Role of Ca2+/calmodulin-dependent kinases in skeletal muscle plasticity
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Role of Ca\(^{2+}\)/calmodulin-dependent kinases in skeletal muscle plasticity

Eva R. Chin

Research Pharmacology, Pfizer Global Research and Development, La Jolla Laboratories, San Diego, California

Chin, Eva R. Role of Ca\(^{2+}\)/calmodulin-dependent kinases in skeletal muscle plasticity. J Appl Physiol 99: 414–423, 2005; doi:10.1152/japplphysiol.00015.2005.—In skeletal muscle, the increase in intracellular Ca\(^{2+}\) resulting from motor activation plays a key role in both contractile activity-dependent and fiber type-specific gene expression. These motor activation-dependent signals are linked to the amplitude and duration of the Ca\(^{2+}\) transients that are decoded downstream by Ca\(^{2+}\)-dependent transcriptional pathways. Evidence is mounting that the Ca\(^{2+}\)/calmodulin-dependent kinases (CaMKs) such as CaMKII play an important role in regulating oxidative enzyme expression, mitochondrial biogenesis, and expression of fiber type-specific myofibrillar proteins. CaMKIV has been shown to promote mitochondrial biogenesis and a mild fast-to-slow fiber type transition but has recently been shown to not be required for activity-dependent changes in muscle phenotype. CaMKII is known to decode frequency-dependent information and is activated during hypertrophic growth and endurance adaptations and also is upregulated during muscle atrophy. CaMKII has also been shown to remain active in a Ca\(^{2+}\)-independent manner after acute and prolonged exercise, and, therefore, is implicated as a mechanism for muscle memory. This mechanism can sense altered functional demands and trigger activation of an adaptational response that is dose dependently related to the activation level. This class of enzymes may therefore be the ideal decoders of information encoded by the intensity, frequency, and duty cycle of muscle activation and thus translate level of muscle activation into phenotypic adaptations through regulation of important muscle genes.

SKELETAL MUSCLE has a remarkable capacity to adapt to altered functional demands. Increased load bearing (i.e., resistance training) and increased duration of activation (i.e., endurance training) result in adaptation of the muscle by triggering different downstream target genes to induce muscle hypertrophy, fiber type transitions, or mitochondrial biogenesis. Likewise, decreased loading due to inactivity or injury triggers signaling events that induce muscle atrophy. Intracellular calcium plays an important role in signal transduction in all cell types, and its role in signaling muscle adaptation has become of great interest to physiologists. In skeletal muscle, it is well established that Ca\(^{2+}\) plays an essential role in the contraction-relaxation cycle. More recently, the role of Ca\(^{2+}\) in regulating activity-dependent muscle gene expression and in explaining muscle fiber type heterogeneity has been recognized as being dually important (6, 36). Neural activation of skeletal muscle results in the release of acetylcholine from the neuromuscular junction and depolarization of the plasma membrane, which activates force production by a process known as excitation-contraction coupling (see Fig. 1). The frequency and duration of stimulation determine the amplitude and duration of the Ca\(^{2+}\) transients and, as a result, the level of force output by the muscle (52). Similarly, it is thought that the amplitude and duration of the Ca\(^{2+}\) transient will also determine the set of genes expressed, thus providing a mechanism for tightly coupling the extent of muscle excitation to regulation of transcription (i.e., excitation-transcription coupling). It is this mechanism that may tightly link muscle gene expression to the activation history of the cell, thus providing a basis by which muscles can adapt, at the molecular level, to the functional demands placed on them.

ROLE OF Ca\(^{2+}\) SIGNALING IN EXCITATION-CONTRACTION COUPLING AND EXCITATION-TRANSCRIPTION COUPLING

It is well established that the preceding pattern of muscle excitation plays an important role in determining the pattern of genes expressed, which in turn affects the contractile behaviour of a given muscle (see Ref. 38). The classic cross-innervation experiments of Vrbova (49), where fast muscle was innervated with a slow motoneuron, as well as models using neural or direct muscle stimulation to transform fast skeletal muscle to a slow type, have demonstrated that muscle fibers can alter their expression of contractile, metabolic, and membrane-bound pump proteins in response to altered input (see Ref. 37 for review). Physical activity, either through increased loading (i.e., resistance training) or increased repetitive stimulation (i.e., endurance training) and decreased physical activity due to disuse atrophy, injury, or age-related muscle wasting can also alter skeletal muscle phenotype by changing the proteins expressed in individual fibers. Although the ability to alter muscle fiber type characteristics has been well established over the past few decades, the molecular mechanism(s) underlying these adaptive responses are not as well understood. In skeletal muscle, there is also a broad heterogeneity between skeletal muscle fiber types. Skeletal muscles consist of two main fiber types, slow (type I or slow oxidative) and fast (type II or

Address for reprint requests and other correspondence: E. R. Chin, Research Pharmacology, Pfizer Global Research & Development, La Jolla Laboratories 10724 Science Center Dr., San Diego, CA 92121 (Email: eva.r.chin@pfizer.com).
glycolytic with varying range of oxidative potential), which vary in their contractile speed, metabolic profile, and fatigue resistance. The main fiber types are determined by the myosin heavy chain isoform expressed, type I, IIa, IIx, and IIb, and also by alterations in various myofibrillar proteins including myosin light chains, troponin (Tn) subunits TnI, TnT, and TnC, and tropomyosin and membrane-bound ion pumps such as the sarcoplasmic/endoplasmic reticulum Ca\textsuperscript{2+} pump (SERCA) proteins. Although the compliment of fiber type-specific genes expressed in a slow vs. a fast fiber program is well understood (see Ref. 42), the regulatory signals that control differential gene expression between fiber types is less well established. It has been hypothesized that the pattern of genes expressed in a fiber type-specific fashion and the changes in gene expression with altered activity or load may be due to the level of electrical activation, mechanical strain on the membrane, alterations in intracellular metabolites (i.e., glucose, glycogen, ATP), ionic species (H\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}), and reactive oxygen species or due to secretion of autocrine and paracrine factors [i.e., insulin-like growth factor 1 (IGF-1) and mechano-growth factor (MGF)]. Currently, there is great interest in the role of intracellular Ca\textsuperscript{2+} and Ca\textsuperscript{2+}-dependent regulation of skeletal muscle gene expression. Although there are both ligand-mediated and excitation-mediated increases in intracellular Ca\textsuperscript{2+}, the focus of the current review will be on the motor activation or excitation-mediated changes in transcription.

**ROLE OF INTRACELLULAR Ca\textsuperscript{2+} IN REGULATING SKELETAL MUSCLE GENE EXPRESSION**

Many Ca\textsuperscript{2+}-sensitive target genes have been identified in skeletal muscle. Downstream target genes that are regulated in a Ca\textsuperscript{2+}-dependent manner include the nicotinic acetylcholine receptor (nAChR) (50), glucose transporter 4 (GLUT4) (34), SR Ca\textsuperscript{2+} ATPase (SERCA1), myosin heavy chain (MHC) isoforms (2, 3), and oxidative enzymes as well as genes that regulate mitochondrial biogenesis (12, 18, 33, 35). These genes are upregulated [GLUT4; MHCIIa to a greater extent than MHC IId/x and MHC IIb; mitochondrial enzymes cytochrome c, cytochrome oxidase subunit 1 (COX1), \(\delta\)-aminolevulinate synthase (ALAS), succinate dehydrogenase (SDH)] or downregulated (nAChR, SERCA1) with increased levels of Ca\textsuperscript{2+}. These genes also have varied expression levels between fibers that have high fatigue resistance (type I and IIa) and those of low fatigue resistance (type IIb), supporting the notion that extent of muscle activation, through the resultant amplitude and duration of Ca\textsuperscript{2+} elevation, determines the pattern and magnitude of gene expression in these fibers.

Support for the Ca\textsuperscript{2+} dependence of mitochondrial enzyme expression has primarily come from studies using cultured myocytes in which intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) is elevated by Ca\textsuperscript{2+} mobilizing agents (i.e., ionophores, caffeine) (18, 33, 35). These studies show increased expression of mitochondrial enzymes at the mRNA and protein level, implicating a transcriptional response to elevated Ca\textsuperscript{2+}. Treatment with the Ca\textsuperscript{2+} ionophore A23187 increased cytochrome c mRNA 1.7-fold in differentiated L6 myotubes and similarly increased cytochrome c promoter activity 2.5-fold (18), supporting the role for intracellular Ca\textsuperscript{2+} in regulating mitochondrial biogenesis. Consistent with this study, treatment of L6 myotubes with the Ca\textsuperscript{2+} ionophore ionomycin or the ryanodine receptor agonist caffeine increased cytochrome c, COX1, ALAS, and citrate synthase protein levels 1.5- to 2.5-fold, which could be inhibited by the Ca\textsuperscript{2+} chelating agent EGTA or the ryanodine receptor antagonist dantrolene (35). In addition to activating expression of these mitochondrial enzyme markers, similar studies have shown increased expression of transcription factors known to play a role in mitochondrial biogenesis, namely mitochondrial transcription factor A (mTFA), nuclear respiratory factors 1 and 2 (NRF1, NRF2) (33), and nuclear factor of activated T cells (NFAT), and the transcriptional coactivator peroxisome proliferator activator gamma (PPAR-\(\gamma\)) coactivator-1\(\alpha\) (PGC-1\(\alpha\)) (41). This further supports the role of Ca\textsuperscript{2+} in regulating mitochondrial gene expression at the transcriptional level. Activation of these transcriptional regulators by Ca\textsuperscript{2+} provides a mechanism whereby increased intracellular Ca\textsuperscript{2+} prepares the muscle for sustained increases in mitochondrial gene expression.

In addition to in vitro studies with cultured myotubes, in vivo studies with transgenic mice that overexpress or have disrupted expression of the Ca\textsuperscript{2+} buffering protein parvalbu-
min (PV) further support the Ca\(^{2+}\) dependence of mitochondrial enzyme expression. Mice with increased expression of PV and, consequently, attenuated levels of [Ca\(^{2+}\)]\(_i\), in activated muscles, have decreased SDH activity, a mitochondrial marker enzyme (12). These data are consistent with increased mitochondrial volume and cytochrome c oxidase activity observed in PV-deficient mice (8). In both the PV overexpression and PV-deficient mice, alterations in muscle oxidative potential were independent of changes in myosin isoform, suggesting differential regulation of mitochondrial and contractile genes when [Ca\(^{2+}\)]\(_i\) is regulated physiologically through altered Ca\(^{2+}\)-regulatory proteins.

In vivo, the increased electrical activity of muscle results in a significant elevation of intracellular Ca\(^{2+}\), and this is thought to be the primary regulator of altered gene expression in skeletal muscle. Under resting conditions, [Ca\(^{2+}\)]\(_i\), measured in isolated single muscle fibers is 30–50 nM (52). In contrast, when muscles are activated to contract, [Ca\(^{2+}\)]\(_i\), reaches 100–300 nM (10) in slow-twitch (type I) fibers activated at frequencies of slow motor units (10–30 Hz; Ref. 21) and even higher to 1–2 \(\mu\)M (52) in fast-twitch fibers (type IIb and IIa) activated at frequencies of fast motor units (80–150 Hz; Ref. 21) (see Fig. 2). Both the amplitude and duration of the Ca\(^{2+}\) transient in skeletal muscle are determined by the motor unit firing frequency. The frequency component, in turn, determines the amplitude of the Ca\(^{2+}\) signal and the resultant force output based on the well-characterized force-Ca\(^{2+}\) relationship (see Fig. 2, C and D). The duration of activation determines the temporal component for both force activation and activation of other Ca\(^{2+}\)-dependent binding proteins and downstream signals. These dramatic increases in amplitude, as well as the duration for which these amplitudes are achieved, are thought to encode signals that will be recognized by different downstream Ca\(^{2+}\)-dependent pathways. The key signaling pathways downstream of the elevation in intracellular Ca\(^{2+}\) that translate this signal into a transcriptional response include the Ca\(^{2+}\)/calmodulin-dependent phosphatase calcineurin (CnA) (11, 32, 55), Ca\(^{2+}\)/calmodulin-dependent kinase II (CaMKII; Refs. 17, 40), Ca\(^{2+}\)/calmodulin-dependent kinase IV (CaMKIV; Ref. 56), and Ca\(^{2+}\)-dependent protein kinase C (PKC; Ref. 18) (see Fig. 3). The focus of this review will be on the Ca\(^{2+}\)-calmodulin-dependent kinases (primarily CaMKII and CaMKIV) and their role in muscle plasticity. For further information on the calcineurin-NFAT and other Ca\(^{2+}\)-dependent pathways, readers are referred to reviews by Berchtold et al. (6), Michel et al. (31), and Olson and Williams (36).

CaMKs

Elevations in intracellular Ca\(^{2+}\) are initially decoded by an intermediate binding protein, CaM. CaM is a 17-kDa protein consisting of four helix-loop-helix protein folding motifs known as EF hands, which are the Ca\(^{2+}\) sensing units. The EF hand consists of an \(\text{NH}_2\)-terminal helix (the E helix), a centrally located Ca\(^{2+}\)-coordinating loop, and a COOH-terminal helix (the F-helix); each motif binds one Ca\(^{2+}\) ion in the central loop region (see Ref. 9). Other EF hand proteins exist in skeletal muscle TnC, which is a Ca\(^{2+}\) sensor involved in regulating force production by allowing actin-myosin interaction on Ca\(^{2+}\) binding, and PV, which acts as a Ca\(^{2+}\) buffer to facilitate rapid relaxation in fast fibers. In contrast to the roles of TnC and PV in regulating contractile activity, CaM is an intermediate in the activation of enzymes and downstream pathways.
signaling pathways, including those that regulate transcription. Calmodulin is a multifunctional signal transducer that undergoes conformational changes before activating other CaM-binding proteins, primarily downstream kinases and phosphatases. The family of serine/threonine protein kinases that are activated in a Ca$^{2+}$ and CaM-dependent manner are collectively known as Ca$^{2+}$/CaM-dependent protein kinases or CaM kinases. The CaM kinases detect and respond to physiologically relevant Ca$^{2+}$ signals based on the affinity of CaM for Ca$^{2+}$ ($K_d = 5 \times 10^{-7}$ M to $5 \times 10^{-6}$ M), which in skeletal muscle translate to levels of Ca$^{2+}$ achieved at stimulation frequencies greater than 30 Hz. The mechanisms by which both frequency and amplitude of the Ca$^{2+}$ oscillations are decoded by CaM include changes in CaM conformation and CaM subcellular localization on binding of Ca$^{2+}$. Decoding of the Ca$^{2+}$ signal at the level of CaM is achieved, in part, by compartmentalization of CaM and its ability to translocate to different regions within the cell on activation. When not activated, CaM is bound to Ca$^{2+}$-independent binding proteins and then is released and translocated within the cell when activation occurs. It is known that CaM becomes localized to mitotic apparatus when cells undergo mitosis (25) and also to translocate to the nucleus in smooth muscle cells and neurons on stimulation events that elevate intracellular Ca$^{2+}$ (16, 26). Studies with green fluorescent protein (GFP)-tagged CaM protein in HeLa cells show that CaM will become concentrated around the mitotic apparatus during cell division (25). Unfortunately little is understood about alterations in CaM localization in skeletal muscle on activation.

The family of CaM kinases is grouped according to whether they are dedicated kinases having a single substrate (i.e., phosphorylase kinase, CaMIIKIII, and myosin light chain kinase) or whether they are multifunctional and have several substrates (i.e., CaMKI, CaMIIKII, and CaMIV). Members of both groups of CaM kinases play important roles in skeletal muscle function, but the multifunctional kinases, particularly CaMKII and CaMIV, are thought to play an important role in muscle plasticity. These multi-functional kinases have multiple isoforms and multiple downstream targets. Specificity of the signal being transduced is therefore determined by the specific isoform activated, its localization and the duration, amplitude and frequency of the Ca$^{2+}$ oscillatory signal. In general, CaMKI and CaMIIKII are ubiquitously expressed, whereas CaMIV has a more limited expression pattern and is found primarily in neural tissue. A summary of the expression pattern and biochemical properties of the multi-functional kinases is shown in Table 1 (modified from Ref. 22). Additionally, CaMKII has multiple isoforms (CaMKII-α, -β, -γ, and -δ), each encoded by separate genes with at least 24 peptides generated by alternate splicing (see Refs. 22, 43) and at least one isoform expressed in every cell type (48). Differences in the mechanism of activation also exist between CaM kinase family members. CaMKI and CaMKIV are regulated by kinases (CaMKII and CaMIV), and by phosphatases, which themselves are regulated by Ca$^{2+}$/CaM binding. Activation of CaMKI and CaMKIV by CaMIIK-α and -β have been shown to increase CREB-mediated transcription in vitro and are thought to be of greatest functional significance in vivo in T-cells and neurons (46). A larger body of literature

<table>
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<tr>
<th>Tissue distribution</th>
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<th>Limited</th>
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<tr>
<td>Ca$^{2+}$ independence</td>
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<td>Yes (to 80%)</td>
<td>Yes (to 20%)</td>
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<tr>
<td>$K_{d,M}$ Unphosphorylated</td>
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<td>20–100 nM</td>
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<td>4 nM</td>
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Table 1. Expression pattern and biochemical properties of the Ca$^{2+}$/calmodulin-dependent kinases.
exists to explain the in vitro activation and molecular regulation of CaMKII and its functional link to in vivo adaptation (see Refs. 7, 47).

CaMKII is a multimeric enzyme composed of 12 subunits arranged as two 6-subunit rings in a hub and spoke configuration (B). Each subunit has a COOH-terminal association domain and NH2-terminal catalytic domains (A) important in its molecular regulation by intra subunit autophosphorylation. The CaMKII structural organization facilitates decoding of the frequency of Ca2+ spikes into distinct amounts of kinase activity by the magnitude of Ca2+. CaM-dependent phosphorylation of CaMKII subunits. After a bout of contractile activity with elevated Ca2+ levels, CaMKII subunits become autophosphorylated and the CaMKII holoenzyme remains active in the absence of the Ca2+ signal (C). Repeated bouts of contractile activity will further activate CaMKII activity and result in autonomous or Ca2+-independent enzyme activity. The relationship between Ca2+ oscillatory frequency and duration and the level of Ca2+-independent activity (autonomy) has been determined in an in vitro system with α- and β-CaMKII (D). A–C are modified from Soderling et al. (47), and D is generated from data in De Koninck and Schulman (15).

Fig. 4. Molecular mechanism of CaMKII activation. The CaMKII holoenzyme has 12 subunits arranged as two 6-subunit rings in a hub and spoke configuration (B). Each subunit has a COOH-terminal association domain and NH2-terminal catalytic domains (A) important in its molecular regulation by intra subunit autophosphorylation. The CaMKII structural organization facilitates decoding of the frequency of Ca2+ spikes into distinct amounts of kinase activity by the magnitude of Ca2+. CaM-dependent phosphorylation of CaMKII subunits. After a bout of contractile activity with elevated Ca2+ levels, CaMKII subunits become autophosphorylated and the CaMKII holoenzyme remains active in the absence of the Ca2+ signal (C). Repeated bouts of contractile activity will further activate CaMKII activity and result in autonomous or Ca2+-independent enzyme activity. The relationship between Ca2+ oscillatory frequency and duration and the level of Ca2+-independent activity (autonomy) has been determined in an in vitro system with α- and β-CaMKII (D). A–C are modified from Soderling et al. (47), and D is generated from data in De Koninck and Schulman (15).
ROLE OF CaMKII IN REGULATING SKELETAL MUSCLE GENE EXPRESSION

The CaMKII pathway is activated by both acute and prolonged activity and thus has been implicated in the activation of mitochondrial biogenesis and in the muscle hypertrophic response. Fluck et al. (17) examined Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent CaMKII activity in muscles after 7 days of stretch overload and after 2 wk of voluntary wheel running. Stretch overload of rooster anterior latissimus dorsi muscle resulted in a 122% increase in muscle protein content associated with a 47% increase in Ca\(^{2+}\)-independent CaMKII activity (17). There was no increase in Ca\(^{2+}\)-dependent or total CaMKII activity, indicating that there was greater activation of the preexisting CaMKII protein. Voluntary wheel running, which induces a threefold increase in lipoprotein lipase expression, a marker of lipid metabolism, resulted in a 43% increase in Ca\(^{2+}\)-independent CaMKII activity with no increase in total CaMKII activity in white vastus lateralis muscle of Sprague-Dawley rats. CaMKII was shown to phosphorylate serum response factor (SRF), which binds to the serum response element (SRE) to activate α-actin gene expression, a marker of the hypertrophic response. Although the similarity in response of CaMKII to endurance training and stretch overload suggests that this pathway is upstream of the specific adaptations to these contractile demands, CaMKII isoform specificity may exist to explain the differential downstream responses. In cardiac muscle, the cardiac-specific nuclear isoform of CaMKII-δB has been implicated in hypertrophy and dilated cardiomyopathy (58). Unfortunately, less is known about skeletal muscle-specific isoforms that may be involved in the hypertrophic response or metabolic adaptations. Nonetheless, the increase in CaMKII autonomous activity indicates sustained activation of this Ca\(^{2+}\) signaling pathway beyond the duration of elevated intracellular Ca\(^{2+}\) during contractile activity and implicates a CaMKII signal in muscle plasticity.

Acute motor activity can also activate CaMKII signaling. Rose and Hargreaves (40) have shown an increase in human muscle Ca\(^{2+}\)-autonomous CaMKII activity from 9% of maximum activity at rest to 17% after 40 min of cycle ergometer exercise at 76% peak oxygen uptake levels (V\(_{O_2}\)peak). At maximum levels of exercise (100% V\(_{O_2}\)peak), Ca\(^{2+}\)-autonomous CaMKII activity increased 70–150%. The increased level of Ca\(^{2+}\)-independent CaMKII activity was not due to an increase in total CaMKII protein but was correlated with increased phosphorylation at Thr\(^{287}\) to 50–70% of basal levels after submaximal exercise (76% V\(_{O_2}\)peak). These data support the idea that existing CaMKII enzyme remains active in vivo even after contractile activity has ceased and intracellular Ca\(^{2+}\) is no longer elevated. Interestingly, there was a greater increase in CaMKII activation at the higher exercise intensity, which could be due to the greater proportion of muscle fibers activated, the different fiber types activated, or the increased concentration of Ca\(^{2+}\) in individual fibers at higher intensities. Although a Ca\(^{2+}\) dose-response effect could not be determined in this study, the intensity-dependent increase in CaMKII activation is consistent with CaMKII being a stimulation-frequency decoder in skeletal muscle.

Consistent with the role of CaMKII in motor activity-dependent adaptations after both acute and prolonged activity, CaMKII activation has been shown to play a role in stimulating muscle glucose uptake (53). The ex vivo exposure of epitrochlearis muscle to concentrations of caffeine that increase cytosolic Ca\(^{2+}\) levels but do not induce contraction resulted in a threefold increase in glucose uptake. This was ~50% of the increase observed with muscle stimulation. The Ca\(^{2+}\)-induced activation of glucose uptake was inhibited by the Ca\(^{2+}\)-CaMK inhibitors KN62 and KN93 but not the inactive analog KN92. Furthermore, contractile activity induced a threefold increase in CaMKII phosphorylation, which was also inhibited by KN62. These data indicate that Ca\(^{2+}\)-CaMKII-dependent signaling is also involved in contractile activity-mediated increases in glucose transport and suggest that CaMKII signaling may coordinate muscle energy supply with energy demand.

The positive correlation of CaMKII activation with muscle hypertrophy and increased oxidative capacity may imply a decrease in CaMKII activity during muscle atrophy. However, it was recently shown that both age-related and denervation-induced muscle atrophy were associated with increased levels of CaMKII-γ (but not CaMKII-α or -β) protein in soleus muscle (13). It is currently unknown whether the upregulation of CaMKII-γ is part of the atrophy signal during muscle wasting or is a compensatory response as the muscle attempts to activate growth pathways to counter the wasting process. Unfortunately, CaMKII activity was not measured in the latter study, and therefore the level of activation of existing CaMKII protein under these atrophy conditions is unknown.

ROLE OF CaMKIV IN REGULATING SKELETAL MUSCLE GENE EXPRESSION

A CaMKIV pathway has also been implicated in the Ca\(^{2+}\)-dependent regulation of muscle gene expression, specifically in the activation of mitochondrial biogenesis and oxidative enzyme expression (56). Overexpression of a constitutively active form of CaMKIV in skeletal muscle resulted in increased mitochondrial volume, increased expression of both mitochondrial and nuclear DNA-encoded mitochondrial enzymes involved in fatty acid metabolism and electron transport, and enhanced recovery from fatigue (56). These mice also showed a subtle (~8%) increase in slow-twitch fibers. Although the downstream target(s) of CaMKIV were not identified, it is known that cAMP response element binding protein (CREB) is phosphorylated in CaMKIV-mediated transcription in neurons and T cells (30). The role of CREB phosphorylation by CaMKIV in regulating skeletal muscle gene expression has not been examined. In the CaMKIV transgenic mice, the increase in mitochondrial content was, however, associated with increased expression of PGC-1α, an important regulator of mitochondrial biogenesis. Transgenic mice that overexpress PGC-1α in skeletal muscle also show a similar phenotype, with increased oxidative fiber content and increased expression of slow/type I fiber genes (27). Thus CaMKIV appears to signal, directly or indirectly, through PGC-1α to increase mitochondrial biogenesis in skeletal muscle. Interestingly, muscle energy depletion using a β-guanidinopropionic acid (GPA) diet to deplete creatine phosphate stores results in increased PGC-1α and CaMKIV expression concomitant with mitochondrial biogenesis (59). Under these conditions, CaMKIV and PGC-1α upregulation are dependent on AMP kinase (AMPK) activity because AMPK dominant-negative mice do not show these mitochondrial adaptations with GPA treatment (59). It is
likely that both the decrease in cellular ATP, which activates AMPK, and the increase in intracellular Ca$^{2+}$ are responsible for signaling an increase in mitochondrial biogenesis and muscle oxidative capacity in response to repetitive muscle activation.

Whereas gain of function studies implicate CaMKIV in mitochondrial biogenesis and slow fiber type formation, loss of function studies have recently questioned the physiological importance of this CaM kinase isoform. Akimoto et al. (1) recently reported that CaMKIV$^{-/-}$ mice have normal fiber type composition in the fast-twitch muscles examined (plantaris, white vastus lateralis) and show an increase in slow myosin (MHC I) in the soleus muscle, a finding opposite of what would be predicted if Ca$^{2+}$ signaling through CaMKIV activated the slow fiber type expression program. Likewise, fast muscles from CaMKIV$^{-/-}$ mice had normal levels of mitochondrial gene expression and responded to both acute and chronic muscle activation by upregulating representative mitochondrial genes (PGC-1α mRNA during acute motor unit stimulation and PGC-1α, MHC IIa, myoglobin, and cytochrome c oxidase IV protein after 4 wk of voluntary running). Because CaMKIV$^{-/-}$ mice responded in a manner similar to wild-type mice, these data challenge the idea that CaMKIV is required for activity-dependent regulation of mitochondrial biogenesis and the type Ib to IIa fiber type transformation and suggest that other protein kinases that share substrates with constitutively active CaMKIV may be the endogenous mediators of this adaptation. Furthermore, these authors provide interesting biochemical data questioning the expression of CaMKIV in skeletal muscle. Previous studies have been unable to detect CaMKIV mRNA in rodent skeletal muscle (13) or protein in human skeletal muscle (40), whereas others have only been able to detect CaMKIV protein by immunoblotting of rodent muscle with very high levels of total protein loading (>100 μg; I. Irrcher, personal communication). Akimoto et al. (1) identified a protein of apparently similar molecular weight as CaMKIV in soleus muscle of both wild-type and (1) identified a protein of apparently similar molecular weight as CaMKIV in soleus muscle of both wild-type and CaMKIV$^{-/-}$ mice that cross-reacts with the CaMKIV antibody. They confirmed that this protein is not CaMKIV. Other isoforms of CaMKII were not upregulated and do not account for the additional CaMKIV band. Although the identity of this protein is not known, these data further question the significance of CaMKIV in the AMPK-stimulated cascade for PGC-1α upregulation as hypothesized by Zong et al. (57) and its role in skeletal muscle plasticity. Identification and characterization of this novel protein or other kinases that activate the substrates activated by CaMKIV (i.e., CREB, PGC-1α) should provide further insight into additional signaling pathways involved in muscle plasticity.

SPECIFICITY OF CaMK SIGNALING: UPSTREAM ACTIVATORS AND DOWNSTREAM TARGETS

Muscle contractile activity-induced increases in intracellular Ca$^{2+}$ and activation of CaMKII activity are implicated in skeletal muscle plasticity and appear to be important in signaling both a hypertrophy and metabolic remodeling response. However, not all bouts of contractile activity and not all increases in intracellular Ca$^{2+}$ result in these adaptations nor can a similar increase in CaMKII activity be responsible for differential adaptations to resistance and endurance training. Rather, a specificity of training mechanism must exist that matches the level of motor unit activation in different muscle groups to their specific adaptations through activation of different downstream signaling pathways and target genes via changes in CaMKII isoform composition, subcellular localization, or substrate specificity. The current body of knowledge has identified some of the key players in these adaptational responses, but future studies are necessary to map the specific contractile stimuli to their respective downstream pathways. Some of the key factors to consider are the intensity and duration of the contractile bout, which in turn determine the amplitude and duration of the intracellular Ca$^{2+}$ transients, the $K_d$ for Ca$^{2+}$ of the downstream Ca$^{2+}$ binding proteins (i.e., CaM vs. TnC, PV, and SERCs), the $K_d$ for Ca$^{2+}$/CaM of the various CaMKII isoforms, the subcellular localization of CaM and the various CaMKII isoforms, as well as the different downstream targets of CaMKII phosphorylation. Activation of Ca$^{2+}$ release from different Ca$^{2+}$ pools is an interesting possibility to explain some of the signaling specificity because different Ca$^{2+}$ sources may activate different compartmentalized sources of CaM and CaM kinases. For example, the CaMKII isoform designated CaMKII-βM is predominantly associated with skeletal muscle SR and is anchored there by a truncated, non-kinase protein α-KAP, which is a splice variant of CaMKII-α (4). Furthermore, the various CaMKII-β splice variants (β, βM, and β′) have similar specific activity but differential sensitivities to Ca$^{2+}$ due to differences in their CaM activation constant and thus initial rates of autophosphorylation (5). Therefore, release of Ca$^{2+}$ from the SR could lead to specific activation of CaMKII-βM, which would trigger a differential downstream response from Ca$^{2+}$ released from plasma membrane-bound L-type Ca$^{2+}$ channels or TRPC3 channels (41) and putative activation of a different CaMKII isoform and the calcineurin-NFAT pathway.

Substrate specificity of the CaMKII isoforms for different downstream targets is also not well understood. On the basis of data in skeletal muscle, the primary target for CaMKII is the serum response factor that binds to serum response element to activate promoters such as skeletal α-actin (17). CaMKII activation of atrial naturetic factor (ANF) in cardiac myocytes is also SRF/SRE dependent (39). Other known substrates of CaMKII include NR2B subunit of the N-methyl-d-aspartate (NMDA) receptor (44), NF-κB (24), cardiac calcium release channel (20), SERCA (57), and CREB (45). Other known intermediates for CaMKII signaling include c-fos, FAP, and AP-1 (51). Thus CaMKII may activate transcription directly through posttranslational modification of transcription factors (i.e., SRF, CREB, c-fos, FAP, AP-1) or may prolong activation stimuli such as Ca$^{2+}$ release by acting on ion channels. A clear delineation of the CaMKII signaling cascade(s) for activation of hypertrophy-responsive genes vs. those inducing mitochondrial biogenesis will be the goal for future research in this area.
examining Ca\textsuperscript{2+}-dependent regulation of muscle gene expression, elevations in intracellular Ca\textsuperscript{2+} are achieved using pharmacological agents in cultured myocytes or the downstream pathways are activated by overexpression of exogenous genes in genetically modified mice. To understand the role that these pathways play in vivo, the physiological relevance of these models need to be considered. Pharmacological tools, primarily Ca\textsuperscript{2+} ionophores (i.e., A23187 and ionomycin) and caffeine, have been shown to upregulate slow fiber specific and oxidative genes. Although this does implicate a Ca\textsuperscript{2+} dependence of the transcriptional response, the cellular consequences may not reflect those occurring during physiological activation of muscle where the Ca\textsuperscript{2+} oscillatory pattern is finely regulated by the motoneuron and may activate release of Ca\textsuperscript{2+} from different Ca\textsuperscript{2+} pools. Prolonged exposure to ionophores results in elevation of [Ca\textsuperscript{2+}]\textsubscript{i} to micromolar levels for prolonged durations, well beyond the physiological range expected during muscle activation. Ohtuka et al. (33) recently demonstrated that continuous exposure to 1 \mu M ionomycin for 5 days (24 h/day), which elevated [Ca\textsuperscript{2+}]\textsubscript{i} to 500–600 nM and increased mitochondrial enzyme expression; however, it resulted in a loss of myocyte protein content and decreased cell viability. Prolonged exposure to caffeine, a ryanodine receptor agonist, also resulted in decreased growth of myotubes and did not activate slow muscle genes (unpublished observations). In contrast, intermittent (5 h/day) exposure to ionomycin or caffeine increased mitochondrial enzyme expression without decreasing protein content (35). Therefore, unphysiologically high or prolonged elevations in [Ca\textsuperscript{2+}]\textsubscript{i} may induce changes that would not normally be observed in vivo. Interestingly, very brief exposure (1 min) of C2C12 myotubes to caffeine activated NFAT nuclear translocation, indicating activation of calcineurin-NFAT signaling, but longer-term exposure depleted nuclear NFAT unless extracellular Ca\textsuperscript{2+} was present and/or non-ryanodine receptor-mediated Ca\textsuperscript{2+} influx was activated (41). The latter suggests that sustained transcriptional signaling in cultured myocytes requires non-SR sources of intracellular Ca\textsuperscript{2+}. The role of this non-SR Ca\textsuperscript{2+} source for transcriptional regulation in adult myocytes or in vivo is not known.

In other studies, CaMKII-dependent regulation of gene expression was dependent on the activation signal that increased intracellular Ca\textsuperscript{2+}. Downregulation of the nAChR in response to elevations in [Ca\textsuperscript{2+}]\textsubscript{i}, induced by A23187 was not suppressed by the CaMKII inhibitor KN93. In contrast, nAChR regulation was suppressed by KN93 when [Ca\textsuperscript{2+}]\textsubscript{i} induced by A23187 was elevated by electrical stimulation (29). These findings illustrate that muscle contractile-induced, CaMKII-dependent regulation of nAChR gene expression cannot be mimicked by ionophore-induced elevations in [Ca\textsuperscript{2+}]\textsubscript{i}, in culture myocytes and suggests that not all methods for elevating intracellular Ca\textsuperscript{2+} will activate the same signal. Whether this discrepancy reflects the release of Ca\textsuperscript{2+} from different pools (i.e., from the SR during contractile activity and from the extracellular pool during ionophore stimulation) or that the finely regulated amplitude and duration of Ca\textsuperscript{2+} oscillations induced by neural stimulation activate different downstream pathways is not known.

Further insight into the role played by different Ca\textsuperscript{2+} pools in skeletal muscle transcriptional regulation has been obtained from recent studies by Rosenberg et al. (41). This group has shown that influx of extracellular Ca\textsuperscript{2+} through non-voltage-dependent transient receptor potential (TRPC3) channels is important in controlling the activation of the calcineurin-NFAT transcription pathway in skeletal muscle. Activation of excitation-contraction coupling by KCl-induced depolarization in C2C12 myotubes resulted in NFAT1c nuclear localization in the absence of extracellular Ca\textsuperscript{2+}. However, when KCl exposure was increased from 1 to 10 min, nuclear NFAT1c was depleted from the nuclear fraction. The presence of extracellular Ca\textsuperscript{2+} in addition to KCl exposure prolonged NFAT1c nuclear localization, indicating that the SR Ca\textsuperscript{2+} pool was important in initiating NFAT1c activation but that extracellular Ca\textsuperscript{2+} pools were required to maintain activation. Furthermore, overexpression of TRPC3 channels increased the influx of Ca\textsuperscript{2+} induced by caffeine and increased NFAT1c activation. These findings implicate a non-SR Ca\textsuperscript{2+} source for sustained transcriptional activation and suggest that different Ca\textsuperscript{2+} pools may be involved in this signal transduction pathway. Interestingly, others (28) have shown that NFAT1c activation is observed in intact adult muscle fibers during intermittent or prolonged 10-Hz stimulation but not during intermittent 50-Hz stimulation, suggesting that finely controlled Ca\textsuperscript{2+} oscillations of low-frequency stimulation can maintain NFAT signaling or that TRPC3 channels are activated under these conditions in vivo. Further investigation will be required to understand how muscle stimulation frequency is related to activation of TRPC3 channels and/or how repetitive release of Ca\textsuperscript{2+} from SR Ca\textsuperscript{2+} stores is linked to activation of TRPC3 or other Ca\textsuperscript{2+} channels. It will also be interesting to determine whether an alternative Ca\textsuperscript{2+} pool is required for CaMK activation or whether the access to different Ca\textsuperscript{2+} pools (i.e., SR Ca\textsuperscript{2+} pool for CaM kinase activation and SR plus TRPC3-mediated extracellular Ca\textsuperscript{2+} pool for calcineurin-NFAT activation) provides a mechanism to explain the synergy between these two pathways in activating oxidative enzyme gene expression (54). In addition to Ca\textsuperscript{2+}-dependent signals, muscle contractile activity may invoke Ca\textsuperscript{2+}-independent signals (i.e., mechanotransduction, ATP depletion, reactive oxygen species) that synergize with Ca\textsuperscript{2+}-dependent signals to fully activate contractile-dependent adaptive responses. Contractile activity is associated with activation of other stress-activated signaling pathways, particularly p38 MAPK and ERK1/2. These signals appear to be independent of muscle energetics and therefore metabolite accumulation because inhibition of the actomyosin ATPase did not suppress p38 MAPK activation in electrically stimulated muscle (14). Passive mechanical tension has also been shown to activate a hypertrophic response through a mammalian target of rapamycin (mTOR)-dependent but phosphatidylinositol 3-kinase (PI3K)- and protein kinase B (Akt)-independent pathway (23) and activation of JNK2. Finally, in addition to understanding the signals that activate transcription, it is important to consider the signals that terminate these transcriptional responses through the activation of nuclear export of key transcription factors. It is generally thought that nuclear export pathways are constitutively active and thus will counter the activation of transcription factors once the initiating stimulus is inactivated (19). The rapid nuclear export of NFAT after KCl-induced activation (<9 min) provides evidence for this active export process in myocytes (41). Only under conditions of stimulus-independent activation (such as Ca\textsuperscript{2+}-independent CaMKII activity) can signaling be prolonged, but even then, regulation of the oppos-
improving kinase or phosphatase will determine the time course for inactivation.

SUMMARY

In summary, there is strong evidence that intracellular Ca\(^{2+}\) signals in skeletal muscle are important not only in excitation-contraction coupling but also in excitation-transcription coupling. Evidence is mounting that the CaMKs play an important role in activating mitochondrial biogenesis and, to a lesser extent, myosin and other contractile protein isoform expression, and muscle hypertrophy. Despite our incomplete understanding of the CaMK signaling pathways in muscle plasticity, the ability of CaMks, particularly CaMKII, to decode the amplitude and frequency of intracellular Ca\(^{2+}\) oscillation makes it an ideal candidate for decoding a specificity of training response. Future insights into the specific CaMKII isoforms involved and their downstream targets in skeletal muscle will help to elucidate the mechanisms by which muscles can adapt to the specific loads placed on them.

It is also clear that multiple signals may converge to fully activate an adaptional response. Transcriptional regulation through other Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent signaling pathways may synergize with CaMK signals to allow for maximum adaptation. Through this well-coordinated system of feedforward and feedback-mediated signaling, skeletal muscle can optimally adapt to the loads placed on it and thus to improve functional capacity to meet the demands of the imposed load.

REFERENCES