Functional Properties of Myosin Isoforms in Avian Muscle

EVERETT BANDMAN[1]

Department of Food Science and Technology, University of California, Davis, One Shields Way, Davis, California 95616

ABSTRACT Sarcomeric myosin is the major skeletal muscle protein and is encoded by a large and complex multigene family whose members are differentially expressed in developing and adult muscle cells. The structure and function of sarcomeric myosins have been extensively analyzed and many myosin genes have now been cloned and sequenced. This manuscript reviews the broad spectrum of myosin research with emphasis on studies in avian systems and discusses how advances in myosin isoform analysis have contributed to muscle and meat science.

(*Key words*: myosin, myosin isoforms, muscle, gene expression, myosin gene family)

1999 Poultry Science 78:729–734

INTRODUCTION

Sarcomeric myosin, the most abundant protein in skeletal muscle, is a well-characterized motor protein that interacts with actin to generate force for muscle contraction. Biochemical studies of muscle myosin go back to the late 1930s when it was shown that muscle contained an important adenosine triphosphatase (ATPase) that was associated with myosin (Engelhardt and Ljubimova, 1939). Over the past 60 yr, muscle researchers have studied myosin's functional and structural properties in order to elucidate the molecular mechanism of muscle contraction and to provide insights into the formation and the ultrastructure of the myofibril, the contractile organelle of skeletal muscle fibers.

Although skeletal muscle myosin is only one branch of the ever expanding superfamily of myosin motors (Sellars *et al*., 1996), it is sequence homology with the motor domain of chicken skeletal muscle myosin (residues 89 to 709) that defines members of this superfamily (Sellars and Goodson, 1995). One of the major advances in myosin motor research has been the solving of the atomic structure of the chicken sarcomeric myosin head, or S1 domain, by x-ray diffraction (Rayment *et al*., 1993). Armed with this information, many laboratories have proposed and are currently testing hypotheses about how changes in myosin structure mediated by actin and adenosine triphosphate (ATP) are translated into force and movement (see review by Howard, 1997).

The literature of myosin research is now too extensive to cover in any single review and the reader is referred to the many excellent works that have recently been published on the structural dynamics of the actomyosin interactions (Cooke, 1997; Baker and Titus, 1998; Goldman, 1998), the role of myosin isoforms in muscle physiology (Schiaffino and Reggiani, 1994; Hilber *et al*., 1997; Pette and Staron, 1997), and mammalian myosin multigene families (Weiss and Leinwand, 1996). The objective of this manuscript is to provide the reader with a broad overview of myosins in developing and mature skeletal muscle cells with emphasis on studies in avian systems. Those interested in a more detailed analysis and discussion of any of the topics covered in this review will find the extensive bibliography a good starting point for their explorations.

MYOSIN STRUCTURE

Skeletal muscle myosin is a large and complex structure that appears in the electron microscope as a long asymmetric molecule with two globular heads attached to a long tail. It consists of two heavy chain subunits (MyHC) of approximately 200 kDa and two pairs of small subunits referred to as regulatory and essential light chains. Although the essential light chains are required for wild type velocity in actin motility experiments, they are not required for actin-activated ATPase activity (Lowey and Trybus, 1995; Waller *et al*., 1995). Curiously, whereas the regulatory light chains play important roles in smooth muscle myosin regulation, their functional role in sarcomeric myosins is unclear. Recent studies suggest that phosphorylation of the light chains may alter thick filament structure

Received for publication August 1, 1998.
Accepted for publication December 8, 1998.
[1]To whom correspondence should be addressed: edbandman@ucdavis.edu

Abbreviation Key: ATP = adenosine triphosphate; ATPase = adenosine triphosphatase; HMM = heavy meromyosin; LMM = light meromyosin; MyHC = myosin heavy chain subunit.

(Levine *et al*., 1991, 1996) providing a basis for the observation that light chain phosphorylation modulates contractile function to enhance performance and efficiency (Sweeney *et al*., 1993).

Most of our understanding of myosin's structural and chemical properties was derived from the study of proteolytic fragments. Treatment of myosin with papain yields two Subfragment 1 (S1) protein fragments and the α-helical coiled-coil rod. The myosin head consists of the N-terminal 840 amino acid residues and two light chains. The S1 contains all the necessary components to generate movement, including the actin-binding interface and the site for ATP hydrolysis. The S1 can be cleaved by trypsin into three fragments (25, 50, and 20 kDa) and the location of functional amino acid residues within these peptides has been extensively studied (for review see Rayment *et al*., 1996). The three-dimensional structure of the chicken S1 revealed that the three fragments do not form individual folding domains, but that the cleavage sites represented flexible loops susceptible for proteolysis.

The rod begins at an invariant proline residue at position 840 in the chicken adult MyHC (Chao and Bandman, 1997). The amino acid sequence of the rod follows the seven amino acid repeat (*a, b, c, d, e, f, g*)n characteristic of α-helical coiled-coil proteins, with *a* and *d* positions occupied predominantly by hydrophobic residues (McLachlan and Karn, 1982). Stability of the coiled-coil is provided by hydrophobic interactions at the interface of the two helices between the side chains of residues at *a* and *d* positions of opposite strands (Hodges *et al*., 1990). Residues at positions *e* and *g* that flank the hydrophobic core are occupied predominantly by charged amino acids that may contribute to further stabilization of the coiled-coil interface both by enhancing the hydrophobic effect and by participating in interhelical salt bridges (O'Shea *et al*., 1991). A putative role of these residues in myosin filament morphology has recently been proposed (Arrizubieta and Bandman, 1998). An additional repeating motif in the myosin rod that has been highly conserved includes a striking repetition of positively and negatively charged amino acid residues at positions *b* and *c* on the outer surface of the coiled-coil spaced 14 residues apart (McLachlan and Karn, 1983). These residues are believed to play a functional role in myosin rod interactions that generate the characteristic 14.3 nm axial stagger of molecules within the myosin filament (Huxley, 1974). An additional conserved feature of sarcomeric myosin rods has been the insertion of four "skip" residues at precise positions within the α-helical coiled-coil sequence (McLachlan and Karn, 1983). The precise function for these residues remains unclear but their positions appear to correlate with bends in the myosin tail observed in the electron microscope (Offer, 1990).

The rod domain can be further subdivided into two peptides, Subfragment 2 (S2) and light meromyosin (LMM) at a highly variable and more flexible region of the rod called the hinge (Lowey *et al*., 1969). If myosin filaments are treated with α-chymotrypsin, proteolysis occurs at the hinge and a soluble, enzymatically active, heavy meromyosin (HMM) fragment, consisting of the S1 and S2 region, is produced along with the LMM (Margossian and Lowey, 1982). The LMM fragment retains the solubility characteristics of the myosin rod and, although soluble at high ionic strength, aggregates under physiological conditions (Harrison *et al*., 1971). The LMM fragment has been the focus of many studies, as LMM aggregates usually are paracrystals with distinct axial banding patterns that exhibit 43- and 14.3-nm repeats that are thought to be related to the 43-nm helical repeat and the 14.3-nm axial translation with which myosin heads protrude from the surface of the thick filament (Stewart and Kensler, 1986). A more extensive review of the morphology and the assembly properties of the myosin rod can be found elsewhere in this volume (Wick, 1998).

MYOSIN MULTIGENE FAMILIES

Sarcomeric MyHC isoform diversity is a common theme in all eukaryotes with striated muscles. With the single exception of *Drosophila melanogaster*, in which a single MyHC gene produces different transcripts through alternative splicing events (Rozek and Davidson, 1983), the development of large and complex multigene families has been responsible for generating and maintaining MyHC diversity, as no other sarcomeric MyHC genes have been found to give rise to more than a single mRNA transcript. Recently, however, evidence for alternative splicing of some nonmuscle and smooth muscle MyHC genes has been presented (Hamada *et al*., 1990), suggesting that at least some subclasses of the myosin superfamily in birds and mammals may utilize differential splicing to generate diversity.

The functional separation of MyHC into two classes, fast and slow isoforms, is reflected in the genomes of species in which sarcomeric MyHC have been cloned and sequenced. Fast MyHC genes from phylogenetically diverse organisms such as fish, amphibia, birds, and mammals (Moore *et al*., 1993) are much more homologous to each other than to slow MyHC genes. The same is true of the slow MyHC genes that have been characterized (Bandman *et al*., 1994).

MyHC gene structure has been preserved throughout evolution. The organization of the rat embryonic (Strehler *et al*., 1986) and chicken embryonic (Molina *et al*., 1987) MyHC genes are very similar. Both genes contain two exons that encode the 5′-untranslated region, the initiating codons of both genes are located in the third exon, and both encode proteins that have 1,940 amino acids. Although intron sequences and sizes are highly variable, all intron positions are exactly conserved with the single exception that the rat exon equivalent to the 40th exon of the chicken gene is divided into two exons 24 bp upstream from the stop

codon. Five of the eight intron positions of a nematode MyHC gene (Strehler *et al*., 1986) have been preserved in rat and chicken genes. This striking conservation of intron positions among vertebrate and invertebrate sarcomeric myosin genes suggests that the ancestral MyHC gene contained at least the common introns.

It is likely that the multigene families of MyHC have arisen from gene duplications and subsequent divergence of each of the members of the gene family. The human fast skeletal MyHC are clustered on Chromosome 11 and the mouse orthologs on Chromosome 17 (Leinwand *et al*., 1983). The α and β cardiac/slow MyHC genes are arranged in tandem in both human and rat genomes at a separate chromosomal locus from that of the fast skeletal MyHC genes (Mahdavi *et al*., 1982). We have recently demonstrated that in the chicken genome, three slow MyHC genes are clustered (Chen *et al*., 1997). Using fluorescent *in situ* hybridization an embryonic fast MyHC probe has mapped the embryonic fast MyHC gene to a microchromosome (Dominguez-Steglich *et al*., 1993). We now have preliminary evidence that similar to mammalian genomes, multiple chicken fast MyHC genes reside at this locus (Bandman, unpublished observations). The estimated number of MyHC-like genes in the chicken, up to 31 (Robbins *et al*., 1986), is considerably greater than the estimated 7 to 10 genes observed in mouse, rat, or human genomes (Leinwand *et al*., 1983b; Wydro *et al*., 1983). However, based on Northern blot analysis with 5′ probes, only 7 of the 31 sequences appear to be related to fast MyHC genes (Kropp *et al*., 1987). An SDS-PAGE was able to resolve six MyHC from developing pectoral muscles (Hofmann *et al*., 1988) whereas high resolution anion-exchange chromatography of myosin S1 fragments isolated from adult chicken fast muscles has been used to identify five protein fractions with distinct primary structures (Rushbrook *et al*., 1997). Subsequent studies using PCR have shown that six of the seven fast MyHC genes identified by Kropp *et al*., 1987 are expressed in the developing pectoral muscle (Rushbrook *et al*., 1998). We have cloned and described the expression of five unique MyHC cDNA that encompass the LMM domain and the 3′-untranslated region (see Moore *et al*., 1992; Tidyman *et al*., 1997). Only two of these cDNA are full length and, thus, it is not possible at the present time to definitively identify and correlate all of the isoforms described in these studies. It is likely that some of the other putative chicken MyHC genes identified on Southern blots (Robbins *et al*., 1986) represent nonmuscle, smooth muscle, and other nonsarcomeric members of the myosin superfamily (Cheney *et al*., 1993).

PLASTICITY OF MYOSIN EXPRESSION IN MUSCLE

Innervation, activity, and thyroid hormone have all been shown to play important roles in specifying which MyHC isoforms are expressed in vertebrate muscle (for review see Bandman, 1985; Schiaffino and Reggiani, 1994); however, the mechanism by which external signals induce changes in MyHC gene expression is not yet clear. In mammals, recent studies have described how thyroid hormone receptors interact with regulatory elements in the mammalian β-cardiac/skeletal MyHC gene to control transcriptional activity (Darling *et al*., 1993). Muscle-specific nuclear protein factors that are involved in the expression of the β-cardiac/skeletal MyHC gene have also been found (Shimizu *et al*., 1992). A review of the regulatory elements characterized in mammalian fast and slow MyHC genes has recently been published (Weiss and Leinwand, 1996). Less is known about the regulation of avian MyHC by extrinsic factors, although recent studies have shown that thyroid hormone modulates MyHC expression in chicken muscle development (Gardahaut *et al*., 1992).

Innervating a fast type muscle with a motoneurone that normally innervates a slow type muscle results in transformation from Type II (fast MyHC containing) to Type I (slow MyHC containing) fibers (Gauthier *et al*., 1983). Changes in fiber type and MyHC expression are also induced by muscle loading. Weighting of the avian wing has been shown to induce muscle hypertrophy and alter MyHC expression in the anterior *Latissimus dorsi*, a slow tonic muscle (Kennedy *et al*., 1986, 1988; Winchester and Gonyea, 1992). Whereas these results suggest mechanical activity plays a crucial role in specifying MyHC expression, no *cis*-acting sequences, *trans*-acting factors, or signal transduction pathways have been yet been implicated in activity-induced fiber type transformations.

It is now well established that chicken fast and slow muscle fibers undergo changes in the expression of MyHC as the fiber matures (Bandman *et al*., 1982; Bader *et al*., 1982). It is also clear that different fast fibers have an intrinsic pattern of MyHC expression during maturation (Bandman and Bennett, 1988). This program of MyHC expression is recapitulated in adult muscle during regeneration following injury (Cerny and Bandman, 1987), although extrinsic factors such as muscle denervation also affect the program of myosin expression (Cerny and Bandman, 1987). Regulation of MyHC gene transcription during muscle development appears to determine MyHC isoform content (Rushbrook *et al*., 1997, 1998; Tidyman and Bandman, 1997).

Whereas the physiological significance of fast and slow MyHC isoforms in adult muscles is well established, the functional significance of MyHC switching during muscle development is unclear. No unique enzymatic or structural properties have been demonstrated for MyHC isoforms expressed in developing muscles. Thus, it is possible that the multigene family encodes many functionally equivalent MyHC, and switching occurs because different regulatory elements are required to ensure expression during specific developmental periods and in physiologically different muscle fiber types. However, it has been proposed that

the unique kinetic properties of muscle fibers composed of a single MyHC are the result of functional differences in myosin isoforms (Hilber *et al*., 1997). Recently, mice, in which specific adult myosin genes have been inactivated, have been developed (Acakpo-Satchivi *et al*., 1997). Studies have shown that although the mouse can compensate for the lack of a specific functional MyHC gene by expressing an alternate isoform and maintaining normal myosin content, these mice nevertheless display severe muscle defects suggesting that MyHC isoforms may have unique roles in the development and function of skeletal muscle (Sartorius *et al*., 1998).

APPLICATIONS OF MYOSIN EXPRESSION IN MEAT SCIENCE

Meat scientists carried out many studies characterizing myosin biochemistry in order to understand how myosin's physiochemical properties contributed to postmortem events in skeletal muscle during the conversion of muscle to meat. Many of the current meat processing technologies, which were developed empirically (for example, the requirement of 2 to 3% salt for binding in comminuted meat products) can now be readily explained with a basic knowledge of myosin's solubility characteristics. Some of the important applications of myosin research both in muscle and meat science are discussed below.

MyHC Expression is a Marker for Describing Muscle Fiber Type

Muscle fibers can be classified into distinct fiber types (Type I and Type II) by histological and immunological criteria (Guth and Samaha, 1969, 1970; Brooke and Kaiser, 1970; Engel, 1974). The biochemical basis for these distinctions results from observations that distinct fast and slow isoforms of MyHC are differentially expressed in Type I and Type II fibers (Zeman and Wood, 1980). Although multiple fast and slow isoforms of myosin are expressed in developing muscle, it has nevertheless been possible to develop antibodies that distinguish Type I and Type II fibers. Subclassification of these fiber types is also possible with both histological and immunocytochemical procedures (Arndt and Pepe, 1975), and monoclonal antibodies specific to fast and slow MyHC isoforms have proven to be crucial probes for delineating muscle fiber type (Crow and Stockdale, 1986).

MyHC Expression is a Marker for Describing Commitment to Avian Muscle Fiber Type

The MyHC produced in a muscle fiber are determined by commitment of stem cells to different myogenic lineages. Chicken and quail myoblasts have been cloned and shown to give rise to homogeneous colonies of myotubes expressing either fast, slow, or both fast and slow MyHC (Miller *et al*., 1985; Miller and Stockdale, 1986a). Because these colonies of myotubes of differing MyHC composition are grown under identical conditions and in some experiments within the same culture dish, Stockdale and coworkers have proposed that different myogenic stem cells give rise to distinct types of fibers and that specification of fiber type occurs within the myogenic lineage prior to terminal differentiation of the stem cell (Stockdale and Miller, 1987). The fact that these different myogenic lineages can be isolated from embryonic chicken muscle at the same time as fibers of differing MyHC composition arise *in vivo* (Crow and Stockdale, 1986; Miller and Stockdale, 1986b), can be viewed as evidence in support of this hypothesis. Recent experiments also demonstrate that commitment to adult satellite cells (myogenic stem cells within differentiated muscle tissue) also can be monitored using MyHC-specific probes (Hartley *et al*., 1991, 1992).

MyHC Content is a Marker for Muscle Protein Accretion and Growth

As myosin is the major protein found in skeletal muscle fibers, muscle growth is often monitored by quantitation of MyHC content. Analyzing myosin protein and mRNA content by SDS-PAGE, ELISA, and Northern blot analyses are often used to quantify muscle hypertrophy (Gore *et al*., 1995).

SUMMARY AND CONCLUSIONS

Although the existence of MyHC isoform diversity has been extensively analyzed and documented, the functional significance of the plethora of sarcomeric myosins remains unclear. Recent studies are beginning to accumulate sufficient evidence that sequence diversity within the S1 will likely be translated into definitive differences in the myosin motor and, thus, physiologically relevant differences in muscle function. The functional significance of sequence diversity in the myosin rod, however, remains more elusive. Ongoing studies of myosin filament assembly, LMM paracrystal formation, LMM solubility, and α-helical coiled-coil dimerization may provide new clues relating myosin rod sequences to function and whether different isoforms have evolved specific roles in myogenesis.

REFERENCES

Acakpo-Satchivi, L.J.R., W. Edelmann, C. Sartorius, B. D. Lu, P. A. Wahr, S. C. Watkins, J. M. Metzger, L. A. Leinwand, and R. Kucherlapati, 1997. Growth and muscle defects in mice lacking adult myosin heavy chain genes. J. Cell Biol. 139:1219–1229.

Arndt, I., and F. A. Pepe, 1975. Antigenic specificity of red and white muscle myosin. J. Histochem. Cytochem. 23:159–168.

Arrizubieta, M.-J., and E. Bandman, 1998. The role of interhelical ionic interactions in myosin rod assembly. Biochem. Biophys. Res. Comm. 244:588–593.

Bader, D., T. Masaki, and D. A. Fischman, 1982. Immunochemical analysis of myosin heavy chain during avian myogenesis *in vivo* and *in vitro*. J. Cell Biol. 95:763–770.

Baker, J. P., and M. A. Titus, 1998. Myosins: matching functions with motors. Curr. Opinion Cell Biol. 10:80–86.

Bandman, E., 1985. Myosin isozyme transitions during muscle development, maturation, and disease. Int. Rev. Cytol. 97: 97–131.

Bandman, E., and T. Bennett, 1988. Diversity of fast myosin heavy chain expression during development of gastrocnemius, bicep brachii, and posterior latissimus dorsi muscles in normal and dystrophic chickens. Dev. Biol. 130: 220–231.

Bandman, E., L. A. Moore, M. J. Arrizubieta, W. E. Tidyman, L. Herman, and M. Wick, 1994. The evolution of the chicken myosin heavy chain multigene family. Pages 131–139 *in*: Molecular Evolution of Physiological Processes, D. Fambrough, ed. Rockefeller Press, New York, NY.

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

Brooke, M. H., and K. K. Kaiser, 1970. Muscle fiber types: how many and what kind? Arch. Neurol. 23:369–379.

Cerny, L. C., and E. Bandman, 1987. Expression of myosin heavy chain isoforms in regenerating myotubes of innervated and denervated chicken pectoral muscle. Dev. Biol. 119:350–362.

Chao, T. H., and E. Bandman, 1997. Cloning, nucleotide sequence and characterization of a full-length cDNA encoding the myosin heavy chain from adult chicken pectoralis major muscle. Gene 199:265–270.

Chen, Q., L. A. Moore, M. Wick, and E. Bandman, 1997. Identification of a genomic locus containing three slow myosin heavy chain genes in the chicken. Biochim. Biophys. Acta: Gene Structure and Expression 1353: 148–156.

Cheney, R. E., M. A. Riley, and M. S. Mooseker, 1993. A phylogenetic analysis of the myosin superfamily. Cell Motil. Cytoskel. 24:215–223.

Cooke, R., 1997. Actomyosin interaction in striated muscle. Physiol. Rev. 77:671–697.

Crow, M. T., and F. E. Stockdale, 1986. The developmental program of fast myosin heavy chain expression in avian skeletal muscle fibers. Dev. Biol. 118:333–342.

Darling, D. S., R. L. Carter, P. M. Yen, J. M. Welborn, W. W. Chin, and P. K. Umeda, 1993. Different dimerization activities of α- and β-thyroid hormone receptor isoforms. J. Biol. Chem. 268:10221–10227.

Dominguez-Steglich, M., J. Robbins, and M. Schmid, 1993. Mapping of the chicken N-CAM gene and a myosin heavy chain gene: avian microchromosomes are not genetically inert reserves of DNA. J. Exp. Zool. 265:395–400.

Engel, W. K., 1974. Fiber-type nomenclature of human skeletal muscle for histochemical purposes. Neurology 24:344–348.

Engelhardt, V. A., and M. N. Ljubimova, 1939. Myosin and adenosinetriphosphatase. Nature 144:668–669.

Gardahaut, M. F., J. Fontaine-Perus, T. Rouaud, E. Bandman, and R. Ferand, 1992. Developmental modulation of myosin expression by thyroid hormone in avian skeletal muscle. Development 115:1121–1131.

Gauthier, G. F., R. Burke, S. Lowey, and A. W. Hobbs, 1983. Myosin isozymes in normal and cross innervated cat skeletal muscle. J. Cell Biol. 97:756–751.

Goldman, Y. E., 1998. Wag the tail: structural dynamics of actomyosin. Cell 93:1–4.

Gore, M. T., R. B. Young, C. R. Bird, C. H. Rahe, D. Marple, J. L. Griffin, and D. R. Mulvaney, 1995. Myosin heavy chain gene expression in bovine fetuses and neonates representing genotypes with contrasting patterns of growth. Proc. Soc. Exp. Biol. Med. 209:86–91.

Guth, L., and F. J. Samaha, 1969. Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. Exp. Neurol. 25:138–152.

Guth, L., and F. J. Samaha, 1970. Procedure for the histochemical demonstration of actomyosin ATPase. Exp. Neurol. 28: 365–367.

Hamada, Y., M. Yanagisawa, Y. Katsuragawa, J. R. Doleman, S. Nagata, G. Matsuda, and T. Masaki, 1990. Distinct vascular and intestinal smooth muscle myosin heavy chain mRNAs are encoded by a single-copy gene in the chicken. Biochem. Biophys. Res. Comm. 170:53–58.

Harrison, R. G., S. Lowey, and C. Cohen, 1971. Assembly of myosin. J. Mol. Biol. 59:531–535.

Hartley, R. S., E. Bandman, and Z. Yablonka-Reuveni, 1991. Myoblasts from fetal and adult skeletal muscle regulate myosin expression differently. Dev. Biol. 148:249–260.

Hartley, R. S., E. Bandman, and Z. Yablonka-Reuveni, 1992. Skeletal muscle satellite cells appear late during late chicken embryogenesis. Dev. Biol. 153:206–216.

Hilber, K., S. Galler, and D. Pette, 1997. Functional differences of myosin heavy-chain isoforms in skeletal muscle. Naturwissenschaften 84:201–204.

Hodges, R. S., N. E. Zhou, C. M. Kay, and P. D. Semchuk, 1990. Synthetic model proteins: contribution of hydrophobic residues and disulfide bonds to protein stability. Peptide Res. 3:123–137.

Hofmann, S., S. Dusterhoft, and D. Pette, 1988. Six myosin heavy chain isoforms are expressed during chick breast muscle development. FEBS Lett. 238:245–248.

Howard, J., 1997. Molecular motors: structural adaptions to cellular functions. Nature 389:561–567.

Huxley, A. F., 1974. Muscle contraction. J. Physiol. 243:1–43.

Kennedy, J. M., S. Kamel, W. W. Tambone, G. Vrbova, and R. Zak, 1986. The expression of myosin heavy chain isoforms in normal and hypertrophied chicken slow muscle. J. Cell. Biol. 103:977–983.

Kennedy, J. M., B. R. Eisenberg, S. K. Reid, L. J. Sweeney, and R. Zak, 1988. Nascent muscle fiber appearance in overloaded chicken slow-tonic muscle. Am. J. Anat. 181: 203–215.

Kropp, K., J. Gulick, and J. Robbins, 1987. Structural and transcriptional analysis of a chicken myosin heavy chain gene subset. J. Biol. Chem. 262:16536–16545.

Leinwand, L. A., R. E. Fournier, B. Nadal-Ginard, and T. B. Shows, 1983a. Multigene family for sarcomeric myosin heavy chain in mouse and human DNA: localization on a single chromosome. Science 221:766–799.

Leinwand, L. A., L. Saez, E. McNally, and B. Nadal-Ginard, 1983b. Isolation and characterization of human myosin heavy chain genes. Proc. Nat. Acad. Sci. USA 80: 3716–3720.

Levine, R.J.C., P. Chantler, R. Kensler, and J. Woodhead, 1991. Effects of phosphorylation by myosin light chain kinase on the structure of *Limulus* thick filaments. J. Cell Biol. 113: 563–572.

Levine, R.J.C., R. W. Kensler, Z. Yang, J. T. Stull, and H. L. Sweeney, 1996. Myosin light chain phosphorylation affects

the structure of rabbit skeletal muscle thick filaments. Biophys. J. 71:898–907.

Lowey, S., and K. Trybus, 1995. Role of skeletal and smooth muscle myosin light chains. Biophys. J. 68:120–127.

Lowey, S., H. S. Slayter, A. G. Weeds, and H. Baker, 1969. Substructure of the myosin molecule. I. Subfragments of myosin by enzymic degradation. J. Mol. Biol. 42:1–29.

Mahdavi, V., M. Periassamy, and B. Nadal-Ginard, 1982. Molecular characterization of two myosin heavy chain genes expressed in the adult heart. Nature (London) 297: 659–664.

Margossian, S. S., and S. Lowey, 1982. Preparation of myosin and its subfragments from rabbit skeletal muscle. Meth. Enzymol. 85:55–71.

McLachlan, A. D., and J. Karn, 1982. Periodic charge distributions in the myosin rod amino acid sequence match cross-bridge spacings in muscle. Nature (London) 299:226–231.

McLachlan, A. D., and J. Karn, 1983. Periodic features in the amino acid sequence of nematode myosin rod. J. Mol. Biol. 164:293–304.

Miller, J. B., and F. E. Stockdale, 1986a. Developmental origins of skeletal muscle fibers: clonal analysis of myogenic cell lineages based on fast and slow myosin heavy chain expression. Proc. Nat. Acad. Sci. USA 83:3860–3864.

Miller, J. B., and F. E. Stockdale, 1986b. Developmental regulation of the multiple myogenic cell lineages of the avian embryo. J. Cell Biol. 103:2197–2208.

Miller, J. B., M. T. Crow, and F. E. Stockdale, 1985. Slow and fast myosin heavy chain content defines three types of myotubes in early muscle cell cultures. J. Cell Biol. 101: 1643–1650.

Molina, M. I., K. E. Kropp, J. Gulick, and J. Robbins, 1987. The sequence of an embryonic myosin heavy chain gene and isolation of its corresponding cDNA. J. Biol. Chem. 262: 6478–6488.

Moore, L. A., M. J. Arrizubieta, W. E. Tidyman, L. A. Herman, and E. Bandman, 1992. Analysis of the chicken fast myosin heavy chain family: Localization of isoform-specific antibody epitopes and regions of divergence. J. Mol. Biol. 225: 1143–1151.

Moore, L. A., W. E. Tidyman, M. J. Arrizubieta, and E. Bandman, 1993. The evolutionary relationship of avian and mammalian myosin heavy-chain genes. J. Mol. Evol. 36:21–30.

Offer, G., 1990. Skip residues correlate with bends in the myosin tail. J. Mol. Biol. 216:213–218.

O'Shea, E. K., J. D. Klemm, P. S. Kim, and T. Alber, 1991. X-ray structure of the GCN4 leucine zipper, a two-stranded coiled-coil coil. Science 254:539–544.

Pette, D., and R. S. Staron, 1997. Mammalian skeletal muscle fiber type transitions. Int. Rev. Cytol. 170:143–223.

Rayment, I., W. R. Rypniewski, K. Schmidt-Base, R. Smith, D. R. Tomchick, M. M. Benning, D. A. Winkelmann, G. Weesenberg, and H. M. Holden, 1993. Three-dimensional structure of myosin subfragment-1: a molecular motor. Science 261:50–58.

Rayment, I., C. Smith, and R. G. Yount, 1996. The active site of myosin. Ann. Rev. Physiol. 58:671–702.

Robbins, J., T. Horan, J. Gulick, and K. Kropp, 1986. The chicken myosin heavy chain family. J. Biol. Chem. 261: 6606–6612.

Rozek, C. E., and N. Davidson, 1983. *Drosophila* has one myosin heavy chain gene with three developmentally regulated transcripts. Cell 32:23–34.

Rushbrook, J. I., J. Huang, C. Weiss, L. Siconolfi-Baez, T.-T. Yao, E. Becker, and M. Feuerman, 1997. Characterization of the myosin heavy chains of avian fast muscles at the protein and mRNA levels. J. Musc. Res. Cell Motil. 18: 449–463.

Rushbrook, J. I., J. Huang, C. Weiss, T.-T. Yao, L. Siconolfi-Baez, and E. Becker, 1998. J. Musc. Res. Cell Motil. 19: 157–168.

Sartorius, C. A., B. D. Lu, L.J.R. Acakpo-Satchivi, R. P. Jacobson, W. C. Byrnes, and L. A. Leiwand, 1998. Myosin heavy chains IIa and Iid are functionally distinct in the mouse. J. Cell. Biol. 141:943–953.

Schiaffino, S., and C. Reggiani, 1994. Myosin isoforms in mammalian skeletal muscle. J. App. Physiol. 77:493–501.

Sellars, J. R., and H. V. Goodson, 1995. Motor proteins II: myosins. Protein Profile 2:1323–1423.

Sellars, J. R., H. V. Goodson, and F. Wang, 1996. A myosin family reunion. J. Musc. Res. Cell Motil. 17:7–22.

Shimizu, N., E. Dizon, and R. Zak, 1992. Both muscle-specific and ubiquitous nuclear factors are required for muscle-specific expression of the myosin heavy chain β gene in cultured cells. Mol. Cell. Biol. 12:619–630.

Stewart, M., and R. W. Kensler, 1986. Arrangement of myosin heads in relaxed thick filaments from frog skeletal muscle. J. Mol. Biol. 192:831–851.

Stockdale, F. E,. and J. B. Miller, 1987. The cellular basis of myosin heavy chain isoform expression during development of avian skeletal muscles. Dev. Biol. 123:1–9.

Strehler, E. E., M. A. Strehler-Page, J. C. Perriard, M. Periasamy, and B. Nadal-Ginard, 1986. Complete nucleotide and encoded amino acid sequence of a mammalian myosin heavy chain gene. J. Mol. Biol. 190:291–317.

Sweeney, H. L., B. Bowman, and J. Stull, 1993. Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. Am. J. Physiol. 264:414–418.

Tidyman, W. E., L. A. Moore, and E. Bandman, 1997. Expression of fast myosin transcripts in developing and dystrophic chicken skeletal muscle. Dev. Dynamics 208: 1–14.

Waller, G. S., G. Ouyang, J. Swafford, P. Vibert, and S. Lowey, 1995. A minimal motor domain from chicken skeletal muscle myosin. J. Biol. Chem. 270:15348–15352.

Weiss, A., and L. Leinwand, 1996. The mammalian myosin heavy chain gene family. Ann. Rev. Cell Dev. Biol. 12: 417–439.

Wick, M., 1999. Filament assembly properties of the sarcomeric myosin heavy chain. Poultry Sci. 78:735–742.

Winchester, P. K., and W. J. Gonyea, 1992. Regional injury and the terminal differentiation of satellite cells in stretched avian slow tonic muscle. Dev. Biol. 151:459–472.

Wydro, R. M., H. T. Nguyen, R. M. Gubits, and B. Nadal-Ginard, 1983. Characterization of sarcomeric myosin heavy chain genes. J. Biol. Chem. 258:670–678.

Zeman, R. J., and D. S. Wood, 1980. Correlative histochemical and physiological measurements in single skinned muscle fibers: Heterogeneity of CA sensitivity in type I fibers. J. Histochem. Cytochem. 28:714–715.