Biochem. J. (1985) 231, 249–261 (Printed in Great Britain)

REVIEW ARTICLE

The myosin alkali light chain proteins and their genes

Paul J. R. BARTON and Margaret E. BUCKINGHAM
Département de Biologie Moléculaire, Institut Pasteur, 28 Rue du Dr. Roux, 75724 Paris, France

Introduction

In all eukaryotes, actin and myosin play a role in the maintenance of cell shape and in cellular movement (e.g. reviewed by Korn, 1978). In the muscles of multicellular organisms actomyosin is the principal structural component of the sarcomere, the unit of the muscle fibre. Movement is effected by the sliding of actin and myosin filaments over each other, with accompanying hydrolysis of ATP providing energy for this process, which is regulated by calcium-sensitive myofibrillar proteins (see Bagshaw, 1982, for review). The myosin molecule consists of two heavy chains (MHC, of M_r about 220000) each with a globular head region where the ATPase activity is situated, and a filamentous, rod-like tail, and four associated light chains (of M_r 15000–30000), two so-called regulatory or DTNB light chains (MLC2) and two alkali light chains (MLC1, MLC3). It is the alkali myosin light chain proteins and their genes which are the subject of this review.

The function of the alkali light chains is not clear. It has been shown that, although these light chains are associated with the globular region of the myosin molecule, probably near the head/tail junction (Flicker *et al.*, 1983; Sellers & Harvey, 1984), they do not modify the ATPase activity of isolated myosin heavy chains (e.g. Wagner & Giniger, 1981; Sivaramakrishnan & Burke, 1982). Other experiments (e.g. Wagner & Weeds, 1977; Winstanley *et al.*, 1979; Trayer & Trayer, 1985) would suggest that they play a role in the interaction of the head region of myosin with actin. It has become clear in the last few years, however, that distinct isoforms of the myosin alkali light chains are present in different muscles and in non-muscle cells, suggesting that these isoforms are associated with the different contractile properties of these tissues. This is also the case for the actins and myosin heavy chains. The presence of different isoforms of the contractile proteins in different adult and developing muscles (for review see Buckingham & Minty, 1983) raises interesting questions, not only about their function, but also about the regulation of the corresponding genes. Recombinant DNA technology has made it possible to isolate the mRNA and gene sequences of families such as that of the myosin alkali light chains, which can be used to address such questions. The contribution of this type of approach to research on muscle, and the interest of muscle as a model system for the molecular biologist in which to ask fundamental questions about gene regulations, are complementary aspects discussed in this review.

The myosin alkali light chain isoforms and their genes

As might be expected from the range of muscle types present in vertebrates, several isoforms of myosin alkali light chain have been identified. In most higher vertebrates so far examined four principal types of light chain pattern are seen on one-dimensional gel electrophoresis (see Fig. 1): (i) fast skeletal muscle contains two alkali light chains $MLC1_F$ and $MLC3_F$; (ii) slow skeletal muscle, depending on species and muscle type, contains one or two $MLC1_S$ isoforms of electrophoretic mobility comparable with that of $MLC1_F$; (iii) cardiac muscle shows a major isoform, $MLC1_V$, derived from ventricular muscle which represents the majority of the cardiac muscle mass. This isoform shows the same electrophoretic mobility as the slow muscle form $MLC1_S$. In mammals atrial muscle contains a specific isoform, $MLC1_A$. (iv) Smooth muscle and non-muscle cells show a single fast-migrating isoform of electrophoretic mobility comparable with that of $MLC3_F$. Fig. 3 shows the relationship between the skeletal and cardiac muscle light chains on two-dimensional gel electrophoresis.

Fast skeletal muscle isoforms. The amino acid sequence of the (rabbit) fast skeletal muscle isoforms $MLC1_F$ and

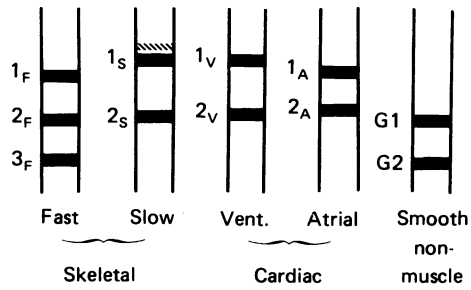

Fig. 1. Schematic representation of one-dimensional electrophoretic patterns of myosin light chain isoforms present in different adult vertebrate muscle types

Myosin light chains are numbered according to their position on the gel. In skeletal and cardiac samples band 2 is the regulatory (DTNB) isoform (MLC2). In smooth/non-muscle tissues the myosin alkali light chain (G2 as described for gizzard muscle) migrates similarly to the $MLC3_F$ isoform of fast skeletal muscle ahead of the regulatory (MLC2 type) light chain (G1). In slow skeletal muscle the relative proportion of the $MLC1_{Sa}$ or $MLC1_{S'}$ isoform is species-dependent.

Abbreviations used: MHC, myosin heavy chain; MLC myosin light chain (subscripts: V, ventricular; A, atrial; F, fast; S, slow; emb, embryonic/foetal); RFLP, restriction fragment length polymorphism; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid).

$MLC3_F$ determined by Frank & Weeds (1974) showed that these isoforms share a common *C*-terminal sequence of 141 amino acids but have distinct *N*-termini of 46 and 8 amino acid residues respectively. This, together with the observation that amino acid substitutions occurring in this common region of $MLC1_F$ between birds and mammals are also found in the $MLC3_F$ sequence (Matsuda *et al.*, 1981*a*), gave the first indication that these two protein isoforms are encoded by a single gene differentially spliced to give the specific *N*-termini. Further evidence in support of this came from cloned recombinant plasmids, containing a DNA sequence (cDNA) complementary to part of the $MLC1_F/MLC3_F$ mRNA sequence, which showed that the 3′ non-coding regions of the $MLC1_F$ and $MLC3_F$ mRNAs are homologous (Robert *et al.*, 1982; Nabeshima *et al.*, 1982). This has now been shown to be the case in the chicken (Nabeshima *et al.*, 1984), mouse (Robert *et al.*, 1984) and rat (Periasamy *et al.*, 1984) where the complete $MLC1_F/MLC3_F$ genes have been isolated and sequenced. The $MLC1_F/MLC3_F$ gene is more complex than envisaged from the protein sequences and appears to involve both the use of two promoters for the $MLC1_F$ and $MLC3_F$ primary transcripts and a complex pattern of splicing to generate the two functional mRNAs. Fig. 2 shows the structure of the mouse $MLC1_F/MLC3_F$ gene, which is the same in overall plan as that found in rat and chicken. The shared 141 amino acid sequence is encoded in a series of four common coding exons and the isoform-specific amino acids are encoded in four separate 5′ exons arranged such that the two exons encoding the $MLC3_F$ *N*-terminus are contained within a 10.8 kilobase intron which separates exons 1 and 2 of $MLC1_F$. The generation of the mRNA encoding $MLC1_F$ and $MLC3_F$ from this gene probably involves splicing of different primary transcripts to give two distinct mRNAs which share a large common region, including the 3′ non-coding sequence, but which differ in their 5′ sequence (coding and non-coding). The $MLC1_F$ and $MLC3_F$ mRNAs are clearly distinguishable by size (for mouse: $MLC1_F$, 1050 nucleotides; $MLC3_F$, 900 nucleotides).

The arrangement of this gene is novel in its use of a combination of both alternative initiation sites and splicing for the generation of two mRNAs, and in the presence of an intron within the 3′ non-coding sequence, which is unusual although not without precedent (for discussion see Robert *et al.*, 1984). Analysis of the DNA sequence flanking the 5′ exons shows the presence of TATA and CAAT sequences required for transcription initiation, which are located upstream of both exon 1 ($MLC1_F$) and exon 2 ($MLC3_F$). The use of two initiation sites to produce two differing proteins from the same gene has not been documented for other genes, although cases of differential initiation and splicing giving rise to different mRNAs encoding the same protein product are known (e.g. mouse amylase, Schibler *et al.*, 1983; *Drosophila* alcohol dehydrogenase, Benyajati *et al.*, 1983), and numerous cases of differential splicing of the same primary transcript to give rise to different protein products are known (e.g. the mouse αA crystallin gene, King & Piatigorsky, 1983). The generation of $MLC1_F$ and $MLC3_F$ mRNAs can be regulated such that $MLC1_F$ is produced independently from $MLC3_F$. For example, in developing skeletal muscle in mammals $MLC1_F$ accumulates before $MLC3_F$ (Sréter *et al.*, 1975; Roy *et al.*, 1979*b*; Gauthier *et al.*, 1982), and this difference in accumulation is seen at the mRNA level as shown by Northern blot analysis of foetal muscle RNA using cloned $MLC1_F/MLC3_F$ DNA probes (Barton *et al.*, 1985*a*), suggesting transcriptional regulation of these mRNAs. Comparison of the promoter regions of these genes from chicken, rat, and mouse has revealed sequences common to all three species, which may be involved in the regulated expression of these genes (Daubas *et al.*, 1985).

The existence of further MLC1-type isoforms in fast

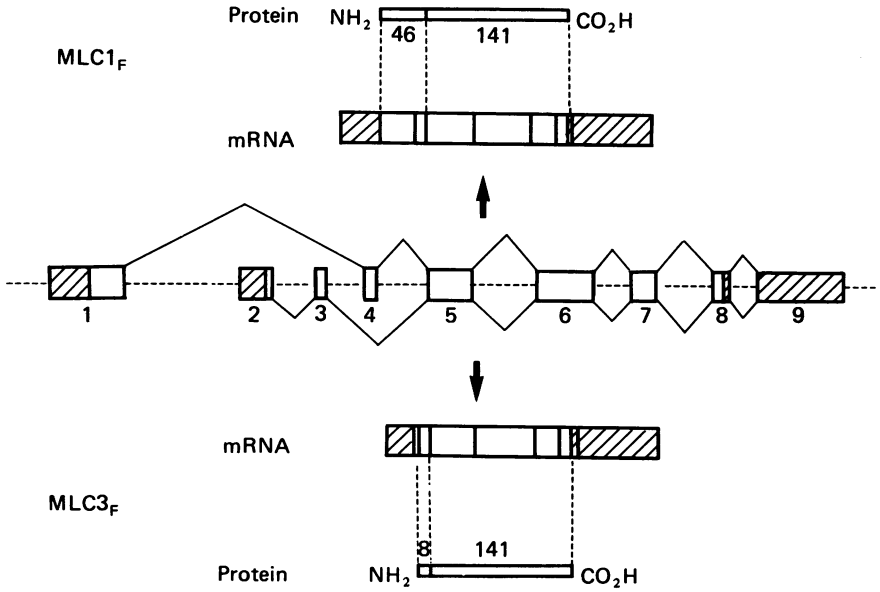

Fig. 2. Schematic representation of the mouse gene encoding the two myosin alkali light chains of fast skeletal muscle

Exon sequences are numbered 1–9 where 1 and 4 are specific to $MLC1_F$ and 2 and 3 to $MLC3_F$. Hatched boxes correspond to non-coding regions in the mRNAs. The number of amino acid residues for the common and specific regions of the $MLC1_F$ and $MLC3_F$ proteins is indicated

skeletal muscles is suggested by the observation that the MLC1 isoform present in the posterior temporalis (jaw closing) muscle in the cat is distinguishable from other MLC1 isoforms on two-dimensional gel electrophoresis (Rowlerson *et al.*, 1981). In the rabbit, the masseter muscle MLC1 isoform comigrates with the slow skeletal muscle form $MLC1_{Sb}$ on two-dimensional gels but is antigenically distinguishable from this isoform (Biral *et al.*, 1982).

Slow skeletal muscle isoforms. The myosin light chain content of slow skeletal muscle shows variation between species and to some extent between different muscles. In mouse the principal slow muscle light chain isoform found, for example, in soleus muscle (which contains mixed fast and slow fibre types but is predominantly slow; see Butler-Browne & Whalen, 1984) is $MLC1_S$. This isoform has been shown to be encoded by the same gene as the ventricular isoform $MLC1_V$ (Barton *et al.*, 1985*b*), and is indistinguishable from $MLC1_V$ on two-dimensional gel analysis in mouse and rat (Barton *et al.*, 1985*b*; Whalen *et al.*, 1978). These isoforms are probably identical, as discussed in detail in the section on cardiac isoforms. Soleus muscle in the rabbit contains two slow muscle isoforms, $MLC1_{Sa}$ and $MLC1_{Sb}$, which are present in similar proportions. The $MLC1_{Sb}$ form has been shown to be indistinguishable from the rabbit cardiac $MLC1_V$ isoform by one-dimensional gel electrophoresis (Lowey & Risby, 1971; Sarkar *et al.*, 1971; Weeds & Pope, 1971), partial amino acid sequence data and peptide mapping (Weeds, 1976), and by two-dimensional gel electrophoresis (Margreth *et al.*, 1980), and probably corresponds to the mouse $MLC1_S/MLC1_V$ protein. A minor additional slow form, $MLC1_{S'}$ (corresponding to $MLC1_{Sa}$ in the rabbit), can be detected in rat soleus (Whalen *et al.*, 1978), but is not readily detectable in mouse (Barton *et al.*, 1985*b*). The exact relationship between $MLC1_{Sa}$ and $MLC1_{Sb}$ is not clear; peptide mapping analysis suggests regions of similar sequence, but amino acid sequence data on the thiol peptides of these isoforms indicate that they are not identical in these regions (Weeds, 1976) and immunological data suggest that $MLC1_{Sa}$ and $MLC1_{Sb}$ have distinct epitopes (Margreth *et al.*, 1980). Analysis of the relationship between the different isoforms of $MLC1_S$ should be possible however by cross-hybridization with the cloned mouse $MLC1_V/MLC1_S$ cDNA and gene probes (see section on cardiac isoforms) and should reveal, for example, whether two mRNAs of $MLC1_S$-like sequence are present in rabbit soleus.

Like many other slow twitch muscles, soleus muscle contains a mixture of fast and slow fibre types, as characterized by cytochemical techniques based on ATPase activity, and by myosin heavy chain content. In mixed fibre muscles such as this, mixed populations of fast and slow type myosin alkali light chain isoforms are present (Whalen *et al.*, 1978; Rubinstein & Kelly, 1978; Gauthier *et al.*, 1982), such that in mouse soleus muscle $MLC1_S$, $MLC1_F$ and $MLC3_F$ isoforms and mRNAs are detectable, although $MLC3_F$ protein is present only in low abundance (Barton *et al.*, 1985*b*) and in chick anterior latissimus dorsi muscle $MLC3_F$ is not detected (Crow *et al.*, 1983). The fact that there is significantly less $MLC3_F$ protein present in the fibres of slow muscles although the $MLC3_F$ mRNA is accumulated raises the intriguing possibility of post-transcriptional regulation. In mixed fibre type muscles it is important to establish whether the slow and fast myosin alkali light chain types are present only in the slow and fast fibres respectively. In general the proportion of fast and slow isoforms in a mixed fibre muscle appears directly related to the proportion of fast and slow fibres, such that mouse or rat diaphragm, which contain a higher proportion of fast fibres, contain proportionately more fast myosin alkali light chain isoforms (Whalen *et al.*, 1978; Gauthier *et al.*, 1982). This suggests that the fast and slow type myosin light chain isoforms are expressed primarily in the respective fibre types. However several studies have indicated that individual fibres may contain mixed myosin alkali light chain types (see Table 1). Type I fibres may fall into two categories: those showing pure slow light chain isoforms, but with varying proportions of $MLC1_{Sa}$ and $MLC1_{Sb}$ in larger mammals, and those showing mixed fast and slow light chains (see Salviati *et al.*, 1982). In fast twitch fibres $MLC1_F$ and $MLC3_F$ are present in similar amounts [in chicken the proportion of $MLC1_F$ to $MLC3_F$ is 1:0.85 (Lowey & Risby, 1971; Takahashi & Tonomura, 1975)]. In contrast, rabbit type IIA fibres appear to contain only a small amount of $MLC3_F$, and some rabbit IIA fibres may also contain $MLC1_{Sb}$ (Salviati *et al.*, 1982). Type IIC fibres are of an intermediate histochemical type and contain mixed myosin heavy chain types and mixed troponins; these fibres contain a true mixture of fast and slow light chains and may represent immature type I fibres (see the section on development).

Table 1. Myosin isoforms in skeletal muscle fibres

The data presented here are based on results with human muscle (Pelloni-Mueller *et al.*, 1976; Pette *et al.*, 1979; Billeter *et al.*, 1981; Salviati *et al.*, 1982, 1984).

Fibre type	Contractile properties	Myosin heavy chain	Myosin alkali light chains
I	Slow	MHC_S	$MLC1_S$ $MLC1_F$ ($MLC3_F$)
IIA	Fast	MHC_{F1}	$MLC1_F$ $MLC3_F$
IIB	Fast	MHC_{F2}	$MLC1_F$ $MLC3_F$
IIC	Mixed	MHC_F MHC_S	$MLC1_F$ $MLC3_F$ $MLC1_S$

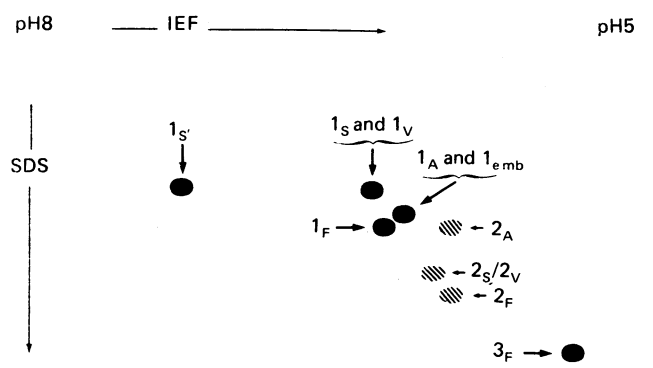

Fig. 3. Schematic representation of a two-dimensional gel (O'Farrell, 1975) showing the relative positions of different myosin light chain isoforms present in rodent striated muscle

Cardiac muscle isoforms. Mammalian cardiac muscle contains two myosin alkali light chains which are the major isoforms present in either the ventricular muscle ($MLC1_V$) or the atrial muscle ($MLC1_A$) (Syrovy *et al.*, 1979; Wikman-Coffelt & Srivastava, 1979). Mammalian ventricular $MLC1_V$, and the chicken cardiac alkali light chain for which the complete amino acid sequence has been determined (Maita *et al.*, 1980), have been shown to have the same electrophoretic mobility, and to share immunological properties, with the major slow skeletal muscle form $MLC1_S$ (Lowey & Risby, 1971; Sarkar *et al.*, 1971; Weeds & Pope, 1971; Obinata *et al.*, 1979; Sartore, 1981). Partial peptide mapping (Weeds, 1976), and two-dimensional gel analysis, of $MLC1_S$ and $MLC1_V$ have shown these isoforms to be indistinguishable on these criteria (Whalen *et al.*, 1978; Dalla Libera *et al.*, 1979; Margreth *et al.*, 1980) (see Fig. 3 for the relationship of the MLC isoforms on two-dimensional gels). Recombinant cDNA clones containing part of the sequence encoding $MLC1_V$ have now been isolated and have been used to define the relationship between $MLC1_V$ and $MLC1_S$ in the mouse. $MLC1_V$ and the slow skeletal muscle form, $MLC1_S$, are encoded by mRNAs which are indistinguishable on the basis of size, and sequence homology with $MLC1_V$ cDNA (Barton *et al.*, 1985*b*). These mRNAs are encoded by a single gene, and although the possibility of differential splicing of this gene giving different mRNAs encoding $MLC1_V$ and $MLC1_S$ cannot yet be excluded, the fact that both the mRNAs and the proteins appear to be identical argues against this. In the case of $MLC1_F$ and $MLC3_F$ both the mRNAs and the proteins are clearly distinguishable by this type of analysis.

Mammalian atrial muscle contains a specific isoform, $MLC1_A$, also found in the Purkinje fibres of the heart (Long *et al.*, 1977; Syrovy *et al.*, 1979). This isoform is not normally found in other adult muscles but is indistinguishable on two-dimensional gels from the foetal skeletal muscle isoform $MLC1_{emb}$ (Whalen *et al.*, 1978, 1982; Dalla Libera, 1981) and the isoform found in foetal ventricular muscle (Price *et al.*, 1980; Cummins *et al.*, 1980). On this basis it has been proposed that $MLC1_A$ and the isoform detected in foetal skeletal and foetal ventricular muscle ($MLC1_{emb}$) are identical; this is also supported by results of peptide mapping (Whalen & Sell, 1980; Cummins, 1982). Using a recombinant cDNA plasmid that encodes part of the $MLC1_A$ protein it has now been demonstrated that in the mouse there is a single gene for the $MLC1_A$ and $MLC1_{emb}$ isoforms, and that the mRNAs found in adult atrial muscle and foetal skeletal muscle are indistinguishable on the basis of size and sequence homology with the cDNA (Barton *et al.*, 1985*a*). As in the case of $MLC1_V$ and $MLC1_S$, this type of analysis at the nucleic acid level does not exclude the possibility of differential splicing or secondary modification differences. Peptide mapping of $MLC1_A$ and $MLC1_{emb}$ from foetal skeletal muscle suggests that these proteins may have different secondary modifications (Dalla Libera & Carraro, 1983) although these differences are not seen in the case of $MLC1_{emb}$ from foetal ventricular muscle (Whalen & Sell, 1980; Cummins, 1982). DNA sequencing of the isolated gene, now in progress, should define the primary structure of the $MLC1_A/MLC1_{emb}$ protein.

Smooth muscle and non-muscle cells. Smooth muscle, such as is found in the digestive tract and uterus, is non-striated and is under involuntary control. One-dimensional analysis shows that it contains a single alkali type light chain (G2 in Fig. 1) of electrophoretic mobility comparable with that of fast skeletal muscle $MLC3_F$ (Burridge, 1974). In chicken the 17 kDa smooth muscle myosin alkali light chain isoform from gizzard has been sequenced and consists of 150 amino acids (cf. chicken $MLC3_F$, 17 kDa, 149 amino acids; $MLC1_F$, 20 kDa, 190 amino acids) (Matsuda *et al.*, 1981*b*). Many non-muscle tissues contain myosin-like proteins (for review see Clarke & Spudich, 1977), and, in the case of such myosin extracted from chicken fibroblast cells, the light chains present have been shown to have the same electrophoretic mobility as the gizzard smooth muscle isoform, although the myosin heavy chains in these two cell types are apparently different (Burridge, 1974). Recent data from cloned recombinant cDNA has shown that smooth muscle and non-muscle alkali myosin light chains are identical except for a few *C*-terminal amino acids and are probably encoded by the same gene (Nabeshima & Nabeshima, 1984). These isoforms have an electrophoretic mobility similar to that of $MLC3_F$ rather than $MLC1_F$ (see Fig. 1) and this similarity between the smooth muscle alkali light chain and $MLC3_F$ is also indicated by the absence of trimethylalanine at the *N*-terminus of these isoforms. Trimethylalanine is present at the *N*-termini of all vertebrate MLC1 isoforms so far examined in skeletal and cardiac muscle, but is absent from $MLC3_F$ in both mammals and birds (chicken) (Henry *et al.*, 1985). Developing chick gizzard contains, in addition to the adult smooth muscle form, an embryonic isoform (L_{23}) expressed during development, but not in the adult (Katoh & Kubo, 1978; Takano-Ohmuro *et al.*, 1983*a*) (see the section on development).

The number and distribution of myosin alkali light chain genes

The different myosin alkali light chain isoforms show a degree of sequence divergence, both at the level of amino acid sequence and of nucleic acid sequence of the mRNAs, which allows different isoforms to be easily distinguished. However, the coding sequence of the mouse fast skeletal muscle $MLC1_F/MLC3_F$ gene shows cross-hybridization with other myosin light chain mRNAs including those encoding $MLC1_V$ ($MLC1_S$) and

$MLC1_A$ ($MLC1_{emb}$) (Barton *et al.*, 1985*a*). Fragments corresponding to these genes, and to a processed type MLC1 pseudogene (see Robert *et al.*, 1984) have been identified. In addition, two unidentified weakly cross-hybridizing fragments are seen which may correspond to other alkali myosin light chain genes such as that encoding the smooth muscle and non-muscle isoform. The presence of a limited number of diverged MLC1 type sequences in the mouse genome is different from the situation for the actins, where Southern blot experiments with an actin coding sequence probe reveal large numbers of strongly cross-hybridizing actin-related gene and pseudogene sequences (Minty *et al.*, 1983). For both actin and myosin heavy chain genes, the degree of sequence conservation is high and when using coding sequence probes conditions of much higher stringency are needed to distinguish between genes encoding different isoforms. The myosin alkali light chain gene family, therefore, consists of a smaller number of more highly diverged sequences than the actin and myosin heavy chain gene families.

Determination of the distribution of the myosin alkali light chain genes within the genome was not possible before cloned copies of the mRNAs became available due to the absence of identified allelic isoforms of these proteins, except in the chicken where electrophoretic variants of $MLC1_F$ have been used to show that the $MLC1_F/MLC3_F$ gene is unlinked to the 'am' gene for muscular dystrophy (Rushbrook *et al.*, 1982; Rushbrook & Somes, 1985). The absence of allelic variants associated with disease or mutant phenotypes has excluded classical genetic approaches, and somatic cell hybridization techniques relating chromosome retention and loss to phenotypic expression are complicated by the multinucleate nature of the myotube cell and by the fact that expression of contractile proteins is suppressed in cell hybrids (see Carlsson *et al.*, 1974) although it can be seen in heterokaryons (Wright, 1982; Blau *et al.*, 1983). Localization of the myosin light chain genes in the mouse has now been established by means of cloned mRNA (cDNA) and gene copies encoding $MLC1_F/MLC3_F$, $MLC1_V$ ($MLC1_S$) and $MLC1_A$ ($MLC1_{emb}$). These have been used to define a series of RFLPs for each of these genes and for the $MLC1_F/MLC3_F$ pseudogene between the mouse species *Mus musculus* (DBA/2) and *Mus spretus*. These RFLPs, which define allelic variants of these genes, have been used to follow the pattern of segregation of the different myosin alkali light chain genes through an F1 backcross between these species (Robert *et al.*, 1985). The pattern of segregation observed shows that the $MLC1_F/MLC3_F$, $MLC1_V$ and $MLC1_A$ genes are not linked and comparison with the segregation pattern of a series of chromosomal markers analysed in the same backcross shows that they are in fact located on different chromosomes (see Fig. 4). Analysis of the segregation pattern of RFLPs for the skeletal muscle and cardiac actin genes, and for the foetal and adult skeletal muscle and adult cardiac muscle myosin heavy chain genes, in the same backcross, shows that the myosin alkali light chain genes analysed are not linked to any of these contractile protein genes with the exception of a loose linkage between the genes for skeletal myosin heavy chains and $MLC1_A$, which are both located on mouse chromosome 11. The myosin alkali light chain genes are also unlinked to the gene encoding the regulatory light chain, $MLC2_F$, as shown by analysis of somatic cell hybrids with cloned gene probes (Czosnek *et al.*, 1982). The $MLC1_F/MLC3_F$ pseudogene is unlinked to the other myosin alkali light chain genes analysed, as expected for a processed type pseudogene presumably derived from the insertion of a mRNA-derived cDNA fragment (see Robert *et al.*, 1984). Regulation of actin and myosin genes expressed in the same phenotype is not, therefore, dependant on close chromosomal proximity. This is not surprising in the case of the different adult isoforms, since most other gene families examined show a dispersion (e.g. the α- and β-globin gene clusters); it is perhaps surprising, however, that the genes expressed in foetal and adult skeletal muscle ($MLC1_A/MLC1_{emb}$ and $MLC1_F/MLC3_F$) are unlinked. Other gene families showing developmentally regulated isoforms, such as embryonic, foetal and adult globin (Bernard *et al.*, 1979),

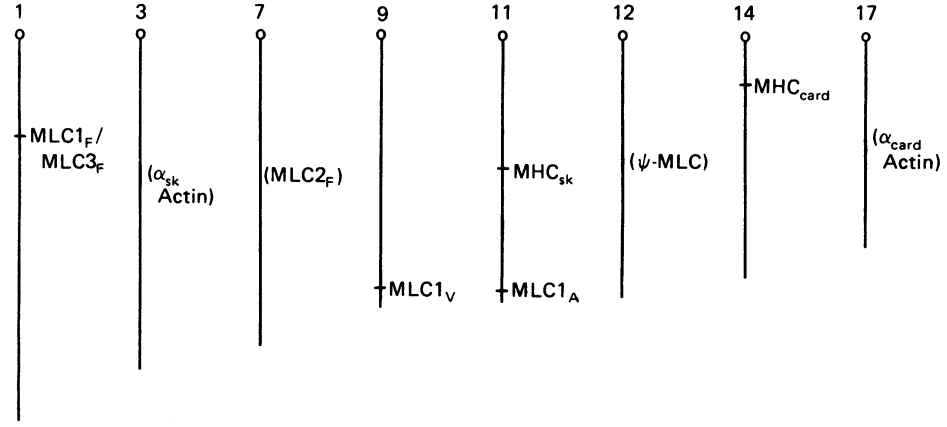

Fig. 4. Distribution of myosin light chain and other contractile protein genes in the mouse genome showing exact position where known, or chromosomal assignment (genes in parentheses)

Data are derived from Robert *et al.* (1985), Czosnek *et al.* (1982, 1983), Leinwand *et al.* (1983) and Weydert *et al.* (1985). Loci represent single genes, except in the case of the MHC genes where MHC_{sk} and MHC_{card} represent clusters. Abbreviations: card, gene(s) expressed in cardiac muscle; sk, gene(s) expressed in skeletal muscle; ψ-MLC, pseudogene sequence derived from the $MLC1_F/MLC3_F$ common coding region.

α-foetoprotein and albumin (Ingram *et al.*, 1981), and embryonic, foetal and adult skeletal muscle myosin heavy chains (Weydert *et al.*, 1985), show close linkage of the foetal and adult genes. The $MLC1_A/MLC1_{emb}$ gene may be of relatively recent evolutionary origin (see the section on evolution) and for this reason might also be expected to be linked to the adult $MLC1_F/MLC3_F$ gene from which it is probably derived.

The location of these genes in other vertebrates is not known, although the availability of cloned probes for these genes should now allow this to be determined for a number of species, including man, either by cross-hybridization or by using existing probes to isolate the corresponding genes from other gene libraries. In *Drosophila*, where the single MLC1-like gene has recently been isolated, this gene has been localized to position 98B by direct *in situ* hybridization to polytene chromosomes (Falkenthal *et al.*, 1984). This gene is unlinked to the genes for *Drosophila* myosin heavy chains (Bernstein *et al.*, 1983), tropomyosin (Bautch *et al.*, 1982), or actin (Fyrberg *et al.*, 1980).

Developmental aspects

The formation of muscle tissue provides a particularly interesting model system for examining changes in gene expression during development. An initial 'switch' takes place from non-muscle to muscle type isoforms of the contractile proteins, followed by a changing pattern of foetal→neonatal→adult phenotypes as the muscle matures. Intriguing questions for a molecular biologist concern firstly the description of the system – what are the changes in isoform type and in the corresponding messenger RNAs, at what developmental stage do they occur, whether there is co-ordination between the different contractile protein families, and secondly how these changes are effected – does the appearance of a new isoform reflect transcriptional activation of the corresponding gene, or a post-transcriptional modification of the mRNA, what molecular factors interact with which nucleic acid sequences to activate individual genes or blocks of genes? The first transition from a non-muscle to a muscle phenotype is best studied in tissue culture. Particularly in the case of skeletal muscle, precursor myoblasts can be isolated and grown as monolayers of mononucleated cells which will fuse together to form multinucleated muscle fibres where muscle isoforms of the contractile proteins accumulate in sarcomeric structures (see for review, Buckingham, 1985). This process has been studied in mammalian and avian muscle cell cultures. In general, muscle isoforms of the different contractile proteins co-accumulate at the onset of fibre formation. This is also observed at the mRNA level, with a reciprocal decrease in the level of non-muscle actin mRNAs (e.g. Caravatti *et al.*, 1982). Some experiments on chromatin conformation would suggest that these are primarily transcriptional events (Carmon *et al.*, 1982). Recent results demonstrating the regulated expression of a skeletal actin gene re-introduced into muscle cells support this idea (Melloul *et al.*, 1984). The initial muscle isoforms expressed in tissue culture tend to reflect a foetal phenotype. Subsequent modifications during fibre maturation may occur to some extent *in vitro*, but studies *in vivo*, principally again with avian and mammalian material, provide the basis for our understanding of these transitions. The myosin and actin multigene families appear to follow different developmental strategies; thus, during the formation of a skeletal muscle, distinct skeletal isoforms of the myosin heavy chains appear sequentially (Whalen *et al.*, 1981) whereas a cardiac isoform of the actins (Vandekerckhove *et al.*, 1985) and myosin alkali light chains (Whalen *et al.*, 1978) accumulate in foetal muscle fibres, although with different kinetics. This is also seen at the level of mRNA accumulation (Weydert *et al.*, 1983; Minty *et al.*, 1982; Barton *et al.*, 1985*a*). It would thus appear that each family is responding differently to the neuronal and hormonal signals which are physiologically important for muscle development (e.g. Gambke *et al.*, 1983).

The description of developmental transitions for the myosin alkali light chains differs between birds and mammals. In mammalian striated muscle (see Table 2), a foetal myosin light chain is present, $MLC1_{emb}$ (Whalen *et al.*, 1978), and has been shown to be similar as a protein (Whalen & Sell, 1980; Price *et al.*, 1980) to, and to be the product of the same gene (Barton *et al.*, 1985*a*) as, the isoform $MLC1_A$ of adult cardiac atria (see the section on adult isoforms). There is no evidence for an equivalent protein in avian muscle. In the foetal skeletal muscle of rodents just before birth the principal alkali light chains present are $MLC1_{emb}$ and $MLC1_F$ (Whalen *et al.*, 1978). $MLC3_F$ only accumulates in significant amounts after birth (Sréter *et al.*, 1975; Roy *et al.*, 1979*b*; Gauthier *et al.*, 1982). Analysis of the corresponding mRNAs, using recombinant DNA probes for these myosin alkali light chain sequences, demonstrates that accumulation of protein isoforms and their mRNAs is closely co-ordinated (Robert *et al.*, 1982; Barton *et al.*, 1985*a*). In human skeletal muscle at birth the foetal $MLC1_{emb}$ isoform has already been replaced by the adult $MLC1_F$, whereas in 3–4-month-old foetuses the former predominates (Strohman *et al.*, 1983; Biral *et al.*, 1984). It is not yet clear what happens at earlier foetal stages in rodent muscle, whether, for example, the early foetal muscle fibres (primary fibre population), which can be distinguished from later foetal fibres (see Ontell, 1982) and may be formed from a distinct precursor myoblast population (Rutz & Hauschka, 1982), accumulate only the $MLC1_{emb}$ isoform. Slow skeletal muscle fibres, present, for example, in the diaphragm or soleus muscle, begin to accumulate significant amounts of slow type myosin alkali light chain isoforms at birth, when a mixture of fast and slow isoforms is initially present (Gauthier *et al.*, 1982) as in type IIC fibres in the adult (Pelloni-Mueller *et al.*, 1976). The $MLC1_{Sa}$ isoform accumulates later than $MLC1_{Sb}$ in these fibres (Gauthier *et al.*, 1982).

When myoblasts from mammalian skeletal muscle are grown in tissue culture the resulting fibres accumulate the foetal and adult fast isoforms. In primary cultures from bovine skeletal muscle (Daubas *et al.*, 1981; Whalen *et al.*, 1982), the slow isoform $MLC1_{S'}$ is also synthesized. No detectable amounts of the other slow isoform $MLC1_S$ are present. The $MLC1_{S'}$ isoform has also, in fact, been detected *in vivo* in human foetal muscle (3–4 months) (Biral *et al.*, 1984), and may therefore be a marker of myotubes at earlier foetal stages, or alternatively a characteristic of a minor muscle fibre population present at this time. The question of whether the same myotube is expressing all the isoforms detected is best addressed by using cloned muscle cell lines. The two mouse lines, C_2 (Yaffé & Saxel, 1977) and T984-Cl 10 (Jakob *et al.*, 1978) synthesize both $MLC1_{emb}$ and $MLC1_F$ (e.g.

Table 2. Myosin alkali light chains in developing skeletal muscle

For references see the text. The designation fast/slow refers to the potential adult fibre type. Minor variations in developmental timing are seen between different fast or slow muscles. It should be noted that the rodent slow muscles studied contain some fast fibres in the adult, unlike those in the chick.

	Mammalian (rodent)			
Age...	18 day foetal		New-born	
Fibre type...	Fast	Slow	Fast	Slow
Isoforms...	$MLC1_{emb}$ $MLC1_F$	$MLC1_{emb}$ $MLC1_F$ $(MLC1_S)$	$(MLC1_{emb})$ $MLC1_F$ $MLC3_F$	$(MLC1_{emb})$ $MLC1_F$ $MLC1_S$

	Avian (chick)			
Age...	5–7 days *in ovo*	8–12 days *in ovo*	13–16 days *in ovo*	
Fibre type...	Fast and slow	Fast and slow	Fast	Slow
Isoforms...	$MLC1_F$	$MLC1_F$ $MLC1_S$	$MLC1_F$ $MLC3_F$ $(MLC1_S)$	$MLC1_S$ $MLC1_F)$

Caravatti *et al.*, 1982). The C_2 line synthesizes some $MLC1_S$, (Blau *et al.*, 1983). Both cell lines accumulate $MLC3_F$. Primary cultures also synthesize varying amounts of this isoform (Whalen *et al.*, 1978; Daubas *et al.*, 1981; Strohman *et al.*, 1983). In the mouse cell lines, the relative proportion of the myosin alkali light chain isoforms is not modified in older myotubes (G. Bugaisky & M. E. Buckingham, unpublished work), whereas in primary cultures the relative proportion of $MLC1_F$ to $MLC1_{emb}$ has been reported to increase after prolonged culture of myotubes (Whalen *et al.*, 1979). The isoforms initially expressed, and the extent to which their expression is subsequently modified *in vitro*, is particularly interesting in view of the absence of hormonal or neuronal modifying influences in tissue culture. Another question which arises in this context is that of the type of myoblast (Rutz & Hauschka, 1982) represented by a cloned muscle cell line. The rat muscle cell line, L6 (L8..., etc) (see Yaffé, 1969), for example, accumulates only the $MLC1_{emb}$ isoform (Whalen *et al.*, 1978). One can ask whether this is typical of a distinct population *in vivo*, perhaps characteristic of early foetal muscle (see Buckingham, 1985). In the L6 muscle cells the gene for the adult myosin alkali light chain isoforms is present, since it can be activated in heterokaryons (Wright, 1982), but no mRNA transcripts are detected with cloned probes (Robert *et al.*, 1982; Barton *et al.*, 1985*a*). This cell line is particularly interesting, in practical terms, for future experiments on transcriptional factors necessary for the activation of myosin alkali light chain genes. One can postulate, for example, that a factor necessary for activation of the $MLC1_F/MLC3_F$ gene is present in the mouse muscle lines and absent from L6 (see the concluding comments).

Avian foetal skeletal muscles, unlike those of mammals, co-accumulate fast and slow myosin alkali light chain isoforms (Obinata *et al.*, 1980; Stockdale *et al.*, 1981*b*; Lowey *et al.*, 1983; Matsuda *et al.*, 1983). This is also seen in the mytubes of primary cultures derived from chick or quail skeletal muscle (Keller & Emerson, 1980; Stockdale *et al.*, 1981*a*; Montarras & Fiszman, 1983; Matsuda *et al.*, 1983). *In ovo*, in a potentially fast (e.g. pectoralis) or slow (e.g. anterior latissimus dorsi) muscle at days 5–7, $MLC1_F$ is the only myosin alkali light chain present. From days 8 to 12 the $MLC1_S$ isoform also accumulates. From day 12 in a potentially fast muscle the adult isoform pattern begins to be established, so that by day 16 $MLC3_F$ has appeared, and by day 19 $MLC1_F$ and $MLC3_F$ are the only alkali light chains present (Crow *et al.*, 1983; Crow & Stockdale, 1984). In adult slow muscles such as the anterior latissimus dorsi, which has some fast fibres, $MLC1_F$ accumulates, but $MLC3_F$ is never detectable (Crow *et al.*, 1983). This phenomenon may be related to the type of innervation of the muscle. With aneural grafts of 11-day quail muscle, the $MLC3_F$ isoform is not detected (Merrifield & Konigsberg, 1985). The slow and fast MLC1 isoforms are both expressed in this situation. The dissociation between $MLC1_F$ and $MLC3_F$ synthesis is particularly interesting in view of the fact that the two proteins are products of the same gene. From studies *in vivo* using anti-(myosin alkali light chain) antibodies it is clear that the same muscle fibre is expressing both fast and slow isoforms in early foetal muscle (Crow *et al.*, 1983), and the question then is whether the same mature fibre subsequently expresses one or the other, or whether the primary fibres are replaced by another myotube population of secondary fibres. It is difficult to provide a clear cut answer to this problem by studies *in vivo*. Clonal analysis of myoblasts from different muscles (pectoral or anterior latissimus dorsi) at different ages shows that all the cells express $MLC1_F$ and $MLC1_S$, and variable, low levels of $MLC3_F$, when they fuse to form myotubes in tissue culture (see Keller & Emerson, 1980). This would suggest again that innervation is not important in the expression of fast or slow myosin alkali light chains initially, and that both isoforms are

indeed synthesized in the same muscle fibre. The precursor myoblasts from different sources seem to have the same potential for light chain expression, suggesting that a different myoblast population is not implicated in the formation of mature fast or slow fibres. Maturation, however, is apparently dependent on factors *in vivo*, such as innervation (see section on manipulated muscle). No permanent cell lines have been derived from avian muscle. However, the use of temperature-sensitive Rous sarcoma virus permits some manipulation of primary cultures, which will differentiate into myotubes at a non-permissive temperature for the virus, and can otherwise be propagated for several generations as mononucleated transformed cells. Such 'transformed' myotubes express the fast and slow myosin alkali light chains, but undergo some modifications in expression after prolonged propagation (Montarras & Fiszman, 1983).

Adult avian cardiac muscle is characterized by the presence of the $MLC1_V$ isoform, which is also the myosin light chain present in foetal hearts. One report suggests that at very early stages (before 10 days) an $MLC1_F$-like protein is present as a minor component in chick hearts (Obinata *et al.*, 1983). This is an intriguing suggestion, since this isoform is generally regarded as skeletal muscle specific. In mammals at present there is no evidence for $MLC1_F$ in foetal cardiac tissue. The mammalian foetal isoform $MLC1_{emb}$ co-accumulates with $MLC1_V$ in developing mammalian ventricles (Cummins *et al.*, 1980; Whalen & Sell, 1980). In the late foetal (20 day) ventricles of rats $MLC1_{emb}$ represents about 25% of total MLC1 protein (Whalen & Sell, 1980), whereas in humans, where earlier stages of gestation have been examined, it represents as much as 50% (e.g. at 20 weeks), falling to 30% at birth (Price *et al.*, 1980). Thus, although cardiac muscle develops more rapidly than skeletal, this foetal isoform is retained for longer in the human ventricle than in human skeletal muscle, where it is no longer detectable at birth (Biral *et al.*, 1984). In mammalian atria the $MLC1_{emb}$ or $MLC1_A$ isoform is the major component, retained in the adult (Cummins *et al.*, 1980; Whalen *et al.*, 1982).

Little information is available about developing smooth muscle. However a foetal myosin light chain has been detected in the developing chicken gizzard (Katoh & Kubo, 1978; Takano-Ohmuro *et al.*, 1983*a*). This alkali light chain has distinct electrophoretic and immunological properties from the light chains of adult striated muscle. Although similar in size it is clearly different from $MLC1_F$, for example. This observation is particularly interesting for two reasons. Firstly, the isoform of adult smooth muscle is of the non-muscle type, the presence of a striated muscle type light chain in this tissue is therefore surprising (see the section on evolution). Secondly, this is the first characterization of a foetal isoform in avian muscle. Although no $MLC1_A$ isoform has been detected, one report indicates that the foetal smooth muscle isoform is also present in chick cardiac and skeletal muscle at early embryonic stages (Takano-Ohmuro *et al.*, 1985).

Myosin alkali light chain expression in diseased and manipulated muscle

The accumulation and abundance of the different isoforms of myosin alkali light chain may be affected by a variety of physiological parameters. It is well documented that nerve contact has an influence on the type of myosin synthesized in skeletal muscles. In general it appears that nerve contact is required by both developing and mature slow twitch muscle to retain expression of the slow muscle isoforms, while fast skeletal muscle is more independent of nerve contact. This is supported by the observation that denervation of developing soleus muscle prevents the development of slow fibres and the synthesis of the slow myosin alkali light chain isoforms. Denervation of the adult soleus results in the partial loss of slow myosin alkali light chain isoforms with their replacement by fast $MLC1_F$ and $MLC3_F$ (Rubinstein & Kelly, 1978). This effect is not seen in denervated fast muscle, although an altered pattern of light chain synthesis, showing an increase in the $MLC1_F/MLC3_F$ ratio, has been observed in denervated chicken pectoralis muscle (Matsuda *et al.*, 1984). The slow muscle phenotype can be induced in fast muscle by chronic low-frequency stimulation and is accompanied by expression of the slow muscle myosin alkali light chain isoforms in, for example, the extensor digitorum longus and tibialis anterior fast skeletal muscles of the rabbit (Roy *et al.*, 1979*a*). This change in expression is seen at the level of mRNA, since translatable $MLC1_S$ mRNA is detectable following stimulation (Heilig & Pette, 1983). Conversion of muscle from fast to slow type can also be achieved by cross-reinnervation of fast skeletal muscle with the nerve originally supplying a slow muscle; to some extent this change may be reciprocal with, for example, fast (extensor digitorum longus) and slow (soleus) muscles being induced to produce slow and fast myosin alkali light chain isoforms, respectively, following the cross-union of their nerves (Sréter *et al.*, 1974; Weeds *et al.*, 1974; Gauthier *et al.*, 1983). However, while transformation of the fast muscle to slow can be complete, transformation of slow muscle to fast type muscle by cross-reinnervation appears to be only partial (Gauthier *et al.*, 1983). In these situations where there is a change in mammalian myosin light chain expression, one might ask whether the foetal $MLC1_{emb}$ isoform appears initially. This appears not to be the case (Brown *et al.*, 1983). This physiological switch in light chain expression provides an elegant model system for the analysis of gene regulation at the nucleic acid level.

Expression of skeletal muscle light chain isoforms is altered in certain pathological conditions and is influenced by thyroid hormone levels. Thyroidectomy results in loss of fast muscle myosin alkali light chain isoforms from the mixed fibre soleus muscle in adult rats (Johnson *et al.*, 1980) and in biceps brachialis muscle from hypothyroid humans, slow isoforms predominate (Salviati *et al.*, 1985). In human atrial muscle showing hypertrophy due to pressure overload there is an induction of expression of the $MLC1_V$ isoform (Cummins, 1982), although this does not occur in rats (Bugaisky *et al.*, 1983). Altered patterns of fast and slow light chains have been noted in human nemaline myopathy where there is a shift towards pure slow type myosin alkali light chains in gastrocnemius muscle (normally mixed fast and slow) (Volpe *et al.*, 1982), and in dystrophic skeletal muscle where slow muscle isoforms appear in muscles normally of pure fast type (e.g. posterior latissimus dorsi muscle in dystrophic chicken) (Bandman, 1984). In dystrophic mice it has been noted that myosin prepared from hind limb muscle contains a reduced level of $MLC3_F$ (Fitzsimmons & Hoh, 1983; John, 1974) and that soleus

muscle contains only the slow muscle isoform ($MLC1_S$) where normally this muscle is of mixed fibre type. Fast muscle of dystrophic mice also contains traces of the slow form $MLC1_S$. In human Duchenne dystrophy it has been shown that foetal myosin is present in the mature muscle and this myosin contains the foetal light chain $MLC1_{emb}$ (Margreth *et al.*, 1984). The presence of the foetal light chain may suggest a developmental deficiency in fibre maturation. In general, in diseased muscle it is difficult to distinguish isoform changes which represent adaptation to the pathological state from those which result directly from a fundamental lesion.

Myosin alkali light chains in other animals; evolutionary aspects

The myosin light chain composition of muscles from a wide variety of animals has been examined, in Chordates (including fish, amphibia, birds and mammals), in Arthropods (including insects and crustacea), in Molluscs (including bivalves and squid) and in the Nematode *Ascaris lumbricoides*. Some caution must be exercised in comparing muscle types between such a variety of organisms, but in general it is clear that all vertebrates so far examined have the same two types of skeletal muscle; fast-type containing $MLC1_F$, $MLC2_F$ and $MLC3_F$, and slow-type containing $MLC1_S$ and $MLC2_S$ (see Fig. 1). Where this has been examined, the major alkali light chain in vertebrate cardiac muscle is probably identical with that found in slow skeletal muscle. In contrast to this, the invertebrates so far examined, with the exception of *Drosophila*, show a single alkali-type myosin light chain which is more closely related in size to the vertebrate $MLC3_F$ and smooth muscle/non-muscle isoforms than to the other vertebrate MLC1-like isoforms (see Fig. 1).

Invertebrate isoforms. One of the most primitive muscle myosins so far examined in detail is that from the Nematode *Ascaris lumbricoides* (Ko *et al.*, 1979) which contains two myosin light chains of M_r 16000 and 18000 (Nakamura *et al.*, 1975), the larger of which can be liberated with DTNB and corresponds to the MLC2 or regulatory light chain. The second light chain shows an electrophoretic mobility comparable with that of the vertebrate $MLC3_F$ and of smooth muscle/non-muscle isoforms. Myosins from molluscs (scallop and squid) and crustacea (crayfish and lobster) show the same pattern of fast migrating alkali light chain (Kendrick-Jones *et al.*, 1976; Ko *et al.*, 1979). In insects the situation is similar: the water bug *Lethocerus codofanus* has two light chains of M_r 17000 and 30000 (MLC2 type), and the dung beetle *Heliocopris japetus* has two of M_r 15000 and 27000 (MLC2 type) (Bullard *et al.*, 1973). *Drosophila* appears more complex, with different isoforms occuring in the tubular and fibrillar muscle types (Takano-Ohmura *et al.*, 1983*b*). Three light chain components are present in these muscles, and although it is at present unclear which of these corresponds to the DNTB regulatory light chain this might suggest a pattern similar to that seen in vertebrate skeletal muscle. A cDNA and the corresponding gene encoding an alkali myosin light chain of *Drosophila* have recently been cloned (Falkenthal *et al.*, 1984), from which the complete sequence of the protein has been determined. This isoform, which corresponds to the fast-migrating major isoform present in adult *Drosophila*, has been shown to be considerably different in sequence from the vertebrate $MLC1_F$, $MLC3_F$ and $MLC1_V$ isoforms, showing, for example, only about 30% homology with the rabbit skeletal muscle forms. Hybridization experiments have shown that this is the only gene encoding myosin alkali light chain-like isoforms in the *Drosophila* genome. The *Drosophila* myosin light chain gene hybridizes to two mRNA species; a major species of 900 nucleotides, corresponding to the cDNA analysed, and a second, minor, mRNA of 1300 nucleotides. The full structural analysis of this gene (Falkenthal *et al.*, 1985) now demonstrates that there are two alternative splicing patterns in the 3′ region of the gene, which result in mRNAs that translate to give proteins with two different *C*-termini. The size difference between these polypeptides does not fully explain the difference in electrophoretic mobility of the putative *Drosophila* myosin alkali light chain proteins, but this may be influenced also by the amino acid composition. Different length mRNAs are also generated as a result of differently placed polyadenylation signals. The splicing pattern of the gene is developmentally regulated so that one myosin alkali light chain protein is present in both larval and adult musculature, while the other is found only in the adult. This phenomenon contrasts with the differential splicing of the 5′ region of the $MLC1_F/MLC3_F$ gene during skeletal muscle development in vertebrates (see Robert *et al.*, 1984; Barton *et al.*, 1985*a*), but can be compared with the preliminary finding that a single gene encodes the vertebrate smooth muscle and non-muscle isoforms which have differing *C*-termini (Nabeshima & Nabeshima, 1984).

Considering the range of muscle types analysed in these different invertebrates, from the fast-contracting insect flight muscles and abdominal flexor muscle in the crayfish to the slow contracting molluscan muscles and the undulating contraction of the Nematode body wall, it is perhaps surprising not to find a greater variety in myosin light chain patterns observed. The principal pattern of myosin light chains seen in invertebrates is like that of vertebrate smooth muscle or non-muscle myosins where a single fast migrating alkali light chain is seen. In addition to this similarity in electrophoretic pattern, it has been noted that these isoforms do not carry trimethylalanine. The isoforms $MLC1_F$, $MLC1_V$ and $MLC1_A$ from all vertebrate species analysed carry this unusual amino acid as the *N*-terminal residue. This modification is not present on vertebrate $MLC3_F$ or the smooth muscle light chain, nor is it found on the invertebrate myosin alkali light chain isoforms of scallop, lobster, squid and clam (Henry *et al.*, 1985). This similarity compares with that seen between invertebrate muscle actin and vertebrate non-muscle type actin (Vandekerckhove & Weber, 1984) although in the case of actin the smooth muscle form is encoded by a different gene from that found in non-muscle cells (Vandekerckhove & Weber, 1978, 1979). The data on the myosin alkali light chains also suggest that the ancestral light chain gene was more similar to the present day non-muscle gene, encoding a protein of size similar to that of the non-muscle/smooth muscle and $MLC3_F$ isoforms rather than that of $MLC1_F$, $MLC1_V$ or $MLC1_A$ (see Fig. 5).

Vertebrate isoforms. Various species of fish have been examined with respect to myosin light chain content (Focant *et al.*, 1976; Watabe *et al.*, 1983*a*,*b*, 1984; Ochiai

et al., 1984; Rowlerson *et al.*, 1985) and have been shown to have the same two patterns as seen in fast and slow skeletal muscle in mammals and birds with $MLC1_F$, $MLC2_F$ and $MLC3_F$ in white muscle, and $MLC1_S$ and $MLC2_S$ in red muscle. Furthermore, in mackerel it has been shown that the cardiac myosin contains the same light chain pattern as the red (slow) skeletal muscle of this fish (Dinh *et al.*, 1985) which is the same as the situation in birds and mammals. In the frog, skeletal muscle myosin contains the standard $MLC1_F$, $MLC2_F$, $MLC3_F$ pattern (Focant & Huriaux, 1980). The pattern of light chains present in the lower vertebrates, or the lower (non-vertebrate) Chordates, is not at present known and the precise order of events leading to the generation of the vertebrate myosin alkali light chain genes is therefore not certain. However, considering that the additional isoforms found in the vertebrate skeletal and cardiac muscle are more closely related to $MLC1_F$ than to $MLC3_F$ in terms of size and the presence of trimethyl-alanine, it seems probable that generation of the $MLC1_F/MLC3_F$ gene structure preceeded the series of duplication and dispersion events that gave rise to the genes encoding $MLC1_V$ ($MLC1_S$) and $MLC1_A$ ($MLC1_{emb}$) (see Fig. 5). It would be of particular interest to establish the myosin light chain content of lower vertebrates, in view of the fact that the first muscle-type actin is detected, for example, in the lamprey (Vandekerckhove & Weber, 1984) and generation of the skeletal muscle $MLC1_F/MLC3_F$ gene probably occurred at the time of vertebrate evolution. It is not clear how the complex $MLC1_F/MLC3_F$ gene structure arose from a simple ancestral gene encoding an $MLC3_F$-like isoform. The arrangement of the 5′ exons and the use of two promoter sequences to generate the $MLC1_F$ and $MLC3_F$ mRNAs make it difficult to envisage a single rearrangement/duplication event. The similarity in the exons 3 and 4 (see Figs. 2 and 5) suggests that these may have arisen by limited duplication of this region, as originally suggested from the protein sequence (Frank & Weeds, 1974). Creation of the slow skeletal/cardiac type gene probably arose by duplication of the entire $MLC1_F/MLC3_F$ gene with subsequent loss of the second ($MLC3_F$) promoter, since the extended $MLC1_F$-like *N*-terminal sequence is present in $MLC1_V/MLC1_S$.

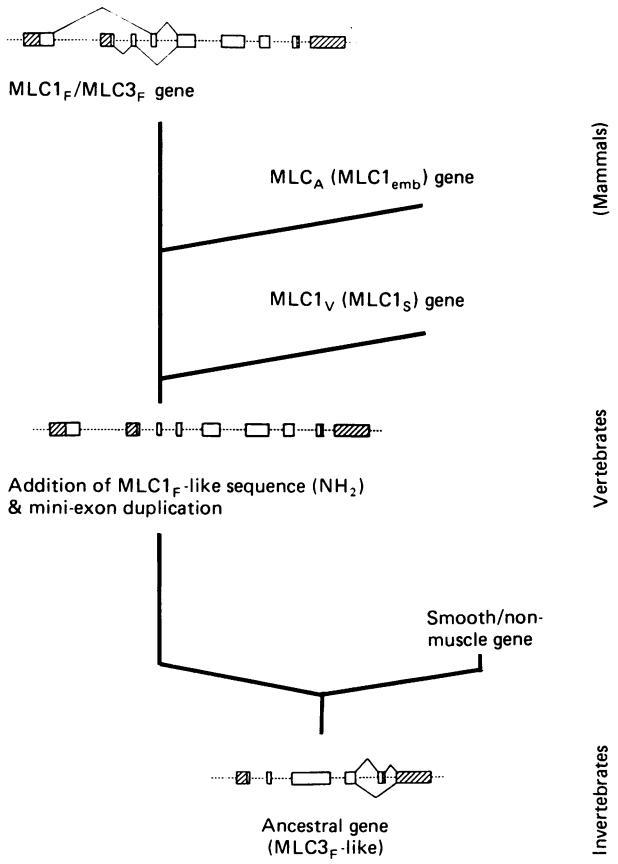

Fig. 5. Proposed evolution of the MLC1 gene family

The structure of the ancestral gene is proposed from the present day invertebrate (*Drosophila*) gene. Closed boxes indicate exon sequences and hatched boxes non-coding sequences. Differential splicing pathways are indicated by lines joining exon sequences.

In higher vertebrates there are fundamental differences in the pattern of expression of the myosin alkali light chain genes during development (see section on development), and in birds and amphibia (frog) there is no equivalent of the mammalian cardiac atrial $MLC1_A$ ($MLC1_{emb}$) isoform (Dalla Libera *et al.*, 1979; Grandier-Vazeille *et al.*, 1983), suggesting that this isoform probably arose after the divergence of mammals and birds. Analysis of the mouse $MLC1_A/MLC1_{emb}$ mRNA and gene show that this isoform is very closely related to $MLC1_F$, also suggesting that it may have arisen by a recent duplication event. Cross-hybridization using the $MLC1_F/MLC3_F$ gene sequence is strongest with the $MLC1_A/MLC1_{emb}$ gene and sequence homology has been detected in the 3′ non-coding regions of these mRNAs (Robert *et al.*, 1982), and regions for which the sequence of the $MLC1_A/MLC1_{emb}$ gene have been completed suggest that this isoform is less diverged from $MLC1_F$ than is $MLC1_V$ (P. J. R. Barton & M. E. Buckingham, unpublished work). An embryonic myosin alkali light chain isoform has been described in developing chick smooth muscle (Katoh & Kubo, 1978; Takano-Ohmuro *et al.*, 1983*a*) which is also expressed in cardiac and skeletal muscle during development (Takano-Ohmuro *et al.*, 1985). This isoform is not expressed in adult chicken atria, which contain the same cardiac isoform as chicken ventricular muscle (Dalla Libera *et al.*, 1979) and the relationship between this isoform and mammalian $MLC1_{emb}$ is not clear at present.

The wider question of the evolutionary relationships within the troponin C super-family, to which the alkali myosin light chains belong, has been examined at the protein level (see Barker *et al.*, 1978). Structural information on other genes from this super-family is becoming available and should allow comparison at the nucleic acid level. For example, the structure and sequence of the genes encoding a rat MLC2 isoform (Nudel *et al.*, 1984) and chicken calmodulin (Simmen *et al.*, 1985) show remarkably similar features to the $MLC1_F/MLC3_F$ gene. Both MLC2 and calmodulin genes show the separation of the ATG (methionine) translational initiation codon from the rest of the coding sequence by an intron sequence. In the case of calmodulin, the first coding exon, after that with the ATG, is separated from the following exons by a large intron, and encodes only the first ten amino-acid residues

of this protein. This arrangement is very similar to that of the $MLC3_F$ exons (see Fig. 3) where the first exon contains the 5′ non-coding sequence and the ATG codon, and where the second exon encodes the first eight amino-acid residues. These observations support the idea that the ancestral myosin alkali light chain gene had an arrangement of exons similar to those encoding $MLC3_F$.

Discussion and perspectives

The contribution of research based on recombinant DNA technology to our understanding of the myosin alkali light chain multigene family, for the moment, mainly concerns the description of what isoforms are where, when. Analysis of the corresponding mRNAs and genes has, for example, clarified the situation for $MLC1_{emb}/MLC1_A$ and $MLC1_V/MLC1_S$ in the mouse. In the near future DNA sequencing of these and other myosin light chain genes should provide information on the protein structure, which in the case of isoforms such as $MLC1_{emb}/MLC1_A$ is completely lacking at present. As discussed in this review, another area in which our knowledge is deficient is that of myosin gene expression in non-muscle and smooth muscle tissues; a recent report of the isolation of non-muscle/smooth muscle myosin alkali light chain coding sequences (Nabeshima & Nabeshima, 1984) indicates that these isoforms should soon be better defined. Research on the $MLC1_V/MLC1_S$ gene sequence may also clarify the relationship between $MLC1_S$ and $MLC1_{S'}$, which is at present unclear.

The use of antibodies, where these are available, has provided many of the answers to questions about the distribution of myosin alkali light chain isoforms in individual fibres in adult and developing muscles. Hybridization *in situ* on tissue sections with nucleic acid probes is a complementary approach (e.g. Levine *et al.*, 1983) which can now be applied to these questions for the myosin alkali light chains and for other muscle proteins for which cloned gene and mRNA probes are available. In general there is more sequence divergence at the nucleic acid than at the protein level because of the degeneracy of the genetic code, and the coding sequences of the myosin alkali light chain isoforms, for example, are easily distinguished under relatively non-stringent hybridization conditions. In addition to the availability and specificity of the nucleic acid probes, this technique offers the advantage that mRNA acid molecules *in vivo* can readily be made accessible to hybridization, and are probably less subject to the effects of steric hindrance than antibodies directed against compact acto-myosin structures.

The characterization of the gene encoding the two fast myosin alkali light chains $MLC1_F/MLC3_F$ points the way to future studies on the regulation of gene expression in this multigene family. The experimental possibilities offered by gene transfer technology, where individual genes or parts of genes can be re-introduced into cells, or indeed into embryos, have just begun to be explored for the contractile proteins. Transcripts from a skeletal muscle actin gene re-introduced into the rat muscle cell line, L6, accumulate as the cells differentiate into muscle fibres, suggesting that the DNA sequences necessary for biological regulation of this gene are present in its immediate 5′ flanking sequences (Melloul *et al.*, 1984). It should be possible, for the myosin alkali light chain genes, to identify the DNA sequences, and the transcriptional factors which interact with them, implicated in their expression.

Comparison of transcriptional signals for these genes with those for other contractile proteins expressed in the same muscle fibre approaches the fundamental question of how a set of genes is activated in a given phenotype; studies on the organization of muscle genes having already ruled out simple 'operon-type' models where co-expressed genes are linked (see Robert *et al.*, 1985). The question of the functional requirement for different isoforms can also be approached by gene manipulation. Here, too, the actins provide an example. Introduction of a cardiac actin gene into a non-muscle cell results in the incorporation of cardiac actin into the cytoskeleton (Gunning *et al.*, 1984). Especially now that it may be possible to neutralize the contribution of transcripts from the endogenous gene (Izant & Weintraub, 1984), re-introduction of different myosin alkali light chain genes into muscle cells in culture should permit examination of the effect of a foreign light chain protein on a muscle sarcomere. Functional studies '*in vivo*' can be extended by the use of modified gene sequences. Ideally, introduction of genetically engineered myosin alkali light chain genes into the embryo, rather than into cultured myotubes, would allow the role of the isoform to be examined during development, and in adult tissues under different physiological conditions. In invertebrates such as the nematode *Caenorhabditis elegans*, where myosin heavy chain mutants have been isolated (e.g. Moerman *et al.*, 1982), or in *Drosophila*, where some flight mutants map to the same locus as the myosin heavy chain gene (Bernstein *et al.*, 1983), complementation type analyses, at least for this contractile protein, should be feasible. In higher vertebrates where heritable mutations in contractile protein genes have not yet been identified, interpretation of the effects of a foreign isoform, supposing that the exogenous gene is expressed correctly in the tissues concerned, is likely to be difficult against a background of the native proteins. A longer term objective in the development of gene transfer technology is to modify the endogenous gene *in vivo* by recombination with exogenous DNA sequences.

This work was supported by grants to M.E.B. from the French Ministry of Research and Technology, the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médical and the Muscular Dystrophy Association (MDA) of America. P.J.R.B. is the recipient of an EMBO fellowship. The authors are grateful to Benoît Robert for valuable discussion. S.C.B. provided comment.

References

Bagshaw, C. R. (1982) Outline Studies in Biochemistry: Muscle Contraction, Chapman and Hall, London

Bandman, E. (1984) Muscle Nerve **7**, 312–326

Barker, W. C., Ketchman, L. K. & Dayhoff, M. O. (1978) in Atlas of Protein Structure (Dayhoff, M. O., ed.), pp. 273–283, National Biomedical Research Foundation, Washington DC

Barton, P. J. R., Robert, B., Fiszman, M. Y., Leader, D. P. & Buckingham, M. E. (1985*a*) J. Muscle Res. Cell Motil. **6**, in the press

Barton, P. J. R., Cohen, A., Robert, B., Fiszman, M. Y., Bonhomme, F., Guenet, J. L., Leader, D. P. & Buckingham, M. E. (1985*b*) J. Biol. Chem. **260**, 8578–8584

Bautch, V. L., Storti, R. V., Mischke, D. & Pardue, M. L. (1982) J. Mol. Biol. **162**, 231–250

Benyajati, C., Spoerel, N., Haymerle, H. & Ashburner, M. (1983) Cell **33**, 125–133
Bernard, R., Little, P. F. R., Annison, G., Williamson, R. & Flavell, R. A. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4827–4831
Bernstein, S. I., Mogami, K., Donady, J. J. & Emerson, C. P., Jr. (1983) Nature (London) **302**, 393–397
Billeter, R., Heizmann, C. W., Howald, H. & Jenny, E. (1981) Eur. J. Biochem. **116**, 389–395
Biral, D., Damiani, E., Volpe, P., Salviati, G. & Margreth, A. (1982), Biochem. J. **203**, 529–540
Biral, D., Damiani, E., Margreth, A. & Scarpini, E. (1984) Biochem. J. **224**, 923
Blau, H. M., Chiu, C. P. & Webster, C. (1983) Cell **32**, 1171–1180
Brown, W. E., Salmons, S. & Whalen, R. G. (1983) J. Biol. Chem. **258**, 14686–14692
Buckingham, M. E. (1985) Essays Biochem. **20**, 77–109
Buckingham, M. E. & Minty, A. J. (1983) in Eukaryotic Genes (Maclean, N., Gregory, S. & Flavell, R. A., eds.), pp. 365–397, Butterworths, London
Bugaisky, L. B., Siegel, E. & Whalen, R. G. (1983) FEBS Lett. **161**, 230–234
Bullard, B., Dabrowska, R. & Winkelman, L. (1973) Biochem. J. **135**, 277–286
Burridge, K. (1974) FEBS Lett. **45**, 14–17
Butler-Browne, G. S. & Whalen, R. G. (1984) Dev. Biol. **102**, 324–334
Caravatti, M., Minty, A. J., Robert, B., Montarras, D., Weydert, A., Cohen, A., Daubas, P. & Buckingham, M. E. (1982) J. Mol. Biol. **160**, 59–76
Carlsson, S. A., Luger, O., Ringertz, N. R. & Savage, R. E. (1974) Exp. Cell Res. **84**, 47–55
Carmon, Y., Czosnek, H., Nudel, U., Shani, M. & Yaffé, D. (1982) Nucleic Acids Res. **10**, 3085–3097
Clarke, M. & Spudich, J. A. (1977) Annu. Rev. Biochem. **46**, 797–822
Crow, M. T. & Stockdale, F. E. (1984) Exp. Biol. Med. **19**, 165–174
Crow, M. T., Olson, P. S. & Stockdale, F. E. (1983) J. Cell Biol. **96**, 736–744
Cummins, P. (1982) Biochem. J. **205**, 195–204
Cummins, P., Price, K. M. & Littler, W. A. (1980) J. Muscle Res. Cell Motil. **1**, 357–366
Czosnek, H., Nudel, U., Shani, M., Barker, P. E., Pravtcheva, D. D., Ruddle, F. H. & Yaffé, D. (1982) EMBO J. **1**, 1299–1305
Czosnek, H., Nudel, U., Mayer, Y., Barker, P. E., Pravtcheva, D. D., Ruddle, F. H. & Yaffé, D. (1983) EMBO J. **2**, 1977–1979
Dalla Libera, L. (1981) Cell Biol. Int. Rep. **5**, 112
Dalla Libera, L. & Carraro, U. (1983) Cell Biol. Int. Rep. **7**, 271–273
Dalla Libera, L., Sartore, S. & Schiaffino, S. (1979) Biochim, Biophys. Acta **581**, 283–294
Daubas, P., Caput, D., Buckingham, M. & Gros, F. (1981) Dev. Biol. **84**, 133–143
Daubas, P., Robert, B., Garner, I. & Buckingham, M. E. (1985) Nucleic Acids Res. **13**, 4623–4643
Dinh, T. N. L., Watabe, S., Ochiai, Y. & Hashimoto, K. (1985) Comp. Biochem. Physiol., in the press
Falkenthal, S., Parker, V. P., Mattox, W. W. & Davidson, N. (1984) Mol. Cell. Biol. **4**, 956–965
Falkenthal, S., Parker, V. P. & Davidson, N. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 449–453
Fitzsimons, R. B. & Hoh, J. F. Y. (1983) J. Physiol. (London) **343**, 539–550
Flicker, P. F., Walliman, T. & Vibert, P. (1983) J. Mol. Biol. **169**, 723–741
Focant, B. & Huriaux, F. (1980) J. Muscle Res. Cell Motil. **1**, 61–72
Focant, B., Huriaux, F. & Johnston, I. A. (1976) Int. J. Biochem. **7**, 129–133
Frank, G. & Weeds, A. G. (1974) Eur. J. Biochem. **44**, 317–334
Fyrberg, E. A., Kindle, K. L. & Davidson, N. (1980) Cell **19**, 365–378
Gambke, B., Lyons, G. E., Haselgrove, J., Kelly, A. M. & Rubinstein, N. A. (1983) FEBS Lett. **156**, 335–339
Gauthier, G. F., Lowey, S., Benfield, P. A. & Hobbs, A. W. (1982) J. Cell Biol. **92**, 471–484
Gauthier, G. F., Burke, R. E., Lowey, S. & Hobbs, A. W. (1983) J. Cell Biol. **97**, 756–771
Gunning, P., Ponte, P., Kedes, L., Hickey, R. J. & Skoultchi, A. I. (1984) Cell **36**, 709–715
Grandier-Vazeille, X., Tetaert, D., Hemon, B. & Biserte, G. (1983) Comp. Biochem. Physiol. **76B**, 263–270
Heilig, A. & Pette, D. (1983) FEBS Lett. **151**, 211–214
Henry, G. D., Trayer, I. P., Brewer, S. & Levine, B. A. (1985) Eur. J. Biochem., **148**, 75–82
Ingram, R. S., Scott, R. W. & Tilghman, S. M. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 4694–4698
Izant, J. G. & Weintraub, H. (1984) Cell **36**, 1007–1015
Jakob, H., Buckingham, M. E., Cohen, A., Dupont, L., Fiszman, M. & Jacob, F. (1978) Exp. Cell Res. **114**, 403–408
John, H. A. (1974) FEBS Lett. **39**, 278–282
Johnson, M. A., Mastaglia, F. L., Montomery, A. G., Pope, B. & Weeds, A. G. (1980) FEBS Lett. **110**, 230–235
Katoh, N. & Kubo, S. (1978) Biochim. Biophys. Acta **535**, 401–411
Keller, L. R. & Emerson, C. P. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 1020–1024
Kendrick-Jones, J., Szentkiralyi, E. M. & Szent-Gyorgyi, A. G. (1976) J. Mol. Biol. **104**, 747–775
King, C. R. & Piatigorsky, J. (1983) Cell **32**, 707–712
Ko, J., Horiuchi, S. & Yamaguchi, M. (1979) J. Biochem. (Tokyo) **85**, 541–548
Korn, E. D. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 588–599
Leinwand, L. A., Fournier, R. E. K., Nadal-Ginard, B. & Shows, T. B. (1983) Science **221**, 766–769
Levine, M., Hafen, E., Garber, R. L. & Gehring, W. J. (1983) EMBO J. **2**, 2037–2046
Long, L., Fabian, F., Mason, D. T. & Wikman-Coffelt, J. (1977) Biochem. Biophys. Res. Commun. **76**, 626–635
Lowey, S. & Risby, D. (1971) Nature (London) **234**, 81–85
Lowey, S., Benfield, P. A., Leblanc, D. D. & Waller, G. S. (1983) J. Muscle Res. Cell Motil. **4**, 695–716
Maita, T., Umegane, T., Kato, Y. & Matsuda, G. (1980) Eur. J. Biochem. **107**, 565–575
Margreth, A., Salviati, G., Dalla Libera, L., Betto, R., Biral, D. & Salvatori, S. (1980) in Plasticity of Muscle (Pette, D., ed.), pp. 193–208, Walter de Gruyter, Berlin, and New York
Margreth, A., Biral, D. & Damiani, E. (1984) Ital. J. Neurol. Sci. Suppl. **3**, 63–70
Matsuda, G., Maita, T. & Umegane, T. (1981*a*) FEBS Lett. **126**, 111–113
Matsuda, G., Maita, T., Kato, Y., Chen, J. I. & Umegane, T. (1981*b*) FEBS Lett. **135**, 232–236
Matsuda, R., Bandman, E. & Strohman, R. C. (1983) Dev. Biol. **95**, 484–491
Matsuda, R., Spector, D. & Strohman, R. C. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 1122–1125
Melloul, D., Aloni, B., Calvo, J., Yaffé, D. & Nudel, U. (1984) EMBO J. **3**, 983–990
Merrifield, P. A. & Konigsberg, I. R. (1985) UCLA Symp. Mol. Cell. Biol. (New Ser.) **29**, in the press
Minty, A. J., Alonso, S., Caravatti, M. & Buckingham, M. (1982) Cell **30**, 185–192
Minty, A. J., Alonso, S., Guénet, J. L. & Buckingham, M. E. (1983) J. Mol. Biol. **167**, 77–101
Moerman, D. G., Plurad, S., Waterston, R. H. & Baillie, D. L. (1982) Cell **29**, 773–781
Montarras, D. & Fiszman, M. Y. (1983) J. Biol. Chem. **258**, 3883–3888
Nabeshima, Y. & Nabeshima, Y. (1984) (abstr.) in International Cell Biology (Seno, S. & Okada, Y., eds.)

Academic Press, Japan, and (abstr.) in Symposium on Myogenesis – Cellular and Molecular Bases, Tokyo
Nabeshima, Y., Fujii-Kuriyama, Y., Muramatsu, M. & Ogata, K. (1982) Nucleic Acids Res. **10**, 6099–6110
Nabeshima, Y., Fujii-Kuriyama, Y., Muramatsu, M. & Ogata, K. (1984) Nature (London) **308**, 333
Nakamura, T., Yanagisawa, T. & Yamaguchi, M. (1975) Biochim. Biophys. Acta **412**, 229–240
Nudel, U., Calvo, J. M., Shani, M. & Levy, Z. (1984) Nucleic Acids Res. **12**, 7175
Obinata, T., Masaki, T. & Takano, H. (1979) J. Biochem. (Tokyo) **86**, 131–137
Obinata, T., Masaki, T. & Takano, H. (1980) J. Biochem. (Tokyo) **87**, 81–88
Obinata, T., Masaki, T., Takano-Ohmuro, H., Tanaka, T. & Shimizu, N. (1983) J. Biochem. (Tokyo) **94**, 1025–1028
Ochiai, Y., Watabe, S. & Hashimoto, K. (1984) Bull. Jpn. Soc. Sci. Fisheries **50**, 1729–1733
O'Farrell, P. H. (1975) J. Biol. Chem. **250**, 4007–4021
Ontell, M. (1982) in Biochemical Development of the Foetus and Neonate (Jones, C. T., ed.), pp. 213–241, Elsevier/North-Holland, Amsterdam
Pelloni-Mueller, G., Ermini, M. & Jenny, E. (1976) FEBS Lett. **67**, 68–74
Periasamy, M., Strehler, E. E., Farfield, L. I., Gubits, R. M., Ruiz-Opazo, N. & Nadal-Ginard, B. (1984) J. Biol. Chem. **259**, 13595–13604
Pette, D., Henriksson, J. & Emmerich, M. (1979) FEBS Lett. **103**, 152–155
Price, K. M., Littler, W. A. & Cummins, P. (1980) Biochem. J. **191**, 571–580
Robert, B., Weydert, A., Caravatti, M., Minty, A., Cohen, A., Daubas, P., Gros, F. & Buckingham, M. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 2437–2441
Robert, B., Daubas, P., Akimenko, M. A., Cohen, A., Garner, I., Guénet, J. L. & Buckingham, M. (1984) Cell **39**, 129–140
Robert, B., Barton, P., Minty, A., Daubas, P., Weydert, A., Bonhomme, F., Catalan, J., Chazottes, D., Guénet, J. L. & Buckingham, M. (1985) Nature (London) **314**, 181–183
Rowlerson, A., Pope, B., Murray, J., Whalen, R. G. & Weeds, A. G. (1981) J. Muscle Res. Cell Motil. **2**, 415–438
Rowlerson, A., Scapolo, P. A., Mascarello, F., Carpene, E. & Veggetti, A. (1985) J. Muscle Res. Cell Motil. **6**, in the press
Roy, R. K., Mabuchi, K., Sarkar, S., Mis, C. & Sréter, F. A. (1979*a*) Biochem. Biophys. Res. Commun. **89**, 181–187
Roy, R. K., Sréter, F. A. & Sarkar, S. (1979*b*) Dev. Biol. **69**, 15–30
Rubinstein, N. A. & Kelly, A. M. (1978) Dev. Biol. **62**, 473–485
Rushbrook, J. I. & Somes, R. G., Jr. (1985) Biochem. Genet. **23**, 17–27
Rushbrook, J. I., Yuan, A. I. & Stracher, A. (1982) Muscle Nerve **5**, 505–514
Rutz, R. & Hauschka, S. (1982) Dev. Biol. **91**, 103–110
Salviati, G., Betto, R. & Danieli Betto, D. (1982) Biochem J. **207**, 261–272
Salviati, G., Betto, R., Danieli Betto, D. & Zeviani, M. (1984) Biochem. J. **224**, 215–225
Salviati, G., Zeviani, M., Betto, R., Nacamulli, D. & Busnardo, B. (1985) Muscle Nerve, in the press
Sarkar, S., Sréter, F. A. & Gergely, J. (1971) Proc. Natl. Acad. Sci. U.S.A. **68**, 946–950
Sartore, S. (1981) Biochim. Biophys. Acta **667**, 143–156
Schibler, U., Hagenbüchle, O., Wellauer, P. K. & Pittet, A. C. (1983) Cell **33**, 501–508
Sellers, J. R. & Harvey, E. V. (1984) J. Biol. Chem. **259**, 14203–14207
Simmen, R. C. M., Tanaka, T., Ts'ui, K. F., Putkey, J. A., Scott, M. J., Lai, E. C. & Means, A. R. (1985) J. Biol. Chem. **260**, 907–912
Sivaramakrishnan, M. & Burke, M. (1982) J. Biol. Chem. **257**, 1102–1105
Sréter, F. A., Gergely, J. & Luff, A. L. (1974) Biochem. Biophys. Res. Commun. **56**, 84
Sréter, F. A., Balint, M. & Gergely, J. (1975) Dev. Biol. **46**, 317–325
Stockdale, F. E., Baden, H. & Raman, N. (1981*a*) Dev. Biol. **82**, 168–171
Stockdale, F. E., Raman, N. & Baden, H. (1981*b*) Proc. Natl. Acad. Sci. U.S.A. **78**, 931–935
Strohman, R. C., Micou-Eastwood, J., Glass, C. A. & Matsuda, R. (1983) Science **221**, 955–957
Syrovy, I., Delcayre, C. & Swynghedauw, B. (1979) J. Mol. Cell. Cardiol. **11**, 1129–1135
Takahashi, M. & Tonomura, Y. (1975) J. Biochem. (Tokyo) **78**, 1123–1133
Takano-Ohmuro, H., Obinata, T., Mikawa, T. & Masaki, T. (1983*a*) J. Biochem. (Tokyo) **93**, 903–908
Takano-Ohmuro, H., Hirose, G. & Mikawa, T. (1983*b*) J. Biochem. (Tokyo) **94**, 967–974
Takano-Ohmuro, H., Obinata, T., Kawashima, M., Masaki, T. & Tanaka, T. (1985) J. Cell. Biol., in the press
Trayer, H. R. & Trayer, I. P. (1985) FEBS Lett. **180**, 170–174
Vandekerckhove, J. & Weber, K. (1978) Eur. J. Biochem. **90**, 451–462
Vandekerckhove, J. & Weber, K. (1979) Differentiation **14**, 123–133
Vandekerckhove, J. & Weber, K. (1984) J. Mol. Biol. **179**, 391–413
Vandekerckhove, J., Bugaisky, G. & Buckingham, M. (1985) J. Biol. Chem., in the press
Volpe, P., Damiani, E., Margreth, A., Pellegrini, G. & Scarlato, G. (1982) Neurology **32**, 37–41
Wagner, P. D. & Giniger, E. (1981) Nature (London) **292**, 560–562
Wagner, P. D. & Weeds, A. G. (1977) J. Mol. Biol. **109**, 455–473
Watabe, S., Dinh, T. N. L., Ochiai, Y. & Hashimoto, K. (1983*a*) J. Biochem. (Tokyo) **94**, 1409–1419
Watabe, S., Hashimoto, K. & Watanabe, S. (1983*b*) J. Biochem. (Tokyo) **94**, 1867–1875
Watabe, S., Dinh, T. N. L. & Hashimoto, K. (1984) Bull. Jpn. Soc. Sci. Fish. **50**, 71–77
Weeds, A. G. (1976) Eur. J. Biochem. **66**, 157–173
Weeds, A. G. & Pope, B. (1971) Nature (London) **234**, 85–88
Weeds, A. G., Trentham, D. R., Kean, C. J. C. & Buller, A. J. (1974) Nature (London) **247**, 135–139
Weydert, A., Daubas, P., Caravatti, M., Minty, A., Bugaisky, G., Cohen, A., Robert, B. & Buckingham, M. (1983) J. Biol. Chem. **258**, 13867–13874
Weydert, A., Daubas, P., Lazaridis, I., Barton, P., Garner, I., Leader, D., Bonhomme, F., Catalan, J., Chazottes, D., Guénet, J.-L., Gros, F. & Buckingham, M. (1985) Proc. Natl. Acad. Sci. U.S.A., in the press
Whalen, R. G. & Sell, S. M. (1980) Nature (London) **286**, 731–733
Whalen, R. G., Butler-Browne, G. S. & Gros, F. (1978) J. Mol. Biol. **126**, 415–431
Whalen, R. G., Butler-Browne, G. S., Sell, S. & Gros, F. (1979) Biochimie **61**, 625–632
Whalen, R. G., Sell, S. M., Butler-Browne, G. S., Schwartz, K., Bouveret, P. & Pinset-Harström, I. (1981) Nature (London) **292**, 805–809
Whalen, R. G., Sell, S. M., Eriksson, A. & Thornell, L. E. (1982) Dev. Biol. **91**, 478–484
Wikman-Coffelt, J. & Srivastava, S. (1979) FEBS Lett. **106**, 207–212
Winstanley, M. A., Small, D. A. P. & Trayer, I. P. (1979) Eur. J. Biochem. **98**, 441–446
Wright, W. E. (1982) In Vitro **18**, 851–858
Yaffé, D. (1969) Curr. Top. Dev. Biol. **4**, 37–77
Yaffé, D. & Saxel, O. (1977) Nature (London) **270**, 725–727