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Skeletal muscle fiber type: using insights from muscle developmental biology to dissect targets for susceptibility and resistance to muscle disease

Jared Talbot and

Department of Molecular Genetics, The Ohio State University, 1060 Carmack Road, Columbus OH 43210, USA

Lisa Maves

Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, 1900 Ninth Avenue, Seattle, WA, USA 98101

Department of Pediatrics, University of Washington, Seattle, WA, USA

Abstract

Skeletal muscle fibers are classified into fiber types, in particular slow twitch versus fast twitch. Muscle fiber types are generally defined by the particular myosin heavy chain isoforms that they express, but many other components contribute to a fiber's physiological characteristics. Skeletal muscle fiber type can have a profound impact on muscle diseases, including certain muscular dystrophies and sarcopenia, the aging-induced loss of muscle mass and strength. These findings suggest that some muscle diseases may be treated by shifting fiber type characteristics either from slow to fast, or fast to slow phenotypes, depending on the disease. Recent studies have begun to address which components of muscle fiber types mediate their susceptibility or resistance to muscle disease. However, for many diseases it remains largely unclear why certain fiber types are affected. A substantial body of work has revealed molecular pathways that regulate muscle fiber type plasticity and early developmental muscle fiber identity. For instance, recent studies have revealed many factors that regulate muscle fiber type through modulating the activity of the muscle regulatory transcription factor MYOD1. Future studies of muscle fiber type development in animal models will continue to enhance our understanding of factors and pathways that may provide therapeutic targets to treat muscle diseases.

Introduction

The skeletal muscle groups of the mammalian body are made up of bundles of muscle fibers. These fibers can be assigned to different identity classifications ("Types"), with characteristic movement rates, response to neural inputs, and metabolic styles^{1,2}. Fiber types are a conserved feature of vertebrate muscle; for instance, adult mouse and fish musculature shows a gradation of myosins^{3,4}, metabolic activity^{5,6}, patterns of innervation^{7,8,9}, and many other distinguishing characteristics^{1,2,10,11}. Skeletal muscle fibers are broadly classified as

"slow-twitch" (type 1) and "fast-twitch" (type 2). Based on differential myosin heavy chain (*MYH*) gene expression, there is further classification of fast-twitch fibers into three major subtypes (types 2A, 2X, and 2B, although humans do not appear to have *MYH4*-expressing type 2B fibers; Figure 1)¹. Hybrid MYH expression in different fibers of a muscle group can allow for even more subtypes (1/2A, 2A/2X, 2X/2B), resulting in an almost continuous range of ATP usage and muscle contraction speeds, from the fastest (type 2B) to the slowest (type 1)^{1,3}. In addition to the standard adult MYHs in Figure 1, there are also other MYHs expressed during development and MYHs that are very restricted to particular muscle groups^{1,2}. Skeletal muscle fibers also vary in energy production. Type 1 and 2A fibers primarily use oxidative metabolism, and type 2X and 2B fibers primarily rely upon glycolytic metabolism. However, even here there is variation, and energy usage is not a strict predictor of fiber type^{1,3}. In addition to MYH expression and cellular metabolism programs, factors contributing to fiber-type identities include multiple components of the sarcomere contractile machinery, such as fast and slow tropomyosin isoforms¹². Recent bioinformatic analyses revealed that structural proteins often use alternative splice forms in different fiber types^{13,14,15}, greatly increasing the diversity of protein forms that differentiate these muscle types. Other bioinformatic analyses have identified numerous microRNAs preferentially expressed in slow or fast muscle, providing potential regulatory mechanisms to impart fiber type identity^{16,17}. Ultimately, the coordinated regulation of fiber-type-specific biochemical and physiological systems gives each fiber type unique functional properties.

In mammalian skeletal muscles, multiple fiber types are generally intermingled within a single muscle group, and different muscle groups have varying proportions of fiber types^{1,2,12,18}. For example, the human soleus leg muscle is predominantly type 1 fibers, whereas the triceps arm muscle is predominantly type 2¹⁸. These proportions are plastic, however, and muscle fibers have the ability to remodel their phenotypes to help muscles adapt to different uses¹⁻³. For example, endurance exercise training can induce a modestly increased proportion of type 1 fibers. Conversely, disease states such as obesity are also associated with altered proportions of fiber types. The diversity in contraction physiology and metabolic activity of different fiber types, along with fiber-type plasticity, not only provide for a wide range of functions but also provide differential susceptibility to certain muscle diseases. In this review, we focus on three areas related to the role of muscle fiber type in muscle disease. First, we describe the muscle diseases that preferentially affect specific muscle fiber types. Second, we describe recent studies that have begun to mechanistically dissect the reasons for muscle fiber type-specific susceptibility and resistance to muscle diseases; in this section we focus on Duchenne muscular dystrophy and the effects of aging. Third, we discuss molecular pathways that can reprogram and specify muscle fiber types. In this section we describe how many of the factors that regulate muscle fiber type identities carry out their effects, with a focus on factors that act via the muscle regulatory transcription factor MYOD1. We conclude by calling for further investigation into the transcriptional control of muscle fiber type, which may offer insights into the causes of fiber type-specific susceptibility in muscle diseases.

MUSCLE DISEASES AFFECTING MUSCLE FIBER TYPE

There are many inherited myopathies and other acquired muscle-related disorders that preferentially affect specific skeletal muscle fiber types. We summarize these disorders and fiber-type effects in Table 1. It largely remains unclear why certain muscle diseases preferentially affect particular fiber types. Because fiber-type specific defects are not observed in all myopathies, non-specific muscle degeneration cannot account for the absence of particular fiber types in some diseases. Thus, understanding the fiber-type-specific effects of these muscle disorders may provide important insights into muscle disease pathologies and potential treatments. Indeed, as we describe below, for some of these diseases, the manipulation of muscle fiber type is a candidate therapeutic approach.

Genetic myopathies

Duchenne muscular dystrophy—Duchenne muscular dystrophy (DMD) is the most common childhood muscular dystrophy; this disease is caused by mutations in the *DMD* gene that encodes dystrophin¹⁹. In human limb muscle samples, Webster et al. showed that type 2 fibers (then referred to as type 2B, but likely type 2X) are the first fibers to degenerate and are eventually lost in DMD patients, whereas type 1 fibers are affected relatively late²⁰. Subsequent studies on muscle biopsies from DMD patients confirmed these findings^{21,22}. The type 1 fibers remaining in DMD patients are not all normal because they can co-express embryonic or fetal MYHs along with slow MYH, indicating that those fibers have undergone degeneration and regeneration, but these effects in type 1 fibers are not as severe as those observed in type 2 fibers^{20,21}. Studies in mouse and dog models of DMD have shown that muscle groups with high proportions of fast muscle, and in particular the glycolytic type 2 fibers, are also preferentially affected, both structurally and functionally^{23,24,25,26,26}. Webster et al. proposed that promoting slow muscle fiber function could be a therapeutic approach to delay the progression of DMD²⁰. We will review more recent studies that have addressed this issue below (see DISSECTING MUSCLE FIBER-TYPE RESISTANCE AND SUSCEPTIBILITY TO MUSCLE DISEASE).

Facioscapulohumeral muscular dystrophy—Facioscapulohumeral muscular dystrophy (FSHD) is a progressive muscular dystrophy characterized by weakness and wasting of the facial, shoulder and upper arm muscles. FSHD is strongly associated with, and likely caused by, aberrant activation of *DUX4*, which encodes a double homeobox transcription factor that is normally repressed in skeletal muscle²⁷. An examination of muscle fibers from patient biopsies showed that maximum force-generating capacity is reduced in type 2 FSHD fibers but not in type 1 fibers²⁸. Consistent with these findings, an earlier study of FSHD patient muscle biopsies used histochemical, transcriptomic, and proteomic analyses to show an increased proportion of type 1 fibers in FSHD patients, suggesting that type 2 fibers are more susceptible to FSHD²⁹.

Myotonic dystrophy—The myotonic dystrophies Type 1 and Type 2 (DM1 and DM2) are among the most common adult-onset muscular dystrophies. They share clinical phenotypes including multisystem involvement, muscle atrophy, and muscle weakness. Both types of myotonic dystrophies are caused by microsatellite expansions; DM1 is caused by

microsatellite expansions in *DMPK*, and DM2 is caused by microsatellite expansions in *CNBP*³⁰. Despite these shared attributes, DM1 and DM2 exhibit different fiber-type effects. DM1 shows type 1 fiber atrophy and a high frequency of type 1 fibers with central nuclei, whereas DM2 shows type 2 fiber atrophy and high frequency of type 2 fibers with central nuclei^{31,32}. In DM2, the type 2 fibers that remain can show hypertrophy³². Also, in DM1, sarcomeric force generation is reduced in both fiber types but particularly in type 1³³. The central nuclei may be indications of incomplete maturation of type 1 fibers (in DM1), or type 2 fiber hypertrophy (in DM2)³².

Congenital fiber type disproportion—Congenital fiber type disproportion (CFTD) is a congenital myopathy that is diagnosed when type 1 fibers are found in predominant proportions, are consistently much smaller than type 2 fibers, and there is no other histologic muscle structural abnormality³⁴. Mutations in several genes have been linked to CFTD, including *ACTA1*, *LMNA*, *MYH7*, *RYR1*, *TPM2*, and *TPM3*^{34–36}. It is not clear how mutations in these genes lead to fiber size disproportion, particularly because many of these genes are expressed in both fiber types. Mutations in these genes are also associated with other myopathies that do not exhibit fiber size disproportion³⁴.

Myosinopathies—Myosinopathies are muscle diseases caused by mutations in myosin heavy chain genes; certain myosinopathies show atrophy of specific fiber types³⁷. For example, mutations in slow *MYH7* can lead to a broad spectrum of skeletal muscle and cardiac myopathies and have been linked with fiber-type disproportion (including CFTD and Laing distal myopathy), which consistently show smaller diameter type 1 fibers but variable predominance of type 1 fibers^{38,39,40,41}. Mutations in fast *MYH2* lead to loss of type 2A muscle fibers accompanied by fatty infiltration³⁸. Patients with these *MYH2* mutations show mild muscle weakness along with weakness of the facial and eye muscles. The fiber-type specificity of these particular myosinopathies are easily understood, because *MYH2* is expressed in type 2A fibers, and *MYH7* is expressed in type 1 (Figure 1).

Pompe disease—Pompe disease is a glycogen storage disease caused by defects in acid alpha-glucosidase (GAA)³⁹. With deficiency or absence of GAA, glycogen accumulates in lysosomes, leading to lysosome swelling, blocking of lysosome-autophagosome fusion, and accumulation of autophagic vesicles. Skeletal and cardiac muscle is most affected by Pompe disease. Loss of GAA activity leads to the infantile form, which presents severe muscle weakness and fatal hypertrophic cardiomyopathy. Partial loss of GAA activity leads to later onset of progressive skeletal muscle weakness. Mouse models of Pompe have been generated through targeted disruptions of the *Gaa* gene. These *Gaa* mutant mouse models show features of both the infantile and late-onset forms of the disease. In *Gaa* knock-out mice that mimic the late-onset form, type 2 fibers exhibit decreased fiber size and massive autophagic build-up, whereas type 1 fibers, even though they exhibit induction of autophagy, do not show decreased size or autophagic buildup^{40,41}. Studies thus far in human Pompe patient biopsies have not detected a selective fiber-type effect³⁹, although see van den Berg et al⁴². While in mice, type 1 fibers respond better to enzyme replacement therapies than type 2 fibers do^{43,44}, the results in human studies have been mixed. It remains to be seen whether fiber shifting will become an effective therapy for Pompe disease.

Other muscle wasting disorders

The effects of acquired muscle wasting and metabolic disorders, in particular type 2 diabetes, obesity, and aging, on specific fiber types have been the subject of many excellent reviews^{1,2,45,46}. Here we focus on studies of human subjects and summarize some of the major findings.

Obesity and type 2 diabetes—Obesity and type 2 diabetes are characterized by severe insulin resistance in skeletal muscle^{1,2}. Obese individuals and individuals with type 2 diabetes both show reduced proportions of type 1 fibers and increased proportions of type 2X fibers^{47,48,49,50,51}. The proportion of type 1 fibers correlates with insulin responsiveness⁵². Furthermore, muscle biopsies from diabetic patients and from patients with a family history of type 2 diabetes showed reduced expression of the oxidative metabolism program, consistent with a shift to type 2X fibers^{53,54}. However, while studies consistently link decreased oxidative enzyme activity with obesity and type 2 diabetes, not all studies find changes in fiber-type proportions⁵⁵. Further research is needed to clarify whether and how changes in metabolic programs or in fiber-type distributions might increase susceptibility to obesity and type 2 diabetes.

Muscle inactivity—Loss of or reduced muscle use has been shown to cause significant atrophy of all muscle fiber types but particularly of type 1 fibers, accompanied by a fiber-type shift from type 1 and 2A fibers to type 2X^{56,57,58,59}. For instance, both spinal cord injury and bed rest shift fibers towards faster phenotypes, though there have been some variations to these findings⁴⁵. For example, Ditor et al. observed the most pronounced atrophy in type 2A fibers in spinal cord injury subjects⁶⁰. Bed rest appears to most strongly induce type 1 fiber atrophy, and an increase in hybrid fibers appears after bed rest⁵⁹. Experiments in rat models have shown that denervation can cause fiber-type specific atrophy but in different fiber types depending on the muscle group examined⁴⁶. Taken together, the type of injury, the muscle group affected, and the time since injury or rest may all influence how specific muscle fiber types are affected⁴⁵.

Aging/sarcopenia—The loss of skeletal muscle mass and strength due to aging, called sarcopenia, is characterized by a selective reduced size and greater atrophy of type 2 fibers^{61,62}. These effects on fiber type morphology correlate with reduced expression of MYH2 (type 2A) and MYH1 (type 2X), whereas MYH7 (type 1) expression was not affected^{63,64}. Other examples of muscle wasting, such as cancer cachexia, fasting, and sepsis, show similar effects on muscle fiber type⁴⁶. In these muscle disorders type 2x fibers are particularly susceptible to atrophy; one possible reason for this increased susceptibility is their lower expression of *PPARGC1A* (also known as PGC-1 α), relative to type 1 and 2A fibers⁶⁵. In humans, *PPARGC1A* is positively correlated with the proportion of type 1 fibers⁶⁶. In mouse models, *PPARGC1A* promotes slow muscle fiber type and oxidative metabolism and can repress muscle atrophy^{67,68}. We discuss *PPARGC1A* in more detail below.

Heart failure and COPD—Diseases such as chronic heart failure and chronic obstructive pulmonary disease (COPD) can lead to systemic effects on peripheral skeletal muscle. In

patients with chronic heart failure and COPD, limb muscle exhibits a shift in fiber type proportions, from type 1 to type 2, with corresponding changes in MYH expression^{69,70}. These changes are similar to those seen in muscle inactivity disorders (see above). However, respiratory muscles, like the diaphragm, show the opposite effects, with a fiber-type shift of type 2 to type 1⁶⁹. In COPD, reduced type 1 fiber proportions in leg muscle are strongly associated with disease severity; nonetheless, patients can show much variability in fiber size and fiber-type proportions⁷¹. Gouzi et al. showed that COPD patients could be classified into 2 groups, one with muscle fiber atrophy and severe loss of type 1 fibers, and a second group with reduced type 1 fiber proportions and preserved fiber size⁷². Such classifications may improve both the ability to predict patients' responses to interventions and our understanding of disease mechanisms. These studies underscore the significance of understanding muscle fiber-type-related phenotypes in muscle diseases.

DISSECTING MUSCLE FIBER-TYPE RESISTANCE AND SUSCEPTIBILITY TO MUSCLE DISEASE

Inducing slow muscle fiber type to ameliorate DMD

As described above, the finding that DMD preferentially affects type 2 fibers led Webster et al. to suggest that possible DMD therapies could work by “selectively promoting slow muscle fiber function”²⁰. More recent studies using the *Dmd*^{mdx} mutant mouse model for DMD found that genetic or pharmacological manipulations that ameliorate symptoms also activate a slow muscle phenotype⁷³. One prominent example of a factor whose expression can ameliorate DMD while promoting a slow muscle phenotype is PPARGC1A. PPARGC1A is a transcriptional coactivator for nuclear receptors and other transcription factors, that acts as a master regulator of the slow muscle contractile and oxidative metabolism gene expression programs^{67,74}. Transgenic overexpression of PPARGC1A in mice ameliorates muscle structural and functional defects caused by *Dmd*^{mdx} mutation⁷⁵. In this context, PPARGC1A upregulates gene expression programs for the slow muscle contractile machinery, oxidative metabolism, and the neuromuscular junction^{67,75}. All of these PPARGC1A targets could provide benefits to DMD muscle. These studies support the hypothesis that promoting a slow muscle fiber type provides resistance to DMD⁷³. However, it is not functionally clear why slow muscles are resistant to DMD; one possible explanation is that type 1 fibers express higher levels of utrophin⁷⁶. Utrophin is structurally very similar to dystrophin, can compensate for the loss of dystrophin, and its upregulation correlates with the induction of slow muscle by factors that ameliorate the *Dmd*^{mdx} mouse, including PPARGC1A^{75,77,78}. These features have made utrophin a strong candidate for a therapeutic target for DMD^{78,79}.

Two recent studies have directly tested whether utrophin, a component of the slow muscle program, is required for amelioration of DMD. Chan et al. test whether utrophin upregulation is required for the ability of PPARGC1A overexpression to ameliorate the muscle defects in mdx mice⁸⁰. They take advantage of the mouse *Dmd*^{mdx};*Utrn* double mutant, which lacks both dystrophin and utrophin and has a more severe muscle degeneration phenotype than the *Dmd*^{mdx} mutant^{81,82}. They show that transgenic overexpression of PPARGC1A in the *Dmd*^{mdx};*Utrn* mice robustly ameliorates the muscle

structural and functional defects, even in the absence of utrophin. They also show that transgenic overexpression of PPARGC1B, a PPARGC1A homolog, ameliorates the *Dmd^{mdx}* model similarly to PPARGC1A, even though PPARGC1B does not induce upregulation of utrophin or the neuromuscular junction program. Although the Chan et al. study did not directly test fiber types after PPARGC1 overexpression, they did find that PPARGC1 increases oxidative muscle enzymes, independent of utrophin. This work shows that PPARGC1A and PPARGC1B can ameliorate mouse DMD independently of utrophin or the neuromuscular junction program, indicating that other components of the slow muscle phenotype provide resistance to DMD.

In a second study, Al-Rewashdy et al. also test whether utrophin is required for amelioration of mouse DMD⁸³. Previous studies have shown that activation of the 5' adenosine monophosphate-activated protein kinase (AMPK) pathway with the pharmacological agent AICAR can ameliorate mdx muscle defects^{84,85}. AMPK activation by AICAR induces a slower, oxidative fiber phenotype as well as increased utrophin and PPARGC1A expression^{84,85}. Al-Rewashdy et al. treated *Dmd^{mdx};Utrn* mice with AICAR to test the requirement for utrophin in the amelioration of DMD⁸³. They found that for some metrics, *Dmd^{mdx}* and the *Dmd^{mdx};Utrn* mice both responded positively to AICAR; in both genotypes, AICAR treatment induced oxidative gene expression, an increased proportion of type 2A (slower) fibers, and slower muscle contractile kinetics. However, for other metrics only mdx mice responded to AICAR; this drug was not able to improve muscle cell membrane structure or muscle function in the *Dmd^{mdx};Utrn* mice. Therefore, this study shows that upon pharmacological induction of the slow muscle program, utrophin is required for resistance to DMD.

These studies provide excellent examples of using genetics and pharmacology in animal models to dissect how pathways that modulate fiber type rescue the DMD phenotype. Since PPARGC1 increases oxidative enzymes and AICAR increases type 2A (oxidative) muscle, fiber shifting may be a shared mechanism for both types of utrophin-independent DMD rescue; future work will need to directly test this potential connection. However, these studies provide conflicting evidence as to how important utrophin upregulation is to mediate the physiological effects of pathways that ameliorate DMD. One difference between these two studies is that the Chan et al. study induces PPARGC1A expression earlier, which may improve muscle development prior to the phenotypic effects of DMD^{80,83}. Additional studies have suggested that PPARGC1A and AMPK may improve the *Dmd^{mdx}* phenotype through enhancing mitochondrial or satellite cell functions^{86,87}. Such studies provide additional candidate factors that can be tested for their roles in mediating the rescue of DMD.

Enhancing fast muscle growth to ameliorate muscle aging effects

Like DMD, aging preferentially causes atrophy of type 2 glycolytic muscle fibers, in humans and in mice^{61,88}. Because skeletal muscle is the primary site of insulin-mediated glucose metabolism, muscle atrophy may potentially lead to metabolic disorders. Indeed, in addition to type 2 fiber atrophy, aging is associated with increased susceptibility to metabolic dysfunction, such as type 2 diabetes⁸⁹. As in other muscle diseases described

here, it is not clear how the aging process leads to type 2 fiber atrophy. It is also not known whether type 2 fiber atrophy in aging causes the increased insulin resistance observed during aging.

As with DMD, type 1 fibers seem more resistant to aging. There have been efforts to promote slow muscle fibers to ameliorate the effects of aging on metabolic dysfunction^{74,90}. Again, PPARGC1A has been a central candidate factor in these efforts, because it can repress muscle atrophy but its expression normally declines with age^{68,74}. However, studies in different mouse models of aging have shown mixed results in the ability of PPARGC1A overexpression to protect against the effects of aging atrophy^{91,92}.

Recently, Akasaki et al. investigated the relationship between type 2 fiber-specific loss and muscle aging, using transgenic AKT1 overexpression in mice⁹⁰. The phosphatidylinositol 3-kinase/AKT1/MTOR signalling pathway controls muscle fiber size: AKT1 activation causes muscle fiber hypertrophy, and AKT1 inactivation leads to muscle atrophy^{93,94,95}. Aging is associated with impaired AKT1 activation⁹⁰. In order to directly test the effects of fast/glycolytic muscle fiber growth in young versus middle-aged mice, Akasaki et al. took advantage of a transgenic mouse model, in which a type 2B fiber-specific transgene drives doxycycline-inducible constitutively active AKT1^{90,96}. Middle-aged mice normally show less muscle mass compared to young mice. AKT1-expressing middle-aged mice show similar muscle mass as young mice and show hypertrophy of glycolytic type 2B fibers, the fibers that are preferentially lost during aging in mice. The AKT1-expressing mice show decreased expression of muscle atrophy genes, reduced expression of PPARGC1A, and increased expression of glycolytic metabolism genes, consistent with the selective growth of the type 2B fibers. AKT1 expression thus causes selective skeletal muscle hypertrophy and ameliorates the loss of muscle associated with aging. In addition, the transgenic AKT1 expression improved the metabolic phenotype of the middle-aged mice, including improved glucose metabolism. This work shows that loss of muscle mass is indeed causally related to metabolic dysfunction in aging. Therefore, interventions to preserve or enhance fast/glycolytic muscle during aging-related muscle atrophy may delay the onset of metabolic dysfunction.

This study indicates a strategy to treat sarcopenia by modulating fiber types in an opposite direction than is suggested for treating DMD. Efforts for DMD therapies have focused on enhancing the slow muscle phenotype, which are more resistant to DMD (see above). Even though type 2 fibers are more susceptible to aging, the work from Akasaki et al. shows that enhancing these type 2 fibers is a potential therapeutic approach to delay the onset of metabolic dysfunction⁹⁰.

MOLECULAR PATHWAYS REGULATING PLASTICITY AND SPECIFICATION OF FIBER TYPES

Taken together, the studies described here support a hypothesis that modulating the proportion of distinct skeletal muscle fiber types may be a viable therapeutic approach for many muscle diseases. To achieve this goal, we need to understand the genetic cascades that rebuild damaged muscle in adults, the signalling pathways that can alter fiber type in

established muscle, and the developmental processes that generate muscle fiber types in embryos. Muscle fiber type specification begins in embryos prior to neural input, but, after birth, neural influence can shift muscle fibers to an overall slow or fast phenotype⁹⁷. The ability to shift between fiber types is referred to as "fiber plasticity"^{1,2}. These two processes (specification and plasticity) employ many shared genes, so the best therapeutic strategies may involve genes from either process. Many excellent reviews have already enumerated the many factors and pathways involved in muscle fiber-type specification and plasticity^{1,2,73}. Here, we will briefly review factors that may be particularly useful for fiber-shifting therapies. In particular, recent studies have revealed many factors that regulate muscle fiber type through modulating the activity of the muscle regulatory transcription factor MYOD1. Figures 2 and 3 illustrate some of the major factors in fiber type specification and plasticity.

Muscle fiber type plasticity

The ability to reprogram existing muscle fibers from one type to another may benefit muscle disease treatments^{2,73}. One established way to reprogram muscles in model organisms is to change the neural inputs to muscles⁹⁷. In response to neural stimulation rates, muscle fibers can be reprogrammed from fast-to-slow or vice versa. This was first discovered by Buller et al.⁹⁸; subsequent work has confirmed and expanded on this finding⁹⁹. Although denervated muscle adopts a fast muscle phenotype, other studies indicate that fast muscle is an actively patterned state⁹⁷. Fitting an active-patterning hypothesis, fiber type correlates with exercise type in athletes^{100,101}, suggesting that physical training may reprogram muscle fibers. Fitting this hypothesis, multiple studies have found that fiber types can be modestly shifted through intensive exercise training regimes^{102,103,104,105,106}. However, the responses to training are modest at best, and only some training studies have been able to induce fiber shifts, prompting calls to better understand how training influences fiber type shifts¹⁰⁷. Although further investigation is needed to identify the best strategies, these findings demonstrate that muscle fibers can be reprogrammed in either direction in existing tissues.

Pathways regulating fiber-type plasticity have been comprehensively reviewed elsewhere^{1,2,73}. Two of the major pathways in fiber-type plasticity are calcineurin signalling and, as discussed above, AMPK signaling (Figure 2). Calcineurin (PPP3CA), a calcium-regulated serine/threonine phosphatase, is a key factor in mediating the muscle fiber-type response to neural input^{2,108}. Calcineurin dephosphorylates and activates NFAT transcription factors, and many studies have used loss- and gain-of-function approaches to show that calcineurin/NFAT signalling plays a major role in promoting a slow, and repressing a fast, muscle fiber type^{1,2,108}. However, different combinations of NFAT factors are required to promote fast muscle fiber types¹⁰⁹. A recent study showed that NFATC1 is required in mice for the proper proportions of slow and fast fibers and for fast-to-slow fiber-type switching in response to exercise¹¹⁰, and we discuss roles of NFAT factors more below. The calcineurin/NFAT pathway is thus a potential therapeutic target for modulating slow and fast fiber types. Indeed, modulation of calcineurin signaling benefits mouse muscular dystrophy models⁷³. Like calcineurin signalling, loss- and gain-of-function manipulations have demonstrated a critical role for AMPK signaling in promoting the slow, oxidative muscle fiber type^{1,73,111}. One of the major mediators of AMPK signalling is PPARGC1A, a potent activator of slow muscle identity, as discussed above^{1,67,73,74,112}. In mice, PPARGC1A is expressed in slow

fibers at a much higher level than in fast fibers, and when PPARGC1A is overexpressed in fast muscles at physiological levels, it activates markers of slow muscle identity and oxidative metabolism^{55,63}. Mice with skeletal muscle-specific knock-out of PPARGC1A show reduced endurance capacity and a shift from type 1 and 2A toward type 2X and 2B muscle fibers⁷⁵. Although exercise-induced mitochondrial biogenesis is inhibited in skeletal muscle *Ppargc1a* knock-out mice, exercise-induced type 2B-to-2A fiber shifts are not affected in the skeletal muscle of *Ppargc1a* knock-out mice, suggesting that exercise-induced fiber-type transformation can be independent of PPARGC1A¹¹³. Nevertheless, as discussed above, PPARGC1A overexpression has shown therapeutic benefits in the *Dmd^{mdx}* mouse model for DMD.

Muscle fiber type specification

Skeletal muscle fiber types are first specified during embryonic development^{114,115}. Both mammalian and zebrafish animal models have provided insight into muscle fiber specification factors. In embryonic zebrafish muscle and in developing mouse limb muscle, Hedgehog signaling promotes slow fiber identity, though perhaps using different downstream mechanisms in the two organisms^{115,116,117}. The homeodomain transcription factor SOX6 acts in fast muscle to prevent the onset of slow-type genes, in both mammalian and zebrafish muscle^{114,115}. The homeodomain transcription factors SIX1 and SIX4 are required during embryogenesis and into postnatal development to promote a fast muscle program and repress a slow muscle program^{118,119,120}. A recent study demonstrated that hedgehog overexpression partially rescues the *Dmd^{mdx}* mouse¹²¹, although whether muscle fiber types are altered in this *Dmd^{mdx}* rescued state has not yet been addressed. *Sox6* is antagonized by a microRNA, *miR-499*¹²², and zebrafish *six1* is post-transcriptionally inhibited by *miR-30a*¹²³. Delivery of *miR-499* may be a straightforward means to increase intermediate fiber types in diseased muscle, and therapies that inhibit SIX function in muscle, such as delivery of *miR-30a*, would be predicted to induce a fast-to-slow fiber type shift. Taken together, these studies provide examples of how investigations of developmental fiber type specification can provide candidate factors and pathways for the potential therapeutic modulation of fiber type.

For simplicity, we have presented muscle specification and plasticity as two separate processes, but the two processes have many commonalities, including shared gene networks. For example, the developmental fast muscle specification factor SIX1, when co-expressed with its cofactor EYA1, is sufficient to reprogram adult slow muscle fibers into fast muscle fibers, indicating that SIX1 is also involved in muscle fiber-type plasticity¹²⁴. The shared pathways between specification and plasticity suggest that therapies intended to convert fibers to a given fiber type may benefit muscles in two ways: by specifying newly-formed fibers to adopt a desired type, and by reprogramming existing fibers to that same desired type.

Modulation of MYOD1 activity in muscle fiber type development

In many cases, an additional commonality between factors involved in muscle fiber-type specification and in fiber-type plasticity is the regulation of MYOD1 activity. MYOD1 is a basic helix-loop-helix (bHLH) transcription factor, a member of the myogenic regulatory

factor (MRF) family (also including MYF5, MYF6, and myogenin); MYOD1 is necessary and sufficient for driving skeletal muscle specification and differentiation^{125,126}. In muscle fiber-type differentiation, MYOD1 has been particularly linked with fast muscle differentiation, because in mouse muscle MYOD1 is expressed more strongly in fast fibers than in slow fibers, and because gain- and loss-of-function experiments have shown that MYOD1 promotes faster and more glycolytic fiber types^{127,128,129,130}. However, these, and other, studies have also found that MYOD1 is involved in promoting muscle differentiation more generally, influencing both slow and fast fiber development; this indicates a nuanced relationship between MYOD1 and fiber type^{127,128,131}. MYOD1 controls skeletal muscle differentiation through direct DNA binding and transcriptional regulation of broad gene expression programs, including transcriptional regulation of genes encoding skeletal muscle contractile proteins^{132–136}. MYOD1's DNA binding and transcriptional activity can be modulated, positively and negatively, by many factors^{126,136}, including many of the factors involved in fiber-type plasticity and specification mentioned above (Figure 3).

Many recent studies have identified transcription factors that can push MYOD1 towards promoting fast or slow muscle fates. Several transcription factors have been found to stimulate fast muscle identity via MYOD1 (Figure 3A)^{131,137,138,139}, while others cause MYOD1 to promote slow muscle fates (Figure 3B)^{132,140,141}. Some of these factors, such as NFATC1 and FHL3, also have converse roles in repressing the ability of MYOD1 to activate fast or slow muscle genes, respectively (Figure 3C–D)^{110,139,142}. These examples reveal how different factors can work through a variety of mechanisms to modulate MYOD1 activity and influence muscle fiber-type gene expression, for example, through binding in a protein complex with MYOD1 on DNA (PBX)^{131,137}, through inhibiting MYOD1 activity (NFATC1)¹¹⁰, or through binding DNA in parallel with MYOD1 and synergizing with MYOD1 activity (EBF3)¹³⁸.

In addition to the examples illustrated in Figure 3, there are many other fiber-type factors that likely interact with MYOD1 to modulate its activity in regulating fiber-type differentiation. For example, SIX1 and SIX4 proteins, which promote fast muscle development, directly bind many MYOD1 targets, including fast muscle differentiation genes^{120,143,144}. In Figure 3, we have presented MYOD1 regulators separately, and, in most cases, acting at distinct target genes. It will be important to determine how these different regulators converge to modulate MYOD1, not only at specific target genes but also in broader fiber-type gene expression programs. Because MYOD1 appears to be a central nexus controlling both slow and fast fiber-type differentiation, understanding MYOD1 regulation may provide insight into how to manipulate muscle fibers toward specific slow or fast identities.

Conclusion

Muscle diseases often affect particular muscle fiber types more strongly than others, indicating that different muscle diseases result not from generic muscle degeneration, but from specific defects in diseased tissue. Although we can describe which muscle fibers are most affected by a particular disease, in most cases we can't explain why certain fiber types are particularly susceptible to individual diseases. In a few cases, we can make clear

connections; for instance, *MHY7* and *MYH2* are specific markers of type 1 and type 2A muscle fibers, respectively, and humans carrying mutations in these myosins have particular loss of type 1, or type 2A, muscle fibers. We expect that continued efforts to understand the fiber-type-specific effects of muscle disorders will provide important insights into fiber-type specific degeneration found in other muscle diseases. For both genetic and acquired muscle disorders, improved classifications of patients' skeletal muscle fiber defects in relation to their other phenotypes should lead to improvements in diagnosis, in the ability to predict patients' responses to interventions, and in our understanding of disease mechanisms. Taken together, the studies reviewed here highlight the impact of muscle fiber-type-related phenotypes in muscle diseases.

Although it is well known that muscle diseases often affect particular fiber types, it remains unclear which characteristics of muscle fiber type contribute to the susceptibility and resistance of certain fiber types to muscle disease. Individual fiber types may be affected in diseases because of factors intrinsic to that fiber type's structural integrity, interaction with other cell types, or developmental effects. We are still discovering the developmental pathways that control fiber-type specification and influence fiber-type plasticity, and current approaches using animal models and muscle cell culture models can continue to help with these efforts. Since fiber-type identity is controlled by many factors, including unknown factors, we encourage the increased use of transcriptome analyses when studying altered fiber-type, such as in the studies by Quiat et al.¹⁴⁵ and Yao et al.¹⁴⁶. For example, quantitative RT-PCR studies in *Ebf3* and *Nfatc1* mutant mouse muscle was useful in demonstrating fiber-type-specific effects in these mutants^{110,138}, but RNA-seq could provide even broader insight into the fiber-type gene expression programs affected in these mouse models. We would also encourage increased use of the zebrafish animal model for muscle fiber-type studies. Zebrafish can provide a system for gaining increased animal numbers for the studies of double or even quadruple genetic mutants, which can allow insight into the interactions of factors involved in fiber-type identities. In addition to being an excellent model for muscle development, zebrafish provide excellent models of human muscle diseases^{147,148}, but zebrafish have not yet been utilized to understand the roles of muscle fiber type in specific muscle diseases. For instance, using zebrafish, muscle diseases could be investigated in living embryos that transgenically mark muscle fiber types during degeneration. With genome editing technologies such as the CRISPR-Cas system, along with its other advantages, the zebrafish system is well positioned to help address not only the functional interactions between fiber-type-identity factors, but also the relationships between fiber types and muscle diseases.

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Figure 1. Skeletal muscle fiber types. (A) Section of human muscle, where fiber types have been differentiated using ATPase staining after pre-incubation at pH 4.6. (B) Illustration showing healthy muscle fibers. Connective tissue (green) interacts with the dystrophin-related complex via basal lamina. Muscle fiber nuclei (orange) are found in peripheral positions. Different fiber types, including type 1 (red), type 2A (pink), and type 2X (purple), can be intermingled within a single mammalian muscle. (C) Particular muscle groups can also be enriched for slow (Soleus) or fast (extensor digitorus longus [EDL]) muscle. In mouse, an

additional fast fiber type, 2B (blue) is present. (D) In zebrafish trunk musculature, different fiber types are segregated, with the slowest fibers situated laterally, and fast fibers situated medially. (E) Key properties of fiber types, with the color code highlighting the graded shift from slow to fastest fibers. To simplify fiber typing, we operationally define these types by their myosin heavy chain (MYH) expression, however many other factors also distinguish fiber types. For instance, metabolic programs also contribute to muscle fiber phenotype.

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Figure 2.

Illustration of some of the known pathways that specify slow (red) or fast (blue) muscle fiber identity during developmental specification or during fiber plasticity. Although the pathways for plasticity are drawn separately from developmental pathways, some factors, such as *SIX1*, are used during both processes.

Figure 3.

Schematic examples of factors that modulate MYOD1 activity in the regulation of fiber-type-specific gene expression. Protein factors are represented as colored circles binding to DNA regulatory regions of different genes involved in muscle fiber-type differentiation. References for these examples are provided in the text. These examples are highly schematized and are not comprehensive of all known factors that modulate MYOD1 activity. These examples are meant to represent a range of mechanisms, which are not mutually exclusive, for regulating MYOD1 activity.

TABLE 1

Muscle Disorders With Effects On Specific Skeletal Muscle Fiber Types

Disorder	Fiber-type effects	Reference(s)
Duchenne muscular dystrophy	Type 2X fibers first to degenerate.	20,21,22
Facioscapulohumeral muscular dystrophy	Maximum force-generating capacity reduced in type 2 fibers. Increased proportion of type 1 fibers.	28,29
Myotonic dystrophy Type 1 (DM1)	Type 1 fiber atrophy and high frequency of type 1 fibers with central nuclei. Force generation reduced more in type 1 fibers.	31,32,33
Myotonic dystrophy Type 2 (DM2)	Type 2 fiber atrophy, type 2 fiber hypertrophy, and high frequency of type 2 fibers with central nuclei.	31,32
Congenital fiber type disproportion	Predominant proportions of type 1 fibers that are consistently much smaller than type 2 fibers.	34
Myosinopathies	<i>MYH7</i> mutations can cause smaller diameter type 1 fibers. <i>MYH2</i> mutations lead to loss of type 2A fibers.	38,149,150,151
Pompe disease	In mouse model, type 2 fibers smaller with massive autophagic build-up.	40,41
Obesity and type 2 diabetes	Reduced proportions of type 1 fibers and increased proportions of type 2X fibers.	47,48,49
Muscle inactivity (spinal cord injury, bed rest)	Type 1 fiber atrophy. Fiber-type shift from type 1 and 2A to 2X.	56,58
Aging/sarcopenia	Type 2 fiber loss and atrophy. Smaller diameter type 2 fibers.	61,62
Heart failure, chronic obstructive pulmonary disease	Fiber-type shift from type 1 to type 2 (limb muscles). Fiber-type shift from type 2 to type 1 (diaphragm).	69