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Signaling Pathways in Skeletal Muscle Remodeling

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Key Words

myofiber, MEF2, calcineurin, calcium-dependent protein kinase, exercise adaptation, hypertrophy

Abstract

Skeletal muscle is comprised of heterogeneous muscle fibers that differ in their physiological and metabolic parameters. It is this diversity that enables different muscle groups to provide a variety of functional properties. In response to environmental demands, skeletal muscle remodels by activating signaling pathways to reprogram gene expression to sustain muscle performance. Studies have been performed using exercise, electrical stimulation, transgenic animal models, disease states, and microgravity to show genetic alterations and transitions of muscle fibers in response to functional demands. Various components of calcium-dependent signaling pathways and multiple transcription factors, coactivators and corepressors have been shown to be involved in skeletal muscle remodeling. Understanding the mechanisms involved in modulating skeletal muscle phenotypes can potentiate the development of new therapeutic measures to ameliorate muscular diseases.

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INTRODUCTION

Skeletal muscle is composed of heterogeneous specialized myofibers that enable the body to maintain posture and perform a wide range of movements and motions. It is this diversity of myofibers that enables different muscle groups to fulfill a variety of functions. In addition to its obvious roles in motility, skeletal muscle plays a central role in the control of whole-body metabolism. These seemingly different functions are controlled by signaling pathways that enable muscle fibers to respond to the changing metabolic and functional demands of the organism.

Calcineurin: a heterodimeric protein phosphatase (PP2B) comprised of calmodulin-binding catalytic A and regulatory B subunits

The premise that myofibers remodel and modify their phenotype was demonstrated over 45 years ago when cross-reinnervation studies were shown to alter the contractile properties of myofibers (1). Similarly, skeletal muscle responds to exercise training by remodeling the biochemical, morphological, and physiological states of individual myofibers. The remodeling process provides an adaptive response that serves to maintain a balance between physiological demands for contractile work and the capacity of skeletal muscle to meet those demands. Many of the remodeling responses involve activation of intracellular signaling pathways and consequent genetic reprogramming, resulting in alterations of muscle mass, contractile properties, and metabolic states.

Advances in genetic engineering have allowed the introduction or depletion of factors within the myofiber, facilitating the evaluation of signaling factors during muscle remodeling. In particular, myofiber transformation has successfully been achieved in transgenic mouse models using muscle-specific promoters to drive expression of calcineurin (protein phosphatase 2B) and various calcium-dependent kinases. Activation of specific signaling pathways in myofibers has profound effects, not only on contractile proteins, but also on alterations of metabolic states leading to changes in muscle performance. Because of space limitations, this review does not discuss the pathways controlling muscle development (2) nor the contribution of satellite cells to skeletal muscle regeneration (3) but focuses on the signaling mechanisms that modify myofiber function with emphasis on clinical significance and therapeutic approaches to ameliorate muscle diseases.

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PROPERTIES OF MYOFIBERS

The musculature of the body is composed of a variety of muscle groups, such as soleus, extensor digitorum longus, and plantaris. Each muscle group is comprised of heterogeneous myofibers that differ in their biochemical, physiological, and metabolic parameters. The myofiber content is a determinant of muscle heterogeneity in contraction speed and fatigue resistance. A striking feature of the myofiber is the ability to transform and remodel in response to environmental demands.

Myofiber Diversity

Although histologically skeletal muscle appears uniform (**Figure 1*a***), it is comprised of myofibers that are heterogeneous with respect to size, metabolism, and contractile function (4). On the basis of specific myosin heavy-chain isoform expression, myofibers are classified into type I, type IIa, type IId/x, and type IIb fibers, with types I and IIa exhibiting oxidative metabolism and types IIx and IIb being primarily glycolytic (5, 6). Type I myofibers, also termed slow-twitch fibers, exert a slow contraction owing to the ATPase activity associated with the type I myosin. Slow-twitch myofibers are rich in mitochondria, have more capillaries surrounding each fiber, exhibit oxidative metabolism, have a low velocity of shortening, and have a high resistance to fatigue. Type II fibers, termed fast-twitch myofibers, exert quick contractions and fatigue rapidly. The slow oxidative fibers are required for maintenance of posture and tasks involving endurance, whereas fast-glycolytic fibers are required for movements involving strength and speed. Different myofiber subtypes are detected during embryonic life (7), and patterning of fiber types within major muscle groups is established postnatally (8).

Slow-twitch myofibers: muscle fibers that express type I myosin heavy chain

Fast-twitch myofibers: muscle fibers expressing type IIa, type IId/x, and type IIb myosin heavy chain

In addition to the variability seen in myosin heavy-chain gene expression, fiber-type differences are observed with the expression profile of other muscle proteins, such as tropomyosin, myosin light chain, parvalbumin, phospholambin, and sarcoplasmic reticulum calcium ATPase (SERCA). Although there are multiple levels of distinction among myofibers, classically, fiber type is defined on the basis of its myosin heavy-chain isoform expression profile. Fiber type is determined using assays that delineate the differences in ATPase activity that correlate with specific myosin heavy-chain isoforms. The basis of the

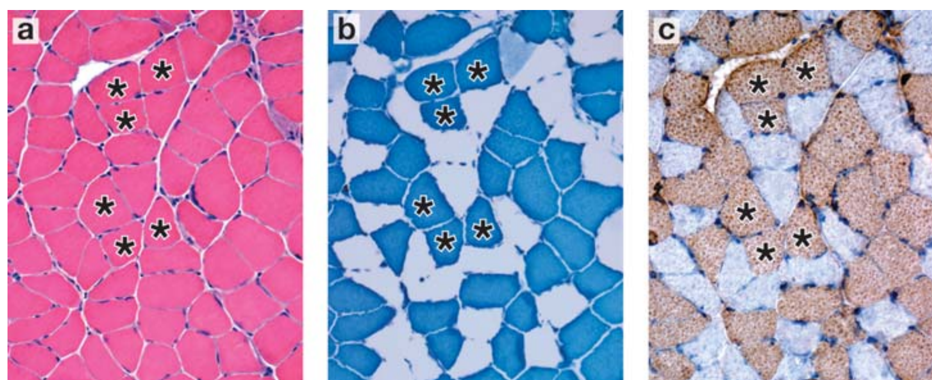

Figure 1

Heterogeneous distribution of skeletal muscle fibers. Fiber-type analysis of serial transverse sections of mouse soleus by (*a*) hematoxylin and eosin stain showed a checkerboard pattern of fibers, (*b*) metachromatic dye-ATPase method showed type I fibers (stained *dark blue*) and type IIA (stained *light blue*), and (*c*) immunohistochemistry using a monoclonal antibody recognized type I myosin heavy chain. The asterisks mark the same type I fibers in each panel.

Motor neuron: a neuron that innervates muscle fibers

Signal transduction: an extracellular signal stimulates a receptor, activating a messenger and changing gene expression and phenotype

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reaction is the deposition of insoluble salts of inorganic phosphate cleaved from ATP by myofibrillar ATPase(s) followed by substitution of the phosphates with less soluble chromogenic salts (9) (**Figure 1*b***). Immunohistochemistry using monoclonal antibodies that recognize isoform-specific myosin heavy chain is another method used to determine fiber type (**Figure 1*c***).

Slow-twitch oxidative myofibers (type I) are involved in sustained, tonic contractile events and maintain intracellular calcium concentrations at relatively high levels (100–300 nM) (10, 11). In contrast, fast-twitch glycolytic myofibers (type IIb) are used for sudden bursts of contraction and are characterized by brief, high-amplitude calcium transients and lower ambient calcium levels (less than 50 nM) (12). These properties of skeletal muscle fibers are dependent on the pattern of motor nerve stimulation, such that tonic motor neuron activity at low frequency (10–20 Hz) promotes the slow fiber phenotype, whereas phasic motor neuron firing at high frequency (100–150 Hz) results in fast fibers.

Myofiber Adaptability

The ability of skeletal muscle to remodel and change phenotypically can be demonstrated by cross-innervation experiments in which slow-twitch muscle (soleus) reinnervated with nerve fibers that normally supply fast-twitch muscle (flexor digitorum longus) results in an increase in contractile speed. Conversely, innervation of fast-twitch muscle with nerve fibers normally found on soleus muscle causes slower contraction (1). These studies established that specific impulse patterns delivered by motor neurons exert a phenotypic influence on the muscles they innervate and that myofibers are capable of remodeling. Further studies using electrical stimulation to modify neural activity delivered to a target muscle corroborate the cross-reinnervation data by showing predicted changes in myosin isoforms (13, 14). Exercise training also induces changes in skeletal muscle by transforming the myofibers to an increased oxidative metabolism and inducing fiber-type transitions from type IIb → type IId/x → type IIa → type I. To everyone's chagrin, upon cessation of exercise training these myosin heavy-chain isoform transitions and metabolic changes are reversed.

Neuronal stimulation reprograms gene expression in the myofiber primarily by using calcium as a second messenger. The input received from motor neurons via acetylcholine receptors generates a depolarization of the membrane, which reaches the sarcolemma transverse (T)-tubular membrane (15). The voltage-operated calcium channel or L-type calcium channel (the dihydropyridine receptor) in the T-tubules interacts with a skeletal muscle-specific sarcoplasmic reticulum calcium-release channel, the ryanodine receptor (RyR1) (16). This physical interaction causes the RyR1 to open and release calcium from the sarcoplasmic reticulum. The changes in intracellular calcium concentrations determine muscle contraction and activate signaling pathways. The process of myofiber transformation is regulated by multiple signaling pathways, many of which converge on each other, culminating in the activation and, perhaps, repression of a myriad of genes involved in remodeling of skeletal muscle.

SIGNALING PATHWAYS IN MYOFIBER REMODELING

Myofibers respond to physiological and pathological signals by transforming and remodeling to adapt to the environmental demands. This adaptation is accomplished through signal transduction by which an extracellular signal interacts with receptors at the cell surface, activating factors in signaling pathways and ultimately remodeling the myofiber by effecting a change in gene expression.

Myocyte Enhancer Factor-2 and Histone Deacetylases

It is well recognized that the myocyte enhancer factor-2 (MEF2) transcription factors, in conjunction with multiple myogenic regulatory factors, play a dominant role in muscle formation by activating muscle-specific genes and that the MEF2/histone deacetylase (HDAC) signaling pathway plays an important role in the transformation of myofibers in response to intracellular calcium fluctuations incurred by the effects of external physiological signals (**Figure 2**). MEF2 is a muscle-enriched transcription factor that binds to an A/T-rich DNA sequence in the control regions of numerous muscle-specific genes (17). There are four vertebrate *MEF2* genes, *MEF2A*, *-B*, *-C*, and *-D*, which are expressed in distinct, but overlapping, patterns during embryogenesis and in adult tissues. In the mouse, *Mef2c* gene expression is detected in developing skeletal muscle concomitant with activation of the skeletal muscle differentiation gene program. High levels of MEF2 proteins are clearly detectable in developing muscle lineages during embryogenesis (18). MEF2A protein appears as cells enter the differentiation pathway, and MEF2C is expressed late in the differentiation program. Studies in primary cell cultures of human skeletal muscle cells showed that various stimuli, such as addition of insulin, hydrogen peroxide, osmotic stress, and activation of AMP-activated protein kinase (AMPK) resulted in activation of MEF2D DNA binding (19).

Myocyte enhancer factor-2 (MEF2): a family of transcription factors that activates muscle-specific genes

HDAC: histone deacetylase

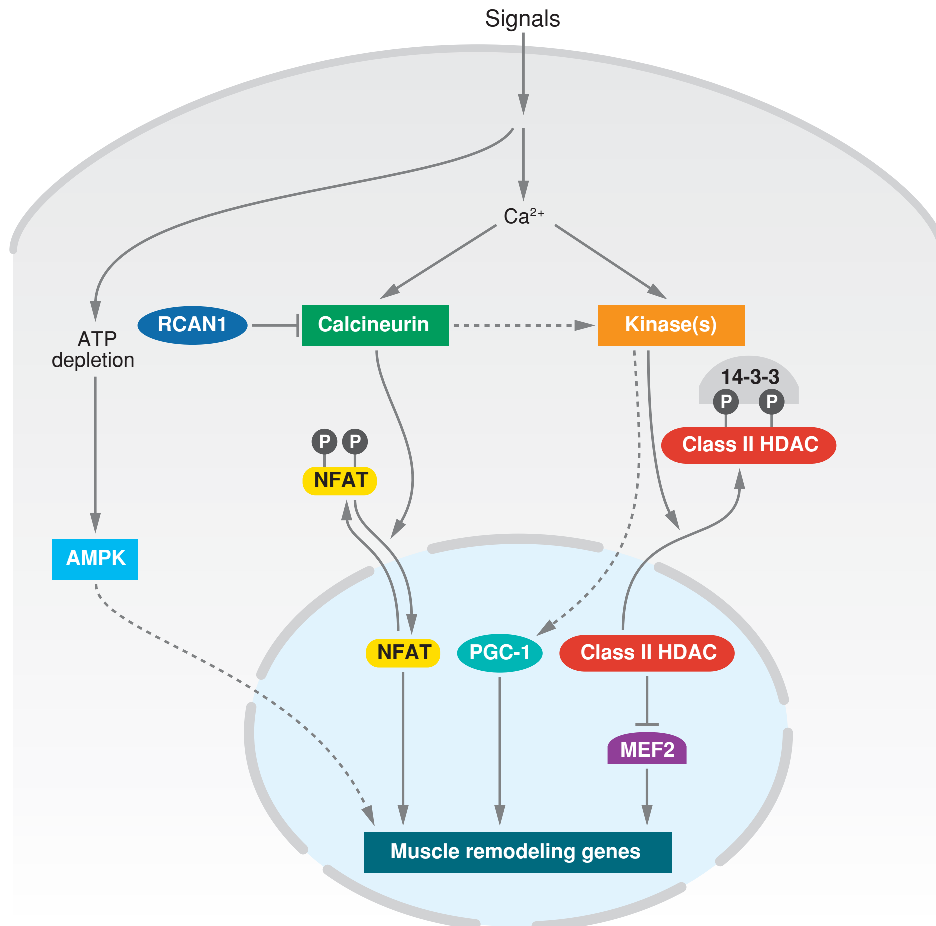

Figure 2

Signaling pathways activate skeletal muscle remodeling genes. In response to physiological demands, intracellular calcium concentration is elevated, activating the calcineurin/nuclear factor of activated T cells (NFAT) and MEF2/HDAC signaling pathways. In response to workload, ATP is depleted activating AMPK.

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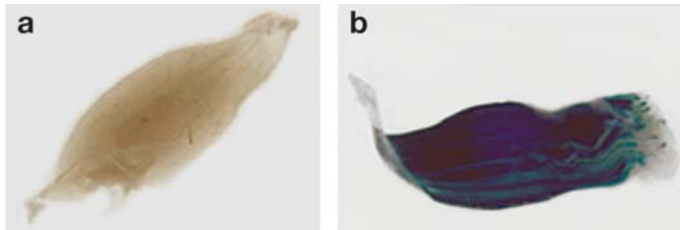

Figure 3

Exercise stimulates transcriptional activation of MEF2. (*a*) Soleus from sedentary MEF2 indicator mouse was stained with X-gal to detect *lacZ* expression. (*b*) Soleus from MEF2 indicator mouse subjected to three days of voluntary wheel running was stained with X-gal to detect *lacZ* expression.

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To monitor the transcriptional activity of MEF2 in vivo, a transgenic MEF2 sensor mouse that harbors a *lacZ* transgene under the control of three tandem copies of the MEF2 consensus DNA-binding site was generated (20). During embryogenesis, these mice express *lacZ* in developing cardiac, skeletal, smooth muscle, and neuronal cells. After birth, transgene expression is downregulated, although MEF2 protein levels are high, suggesting that MEF2 activity is repressed. A series of studies showed that MEF2 activity is controlled through association with class II HDACs, which bind to MEF2 and repress MEF2 activity (21–25). In response to various signals, HDAC kinases are activated and phosphorylate these HDACs, creating a docking site for intracellular chaperone protein 14-3-3 to bind HDAC and mask the nuclear localization sequence as well as induce a conformational change in HDAC that unmasks a nuclear export sequence, causing HDAC to exit from the nucleus and promoting MEF2 activity (26–28). Signal-dependent release of class II HDACs from MEF2 appears to play a role in skeletal muscle differentiation (22). Transitioning myofibers to a slow-twitch phenotype, using 10-Hz electrical stimulation, translocates HDAC4 from the nucleus to the cytoplasm and increases MEF2 activity, further supporting the role of class II HDACs in signaling pathways during skeletal muscle remodeling (29).

To identify factors that stimulate MEF2 activity, MEF2 sensor mice were subjected to voluntary wheel running and electrical stimulation of the sciatic nerve (30). These stimuli have been shown to promote a substantial degree of fiber-type transformation (type IIb to IIa to I) and to upregulate expression of proteins associated with oxidative metabolism, such as myoglobin. Following these exercise regimens, MEF2 is activated, as demonstrated by the expression of *lacZ* in the MEF2 sensor mice (**Figure 3**).

Further studies on MEF2 activation by exercise showed that this response is blocked when cyclosporine A, an inhibitor of the serine/threonine protein phosphatase 2B, calcineurin, is administrated. In addition, crossing transgenic mice overexpressing activated calcineurin in skeletal mice with the MEF2 sensor mice showed that MEF2 activity was dramatically activated by calcineurin signaling (30, 31). Furthermore, it was shown that the activation of both the MEF2 and calcineurin pathways promotes expression of muscle-specific genes, including *myoglobin*, *myosin heavy chain*, and *slow troponin I* (32, 33). These findings revealed cross talk between the MEF2/HDAC and calcineurin signaling pathways and delineated a molecular pathway in which calcineurin and MEF2 participate in the adaptive mechanisms by which myofibers acquire specialized contractile and metabolic properties as a function of changing patterns of muscle contraction induced by exercise (**Figure 2**).

Although many MEF2 gene targets are known, signaling pathways downstream of MEF2 are largely unknown. Analysis of the gene expression profile of mice lacking *Mef2c* identified a decrease in expression of a novel MEF2-regulated gene encoding a muscle-specific protein kinase Stk23/Srpk3, belonging to the serine arginine protein kinase (SRPK) family, which phosphorylates serine/arginine repeat-containing proteins (34). The *Srpk3* gene is specifically expressed in the heart and skeletal muscle from embryogenesis to adulthood and is controlled by

a muscle-specific enhancer with an essential MEF2-binding site. When the *Srpk3* gene is disrupted in mice, myofibers show an increase in centrally placed nuclei, a characteristic of many myopathies, and disorganized intermyofibrillar network in type II fibers. Overexpression of *Srpk3* in skeletal muscle causes severe myofiber degeneration and early lethality. These findings show that SRPK-mediated signaling plays important roles in muscle growth and homeostasis downstream of muscle-specific transcription regulated by MEF2.

Calcineurin/Nuclear Factor of Activated T Cells

Calcineurin, a heterodimeric protein phosphatase comprised of a calmodulin-binding catalytic A subunit and a calcium-binding regulatory B subunit, is specifically activated by sustained, low-amplitude calcium waves and is a sensor of contractile activity by sensing calcium fluctuations (35, 36). Signaling is initiated by sustained, low-amplitude calcium waves allowing calcium to bind calmodulin, which activates calcineurin via the regulatory subunit (37). Upon activation, calcineurin dephosphorylates nuclear factor of activated T cells (NFAT), resulting in translocation of NFAT from the cytoplasm to the nucleus where it associates with other transcription factors to activate specific sets of calcium-dependent target genes (38) (**Figure 2**). Among the transcription factors that may serve as an important partner for NFAT proteins in myocytes is MEF2 (17, 30, 31).

Overexpression of activated calcineurin in myoblasts modulates myofiber gene expression by activating a subset of genes, which are associated with type I myofibers, such as myoglobin and troponin I slow (32, 39). To examine the effect of calcineurin in vivo, transgenic mice, harboring a muscle creatine kinase promoter driving activated calcineurin, were generated and shown to upregulate endogenous oxidative proteins, such as myoglobin, in a dose-dependent manner and drive fast to slow myofiber transformation (30, 40), supporting the role of the calcineurin/NFAT pathway in myofiber remodeling. Additional evidence of the role of the calcineurin/NFAT pathway in fiber-type specificity is seen by a reduction in oxidative/slow type I fibers in mice lacking calcineurin A isoforms alpha or beta (41). In addition, conditional calcineurin β1 knockout mice, lacking the calcium-binding regulatory subunit specifically in skeletal muscle, display dramatic deficiencies in both myosin heavy-chain type I and IIa protein expression and a decrease in the number of slow fibers (42). These results further demonstrate that calcineurin activity regulates the slow fiber program. Notably, the conventional calcineurin A alpha or beta knockout mice show a reduction in muscle weight; in contrast, no significant weight reduction was seen with the skeletal muscle-specific conditional knockout calcineurin β1 mice. The difference observed in muscle mass between these two calcineurin knockout lines is most likely attributable to the elimination of calcineurin in all cells, including myogenic progenitors and myoblasts, in the conventional calcineurin knockout mice, whereas elimination of calcineurin is restricted to postdifferentiated myofibers in the mice lacking calcineurin β1 specifically in skeletal muscle.

Further evidence for a role of calcineurin in maintenance of the slow fiber phenotype is seen in the treatment of rats with cyclosporine A, an inhibitor of calcineurin activity, which results in an induction of glycolytic enzymes and a decrease in slow type I contractile proteins with a transformation toward a fast phenotype (32, 43). These findings are further supported by a study showing that calcineurin inhibitors block upregulation of type I isoforms of myosin in a muscle regenerating system (44) and in a primary skeletal muscle cell culture (45), showing that calcineurin activity is required for induction and maintenance of the slow type I myofiber gene program. Furthermore, studies in mice have shown that NFAT activity is higher in slow compared to fast muscles

NFAT: nuclear factor of activated T cells

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Skeletal muscle hypertrophy: an increase in the size of muscle fiber, which increases muscle mass

(46). Introduction of a synthetic peptide inhibitor of calcineurin-mediated NFAT activation into the soleus leads to downregulation of slow myosin heavy-chain expression and an upregulation of myosin heavy-chain type IId/x (46). These results indicate that NFAT activity is required for maintenance of slow myosin heavy-chain gene expression and potentially is involved in the repression of the fast myosin heavy-chain IIx gene. Although the mechanism whereby calcineurin signaling induces the slow fiber gene via NFAT and MEF2 seems clear, it remains unclear how the fast fiber gene program is repressed by such signals.

Overexpression of a protein inhibitor of calcineurin, RCAN1 (previously known as MCIP-1) (47), has been shown to inhibit calcineurin activity in vivo. Stable mouse lines containing a conditional RCAN1 transgene were generated and crossed with a transgenic mouse line containing a skeletal muscle-specific promoter driving Cre recombinase. This strategy results in the expression of RCAN1 in skeletal muscle by using skeletal muscle-specific Cre recombinase to excise a region of DNA and place RCAN1 cDNA in the open reading frame. Using this Cre-ON approach, it was shown that the skeletal muscle of the mice overexpressing RCAN-1 has a decrease in calcineurin activity compared to wild-type mice (47), and most notably, these transgenic mice lack type 1 fibers. These findings show that calcineurin activity is essential for maintaining type I fibers.

In contrast, inconsistencies with the calcineurin/NFAT pathway model were shown by slow fiber-specific expression of a reporter gene (luciferase) controlled by expression of mutated forms of the slow troponin I promoter that lack NFAT- or MEF2-binding sites (48), and another group (49) showed that in vivo injections of a plasmid expressing activated calcineurin did not activate the slow myosin light chain promoter in soleus or extensor digitorum longus muscle. In addition, mice lacking NFATc2 or -c3 exhibit reduced muscle fiber size or number, respectively, but no significant change in the proportions of fiber types (50, 51). However, multiple studies have clearly shown and confirmed that skeletal muscle hypertrophy is not dependent on calcineurin activity (42, 52, 53).

Calsarcins, a family of sarcomeric proteins, have been identified as regulators of calcineurin by interacting with calcineurin and colocalizing with the z-disk protein, alpha-actinin (54). Cell culture experiments demonstrate that calsarcin-1 suppresses calcineurin activity in vitro. Mice deficient in calsarcin-1 showed that in vivo calcineurin activity and signaling are enhanced in striated muscle, indicating that the absence of calsarcin-1 relieves calcineurin inhibition (55). Consistent with the hypothesis that calcineurin activity promotes type I fibers, calsarcin-1 deficient mice show an increase in type I fibers. Through protein interactions, calsarcins serve to tether calcineurin to the sarcomere, placing it in proximity to a unique intracellular calcium pool where it can interact with specific upstream activators and downstream substrates. These findings identify the sarcomere as a site of regulation of the calcineurin/NFAT signaling pathway, via calsarcin-1, and implicate the sarcomere as an active modulator of myofiber remodeling at the level of gene transcription.

Calcium/Calmodulin-Dependent Protein Kinase, Protein Kinase C, and PKCmu/Protein Kinase D

Class II HDACs (HDAC4, HDAC5, HDAC7, and HDAC9) are highly expressed in skeletal muscle and directly bind MEF2, repressing expression of MEF2-dependent genes. It has been shown that binding of class II HDACs to MEF2 is mediated by 18 conserved amino acids in the amino-terminal extensions of class II HDACs, a domain that is lacking in class I HDACs (56). Phosphorylation of class II HDACs results in their export from the nucleus and activation of MEF2-dependent genes (22), leading to muscle remodeling. Because of the critical

role of HDAC phosphorylation in regulating myocyte differentiation and remodeling, there has been intense interest in identifying the kinase(s) responsible for class II HDAC nuclear export and inactivation in vivo. In vitro studies have shown that signaling by calcium/calmodulin-dependent protein kinase (CaMK) results in phosphorylation of class II HDACs, promoting shuttling of HDACs from the nucleus to the cytoplasm and activation of MEF2 (22). Further evidence supporting the role of CaMK in skeletal muscle remodeling is seen when addition of a CaMK inhibitor, KN-62, blocks HDAC-green fluorescent protein (GFP) translocation from the nucleus to the cytoplasm in response to slow fiber-type electrical stimulation in isolated myofibers (29). In addition, CaMKII is known to be sensitive to the frequency of calcium oscillations (57) and is activated during hypertrophic growth and endurance adaptations (58). The notion that CaMK is involved in muscle remodeling is supported by ectopically overexpressing CaMKIV in skeletal muscle and observing an increase in type I fibers. However, CaMKIV is not expressed endogenously in skeletal muscle, and mice lacking CaMKIV have normal fiber-type composition with an increase in slow myosin heavy-chain isoform in the soleus muscle (59). Therefore, although exogenous CaMKIV promotes transformation of myofibers to a slow phenotype, it is unlikely that CaMKIV plays a role in physiological skeletal muscle remodeling. Furthermore on the basis of a biochemical assay for the HDAC kinase, there appears to be another HDAC kinase that is induced in response to calcineurin signaling (at least in the heart); this kinase is resistant to CaMK inhibitors and does not bind to calmodulin (60).

To further define the signaling pathways leading to the phosphorylation of class II HDACs, the potential of multiple kinase pathways to stimulate HDAC5 nuclear export was examined and showed that the protein kinase C (PKC) pathway promotes nuclear export of HDAC5 by stimulating phosphorylation of the 14-3-3 docking sites (61). Further studies showed that PKCmu/protein kinase D (PKD) acts as a downstream effector kinase of PKC and stimulates the nuclear export of HDAC5. On the basis of expression of PKD in skeletal muscle, in vitro studies, and transgenic mouse lines (M.S. Kim, R. Bassel-Duby, and E.N. Olson, unpublished data), we speculate that PKD is an important skeletal muscle HDAC kinase.

PKC: protein kinase C

PKD: PKCmu/protein kinase D

Exercise studies performed in humans showed that atypical PKC isoforms (aPKCzeta, -lambda, -mu), but not conventional PKC isoforms (cPKCalpha, -beta1, -beta2, and -delta), are activated by exercise in contracting muscle (61a, 61b). These findings are consistent with the transgenic mouse data showing a role for PKDmu/PKD in skeletal muscle remodeling and suggesting a potential role for atypical PKC in the regulation of skeletal muscle function and metabolism during exercise in both mice and humans.

Involvement of skeletal muscle signaling pathways is seen with other members of the PKC family. PKC-theta, a member of the novel PKC subfamily, is the predominant PKC isoform expressed in skeletal muscle (62, 63). In adult skeletal muscle, PKC-theta is expressed primarily in type II glycolytic fibers (64). Studies using lipid emulsion infusion in rats showed that activation of PKC-theta is associated with skeletal muscle insulin resistance (65, 66). Most recently, mice lacking PKC-theta were shown to be protected against fat-induced defects in skeletal muscle insulin signaling (67), indicating that PKC-theta is a crucial component mediating fat-induced insulin resistance in skeletal muscle.

Peroxisome Proliferator-Activated Receptor Delta and Peroxisome-Proliferator-Activated Receptor Gamma Coactivator-1 alpha

Enhanced oxidative capacity and metabolic efficiency of skeletal muscle is seen

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PGC-1α: peroxisome-proliferator-activated receptor-gamma coactivator-1

PPAR: peroxisome proliferator-activated receptor

mTOR: mammalian target of rapamycin

IGF-1: insulin-like growth factor

following exercise training, in part owing to a dramatic increase in mitochondrial content resulting from changes in the expression of genes that increase mitochondrial biogenesis. The transcriptional coactivator peroxisome-proliferator-activated receptor-gamma coactivator-1 (PGC-1α) is considered a master regulator of mitochondrial gene expression and has been shown to activate mitochondrial biogenesis and oxidative metabolism (68–70). PGC-1α, expressed in brown fat and skeletal muscle, is preferentially enriched in type I myofibers. Studies performed in humans and rodents show that endurance exercise induces PGC-1α mRNA and protein expression (71–74). Skeletal muscle-specific overexpression of PGC-1α in transgenic mice resulted in an increase in type I fibers in white vastus and plantaris muscles (75). These transgenic mice also exhibited an increase in proteins involved in metabolic oxidation and, most importantly, displayed an increase in muscle performance and a decrease in muscle fatigue. Using fiber-type-specific promoters, it was shown that PGC-1α activates transcription in cooperation with MEF2 proteins and serves as a target for calcineurin signaling, which has been implicated in slow fiber gene expression. These findings indicate that PGC-1α is a principle factor modulating muscle fiber type and outline a combinatorial effect of activation of multiple signaling pathways evoked during skeletal muscle remodeling.

Peroxisome proliferator-activated receptor (PPAR) delta is a major transcriptional regulator of fat burning in adipose tissue through activation of enzymes associated with long-chain fatty-acid β-oxidation (76) and is the predominant PPAR isoform present in skeletal muscle. PPAR delta was overexpressed in skeletal muscle, resulting in a fiber-type switch to increase the number of oxidative myofibers (77), and the mice with activated PPAR delta showed an increase specifically in type I fibers and the ability to continuously run up to twice the distance of wild-type littermates (78). Because PPARs associate with PGC-1, it is conceivable that exercise induction of PGC-1α may activate PPAR delta and induce myofiber remodeling.

Ras/Mitogen-Activated Protein Kinase

High-intensity exercise (79) and electrostimulation (80) have been shown to activate the Ras/mitogen-activated protein kinase (MAPK) pathway. In vivo studies showed that Ras-dependent pathways affect both fiber size and fiber type (81). Introduction of exogenous MAPK-activating Ras (RasV12S35) into denervated regenerating muscle fibers induced the expression of type I myosin heavy chain but did not affect myofiber size. The Ras/MAPK pathway mediates the switch in a myosin heavy-chain gene induced by slow motor neurons in regenerating muscle. In contrast, activation of the PI3K/protein kinase B (Akt) pathway by Ras induces muscle growth but does not alter fiber-type distribution, corroborating the studies performed with overexpression of activated Akt in skeletal muscle (82).

Insulin-Like Growth Factor, Akt, and Mammalian Target of Rapamycin

As exemplified by the physique of a bodybuilder, skeletal muscle can adapt to workload by changing myofiber size. Studies using a functional overload model of the rat plantaris showed that the Akt/mammalian target of rapamycin (mTOR) signaling pathway is activated during hypertrophy (52), corroborating studies that showed hypertrophy of cultured myoblasts in response to insulin-like growth factor (IGF-1) to be dependent on a PI3K/Akt/mTOR pathway (83) (**Figure 4**). Transgenic mice overexpressing constitutively active Akt, specifically in skeletal muscle, showed an increase in muscle mass owing to an increase in muscle fiber size (84). Direct and indirect targets of Akt (also referred to as protein kinase B) include mTOR and glycogen synthase kinase 3.

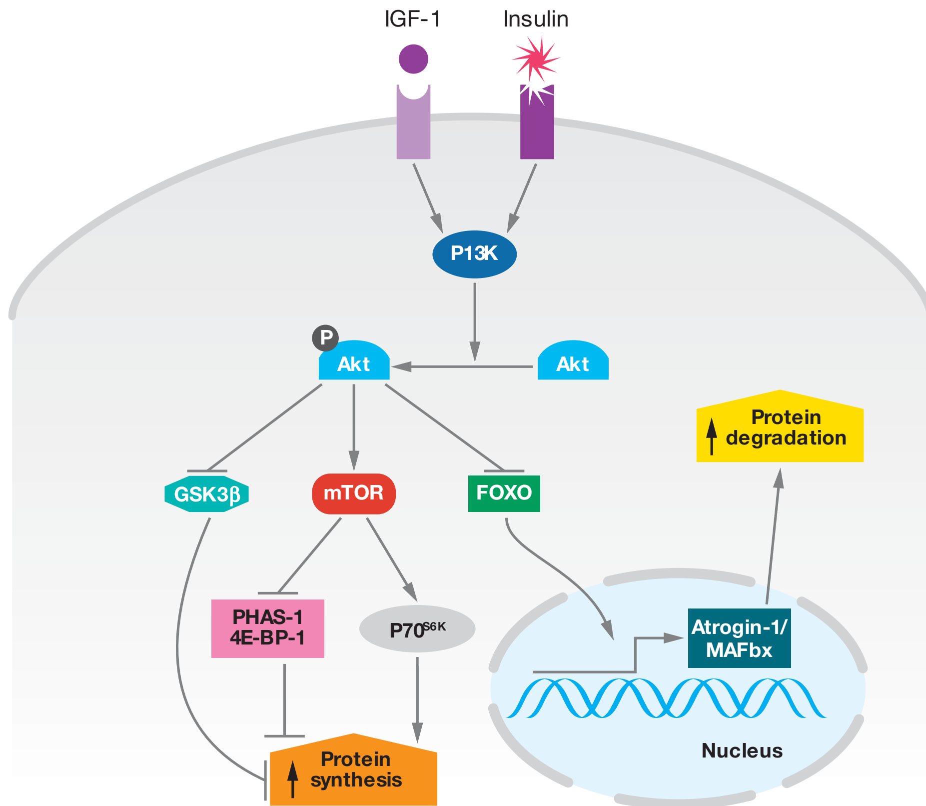

Figure 4

Signaling pathways in hypertrophy and atrophy. In response to IGF, the Akt/mTOR signaling pathway is activated. Phosphorylated Akt phosphorylates FOXO, inhibiting FOXO nuclear entry. Activation of mTOR by Akt promotes protein synthesis and increases muscle mass, resulting in hypertrophy. In disease states, Akt is not activated, and unphosphorylated FOXO enters the nucleus and induces the muscle atrophy F-box (MAFbx)/atrogin-1/ expression gene, promoting muscle atrophy.

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mTOR is a kinase, sensitive to rapamycin, whose downstream targets, p70^{S6K} and PHS-1/4E-BP1 increase protein translation initiation and elongation, promoting protein synthesis. Plantaris muscle from rats subjected to muscle overload and treated with rapamycin, an inhibitor of mTOR activity, showed similar activation of Akt in response to increased workload but did not show any change in myofiber size or weight, demonstrating that activation of mTOR is necessary for skeletal muscle hypertrophy.

CLINICAL SIGNIFICANCE

Understanding the signaling pathways that control myofiber remodeling is pertinent to several important human diseases, including inherited myopathies, systemic metabolic diseases, and common cardiovascular disorders. In muscular dystrophy, certain fibers are preferentially affected with degenerative changes; in diabetes, skeletal muscle contributes to exercise intolerance; and in heart failure patients, skeletal muscle atrophy is associated with a subgroup of patients at extremely high risk. The factors in signaling pathways in muscle remodeling may be viable therapeutic targets for the treatment of skeletal muscle disease.

Muscular Dystrophy

Duchenne muscle dystrophy (DMD) is a debilitating, life-threatening X-linked recessive muscular disorder, caused by mutations in the dystrophin gene. A strategy used to alleviate DMD involves upregulation of utrophin, an autosomal homolog of dystrophin. Activation of calcineurin stimulates the expression of utrophin through an NFAT site in the utrophin promoter (85). Moreover,

Skeletal muscle atrophy: a decrease in myofiber size, ultimately generating a decrease in total muscle mass

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GLUT4: glucose transporter 4

overexpressing activated calcineurin in skeletal muscle of *mdx* mice, lacking the dystrophin gene, results in an increase in utrophin expression, an increase in oxidative fibers, and a decrease in pathology, suggesting that expression of exogenous calcineurin in skeletal muscles provides substantial beneficial effects on dystrophic muscle fibers (86). In addition, it was observed that in skeletal muscle of DMD patients, fast myofibers are preferentially affected with degenerative changes, whereas slow myofibers are relatively spared (87). Introduction of calcineurin in skeletal muscle not only activates utrophin expression but should also promote the formation of type I fibers, displacing the fast fibers that are more prone to damage. It will be of interest to examine whether the induction of slow type I fibers is sufficient to ameliorate DMD. It is encouraging that overexpressing IGF-1 within skeletal muscles reduces the severity of the dystrophy, demonstrating that modification of the myofiber has beneficial therapeutic effects in DMD (88).

Type 2 Diabetes Mellitus and Obesity

Skeletal muscle accounts for the majority of insulin-stimulated glucose uptake in humans and rodents. The insulin signaling pathway in skeletal muscle is controlled by a series of phosphorylation events linking initial activation of the insulin receptor to downstream substrates and ultimately translocating glucose transporter 4 (GLUT4) to the plasma membrane to bind and uptake glucose. A major contributing factor to the progressive development of type 2 diabetes is reduced insulin-stimulated whole-body glucose disposal, with the greatest defects attributed to skeletal muscle. Impaired insulin signal transduction (89) and defects in GLUT4 trafficking (90) are associated with skeletal muscle insulin resistance in individuals with type 2 diabetes. Fiber-type specific differences are seen in the insulin signal transduction pathway. In human skeletal muscle, insulin-stimulated glucose transport directly correlates with the percentage of slow-twitch muscle fibers, suggesting that a reduced skeletal muscle type I myofiber population may be one component of a multifactorial process involved in the development of insulin resistance (91). In fact, slow-twitch oxidative skeletal muscle has greater insulin binding capacity as well as increased insulin receptor kinase activity and autophosphorylation compared with fast-twitch glycolytic skeletal muscle (92). Furthermore, muscles with a greater percentage of oxidative myofibers have a higher content of GLUT4 (93). Overexpression of activated calcineurin in skeletal muscle of transgenic mice evokes an increase in type I myofibers and leads to improved insulin-stimulated glucose uptake (in association with increased expression of the insulin receptor, Akt, and GLUT4) compared to wild-type littermates (94). Interestingly, such mice are protected against glucose intolerance when maintained on a high-fat diet. These results validate calcineurin as a target to improve insulin signal transduction, enhance GLUT4 to correct glucose transport defects, and improve glucose homeostasis in diabetic individuals.

A non-insulin-dependent pathway regulating glucose transport and GLUT4 translocation to the plasma membrane and T-tubules in skeletal muscle involves AMPK a heterotrimeric protein that senses increases in AMP-to-ATP and creatine-to-phosphocreatine ratios via a mechanism that involves allosteric and phosphorylation modifications (95). AMPK is activated in skeletal muscle in response to exercise, phosphorylating target proteins along diverse metabolic pathways, resulting in an increase of ATP-generating pathways, such as glucose uptake and fatty-acid oxidation (96, 97). Studies using AICAR, a pharmacological activator of AMPK, in addition to transgenic overexpression of dominant-negative mutants of AMPK showed conclusively that AMPK activation increases skeletal muscle glucose transport by translocating GLUT4 to the membrane, comparable to the effect seen with exercise. These findings point to the AMPK pathway

as a potential target for therapeutic strategies to restore metabolic balance to type 2 diabetic patients.

Differences in muscle fiber composition may also play a role in determining susceptibility to dietary obesity. Skeletal muscle in obese individuals exhibits reduced oxidative capacity, increased glycolytic capacity, and a decreased percentage of type I fibers (91, 98). PPARs comprise a family of nuclear hormone receptors that mediate the transcriptional effects of fatty acids and fatty-acid metabolites. Transgenic mice overexpressing PPAR delta in skeletal muscle exhibited an increase in oxidative myofibers and a reduction in adipocyte size (99). Treatment of mice with the PPAR delta agonist GW501516 promoted an increase in expression of genes involved in oxidative fibers and mitochondrial biogenesis (78), and transgenic mice overexpressing activated PPAR delta in skeletal muscle kept on a high-fat diet gained 50% less weight than wild-type littermates, implying that expression of PPAR delta in skeletal muscle has a protective role against obesity.

Muscle Atrophy

Muscle atrophy is defined as a decrease in myofiber size, ultimately generating a decrease in total muscle mass, resulting from disuse, disease, or injury. Sarcopenia is an age-related chronic loss of muscle and strength; and cachexia is a form of muscle atrophy associated with muscle disease or damage to the nerve associated with the muscle, commonly leading to severe muscle wasting. Atrophic myofibers have a smaller cross-sectional area than normal myofibers and generate a reduced force. However, they generally do not undergo apoptosis but retain most of the structural features of normal muscle. There is much interest in understanding the signaling pathways that mediate atrophy in order to design therapies to inhibit these pathways and ultimately to alleviate muscle atrophy. Gene expression profiling of muscles harvested from multiple atrophy mouse models identified two genes, *muscle ring finger* (*MuRF*)*1* and *muscle atrophy F-box* (*MAFbx*)/*atrogin-1*, to be upregulated in atrophied muscle (100–102), and genetic deletion of these genes partially alleviated muscle atrophy. Both MuRF1 and atrogin-1/MAFbx proteins are E3 ubiquitin ligases responsible for the substrate specificity of ubiquitin conjugation as part of the ATP-dependent ubiquitin-proteosome proteolysis pathway involved in protein breakdown and degradation, which may conceivably result in a decrease of myofiber size. Interestingly, FOXO transcription factors, substrates of Akt, have been shown to induce atrogin-1/MAFbx expression (103, 104), connecting the molecular mediators of atrophy and the IGF-1/PI3K/Akt hypertrophy pathway (**Figure 4**). In the presence of IGF-1, PI3K/Akt is activated and phosphorylates FOXO, preventing it from entering the nucleus to activate atrophy-related genes. Skeletal muscle hypertrophy, following administration of IGF-1, is mediated by an increase in protein synthesis owing to Akt-induced phosphorylation, activation of mTOR, as well as a lack of MAFbx/atrogin-1 expression caused by Akt-induced phosphorylation of FOXO and nuclear exclusion. Muscle disuse leads to a reduction in PI3K/Akt activity and a decrease in FOXO phosphorylation, triggering nuclear import of FOXO and activation of the atrogin-1/MAFbx.

MuRF: muscle ring finger

NF-κB, a mediator of cytokine tumor necrosis factor (TNF) alpha during the inflammatory response, is activated during muscular disuse. Myofibers treated with TNF plus interferon-gamma fail to maintain contractile activities and show significant reductions in both MyoD and myosin heavy-chain gene expression, suggesting NF-κB involvement in cachexia by suppression of muscle-specific gene expression (105). Other studies using two separate mouse models, one designed to activate NF-κB and the other to inhibit NF-κB activity selectively in skeletal muscle, demonstrated that activation of the NF-κB pathway is sufficient to induce severe skeletal atrophy, resembling cachexia (106).

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Interestingly, it was shown that activation of NF-κB in muscle promotes proteolysis, as evidenced by elevated MuRF1 transcripts and protein levels, but does not activate cytokine signaling (106). Blocking the NF-κB pathway was shown to ameliorate muscle atrophy, suggesting new drug targets for clinical intervention during cachexia and other skeletal muscle atrophies.

Short periods of myofiber dennervation and muscle disuse provoke muscle atrophy, which in certain cases is reversible, leading to the concept of compensatory mechanisms to sustain myofiber composition following limited episodes of inactivity. Expression of Runx1, a DNA-binding protein, is strongly induced following myofiber denervation (100, 107). Using mice lacking Runx1 specifically in skeletal muscle, it was shown that expression of Runx1 is required to sustain denervated muscles from undergoing autophagy and severe muscle wasting (108).

A novel transcription factor named MusTRD1 (muscle TFII-I repeat domain-containing protein 1) was isolated because of its ability to bind the enhancer region of the troponin I slow gene (109). There are 11 mouse MusTRD isoforms, and studies have shown that MusTRD1 can act as a repressor of the troponin I (TnI) slow enhancer. It is hypothesized that modulation of the MusTRD isoform content within muscle fibers provides a means of differentially regulating downstream target genes in muscles of different fiber composition.

Anabolic Steroids

A timely issue in muscle remodeling is the use of androgens as anabolic agents to increase skeletal muscle mass and reduce body fat. Testosterone effects on skeletal muscle mass are dose dependent, with administration of supraphysiological doses leading to a substantial increase in muscle size and strength. Androgen receptors reside in muscle cells and most likely mediate the response to androgens. Interestingly, studies determining the effects of testosterone on muscle performance showed that testosterone administration is associated with an increase in leg power and strength but showed no change in muscle fatigability and no change in specific tension, indicating that testosterone-induced gains in muscle strength are reflective of an increase of muscle mass (110). The increase in muscle mass is hypertrophic growth, as it is associated with an increase in myofiber cross-sectional area, observed both in type I and type II myofibers (111) and is not due to an increase in the number of myofibers. No significant transition of myofiber specificity is seen because the relative proportion of type I and type II fibers does not change after administration of testosterone. In addition, no change is observed in the number of fibers per unit of muscle; however, an increase in myonuclear number is apparent and is hypothesized to be attributable to fusion with satellite cells (111). A study showing the long-term effects (about 10 years) of anabolic steroids on high-level power-lifter athletes showed a larger myofiber area with more myonuclei per fiber and more centralized nuclei in athletes using steroids (112). In addition, no differences were seen with regard to fiber-type proportions; however, the type I fibers had 61% larger area, and the type II fibers had a 44% larger area than athletes without steroids.

CONCLUSIONS

We are advised by physicians, family members, and various government agencies to improve our health status by exercising. During exercise, the motor neuron is stimulated, resulting in activation of multiple signaling pathways in the myofibers and remodeling skeletal muscle to adapt to the physiological demand. Great strides have been made in animal models to understand the signaling pathways involved in muscle remodeling. However, whether these signaling pathways are physiologically valid in humans needs to be confirmed, and the identity of additional transcription factors and target genes remains

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to be determined. Using multiple approaches, it has been elegantly shown that various components of signaling pathways promote fiber-type transitions, and it will be challenging and informative to determine how these pathways intercalate and connect to remodel myofibers. Once further advances are made, we will depend on somatic cell delivery systems to target muscle fibers and provide components that have been shown to reduce the severity of muscular and metabolic diseases. In the future, exercising might mean taking a "pill" to activate skeletal muscle remodeling via signaling pathways. But for now, it is no pain, no gain. Keep on running! Remember, it is better to burn out than fade away.

SUMMARY POINTS

1. Skeletal muscle is comprised of a complex array of heterogeneous muscle fibers that differ in their physiological and metabolic parameters.
2. In response to environmental demands, skeletal muscle remodels and changes phenotypically in order to sustain muscle performance.
3. Skeletal muscle remodels by activating signaling pathways to reprogram gene expression.
4. Changes in calcium-dependent signaling pathways play key roles in regulating muscle growth and metabolism.
5. Genetic and pharmacological modulation of skeletal muscle signaling pathways offer therapeutic opportunities for the treatment of muscle diseases.

FUTURE ISSUES TO BE RESOLVED

1. Many of the defined signaling pathways in skeletal muscle remodeling have been determined using transgenic or knockout mouse models. Confirmation is needed to determine whether these signaling pathways are physiologically valid and are involved in humans.
2. Although many signaling pathways have been identified in remodeling skeletal muscle, it remains unclear how these pathways are initiated by the motor neuron and how the pathways are intercalated.
3. The identity of additional transcription factors and target genes that are involved in skeletal muscle remodeling remains to be determined.
4. Discovery of a skeletal muscle somatic cell delivery system is needed to target muscle fibers and provide components that have been shown to reduce the severity of muscular and metabolic diseases.

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