

Activity-Dependent Signaling Pathways Controlling Muscle Diversity and Plasticity

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A variety of fiber types with different contractile and metabolic properties is present in mammalian skeletal muscle. The fiber-type profile is controlled by nerve activity via specific signaling pathways, whose identification may provide potential therapeutic targets for the prevention and treatment of metabolic and neuromuscular diseases.

Skeletal muscles comprise a spectrum of fiber types with different structural and functional properties. The fiber-type profile of different muscles is initially established during development independently of neural influences, but nerve activity has a major role in the maintenance and modulation of fiber-type properties in adult muscle. The molecular mechanisms of activity-dependent muscle fiber plasticity have been the object of intensive investigation, and several signaling pathways have been identified that appear to mediate the effects of activity on the muscle phenotype. This field of study is no longer confined to muscle physiology and sport sciences but is becoming central in clinical medicine and is likely to expand in the near future, since muscle activity has a beneficial effect in the prevention of many chronic diseases, and a better understanding of the mechanism of activity-dependent muscle changes may help to identify potential therapeutic targets.

The present short review will focus on *in vivo* studies with a view of relating the new mechanistic studies on signaling pathways to previous knowledge based on descriptive studies on the response of skeletal muscles to changes in activity patterns. In the first part, we will summarize basic notions on muscle fiber-type diversity and plasticity, which are especially relevant to the interpretation of signaling studies. In the second part, we will consider selected signaling pathways, focusing on the calcineurin (Cn)-NFAT pathway and on PPAR β/δ and PGC-1 α and -1 β . Other reviews in the last few years have covered various aspects of this subject (7, 10, 47, 68, 87).

Diversity and Plasticity of Fiber Types in Mammalian Skeletal Muscle

The simplistic distinction between fast-white muscles, specialized for phasic activity, and slow-red muscles, specialized for more continuous activity, was first called into question around 1968–1970, when the existence of different fast muscle fibers was recognized. Correlated histochemical-physiological studies of individual motor units (25) and ultrastructural studies (79) showed that fast-twitch fibers show large variations in mitochondrial content and level of succinate

dehydrogenase (SDH), and this correlates with a variable resistance to fatigue. At the same time, histochemical procedures for myosin ATPase led to the identification of two fast fiber populations, called type IIa and IIb, distinct from the slow type I fibers (13, 31), and correlated physiological-histochemical studies on individual motor units confirmed the existence of fatigue-sensitive fast motor units composed of IIb fibers staining weakly for SDH and fast fatigue-resistant units composed of IIa fibers staining strongly for SDH (14). Biochemical analyses showed that both fast-white and fast-red muscles have a high level of glycolytic enzymes despite the different oxidative enzyme complement: this led to the classification of slow oxidative, fast-twitch oxidative glycolytic and fast-twitch glycolytic muscle fibers (67). However, the nomenclature based on myosin ATPase staining (type I, IIa, and IIb) became the most used in the muscle field.

Subsequent studies led to the identification of a third fast fiber type with myosin heavy chain (MHC) composition different from IIa and IIb fibers. These fibers were called type IIx (77, 78, 81) or type IIc (8, 93), and the identity of IIx and IIc MHC was demonstrated by Western blotting analysis (41). However, it was not established whether this MHC is a different isoform or results from posttranslational modification of other MHCs until a distinct MHC-IIx transcript was identified in type IIx fibers (24). In rat skeletal muscle, type IIx fibers have strong SDH staining (78), and their maximal velocity of shortening is intermediate between that of IIa and IIb fibers (11, 12). Immunohistochemical and *in situ* hybridization analyses of muscle sections (24, 29) and biochemical and physiological studies of single fibers (11, 69) confirmed the existence of a spectrum of fiber types with pure or hybrid MHC composition, according to the scheme: type I \leftrightarrow I/IIa \leftrightarrow IIa \leftrightarrow IIa/IIx \leftrightarrow IIx \leftrightarrow IIx/IIb \leftrightarrow IIb. The situation is different in most human skeletal muscles in which MHC-IIb is not expressed: fibers typed as IIb based on ATPase staining are in fact IIx fibers based on MHC composition (85). In addition, in contrast to rat and mouse IIx fibers, human IIx fibers have the lowest level of SDH activity compared with all other fiber types (FIGURE 1A). Therefore, it is not justified to extrapolate results from transgenic mice to

human muscles assuming that each fiber type has similar properties in different species.

During postnatal development and regeneration, a default nerve activity-independent pathway of muscle fiber differentiation, which is controlled by thyroid hormone, leads to the activation of a fast gene program (15, 26). In contrast, the postnatal induction and maintenance of the slow gene program is dependent on

slow motoneuron activity. The muscle fiber-type profile undergoes further changes during postnatal life. At birth, most mouse fast muscles contain a significant proportion of slow type I fibers, most of which disappear during subsequent stages (1, 98) (FIGURE 1B). This finding must be taken into account for a correct interpretation of fiber-type changes in transgenic mice, such as those overexpressing activated Cn or PGC-1 α (see below).

Fiber-type switching can be induced in adult skeletal muscle by changes in nerve activity as shown by nerve cross-union and electrical stimulation studies (see Refs. 50, 70). A slow-to-fast switch in the direction I \rightarrow IIa \rightarrow IIx \rightarrow IIb can be induced by phasic high-frequency electrical stimulation, resembling the firing pattern of fast motoneurons (FIGURE 2A), whereas a fast-to-slow switch in the opposite direction IIb \rightarrow IIx \rightarrow IIa \rightarrow I can be induced by tonic low-frequency electrical stimulation, resembling the firing pattern of slow motoneurons. However, intrinsic differences between muscles and fiber types may limit the range of possible adaptations; thus fast muscles have the capacity to adapt in the range IIb \leftrightarrow IIx \leftrightarrow IIa and slow muscles in the range I \leftrightarrow IIa \leftrightarrow IIx (5). However, changes in the thyroid state can expand this range; for example MHC-IIb can be induced in slow muscles by the combined effect of hyperthyroidism and reduced activity consequent to mechanical unloading (16), whereas MHC I can be induced in fast muscles by hypothyroidism combined with chronic low-frequency stimulation or overloading (17, 38). The time factor is also important in promoting fiber-type transitions by hyperactivity or inactivity. Low-frequency stimulation for 2 mo does not lead to significant expression of MHC I in fast rat muscles (5, 92), but a fast-to-slow switch has been detected after 4 mo (100). A complete disappearance of type I fibers has been demonstrated in human skeletal muscle after long-term spinal cord injury (30) and in rat slow muscles 60–90 days following spinal cord isolation (36).

Developing and regenerating skeletal muscles appear to have a greater plasticity with respect to fiber-type switching compared with adult muscle. For example, the compensatory hypertrophy of the extensor digitorum longus (EDL) muscle induced by ablation of the synergistic tibialis anterior in newborn rats is accompanied by an increase in type I fibers and a complete switch of the fast fibers from type IIb to IIa/IIx, with correspondingly increased SDH staining (FIGURE 2B) (80). In contrast, no significant change in EDL fiber-type profile was observed when the same experiment was performed in adult animals. These findings should be taken into account when the effects of perturbations of signaling pathways in transgenic mice are interpreted, since changes in fiber-type composition could be due to the effect of the transgenes during early developmental stages and not in the adult. However, the response to functional overload

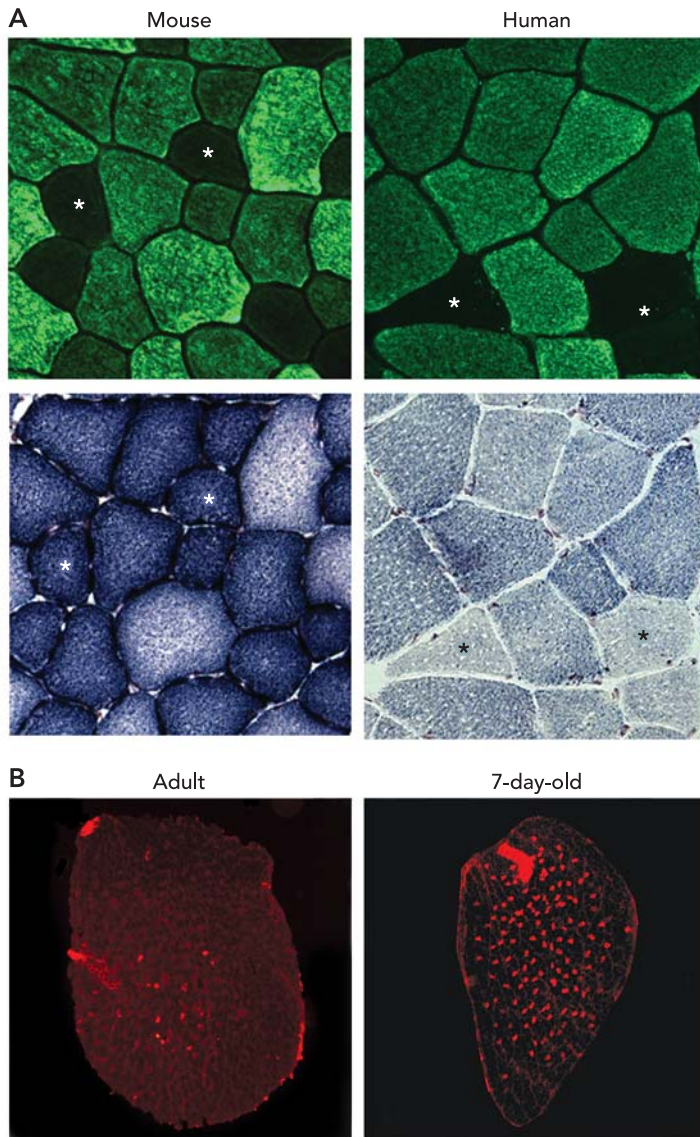


FIGURE 1. Variations in muscle fiber-type properties and composition according to species and stage of development

A: different metabolic properties of type IIx fibers in human and mouse skeletal muscle. Serial sections of mouse tibialis anterior muscle (left) and human vastus lateralis (right) were stained for succinate dehydrogenase (SDH) histochemical activity (bottom) or with an anti-MHC antibody (BF-35; see Ref. 78) that reacts with type I, IIa, and IIb fibers but not IIx fibers (top). Note that type 2X fibers (marked by asterisks in sections stained for SDH) are highly oxidative in mouse muscles, whereas they are the least oxidative fibers in human skeletal muscle. Also note that the level of oxidative enzymes is much higher in mouse compared with human muscle fibers. **B:** type I fibers disappear during postnatal development in mouse fast skeletal muscles. Sections of mouse plantaris muscle were stained with an antibody specific for MHC I (BF-D5; see Ref. 78). Note that type I fibers, which are well represented in the 1-wk-old mouse muscle, have almost completely disappeared in the adult plantaris. The section of 1-wk-old muscle is shown at higher magnification (double that of adult muscle) to better visualize type I fibers.

may vary in different experimental settings: for example, significant changes in the fiber-type profile do take place in the adult plantaris muscle after removal of the synergistic gastrocnemius.

Signaling Pathways Involved in Activity-Dependent Fiber-Type Remodeling

Ideally, the following conditions need to be fulfilled to validate the role of a signaling pathway in mediating the response to muscle activity.

1) The level of activity or expression of the postulated component and the response of downstream effectors (e.g., nuclear translocation of a transcription factor or activation of a reporter) should be compatible with the proposed role, for example should increase in response to appropriate patterns of electrical stimulation.

2) Gain-of-function approaches, such as overexpression of a constitutively active mutant, should cause activation of downstream effectors or target genes with effects similar to those induced by muscle activity.

3) Loss-of-function approaches, such as specific pharmacological inhibitors or knockout/knockdown of the postulated component or overexpression of dominant negative mutants, should block the response to muscle activity.

All these criteria are rarely satisfied, and the role of some pathways is only based on overexpression experiments in transgenic mice, a potentially misleading approach because 1) nonphysiological super-activation of a signaling pathway can stimulate or repress downstream effectors that are not affected under physiological conditions, and 2) a variety of compensatory adjustments may be induced when the transgene is overexpressed since early developmental stages. Several lines of evidence indicate that the Cn-NFAT pathway is the best candidate for an activity-dependent signaling pathway responsible for the maintenance of the slow gene program in adult slow muscles and the induction of this program in regenerating slow muscle. However, although the role of this pathway on myosin gene expression is well established, its role on the regulation of the muscle metabolic profile is less clear. Two factors that seem to play a major role in the control of the oxidative fiber-type profile are PPAR β/δ and PGC-1 α and -1 β . A scheme of the major pathways involved in activity-dependent regulation of the fiber-type profile is illustrated in **FIGURE 3**.

Cn-NFAT signaling

Cn is a Ca²⁺/calmodulin-regulated protein phosphatase that acts on the transcription factors of the NFAT (nuclear factor of activated T cells) family inducing their translocation to the nucleus and transcriptional activation. Cn is a heterodimer of catalytic (CnA) and regulatory (CnB) subunits. Both Cn subunits and NFATs comprise various isoforms. Skeletal muscles express CnB1,

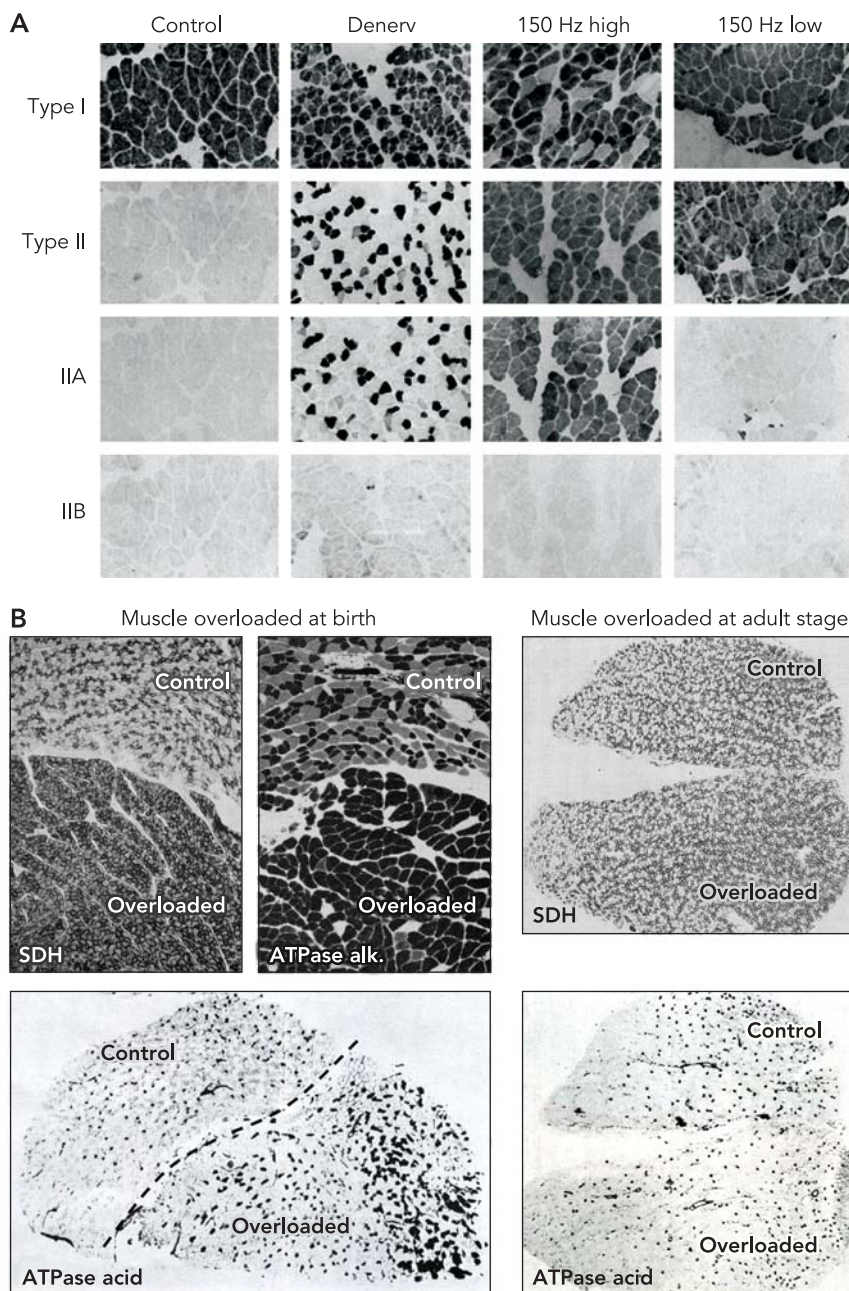


FIGURE 2. Muscle plasticity in adult and developing skeletal muscle
A: changes in MHC composition induced by inactivity and fast-type activity in slow skeletal muscle. Sections of control, denervated, and stimulated rat soleus muscles were stained with antibodies specific for type I (slow), all type II, type Ila, or type I Ib MHCs. In the group of rats used in these experiments, the control soleus was homogeneously composed of type I fibers. Note that expression of MHC Ila is induced in a proportion of fibers by denervation. Stimulation using the “150-Hz low amount” pattern (probably resembling the firing pattern of type I Ib motoneurons) leads to expression of MHC Ix (reactivity with anti-type II MHC antibody, no reactivity with anti-Ila and anti-I Ib antibodies), whereas stimulation using the “150-Hz high amount” pattern (probably resembling the firing pattern of type I la motoneurons) leads to abundant expression of both MHC Ila and Ix. However, a significant amount of MHC I persists in most stimulated fibers. Modified from Ref. 5. **B:** changes in fiber-type profile induced by functional overload in neonatal but not in adult skeletal muscle. Functional overload was induced in the rat EDL muscle by removal of synergistic tibialis anterior muscle. When the experiment is performed in newborn animals (left), the overloaded EDL shows increased SDH activity, almost complete disappearance of type I Ib fibers, identified by intermediate staining in the ATPase reaction following alkali preincubation, and increased proportion of type I fibers, staining dark in the ATPase reaction following acid preincubation. In contrast, when the same experiment is performed in adult rats (right), the EDL muscle undergoes hypertrophy but shows no significant change in the fiber-type profile. Modified from Ref. 80.

but not CnB2, and both CnA α and CnA β , but not cary. All the four Cn-dependent NFAT isoforms, called NFATc1–c4, are expressed in skeletal muscle.

Cn signaling. Fast muscles from transgenic mice overexpressing activated Cn under the control of muscle creatine kinase promoter have a slightly increased number of slow type I fibers (61) and an increased proportion of type IIa fibers with a concomitant decrease of type IIb fibers (18). A MHC fast-to-slow switch in the direction IIb \rightarrow IIx \rightarrow IIa \rightarrow I is shown by the finding that soleus muscle, which normally contains only type IIa and I MHC, has decreased MHC IIa and increased MHC I, whereas plantaris muscle, which normally contains IIb > IIx > IIa and very rare type I fibers, has decreased MHC IIb and increased MHC IIx, MHC IIa, and MHC I (90). The finding of a slight increase in slow fibers in fast muscles of Cn transgenic mice is not necessarily the result of a transformation of fast into slow fibers but may simply reflect a block in the postnatal disappearance of slow fibers that occurs in most rat and mouse fast muscles, as discussed above. In fact, in the plantaris muscle from Cn transgenic mice, the amount of MHC I was about 6% of all MHCs compared with about 1% in controls (90), but MHC I is known to decrease in the same muscle from about 6% in 7-day-old mouse to about 1% in adult (1). Overexpression of activated Cn also induces increased expression of myoglobin (61) and of enzymes responsible for mitochondrial oxidative phosphorylation and lipid metabolism (48, 75). This effect on the metabolic profile may be due to the upregulation of the transcription factor

PPAR β/δ and of the transcriptional coactivator PGC-1 α induced by activated Cn (48).

The physiological relevance of Cn *in vivo* is demonstrated by several studies using different loss-of-function approaches. A partial slow-to-fast fiber-type switching is induced in the adult soleus muscle of rats treated with the Cn inhibitor cyclosporine A (22). CnA α - and CnA β -null mice show a downregulation of the slow gene program in skeletal muscles (65), and mice with muscle-specific deletion of the Cn regulatory subunit CnB1 show impaired fast-to-slow fiber-type switching following functional overloading of the fast plantaris muscle (64). The upregulation of MHC I induced by slow motoneurons or by low-frequency stimulation in regenerating rat soleus muscle is prevented by the Cn inhibitors cyclosporin A and FK506, and by transfection with the Cn inhibitory protein domain from cain/cabin-1 (83). Cn is also involved in the maintenance of the slow muscle gene program because transfection with cain causes a switch from MHC I to MHC IIx gene expression in adult rat soleus muscle, as determined by *in situ* hybridization analysis (83).

The role of Cn activity in the activity-dependent regulation of the slow gene program is supported by the effect of the Cn modulatory protein DSCR1 (alias MCIP1 alias calcipressin alias RCAN1). Overexpression of DSCR1/MCIP1 inhibits Cn signaling (28, 73) through direct binding of its COOH-terminal domain to the enzyme active site (21). Transgenic mice overexpressing DSCR1 in skeletal muscle since early developmental stages show a normal muscle

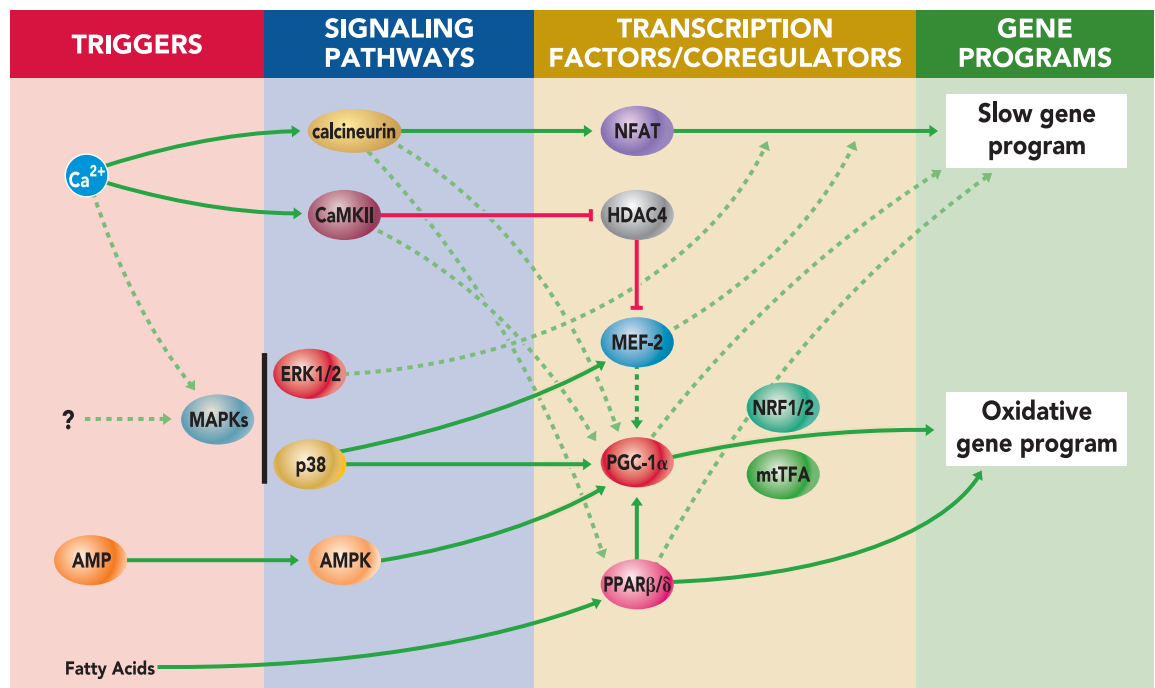


FIGURE 3. Scheme of the signaling pathways and transcriptional factors and coregulators involved in the control of slow gene program and oxidative gene program
The scheme highlights the major role of the calcineurin (Cn)-NFAT pathway in the regulation of the slow gene program and the role of PPAR β/δ and PGC-1 α and -1 β in the regulation of the oxidative gene program. Dotted lines indicate less established pathways.

fiber-type profile at birth (62). However, they begin losing slow fibers at *postnatal day 7*, and by *day 14* all type I fibers in the soleus muscle have switched to type IIa. This shows that Cn signaling is not required for the initial diversification of fast and slow fibers in the embryo but is necessary for the nerve activity-dependent maintenance of slow fibers in postnatal life. On the other hand, no significant change was detected in the type IIa/IIx/IIb profile in fast muscles of transgenic mice nor in the myoglobin, cytochrome c, and PGC-1 α expression in both fast and slow muscles, suggesting that basal muscle oxidative capacity is largely independent of Cn activity (62). It would be of interest to know whether the increase in oxidative enzymes induced by exercise or functional overload is blunted in animals overexpressing DSCR1.

NFAT signaling. Several lines of evidence indicate that the transcription factors of the NFAT family act as nerve activity sensors in skeletal muscle and control activity-dependent fiber-type specification. First, an NFATc1-GFP fusion protein expressed in isolated fibers from adult mouse flexor digitorum brevis, a predominantly fast-twitch muscle, shows a cytoplasmic localization in unstimulated fibers but translocates to the nucleus in fibers stimulated with a low-frequency pattern typical of slow motor units (45). In vivo studies showed that NFATc1-GFP has a predominantly cytoplasmic localization in the fast tibialis anterior muscle but a predominantly nuclear localization in the slow soleus muscle (95). NFATc1 nuclear import is rapidly induced in fast tibialis anterior muscle fibers by low-frequency electrical stimulation (FIGURE 4), whereas nuclear export is rapidly induced in slow soleus muscle fibers by inactivity consequent to denervation or anaesthesia (95). GSK3 β and CK1/2 appear to be the major kinases that regulate NFATc1 nuclear export after muscle activity in isolated muscle fibers from adult skeletal muscle (84).

Second, NFAT transcriptional activity monitored by different NFAT reporters is higher in slow than in fast muscles (54, 65). Furthermore, NFAT activity is decreased by denervation in slow muscles and is increased by electrostimulation of denervated muscles with a continuous low-frequency impulse pattern, mimicking the firing pattern of slow motoneurons, but not with a phasic high-frequency pattern typical of fast motoneurons (54).

Third, constitutively active NFATc1 is able to induce MHC I expression in regenerating denervated soleus muscle and also, although with less efficiency, in regenerating EDL muscle (54). Constitutively active NFATc1 transfected in adult fast muscle fibers stimulates a MHC I promoter and inhibits a MHC IIb promoter but is not able to induce MHC I expression.

NFATc2-null and NFATc3-null mice have essentially normal fiber-type composition (35, 37); however, this may be due to a redundant role of NFAT isoforms. In fact, the peptide inhibitor VIVIT, which blocks the

interaction of all NFATs with Cn, prevents the expression of MHC I induced by slow motoneuron activity in regenerating rat soleus muscle and causes a rapid downregulation of MHC_{slow} and upregulation of MHC IIx and MHC IIb transcripts in adult rat soleus (54).

The transcriptional activity of NFAT is known to require interaction with other transcription factors. Based on the response of a MEF2-dependent reporter, MEF2 has been postulated to interact with NFAT in the control of the slow gene program (102), and MEF2 can be regulated by CaMKII activity via HDAC (55). Studies on isolated fibers from adult skeletal muscle showed that slow-like but not fast-like stimulation is able to induce autophosphorylation and nuclear translocation of CaMKII, nuclear export of HDAC4, and activation of a MEF2 reporter (46). CaMKII is expressed in skeletal muscle and is stimulated by muscle activity (72). Another potential interactor of NFAT is represented by AP-1, which is an NFAT transcriptional cofactor involved in the induction of the immune response and is regulated by the ERK1/2 MAPKs. Cn-NFAT and MEK1-ERK1/2 are also interdependent in cardiomyocytes, where they interact in the regulation of cardiac gene expression and cardiac growth (76). ERK1/2 are activated during exercise and also during contraction of isolated muscle (40). A role of ERK1/2 in the regulation of fiber type-specific gene programs has been suggested by the observation that a constitutively active Ras mutant that is known to selectively activate ERK1/2 (RasV12S35) induces MHC

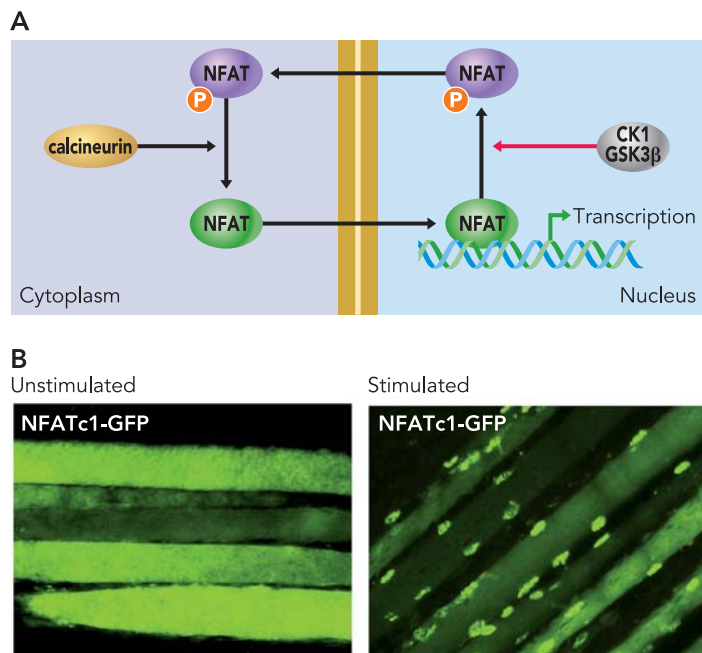


FIGURE 4. Full legend text

A: the scheme illustrates the nucleocytoplasmic shuttling of NFAT. Dephosphorylation of NFAT by calcineurin causes NFAT nuclear import, whereas phosphorylation of NFAT by GSK3 β and CK1/2 causes NFAT nuclear export. **B:** nuclear translocation of NFATc1 induced by electrical stimulation in fast muscle fibers. Rat tibialis anterior muscle was transfected with a plasmid coding for NFATc1-GFP fusion protein and 7 days later was stimulated for 2 h with a low-frequency impulse pattern resembling the firing pattern of slow motoneurons. Adapted from Ref. 94.

I expression in regenerating denervated rat soleus muscle (60). Conversely, a Ras-dominant negative mutant blocks the upregulation of MHC I induced by slow motoneuron activity in regenerating soleus. On the other hand, a Ras mutant that selectively activates the PI3K-Akt/PKB pathway (RasV12C40) affects cell growth but not fiber-type specification in the same system (60).

PGC-1 α and -1 β and PPAR β/δ

The transcription factor PPAR β/δ and the transcriptional co-activators PGC-1 α and -1 β have recently emerged as major players in the control of the oxidative metabolism of skeletal muscle fibers.

PGC-1 α and -1 β . PGC-1 α , which was originally identified as an interactor of PPAR γ in brown adipose tissue, is a transcriptional co-activator that can potentially stimulate transcriptional activity by interacting with transcription factors and basal transcriptional machinery (71). PGC-1 α stimulates mitochondrial biogenesis and oxidative enzymes in different cell types by inducing the expression of nuclear respiratory factors (NRF)-1 and 2, which control the transcription of many mitochondrial genes, and by co-activating the transcriptional activity of NRF-1. NRF-1 and -2 are in turn able to stimulate the expression of the mitochondrial transcription factor A (mtTFA), a mitochondrial protein essential for the replication and transcription of mitochondrial DNA.

PGC-1 α is expressed at higher levels in slow than fast muscles and is readily induced by exercise in both rodents and humans (6, 74, 91, 103). Overexpression of PGC-1 α in fast muscle fibers stimulates mitochondrial biogenesis and synthesis of oxidative enzymes and increases the proportion of type I fibers, thus making the muscles more resistant to fatigue (44). However, exercise in adult animals does not induce significant fast-to-slow fiber-type switch, and the increase in type I fibers in PGC-1 α transgenic mice might be due to the maintenance of the slow fibers present in neonatal fast muscles or to effects of the transgene during early developmental stages rather than in the adult, as discussed above. Exercise can also induce a rapid activation of PGC-1 α , possibly due to p38 MAPK-dependent phosphorylation, which leads to nuclear translocation of PGC-1 α and increased transcription of mitochondrial enzymes even before an increase in PGC-1 α expression level (101).

No significant difference in fiber-type distribution was observed in the soleus muscle from PGC-1 α knockout mice, but the level of transcripts of mitochondrial ATP synthase and cytochrome oxidase subunits was decreased (3). Fast muscles from knockout mice showed an increased level of slow genes, such as slow troponin I and SERCA2: this paradoxical effect was interpreted as the result of the hyperactivity of these animals secondary to alterations in the central nervous system (3). If this interpretation is correct, this would rule out a significant role of PGC-1 α in mediating activity-dependent changes in fiber-type profile. Slow mus-

cles in another PGC-1 α knockout model, in which mice have reduced locomotor activity, show diminished mitochondrial number and respiratory capacity; however, the fiber-type profile was not analyzed (43).

PGC-1 α is controlled by multiple pathways, including CaMK (32), MEF2 (23), Cn (32), p38 MAPK (2), and AMP-activated protein kinase (AMPK) (89, 91, 104). AMPK is a major regulator of muscle energy metabolism acting as a fuel and energy status sensor (see Ref. 34). AMPK activation leads to increased levels of mitochondrial oxidative enzymes (99), and this effect is probably mediated via PGC-1 α , since mice expressing a dominant-negative mutant of AMPK in muscle show no change in PGC-1 α and mitochondrial content after treatments that lead to AMPK activation and increased PGC-1 α and mitochondrial content (104). Two upstream kinases, LKB1 and CaMKK, are involved in the activation of AMPK (34). Mice expressing a dominant negative form of AMPK are intolerant to exercise (58), and the same is true for mice with muscle-specific LKB1 deficiency that causes lower levels of cytochrome c and PGC-1 α protein expression in the red region of the quadriceps (94).

PGC-1 β , a homolog of PGC-1 α , is also expressed at high levels in skeletal muscle and has been reported to be especially expressed in type IIx fibers (4). In contrast with PGC-1 α , PGC-1 β expression is not changed by exercise or denervation (39). Overexpression of PGC-1 β in transgenic mice stimulates mitochondrial biogenesis and oxidative enzymes with an increase in type IIx fibers (4). PGC-1 β -null mice show reduced expression of oxidative phosphorylation genes and mitochondrial content in soleus muscle; however, the fiber-type profile in skeletal muscle was not examined (42, 86, 96).

PPAR β/δ . The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that bind DNA as heterodimers with retinoid X receptors (RXRs). The three PPAR isoforms in mammals, α (NR1C1), β/δ (NR1C2), and γ (NR1C3), are activated by lipids and affect lipid metabolism in different tissues. PPAR β/δ , which is several-fold more abundant than either PPAR α or PPAR γ in skeletal muscle (59), is expressed at higher levels in slow/oxidative compared with fast/glycolytic muscles (97) and is induced by endurance training in mice (52) and by acute exercise in humans (53). Muscle-specific overexpression of wild-type or constitutively active PPAR β/δ leads to a more oxidative fiber-type profile with increased mitochondrial DNA, upregulation of some slow contractile protein genes, and increased resistance to fatigue (52, 97). These effects appear to be a direct effect of PPAR β/δ activation, as levels of PGC-1 α remain unchanged (97). Similar effects can be produced by treatment with specific PPAR β/δ agonists, such as GW501516. The physiological relevance of these results has been validated by the finding that muscle-specific knockout of PPAR β/δ causes a slow-to-fast fiber-type switching

with downregulation of MHC I and upregulation of MHC IIb transcript levels in the gastrocnemius muscle, and a concomitant switch toward a less oxidative fiber-type profile with downregulation of PGC-1 α , mtTFA, and many genes involved in oxidative phosphorylation, although the mitochondrial DNA content was unchanged (82).

Clinical Implications: Activity-Dependent Signaling Pathways as Potential Therapeutic Targets

The identification of the signaling pathways that control muscle fiber-type diversity may provide potential new targets for the treatment and prevention of different disorders ranging from metabolic to neuromuscular diseases. Two specific conditions that might benefit from a shift in the muscle fiber-type profile are Type 2 diabetes and muscular dystrophy.

Insulin resistance and Type 2 diabetes

Type 2 diabetes and its complications, including cardiovascular disease, represent a prevalent and expanding health threat in modern societies. The beneficial role of physical exercise in preventing insulin resistance, often associated with obesity and Type 2 diabetes, is well established (see Ref. 27). Exercise promotes a shift to a more oxidative fiber-type phenotype, thus reducing insulin resistance because glucose transport and lipid oxidation are more efficient in oxidative fibers. Conversely, insulin resistance and Type 2 diabetes are accompanied by a reduction in muscle mitochondrial content and oxidative enzymes (49, 56, 66), and this is also found in healthy offspring of patients with Type 2 diabetes (57). Experimental studies support the concept that skeletal muscle reprogramming to an oxidative phenotype can protect against the development of insulin resistance and Type 2 diabetes. For example, transgenic mice overexpressing activated Cn in skeletal muscles show reduced insulin resistance after high-fat diet (75). Mice overexpressing activated PPAR β/δ or treated with the PPAR β/δ -selective agonist GW501516 also showed reduced weight increase and improved glucose tolerance after high-fat diet (see Refs. 9, 51). In contrast, muscle-specific disruption of PPAR β/δ causes a shift to a less oxidative fiber-type profile that precedes the development of obesity and Type 2 diabetes (82). PGC-1 α has also been suggested to prevent diabetes via changes in oxidative energy metabolism; however, PGC-1 α -null mice are surprisingly less susceptible to diet-induced insulin resistance than wild-type controls (43). AMPK activation and anti-diabetic drugs that act via AMPK activation, such as metformin and rosiglitazone, also have a beneficial effect in the prevention and treatment of insulin resistance and diabetes (see Ref. 34). Taken together, these results suggest that increasing the proportion of oxidative

muscle fibers by stimulating the Cn, AMPK, PGC-1 α , and PPAR β/δ pathways may improve insulin-stimulated glucose transport and help to overcome the mitochondrial dysfunction and metabolic defects associated with insulin-resistant states.

“The identification of the signaling pathways that control muscle fiber-type diversity may provide potential new targets for the treatment and prevention of different disorders...”

Muscular dystrophy

A fiber-type shift in the direction IIB \rightarrow IIX \rightarrow IIA \rightarrow I has been suggested to mitigate the progression of muscular dystrophy. The dystrophin-deficient *mdx* mouse, a model of Duchenne muscular dystrophy, shows attenuated dystrophic pathology and improved resistance to contraction-induced injury when crossed with mice expressing an activated Cn mutant (18, 88). On the other hand, targeted inhibition of Ca²⁺/calmodulin signaling exacerbates the dystrophic phenotype in *mdx* mouse muscle (19). This effect may be explained by the fact that the transition to a more oxidative muscle fiber phenotype induced by Cn activation is accompanied by increased utrophin A expression that could compensate for the lack of dystrophin (20). Overexpression of PGC-1 α has also been found to partially rescue *mdx* pathology (33). However, an entirely opposite effect was described in δ -sarcoglycan-null mice, a model of limb-girdle muscular dystrophy characterized by a severe dystrophic phenotype. Genetic disruption of Cn signaling using two different genetic approaches (inactivation of CnB or CnA β) actually improved skeletal muscle pathology and cardiac disease (63). Conversely, overexpression of activated Cn caused worsened muscle pathology in the δ -sarcoglycan-null background (63). It remains to be established what might be the effect of Cn inhibition in human Duchenne muscular dystrophy, a disease genetically similar to *mdx* mice but pathologically and clinically more akin to δ -sarcoglycan-null mice. This issue is especially important since efficient Cn inhibitors are available, such as cyclosporine A and FK506, and others are being developed. ■

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