

Cellular and Molecular Bases of Skeletal Muscle Fiber Diversity

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Abstract

Vertebrate muscles display different physiological properties such as energy utilization, tension generation, and rate of shortening during contraction. Although these differences exist along a spectrum, striated muscle fibers can be broadly classified into two groups: fast- and slow-contracting. Differences in the rate of contraction derive primarily from the expression of different isoforms of myosin heavy chain protein, so fast- and slow-fibers can be identified on the bases of differential staining for myosin ATPase or with monoclonal antibodies that recognize either fast or slow isoforms. The mechanisms that control whether a muscle fiber develops as a fast or slow fiber are a complex interplay between the cellular origins of the fiber and interactions of the developing fiber with the nervous system, and in some muscles, the endocrine system. The MyoD family of myogenic regulatory factors (MRFs), like muscle-specific proteins, are differentially expressed in fast and slow muscles. However, knockout studies which demonstrate that normal fiber-types develop in the absence of members of this family, make it unlikely that these transcription factors provide a simple explanation for fiber diversification into fast and slow type. This review will focus on the mechanisms involved in the formation of slow or fast skeletal muscle fibers.

Key words: myogenesis, skeletal muscle, myosin heavy chain, myogenic regulatory factors, fiber-type.

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The metazoan body is composed of many different cell types acting in concert to form an individual. It has long been a goal of developmental biologists to understand the mechanisms which lead to the creation of diverse cell types from a single precursor. The phenotypic differences which characterize cells are a result of differential gene expression. While post-transcriptional regulation can be important, current research into how different cell types arise during development has focused primarily on regulation of transcription. The formation of different skeletal muscle cell types illustrates this principle.

Striated muscle fibers first appear in the somites and are among the earliest tissue types to arise during vertebrate development. Recent evidence suggests that specification of cells to a striated muscle cell fate, or lineage, may occur before or at the time of gastrulation [27]. But fiber diversification, recognized by differential expression of fast and slow isoforms of myosin heavy chain (MyHC) and other contractile proteins, is first seen in the organized skeletal muscle structures, the myotome [60, 65], and as the first fibers form in the embryonic limbs [15, 19]. In both structures distinctly different skeletal muscle fiber-types can be recognized [19, 45]. MyHC protein expression plays the predominant role in establishing the various muscle fiber

phenotypes described in embryonic, fetal, neonatal, and adult development, and these patterns are correlated with functional differences as the isoform(s) of MyHC expressed in the muscle or fiber are highly related to the rate of contraction of individual fibers [5, 52, 58].

Striated muscle fibers express sets of contractile proteins in carefully regulated stoichiometric amounts. It is the expression of contractile proteins belonging to families of related isoforms, which produces muscle fibers of distinct fiber types. For convenience, members of the MyHC family have been assigned to either a slow or fast group depending on their expression in slow or fast contracting muscle fibers. Several isoforms of MyHC can be expressed contemporaneously within a single fiber [18], and different members of the fast class may be expressed within an individual muscle at different times in development [2, 3, 18, 68]. A complex pattern of fast and slow MyHC gene expression in different muscle fibers, and transitions among isoforms within each class during development, generates the diversity of muscle fiber-types observed in the adult. The challenge is to identify the mechanism(s) that reproducibly produce the precise patterns of fiber distribution seen within the vertebrate body and within each specific muscle.

The basis for muscle fiber diversification into fast and slow MyHC expressing fibers has been attributed to a number of factors, including differences in cellular origins during embryonic and fetal development, differences in innervation, and differences in response to hormones. The extent to which each of these contribute to fiber-type may vary during different phases of development. However, the underlying molecular mechanism(s) responsible for diversification into the two basic phenotypes of fibers, fast and slow, remains unknown. A family of genes encoding the myogenic regulatory factors, the MyoD family of genes (MRFs), has been proposed as a possible basis of this diversification. This family of transcription factors plays a key role in initiating and sustaining the skeletal muscle cell phenotype (reviewed in [8, 43, 67]). Each MyoD family member has the ability to convert non-muscle cell types to skeletal muscle cells that express myosin. The importance of cell lineage, innervation, hormones, and the myogenic regulatory family of genes to the formation of distinct skeletal muscle fiber phenotypes will be considered.

Cellular Origins of Striated Muscle Fiber Types

Fibers of diverse types are recognized in the embryo as soon as identifiable skeletal muscles first appear. Therefore, the cellular origins of fiber diversity were sought within the early (embryonic) period of vertebrate development [62]. Muscle fiber diversity arises, in part, from intrinsic differences among skeletal myoblast precursors to fibers [39, 57]. Myoblasts isolated from early avian muscle and grown clonally *in vitro* produce colonies in which the fibers that form express a uniform MyHC phenotype. Most colonies form fibers that express only fast MyHC(s), while a smaller percentage form fibers that express both fast and slow MyHC isoforms [39, 57]. That a similar mechanism of diverse fiber-type formation is operative within the embryo is supported by the observations that distinct fast and slow fibers form in the limbs prior to innervation [45]; that fiber-type formation in the embryo occurs in the absence of functional innervation [19, 25]; and that clones of different types of myoblasts when transferred to embryonic limb buds form fibers of only one specific type [21].

It is important to recognize that the least common type of muscle fiber in most vertebrate muscles are those that express slow isoforms of MyHC. Most muscles and muscle fibers express only fast isoforms of MyHC. It appears that slow MyHC-expressing fibers originate very early in embryonic development and that few new slow fibers form beyond the embryonic or fetal period of development [19]. In some remarkable observations in the developing zebrafish, the early allocation of cells to an exclusive slow fiber fate has been demonstrated [20]. The muscle fibers of the zebrafish that will be slow in the adult form from a specific subset of myoblasts or myocytes located adjacent to the notochord. From this location they migrate early in development to the lateral portion of the trunk and give rise to all the slow fibers of the fish. Thus, in both birds and fish, there is the formation of a small subset of cells early

in development that produce the slow fibers found at later stages of development.

The findings that slow fibers have origins that differ from those of the fast fibers and that the dichotomy into fast and slow fiber fates occurs very early in development suggests two things. That the nervous system is not responsible for establishing this diversity, and that the slow fibers may have different regulatory processes to control myosin expression than are operative in fast fibers. These are borne out by experimental studies. Early work by Butler and Cosmos [11] demonstrated that in the absence of a neural tube the limb buds form and contain muscle fibers with ATPase staining properties of both the fast and slow fiber type. Subsequent work showed that blocking of neuromuscle transmission with curare prior to the first fibers to form in the embryo did not prevent fast and slow fiber appearance [16, 19, 29]. Lance-Jones and colleagues [64] have shown that the myogenic precursor cells that produce slow muscle fibers of the limb migrate from the somite prior to outgrowth of axons from the neural tube and in advance of those that form the fast muscle fibers of the limb. This suggests that commitment to the slow- or fast-fiber phenotype occurs in the somites or during migration from the somite into the limb. These findings support the contention that forces independent of the nervous system or innervation control the emergence of fibers types in birds and fish.

Recent studies on the sequential expression of slow MyHC isoforms are also consistent with the idea that the origin of slow fibers differs from that of fast fibers. The earliest MyHC described in slow fibers of the bird is slow MyHC 3, an isoform also found in the primitive or tubular heart [42, 66]. This isoform is the predominant MyHC in the primary slow fibers of the limb prior to innervation. As fetal fibers (secondary fibers) form, slow MyHC 3 is down regulated and disappears as fetal fibers come to dominate muscles of the limb. Thus, the lineage of slow fiber-types in the avian embryo can be identified as soon as fibers form in the limb musculature. This myosin is also expressed as the heart first forms. Thus the expression of slow MyHC 3 in primary fibers in muscles of the limb of the early embryo and in the pulsatile cardiac tube, suggests primitive cellular origins of these two striated muscles. The expression of the slow MyHC 3 gene in a subset of embryonic muscle fibers provides a genetic marker to study the mechanism(s) regulating the emergence of fiber type.

Although the expression of slow MyHC proteins defines the principal difference between fast- and slow-fiber-types, studies examining the regulation of the genes encoding these isoforms are at a preliminary stage. In mammals, the β -cardiac/slow MyHC gene has been studied in some detail [23, 36, 53], but the elements restricting its expression in skeletal muscle to slow-fibers have not been determined. Studies have begun to dissect regulatory elements restricting the expression of slow MyHC 3 to slow-fiber muscle [42]. Because no difference could be found in the methylation pattern of the avian slow MyHC gene between

fast and slow muscle (unpublished observations), it is likely that specific cis-trans interactions regulate expression of this gene in slow muscle fibers. Analysis of regulatory domains within the promoter of slow MyHC 3 gene may provide a basis for understanding the mechanism that regulates the differential expression of this gene in fast and slow fibers, and thus a mechanistic basis for distinguishing fast from slow fibers.

Quite apart from analyses of fiber diversification as the axial and limbs muscle form, are analyses directed at defining when and where the cell lineages first originate that produce muscle fibers per se and fast and slow muscle cell precursors in particular. George-Weinstein and colleagues have reported that it is possible to isolate skeletal muscle cell precursors from the blastodisc of avian embryos [27]. This observation is of great importance as in the last five years there has been a concerted focus of studies on the early somite as the site of skeletal muscle cell origins. The latter studies, both *in vivo* and *in vitro*, have revealed that the axial structures, the neural tube and notochord, play positive roles in initiating myogenesis within somites, which are not autonomous with regard to myogenesis [9, 40, 48, 50, 61].

These studies indicate that myogenesis in somites, or segmental plate mesoderm, is dependent on factors diffusible from the axial structures. They demonstrate that these structures initiate the emergence of myogenic cells and that adjacent structures, including the overlying ectoderm, and adjacent lateral plate mesoderm modulate the positive influences of the axial structures. Without these interactions somites do not form the muscle fibers of the myotome *in vitro* or *in vivo*. However, if myogenic cells originate before somites or segmental plate mesoderm are formed then the inductive work on somite myogenesis must be re-interpreted. The work of George-Weinstein and colleagues raises an important conceptual twist to these data. It suggests that the myogenic lineage must exist in a repressed state through mesoderm formation rather than that myogenesis initiated as the somites interact with the axial and adjoining structures. It follows that myogenesis remains in a repressed state until these interactions with axial and adjacent structures relieve that inhibition. That this could be the case is suggested by the observations that the MyoD family of genes are expressed in mesoderm prior to somitogenesis and without the presence of the neural tube the expression of MyoD is lost [50]. Resolution of the issue of whether myogenesis is initiated but repressed prior to mesoderm formation and somitization, or initiated *de novo* with somitization, will be important to an understanding of the origins of fiber diversification.

Innervation and the Formation of Fiber Type

Not all muscle protein expression in skeletal muscles is dependent solely on the origin of cells in the embryo that produce these muscles. Studies have shown that during the fetal stage of myogenesis skeletal muscle fiber-type is influenced by innervation [16, 19, 29]. Innervation, which

occurs in limb muscle during the fetal phase of myogenesis, if prevented or disrupted, alters the patterns of MyHC isoforms expressed and the relative proportions of fast and slow fiber-types within muscles change. It is important to recognize that a distinguishing feature between muscle fiber formation in the embryo and in the fetus is that innervation is a requisite in the fetus but not in the embryo. In both birds and mammals, most secondary fibers initially express a fast-fiber phenotype, which under the influence of motor nerves can be changed in slow-fiber muscles [15, 24, 29, 31]. Reinnervation experiments also demonstrate the plasticity of fiber-type in the non-embryonic periods of muscle development. Reinnervation of a slow muscle in the neonate or adult period with a nerve that normally innervates a fast muscle can alter the number of fast and slow fibers within a slow muscle (reviewed in [46]), and within limits can alter the expression of slow and fast MyHC isoforms [32]. It is likely that the cellular origin of a fiber is important in the initial laying down of fibers within nascent muscles of the embryo, and that innervation acting on these and the newly formed secondary fibers of the fetus serves to amplify and augment the embryonic pattern. Neither cell lineage nor innervation alone are sufficient to provide the variety of fiber-types that emerge in all phases of development.

Endocrine Effects on Fiber Type

Hormones, especially thyroid hormone, and androgens, can alter the isoforms of MyHC expressed in skeletal muscle tissue [34]. Perhaps the most dramatic example of the effects of hormones on fiber type is found in the work of Kelley and colleagues [12, 56, 63]. They find that during the post metamorphic period of frog development, testosterone will convert slow-twitch muscle fibers of the larynx into fast-twitch fibers [12, 56, 63]. Others have found that the sexual dimorphic nature of muscles in mammals is rooted in androgenic effects on myosin expression [38]. In mammals, thyroid hormone influences the expression of the β -cardiac MyHC gene [28, 34], the slow MyHC gene of mammals [37]. In the absence of thyroid hormone, the expression of slow MyHC in the rat, is delayed and the levels of expression within skeletal muscles is altered [26, 34]. It appears that the endocrine system influences fiber-type once formed and acts throughout adult life to maintain the types of fibers that emerge earlier in life.

MyoD Family Members and Expression of Muscle Fiber Phenotypes

Because the MyoD family of myogenic regulatory factors are so fundamental to the formation of skeletal muscle cells, these factors become candidates for determinants of skeletal muscle fiber-type. The hypothesis was advanced that distinct skeletal muscle fiber-types result from differences in the expression of the MyoD family members. Studies in birds and mammals have determined temporal and spatial differences in expression patterns of various MyoD family members during early development [6, 17,

44, 49, 55], and MRF expression correlations with fiber-type [33]. Consistent with this hypothesis are results from several laboratories which demonstrate that individual MRF family members differ in their ability to activate expression of muscle-specific genes [1, 4, 13, 69]. For example, MyoD and myogenin are capable of activating myosin light chain 1 gene expression to a higher level than is MRF4 [1]. On the other hand, mice null for the MyoD gene have muscles with a normal complement of fiber types [54]. However, this is a hypothesis that could integrate the various factors which are thought to most influence specification of fiber type.

Within the developing somite, the MRFs display expression patterns distinguishing dorsal/medial and ventral/lateral subdomains [17, 59], and during early mouse myotome formation, MyoD localizes to epaxial muscle precursors adjacent to the neural tube, while myf-5-expressing cells are found in hypaxial muscle precursors along the lateral margin of the myotome. The activation or induction of myogenesis and the continued expression of MRFs in the somite is dependent on the axial structures (neural tube and notochord) adjacent to the developing somite [9, 40, 50, 61]. Recently, it has been shown that signals from the axial structures and lateral structures (lateral plate) can affect expression of MRFs within somites [48, 50]. In particular, it appears that the notochord activates the expression of MyoD in somites of the quail embryo and the MRF cascade that leads to the formation of the muscle fibers of the myotome. Both fast and slow MyHC isoforms are detected shortly after the myotome forms *in vivo*, and following induction of myogenesis in somites by axial structures *in vitro*. Thus, those events that initiate or permit myogenesis to proceed in the somite are also associated with the dichotomous formation of fiber-types.

Innervation influences MyoD expression and secondary fiber-type

Changes in innervation that produce changes in fiber-type also produce dramatic differences in MRF expression in muscles. MyoD, myf-5, and myogenin mRNA levels decline in the hindlimb muscles of mice between embryonic day 15 and 1 week postnatal [33, 44]. This decline occurs during the fetal period of innervation-influenced secondary fiber formation. Experiments have also shown that MRF family member mRNA levels are down-regulated by innervation [10, 22]. On the other hand, adult muscles which have been experimentally denervated rapidly re-express MyoD and myogenin, with transcripts reaching levels 15- and 40-fold higher than those found in innervated control muscles. The increases of MyoD family mRNAs associated with denervation, can be prevented by direct electrical stimulation, suggesting that genes of the MyoD family are responsive to electrical activity or the cell-cell contact with the nerve. Another muscle gene, that encoding the alpha-subunit of the acetylcholine receptor, is down-regulated by innervation and upregulated by MyoD and myogenin [47], but the expression of many

other genes encoding muscle enzymes and contractile proteins does not show a correlation with innervation or MRF family member expression. Thus not only is the nervous system required for the maintenance of MRF expression during the activation of myogenesis, but it continues to play a modulating role in MRF expression via innervation as fibers appear and mature.

There is data to suggest that maintenance, as distinguished from initiation, of fiber-type may be linked to expression of myogenic factors. Hughes and colleagues [33] showed a correlation between innervation, selective accumulation of MyoD and myogenin, and maintenance of fiber-type in adult rat muscle. MyoD transcripts predominate in fast twitch muscles of the rat, while myogenin predominates in slow twitch muscles. Analyses at the cellular level demonstrated enhanced myogenin accumulation within fibers that expressed slow MyHC. This correlation of a higher concentration of myogenin in slow muscles suggested a role for the MRF in maintenance of the slow fiber-type. Other experiments consistent with a role for myogenin in regulating slow MyHC expression are cross-reinnervation experiments of the soleus muscle (the rat soleus contains greater than 90% slow fibers) with a nerve that would normally innervate a fast-fiber muscle. These cross-reinnervated muscles showed a decrease in myogenin mRNA and a concomitant loss of slow MyHC expression [33].

As in the rat, MyoD and myogenin accumulate to different levels in muscles of the bird that differ in fast and in slow fiber content. However, the relative levels of MyoD and myogenin in fast- and slow-fiber muscles are opposite to those found in rodents - MyoD is expressed in greater amounts in slow muscles and myogenin is expressed in greater amounts in fast muscles. In posthatch birds the anterior latissimus dorsi (ALD), a muscle composed entirely of slow-fibers, contains nearly 10-fold more MyoD than does the predominately fast-fiber, pectoralis muscle (Figure 1A). In contrast, the pectoralis has a 3.5-fold greater amount of myogenin compared to the level in the ALD. Marked differences between rodents and birds in the pattern of expression of MyoD family members are also found in early somites of rodents compared to birds. MyoD is the first MRF family member to be detected in quail somites [14, 49], whereas MyoD is not expressed until much later in mouse somites [6, 44, 55]; Myf-5 is the first MRF to be expressed in the somite muscle of the rodent [44].

MRF Expression in Avian Primary and Secondary Muscle Formation

We have investigated the time course of expression of MyoD family members during muscle development *in ovo* to determine if there were correlations between muscle phenotype and MRF expression. Muscles from the embryonic period (ED 6-10) and from the fetal periods of myogenesis (ED 10 to hatching) were analyzed (Figure 1). Fibers formed independently of innervation in the embryonic period while fibers formed in an innervation-depend-

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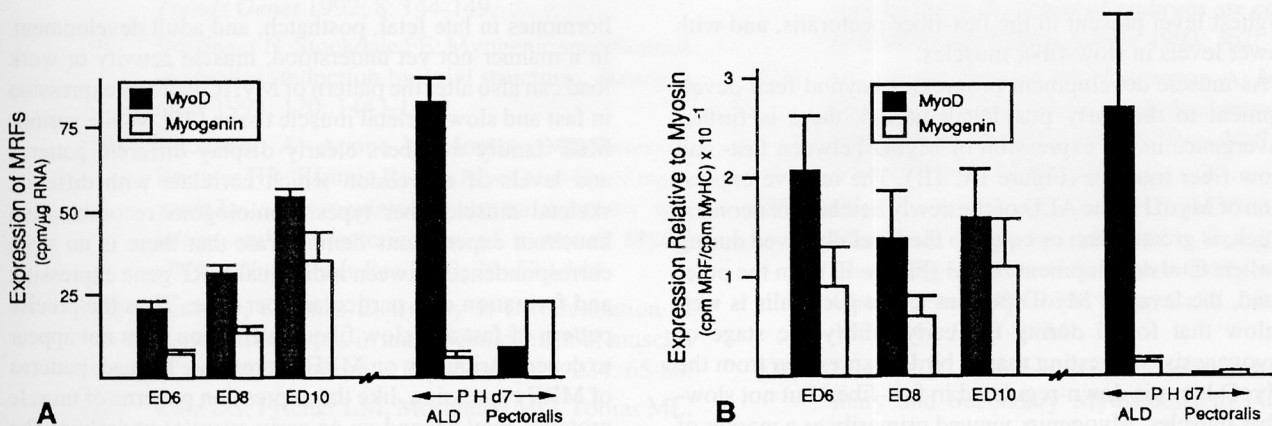


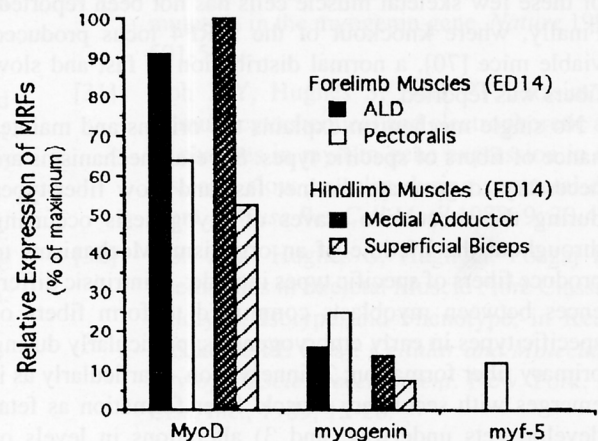
Figure 1. Expression of MyoD and myogenin in embryonic and early post-hatch chicken muscle. **A.** Ten mg of total RNA isolated from chicken hindlimbs and from ALD and pectoralis muscles was hybridized with probes specific for either MyoD or myogenin (a gift of Drs. F. Charles de la Brousse and C. Emerson), each labeled to equivalent specific activity. **B.** Total myosin heavy chain RNA present at each time point was estimated following hybridization with a 2.1 kb fragment of the chicken embryonic fast MyHC cDNA (chicken *cefMyHC*) (a gift of Dr. J. Robbins) encoding a portion of the highly conserved rod region. During the embryonic phase of myogenesis, ED6-10, MyoD and myogenin levels remain constant, and roughly equivalent, relative to the amount of MyHC RNA expressed. ED, embryonic day; PH, posthatch; ALD, anterior latissimus dorsi.

ent manner in the fetal period. The absolute levels of both MyoD and myogenin increase as muscle fibers form between ED6 and ED10 (Figure 1A). During this time period, the extent of myogenesis was determined by measuring MyHC mRNA levels in the developing limbs. When expressed relative to the amount of MyHC, MyoD and myogenin remain constant during embryonic myogenesis (Figure 1B). The equivalent size and specific activity of the two MRF probes allowed us to compare the relative abundance of MyoD and myogenin in developing hindlimb muscle, showing that the ratio of MyoD to myogenin RNA

remains fairly constant at about 2:1 throughout the embryonic period of development (Figure 1B). It should be noted that at this embryonic stage of development only a minority of primary fibers express slow MyHC in the avian hindlimb [45].

By 14 days of development, a time when secondary fiber formation is well underway, and most fibers are innervated, we isolated individual fast- and slow-fiber muscles from the chick embryo and measured the amount of MyoD, myogenin, and myf-5 mRNA present in each (Figure 2). Muscles composed of slow-fibers, whether derived from the forelimb (ALD) or the hindlimb (medial adductor), expressed higher levels of MyoD mRNA than did muscles of the forelimb or hindlimb composed of fast-fibers (pectoralis and superficial biceps). Interestingly, myf-5 was undetectable in slow-fiber muscles at ED14, but was clearly present in the fast-fiber pectoralis and at a very low level in the superficial biceps (a fast-fiber muscle) at this time. Myogenin was present in all four muscles, with the

Figure 2. Expression of MRFs in fast-fiber-type and slow-fiber-type fetal muscles. Ten mg of total RNA from fast-fiber-type muscles (pectoralis and superficial biceps) and slow-fiber-type muscles (ALD and medial adductor) were hybridized with probes specific for MyoD, myogenin, or myf-5 (as in Figure 1), each labeled to equivalent specific activity. Muscles composed of slow-fibers expressed higher levels of MyoD than did muscles composed of fast-fibers. Myf-5 was undetectable in slow-fiber muscles but was present in low amounts in the fast-fiber muscles. Myogenin was present in all four muscles, with the highest level in the fast-fiber pectoralis, and lower levels in slow-fiber muscles. All RNA samples were examined by gel electrophoresis to confirm quantitation and to assure no degradation had occurred. The same RNAs were hybridized with the chicken *cefMyHC* probe to demonstrate that muscle tissue was extracted in each sample (data not shown). ED, embryonic day; PH, posthatch; ALD, anterior latissimus dorsi.



highest level present in the fast-fiber pectoralis, and with lower levels in slow-fiber muscles.

As muscle development progresses beyond fetal development to the early post-hatch period, there is further divergence in the expression of MyoD between fast- and slow-fiber muscles (Figure 1A, 1B). The relative expression of MyoD in the ALD of the newly hatched, or neonatal chick, is greater than or equal to the level observed during earlier, fetal development *in ovo* (Figure 1B). On the other hand, the level of MyoD present in the pectoralis is well below that found during the early embryonic stage of myogenesis, suggesting that in birds, expression from the MyoD locus is down-regulated in fast-fiber, but not slow-fiber muscles. Myogenin, viewed primarily as a marker of muscle cell differentiation [30, 41], is down-regulated in both fast- and slow-fiber muscles of the neonate, but to a greater degree in the ALD than in the pectoralis (Figure 1B). The difference in post hatch levels of myogenin between the pectoralis and ALD muscles could reflect continued formation of muscle fibers as the pectoralis continues to grow, while less growth occurs in the ALD.

While correlative data on the relationship of MRF gene expression to fiber-type continues to accumulate, null mutants of MyoD or myf-5, produced by homologous recombination in transgenic mice, argue against a simple relationship between expression of a single MyoD family member and the formation of particular fiber-types [7, 54]. Mice homozygous for a mutant MyoD allele are viable and fertile, with no apparent skeletal muscle abnormalities. The proportions of fast and slow fiber-types are identical in wild-type and MyoD mutant mice in the predominately fast fiber-type sartorius and predominately slow fiber-type soleus muscles [54]. Although mice homozygous for disruption of the myf-5 gene die at birth, no abnormalities in the number, or relative proportions, of muscle fiber-types were observed in the fetuses of mutant mice [7]. On the other hand, disruption of the myogenin locus prevents differentiation of skeletal muscle fibers and results in embryonic lethals [30, 41]. The differentiation of the few muscle fibers that form in fetuses of myogenin knockout mice, is probably supported by the expression of MRF4 [51], but the type(s) of MyHC expressed, and the fiber-type of these few skeletal muscle cells has not been reported. Finally, where knockout of the MRF4 locus produced viable mice [70], a normal distribution of fast and slow fibers was reported.

No single mechanism explains the origins and maintenance of fibers of specific types. Several mechanisms are necessary to generate distinct fast and slow fiber-types during the successive waves of myogenesis occurring throughout the lifetime of an organism. Mechanisms to produce fibers of specific types include: 1) intrinsic differences between myoblasts committed to form fibers of specific types in early embryogenesis, particularly during primary fiber formation; 2) innervation - particularly as it emerges with secondary muscle fiber formation as fetal development gets underway, and 3) alterations in levels of

hormones in late fetal, posthatch, and adult development. In a manner not yet understood, muscle activity or work load can also alter the pattern of MyHC isoform expression in fast and slow skeletal muscle tissue [35]. While various MRF family members clearly display different patterns and levels of expression which correlate with different skeletal muscle fiber types, homologous recombination knockout experiments demonstrate that there is no strict correspondence between individual MRF gene expression and formation of a particular fiber-type. Thus the precise pattern of fast and slow fiber distribution does not appear to depend primarily on MRF expression. Instead, patterns of MRF expression, like the expression patterns of muscle proteins, may depend on an early event(s) underlying the formation of fast and slow fibers, and may be regulated by innervation such as to maintain or sustain the fiber-type pattern established in the embryo.

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