relation to the independent regulation of $MLC1_F$ and $MLC3_F$ mRNAs which are transcribed from the same gene.

Key words: myosin, light chain, development, muscle.

Introduction

Myosin alkali light chains (MLC) are encoded by a multigene family and show both tissue specificity and developmental regulation (for review, see Barton and Buckingham, 1985). In mammalian striated muscle, there are three principal MLC genes expressed. In adult fast skeletal muscle, the two fast isoforms $MLC1_F$ and $MLC3_F$ are encoded by a single gene (Robert *et al.* 1984; Periasamy *et al.* 1984; Nabeshima *et al.* 1984) and differ only in their *N*-terminal sequence. This gene uses two independent promoters (Strehler *et al.* 1985; Daubas *et al.* 1988) to generate primary transcripts that, following splicing, generate $MLC1_F$- and $MLC3_F$-specific mRNA, which are identical over most of their length but which differ in their 5′ coding and noncoding sequences. Slow skeletal muscle contains an isoform ($MLC1_S$) which is the same (and is encoded by the same gene) as the cardiac isoform present in ventricular muscle in mammals (Barton *et al.* 1985*a*). In birds, this isoform is expressed in both atrial and ventricular muscle (Nakamura *et al.* 1988). During mammalian development there is expression of an embryonic isoform $MLC1_{emb}$ (Whalen *et al.* 1978) in all striated muscles. This isoform persists in the atrial muscle where it is the major alkali light chain also known as $MLC1_A$ (Barton *et al.* 1985*b*, 1988). The situation in birds is different, here an embryonic isoform (L23) is expressed transiently and at low levels in early striated muscle, but is a major isoform of developing smooth muscle and is expressed in brain at all stages (Takano-Ohmuro *et al.* 1985; Kawashima *et al.* 1987). There is no evidence for the expression of the mammalian isoform $MLC1_{emb}$ in either smooth muscle or brain.

The influence of nerve stimulus on muscle fibre type and isoform composition in neonatal and adult muscle is well documented (for review see Pette and Vbrova, 1985). In particular the influence of nerve on myosin light chain expression has been examined in denervated (Matsuda *et al.* 1984), cross reinnervated (Weeds *et al.* 1974) and electrically stimulated muscle (Brown *et al.* 1983; Kirschbaum *et al.* 1989). In these experiments, it has been shown that, if fast skeletal muscle is denervated or stimulated with slow muscle type frequencies, it will gain characteristics of slow muscle and undergo isoform switching. For the myosin light chains this is typified by the re-expression of the slow ($MLC1_S$) isoform and a significant reduction in the accumulation of $MLC3_F$ protein and mRNA (Kirschbaum *et al.* 1989). In this situation, there is a transition in muscle fibre type

from fast to slow resulting in mixed fibre populations with isoform content typical of mixed fibre muscles, i.e. predominantly $MLCl_S$ and $MLCl_F$.

Transitions in the myosin light chain protein content of developing avian muscle have been well documented (Bandman *et al.* 1982; Crow *et al.* 1983; Takano-Ohmuro *et al.* 1985; Kawashima *et al.* 1988) and the influence of nerve contact examined in both neonatal muscle (Matsuda *et al.* 1984) and during early development (Merrifield and Konigsberg, 1987). In mammals, the pattern of myosin light chain gene expression during prenatal development is poorly documented, and the influence of prenatal denervation is unknown. In this paper, we describe the transitions in $MLC1_F$, $MLC3_F$ and $MLC1_A$ ($MLC1_{emb}$) mRNA accumulation in mouse skeletal muscle taken at 15 days and 18 days *in utero* and 12 days after birth. mRNA accumulation was measured by the use of cloned probes and S1-nuclease protection assay. In order to examine the influence of nerve on fetal development we have made use of β-bungarotoxin, a neurotoxin which binds to and destroys peripheral nerves (McCaig *et al.* 1987). Injection of β-bungarotoxin *in utero* destroys nerve fibres and subsequent muscle development therefore occurs in an aneural environment. Secondary muscle fibre formation is dependent on nerve activity, and fetal muscle that has been denervated by β-bungarotoxin injection, contains drastically reduced numbers of secondary fibres (Harris, 1981; Ross *et al.* 1987*a*, 1987*b*). In this study, denervation was effected at day 15 *in utero* and muscle analysed at day 18.

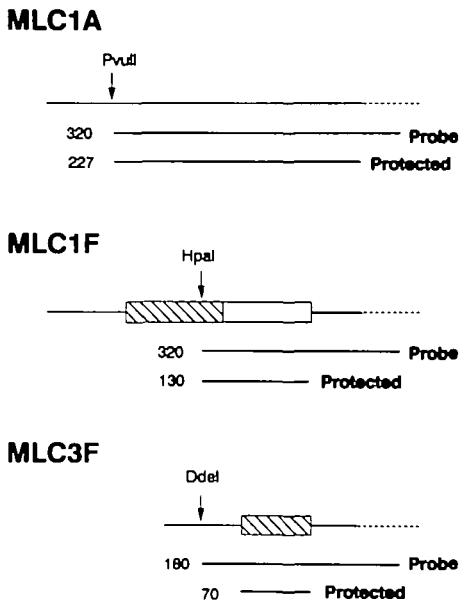

Fig. 1. Myosin alkali light chain specific probes used in S1 analysis. In each case, the DNA fragment used for probe preparation is shown together with the restriction site used, the size and position of the antisense probe generated, and the size and position of the resulting protected fragment. In the case of $MLC1_F$ and $MLC3_F$, these are cloned genomic DNA fragments containing exons 1 and 2 of the mouse $MLC1_F/MLC3_F$ gene which are specific for $MLC1_F$ and $MLC3_F$, respectively. In the case of $MLC1_A$ ($MLC1_{emb}$), this is a cDNA clone covering 3′ coding and noncoding sequence (see text). Exon sequences are indicated as boxes with hatched boxes representing 5′-non-coding sequence. Dotted lines represent M13 vector sequences.

Materials and methods

Denervation and preparation of RNA

C3H pregnant mice, dated from the time of conception, were sacrificed either at day 15 or day 18 for the preparation of fetal skeletal muscle RNA. β-bungarotoxin-treated fetuses were prepared as previously described (Weydert *et al.* 1987). Briefly, pregnant mice were anaesthetised and individual fetuses injected intraperitoneally with 1 μg of β-bungarotoxin (Boehringer) *in utero*. At day 18 mice were sacrificed and RNA extracted from both treated and untreated litter mates. β-bungarotoxin-treated fetuses were clearly paralysed and had markedly reduced muscle mass. Skeletal muscle was dissected from the hind limbs or ribs and frozen in liquid nitrogen. For the preparation of RNA, tissue was homogenized in 4 M-LiCl/8 M-urea and RNA selectively precipitated and re-extracted in LiCl/urea as in Barton *et al.* (1985*a*). RNA was also prepared from normal newborn (12 day C3H) mice. RNA from myotubes formed by the myogenic cell line T984-Cl10 (Jakob *et al.* 1978) was prepared as previously described (Barton *et al.* 1985*a*).

Probes used in S1 protection assay

In order to detect $MLC1_F$ and $MLC3_F$ mRNA transcripts, we have used genomic DNA fragments cloned into M13 which encompass either exon 1 or exon 2 of the $MLC1_F/MLC3_F$ gene (see Robert *et al.* 1984). These exons are specific to $MLC1_F$ or $MLC3_F$ mRNAs, respectively. Clone LC101 contains a genomic DNA fragment encompassing the whole of exon 1 of the $MLC1_F/MLC3_F$ gene inserted into the *Sma*I site of M13 mp8. Synthesis from the universal primer and subsequent restriction at the *Hpa*I site located within the 5′ noncoding sequence generates an antisense strand of about 320 nucleotides of which 130 are complementary to exon 1 (see Fig. 1). Clone LC40 contains the exon 2 of the $MLC1_F/MLC3_F$ gene inserted into the *Sma*I site of M13 mp8. This is the 5′ noncoding exon of $MLC3_F$ mRNA. Synthesis from the M13 universal primer and restriction with *Dde*I generates an antisense strand of 180 nucleotides, 70 of which are complementary to exon sequence. Clone PS6 contains the cDNA insert of the $MLC1_A$ clone pCl10.4 (see Barton *et al.* 1988) subcloned in M13 mp19. Synthesis from the M13 universal primer and restriction with *Pvu*II generates an antisense strand of 320 nucleotides of which 227 are complementary to $MLC1_A$ mRNA.

S1 protection assay

Labelled probes were generated from clones LC101 ($MLC1_F$), LC40 ($MLC3_F$) and PS6 ($MLC1_A$) by synthesis from the universal primer in the presence of α^{32}P dCTP and α^{32}P-dTTP and unlabelled dGTP and dATP. Following synthesis and digestion with the appropriate restriction enzyme (see above), the desired fragment was isolated on 6% acrylamide gel. S1 protection was carried out as described in Barton *et al.* (1988) using 200 ng equivalent of poly(A)$^+$ RNA (in total RNA) per reaction and >80 000cts min^{-1} of probe (Sp.Act. 400 Ci $mmole^{-1}$). The products of S1 digests were analysed on 6% acrylamide gels with appropriate size markers and exposed to X-ray film at −80 °C. Quantification of signal intensity was achieved using various exposure times and scanning densitometry (Bio-Rad).

Results

Detection of $MLC1_A$, $MLC1_F$ and $MLC3_F$ mRNAs

In order to quantify mRNAs encoding $MLC1_A$ ($MLC1_{emb}$), $MLC1_F$ and $MLC3_F$ we have used S1 protection analysis. This technique avoids complications due to cross hybridisation between mRNAs of similar sequence such as the myosin light chains and allows the simultaneous analysis of two or more probes thereby reducing experimental variation to a minimum. Probes specific for $MLC1_A$, $MLC1_F$ and $MLC3_F$ were derived as shown in Fig. 1, and were tested using RNA from myotubes formed by the cell line T984-Cl10 (Jakob *et al.* 1978) which expresses all three isoforms. When hybridised to RNA from this cell line the $MLC1_F$ and $MLC1_A$ probes both show a single protected fragment of the predicted size (Fig. 2). In the case of $MLC3_F$, a series of bands of 70±2 nucleotides is seen. This probe extends past the site of initiation of transcription of $MLC3_F$ mRNA (see Robert *et al.* 1984) and these bands probably result from multiple transcription start sites, as has also been noted in the case of the rat $MLC1_F/MLC3_F$ gene (Strehler *et al.* 1985). Multiple bands around 70 nucleotides in length are seen with all RNA samples analysed here and are therefore not specific to the T984-Cl10 cell line.

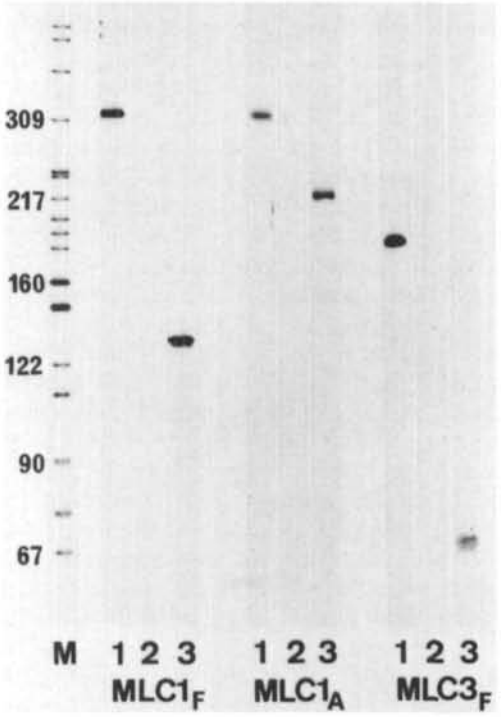

Fig. 2. Detection of $MLC1_F$, $MLC1_A$ and $MLC3_F$ mRNAs by S1 protection. RNA from the myogenic cell line T984-C110 was hybridised with S1 probes derived as shown in Fig. 1. Following hybridisation and digestion with S1 nuclease, protected products were analysed on acrylamide gels (track 3) and compared with both undigested probe (track 1) and probe hybridised with ribosomal RNA (track 2). The size of fragments was determined by reference to a labelled pBR322 *Hpa*II digest (M).

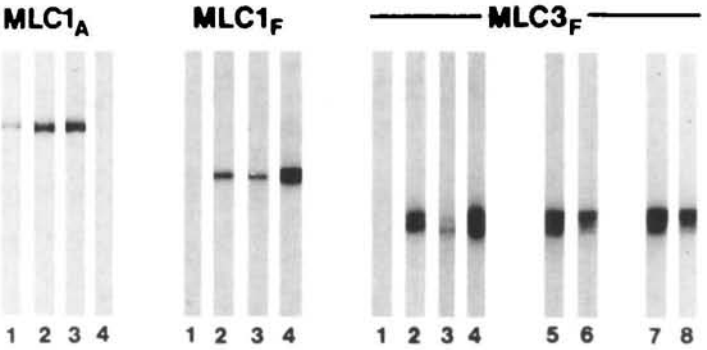

Fig. 3. mRNA accumulation in normal and denervated fetal muscle: RNA from 15-day fetal muscle (track 1) normal 18-day fetal muscle (track 2), β-bungarotoxin-treated 18-day fetal muscle (track 3) and 12-day postnatal muscle (track 4) was analysed for the accumulation of $MLC1_A$ (MLC1emb), $MLC1_F$ and $MLC3_F$ mRNA. Additional normal 18-day fetal muscle samples (tracks 5 and 7) and β-bungarotoxin-treated samples (tracks 6 and 8) are shown for $MLC3_F$.

Myosin light chain mRNA accumulation in normal and denervated muscle

The relative abundance of mRNAs encoding $MLC1_A$ ($MLCl_{emb}$), $MLC1_F$ and $MLC3_F$ was determined in RNA from normal 15 day fetal, 18 day fetal, newborn (12-day-old) skeletal muscle, and from β-bungarotoxin-treated fetuses. Fig. 3 shows the results of a typical experiment showing the accumulation of these mRNA. As the probes are labelled along their length, and as different experiments required differing exposure times to allow optimal analysis by scanning densitometry, we have pooled data from a number of experiments and have corrected in each case for length of protected fragment and for exposure times. The linear relationship of optical density to mRNA concentration was confirmed using two different probes and serial RNA dilutions (data not shown). The pooled data from three independent experiments and two batches of RNA are represented in Figs 4 and 5. Firstly, we quantified the relative accumulation of each mRNA during normal development as shown in Fig. 4. These data are derived from three determinations each of which gave similar (±<10%) values. At 15 days *in utero*, $MLC1_A$ is the most abundant MLC mRNA present; $MLC1_F$ mRNA is detectable at a lower level (ratio $MLC1_A$: $MLC1_F$ 3:1) but $MLC3_F$ mRNA was not detected at this stage. By 18 days, $MLC1_F$ becomes the predominant transcript although $MLC1_A$ is also abundant. $MLC3_F$ mRNA is detectable in 18 day muscle although at a significantly lower abundance than $MLC1_F$ (ratio $MLC3_F$: $MLC1_F$ 1:2.2). By 12 days after birth only $MLC1_F$ and $MLC3_F$ mRNA are detectable and by this stage $MLC3_F$ has become a major transcript which was, in this analysis, in fact more abundant than $MLC1_F$ ($MLC3_F$: $MLC1_F$ 1:0.8).

To examine the effect of denervation by β-bungarotoxin treatment, we have quantified the accumulation of $MLC1_A$, $MLC1_F$ and $MLC3_F$ mRNAs in fetuses treated with toxin at day 15 and allowed to develop to

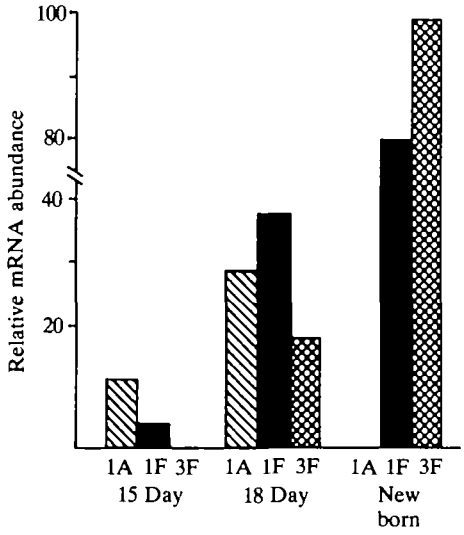

Fig. 4. $MLC1_A$, $MLC1_F$ and $MLC3_F$ mRNA accumulation during normal fetal muscle development. The results are corrected for both relative length of the protected fragment and for autoradiographic exposure times. The relative abundance is expressed in arbitrary units relative to the level of $MLC3_F$ mRNA in new born muscle (100).

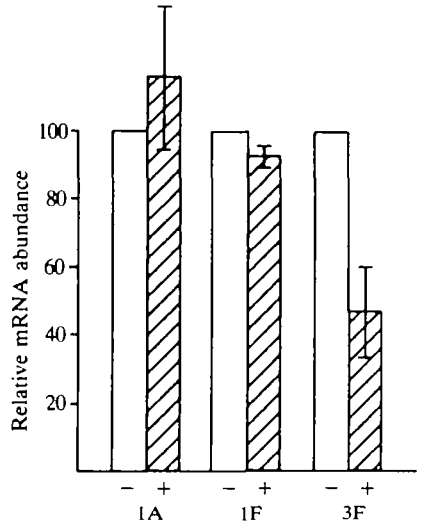

Fig. 5. Effect of denervation of fetal muscle development: $MLC1_A$, $MLC1_F$ and $MLC3_F$ mRNA accumulation was quantified in RNA from 18-day normal and 18-day β-bungarotoxin-treated litter mates. In each case, results are expressed as accumulation in toxin-treated fetuses as a % of that seen in their normal litter mates. Results are from three independent experiments and two independent series of RNA preparations. Bar lines indicate ±1 standard deviation.

day 18. Both toxin-treated fetuses and their normal litter mates were examined. The results of a typical experiment are shown in Fig. 3 and the pooled (corrected) data from all experiments is shown in Fig. 5 where it is expressed as mRNA abundance in treated animals compared to their untreated litter mates. The results indicate that there is a slight (16%) increase in $MLC1_A$ mRNA accumulation in treated fetuses compared to normal, that there is no significant change for $MLC1_F$ but that there is a major decrease in $MLC3_F$ mRNA accumulation which is reduced to less than half that of normal.

Discussion

We have used cloned probes corresponding to the mRNAs encoding the myosin alkali light chains $MLC1_A$ ($MLC1_{emb}$), $MLC1_F$ and $MLC3_F$ to examine the pattern of accumulation of their transcripts during development of normal and denervated fetal muscle. The results show a transition in the abundance of $MLC1_A$ and $MLC1_F$ between day 15 and day 18 *in utero* such that, at day 15, $MLC1_A$ is the major mRNA but, at day 18, $MLC1_F$ is predominant. Accumulation of $MLC3_F$ mRNA is not detected at day 15. By 18 days, $MLC3_F$ is present but remains significantly less abundant than $MLC1_F$, by 12 days after birth, $MLC3_F$ is as abundant as $MLC1_F$.

There is an overall increase in MLC mRNA detected at 18 days compared to 15 days. For $MLC1_A$, the increase (3-fold) parallels the increase in fibre number during this period (e.g. from 1500 to 4800 in the rat diaphragm, Harris, 1981), suggesting that the actual concentration of this mRNA per fibre remains constant over this period. In the case of $MLC1_F$, the increase in mRNA accumulation (10-fold) indicates a net increase in amount per fibre as well as the formation of new (secondary) fibres. These results parallel our earlier study of myosin heavy chain transitions over this period where the embryonic MHC mRNA increased in proportion to fibre type, but the perinatal mRNA showed much more rapid accumulation to become the predominant isoform at 18 days (Weydert *et al.* 1987). The three MLC mRNAs therefore show different patterns of accumulation. $MLC1_A$ is predominant at 15 days, remains constant in concentration during fetal growth and is rapidly lost after birth. $MLC1_F$ is present at low abundance at 15 days and increases with age. $MLC3_F$ mRNA begins to accumulate after 15 days *in utero* but increases in abundance more rapidly than $MLC1_F$ until they are of similar concentration to the adult. The lag in accumulation of $MLC3_F$ mRNA compared to $MLC1_F$ is also seen at the level of protein accumulation where, at 18 days *in utero*, mouse skeletal muscle contains only trace amounts of $MLC3_F$ protein (Barton *et al.* 1985*b*), as is also the case in developing skeletal muscle in the rat (Whalen, 1978) and in chick (Crow *et al.* 1983).

The period between day 15 and day 18 *in utero* is a major phase of secondary muscle fibre formation in the mouse (Ontell and Kozeka, 1984*a*,*b*). At 15 days, primary muscle fibres predominate in skeletal muscle but, by 18 days, secondary muscle fibres form the major part of the muscle mass. Nerve contact is already established during this time period, and nerve electrical activity is essential for correct development and the formation of secondary muscle fibres (Harris, 1981; Ross *et al.* 1987*a*,*b*). In this study, we demonstrate that

the principal effect of denervation by β-bungarotoxin treatment on myosin alkali light chain expression is a reduction of $MLC3_F$ mRNA. The fact that $MLC1_A$ and $MLC1_F$ mRNA accumulation proceed normally in these denervated muscles indicates that correct regulation of the transcripts is nerve-independent. In addition, as the denervated 18 day muscle contains predominantly primary muscle fibres this indicates that $MLC1_A$ and $MLC1_F$ are both expressed, and both correctly regulated, in primary muscle fibres. The switch from $MLC1_A$ to $MLC1_F$ is not therefore simply due to transition from predominantly primary to predominantly secondary fibres. These results parallel earlier studies showing that myosin heavy chain transitions occur in β-bungarotoxin denervated fetal muscle (Weydert *et al.* 1987).

In the case of $MLC3_F$ mRNA accumulation, there is an approximately 50% reduction in denervated muscle compared to normal. Reduction in $MLC3_F$ protein accumulation has been documented in the case of chick limb bud grafts cultured on the chorioallantoic membrane of chick hosts using antibodies to detect $MLC1_F$ and $MLC3_F$ accumulation (Merrifield and Konigsberg, 1987). Here we show that mouse muscle denervated *in situ* by β-bungarotoxin treatment has significantly reduced $MLC3_F$ mRNA accumulation. This situation resembles that of the chick limb bud grafts in that nerve contacts were absent so that these experiments do not distinguish between the relative importance of nerve contact and nerve activity. Experiments comparing the effects of nerve paralysis and nerve destruction on muscle development have revealed few differences in the effects of these treatments (Harris, 1981), suggesting that nerve activity is the most important factor. This indicates that it is nerve activity itself that is important in $MLC3_F$ regulation, and we demonstrate that regulation is effected at the level of mRNA accumulation. Similar indications as to the importance of nerve activity have been provided by experiments using myogenic cell cultures where $MLC3_F$ accumulation is associated with external electrical stimulation (Srihari and Pette, 1981) and contraction (Moss *et al.* 1986), as well as in experiments comparing the effects of paralysis and denervation on MHC accumulation in fetal muscles (Harris *et al.* 1989).

It is of particular interest that $MLC1_F$ and $MLC3_F$ mRNAs accumulate with different kinetics, as they are generated from a single gene (Robert *et al.* 1984; Periasamy *et al.* 1984; Nabeshima *et al.* 1984) by the use of two independent promoters (Strehler *et al.* 1985; Daubas *et al.* 1988). Recent data from nuclear run-on assays carried out in our laboratory, have shown that mRNA accumulation is paralleled by transcriptional activity during normal development, for $MLC1_A$, $MLC1_F$ and $MLC3_F$ (R. Cox, personal communication). The two promoters of the $MLC1_F/MLC3_F$ gene are therefore sequentially activated during development and respond differently to denervation.

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References

Bandman, E., Matsuda, R. and Strohman, R. C. (1982). Developmental appearance of myosin heavy and light chain isoforms *in vivo* and *in vitro* in chicken skeletal muscle. *Devl Biol.* **93**, 508–518.

Barton, P. J. R. and Buckingham, M. (1985). The myosin alkali light chain proteins and their genes. *Biochem. J.* **231**, 249–261.

Barton, P. J. R., Cohen, A., Robert, B., Fiszman, M. Y., Bonhomme, F., Guenet, J-L., Leader, D. P. and Buckingham, M. E. (1985*a*). The myosin alkali light chains of mouse ventricular and slow skeletal muscle are indistinguishable and are encoded by the same gene. *J. biol. Chem.* **260**, 8578–8584.

Barton, P. J. R., Robert, B., Cohen, A., Garner, I., Sassoon, D., Weydert, A. and Buckingham, M. E. (1988). Structure and sequence of the myosin alkali light chain gene expressed in adult cardiac atria and fetal striated muscle. *J. biol. Chem.* **263**, 12669–12676.

Barton, P. J. R., Robert, B., Fiszman, M. Y., Leader, D. P. and Buckingham, M. E. (1985*b*). The same myosin alkali light chain gene is expressed in adult cardiac atria and in fetal skeletal muscle. *J. Muscle Res. Cell Motil.* **6**, 461–475.

Brown, W. E., Salmons, S. and Whalen, R. G. (1983). The sequential replacement of myosin subunit isoforms during muscle type transformation induced by long term electrical stimulation. *J. biol. Chem.* **258**, 14686–14692.

Crow, M. T., Olson, P. S. and Stockdale, F. E. (1983). Myosin light-chain expression during avian muscle development. *J. Cell Biol.* **96**, 736–744.

Daubas, P., Klarsfeld, A., Garner, I., Pinset, C., Cox, R. and Buckingham, M. (1988). Functional activity of the two promoters of the myosin alkali light chain gene in primary muscle cell cultures: comparison with other muscle gene promoters and other culture systems. *Nucleic Acids Res.* **16**, 1251–1271.

Harris, A. J. (1981). Embryonic growth and innervation of rat skeletal muscles. I. Neural regulation of muscle fibre numbers. *Phil. Trans. Roy. Soc. Lond.* B **293**, 257–277.

Harris, A. J., Fitzsimons, R. B. and McEwan, J. C. (1989). Neural control of the sequence of expression of myosin heavy chain isoforms in foetal mammalian muscles. *Development.* In Press.

Jakob, H., Buckingham, M. E., Cohen, A., Dupont, L., Fiszman, M. and Jacob, F. (1978). A skeletal muscle cell line isolated from a mouse teratocarcinoma undergoes apparently normal terminal differentiation *in vitro*. *Expl Cell Res.* **114**, 403–408.

Kawashima, M., Nabeshima, Y-I., Obinata, T. and Fujii-Kuriyama, Y. (1987). A common myosin light chain is expressed in chicken embryonic skeletal, cardiac and smooth muscles and in brain continuously from embryo to adult. *J. biol. Chem.* **262**, 14408–14414.

Kirschbaum, B. J., Simoneau, J-A., Bar, A., Barton, P. J. R., Buckingham, M. E. and Pette, D. (1989). Chronic stimulation-induced changes of myosin light chains at the mRNA and protein levels in rat fast-twitch muscle. *Eur. J. Biochem.* **179**, 23–29.

Matsuda, R., Spector, D. and Strohman, R. C. (1984). Denervated skeletal muscle displays discoordinate regulation for the synthesis of several myofibrillar proteins. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1122–1125.

McCaig, C. D., Ross, J. J. and Harris, A. J. (1987). Embryonic somatic nerve destruction with β-bungarotoxin. *Cell Tissue Res.* **247**, 41–50.

Merrifield, P. A. and Konigsberg, I. R. (1987). Nerve-dependent accumulation of myosin light chain 3 in developing limb musculature. *Development* **101**, 673–684.

Moss, P., Micou-Eastwood, J. and Strohman, R. C. (1986). Altered synthesis of myosin light chains is associated with contractility in cultures of differentiating chick embryo chest muscle. *Devl Biol.* **114**, 311–314.

Nabeshima, Y-I., Fujii-Kuriyama, Y., Muramatsu, M. and Ogata, K. (1984). Alternative transcription and two modes of splicing result in two myosin light chains from one gene. *Nature, Lond.* **308**, 333–338.

Nakamura, S., Nabeshima, Y-I., Kobayashi, H., Nabeshima, Y., Nonomura, Y. and Fujii-Kuriyama, Y. (1988). Single chicken cardiac myosin alkali light-chain gene generates two different mRNAs by alternative splicing of a complex exon. *J. molec. Biol.* **203**, 895–904.

Ontell, M. and Kozeka, K. (1984*a*). The organogenesis of murine striated muscle: a cytoarchitectural study. *Amer. J. Anat.* **171**, 133–148.

Ontell, M. and Kozeka, K. (1984*b*). Organogenesis of the mouse extensor digitorum logus muscle; a quantitative study. *Amer. J. Anat.* **171**, 149–161.

Periasamy, M., Strehler, E. E., Garfinkel, L. I., Gubits, R. M., Ruiz-Opazo, N. and Nadal-Ginard, B. (1984). Fast skeletal muscle myosin light chains 1 and 3 are produced from a single gene by a combined process of differential RNA transcription and splicing. *J. biol. Chem.* **259**, 13 595–13 604.

Pette, D. and Vrbova, G. (1985). Neural control of phenotypic expression in mammalian muscle fibers. *Muscle and Nerve* **8**, 676–689.

Robert, B., Daubas, P., Akimenko, M-A., Cohen, A., Garner, I., Guenet, J-L. and Buckingham, M. E. (1984). A single locus in the mouse encodes both myosin light chains 1 and 3, a second locus corresponds to a related pseudogene. *Cell* **39**, 129–140.

Ross, J. J., Duxson, M. J. and Harris, A. J. (1987*a*). Formation of primary and secondary myotubes in rat lumbrical muscles. *Development* **100**, 383–394.

Ross, J. J., Duxson, M. J. and Harris, A. J. (1987*b*). Neural determination of muscle fibre numbers in embryonic rat lumbrical muscles. *Development* **100**, 395–409.

Srihari, T. and Pette, D. (1981). Myosin light chains in normal and electrostimulated cultures of chicken breast muscle. *FEBS Lett.* **123**, 312–314.

Strehler, E. E., Periasamy, M., Strehler-Page, M-A. and Nadal-Ginard, B. (1985). Myosin light-chain 1 and 3 gene has two structurally distinct and differentially regulated promoters evolving at different rates. *Molec. Cell. Biol.* **5**, 3168–3182.

Takano-Ohmuro, H., Obinata, T., Kawashima, M., Masaki, T. and Tanaka, T. (1985). Embryonic chicken skeletal, cardiac and smooth muscles express a common embryo-specific myosin light chain. *J. Cell Biol.* **100**, 2025–2030.

Weeds, A. G., Trentham, D. R., Kean, C. J. C. and Buller, A. J. (1974). Myosin from cross-reinnervated cat muscles. *Nature, Lond.* **247**, 135–139.

Weydert, A., Barton, P., Harris, A. J., Pinset, C. and Buckingham, M. (1987). Developmental pattern of mouse skeletal myosin heavy chain gene transcripts *in vivo* and *in vitro*. *Cell* **49**, 121–129.

Whalen, R. G., Butler-Browne, G. and Gros, F. (1978). Identification of a novel form of myosin light chain present in embryonic muscle tissue and cultured muscle cells. *J. molec. Biol.* **126**, 415–431.

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