Energy sensing and regulation of gene expression in skeletal muscle

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Freyssenet D. Energy sensing and regulation of gene expression in skeletal muscle. J Appl Physiol 102: 529–540, 2007; doi:10.1152/japplphysiol.01126.2005.—Major modifications in energy homeostasis occur in skeletal muscle during exercise. Emerging evidence suggests that changes in energy homeostasis take part in the regulation of gene expression and contribute to muscle plasticity. A number of energy-sensing molecules have been shown to sense variations in energy homeostasis and trigger regulation of gene expression. The AMP-activated protein kinase, hypoxia-inducible factor 1, peroxisome proliferator-activated receptors, and Sirt1 proteins all contribute to altering skeletal muscle gene expression by sensing changes in the concentrations of AMP, molecular oxygen, intracellular free fatty acids, and NAD⁺, respectively. These molecules may therefore sense information relating to the intensity, duration, and frequency of muscle exercise. Mitochondria also contribute to the overall response, both by modulating the response of energy-sensing molecules and by generating their own signals. This review seeks to examine our current understanding of the roles that energy-sensing molecules and mitochondria can play in the regulation of gene expression in skeletal muscle.

energy-sensing molecules; endurance training; mitochondria

EXERCISE PHYSIOLOGISTS have maintained a longstanding interest in bioenergetics, largely because of the exceptional capacity of skeletal muscle to increase its energy production during exercise. As knowledge of muscle bioenergetics has progressively increased and numerous training-induced adaptations were described, many exercise physiologists have focused on dissecting the molecular events and regulatory mechanisms involved in the cellular response. Particular attention has been paid to exercise-induced extracellular factors, intracellular factors, signaling pathways, and transcription factors, as well as the regulatory influences responsible for modulating the cellular response (reviewed in Ref. 84). A number of studies have suggested that the adaptive response of skeletal muscle to exercise is likely to involve energy metabolism through regulation of key signaling molecules and transcription factors. Figure 1 illustrates the organizational hierarchy of molecular components that are believed to be involved in the energy dependence of nuclear gene expression in skeletal muscle.

The purpose of this review is to examine the role of energy sensing for regulation of nuclear gene expression (transcription and translation) in skeletal muscle and to show how energy sensing may contribute to muscle plasticity. We will first focus on energy-sensing molecules, which are herein defined as proteins capable of transducing variations in energy homeostasis into alterations in gene expression. We will also assess how endurance training can influence the response of energy-sensing molecules and how mitochondria act as sensors for variations in energy homeostasis in skeletal muscle. Specificity of mitochondrial signaling during skeletal muscle regeneration will be also developed. Finally, we will discuss some new candidates that could be tested for potential roles in energy sensing in skeletal muscle.

ROLE OF ENERGY-SENSING MOLECULES IN REGULATING GENE EXPRESSION IN SKELETAL MUSCLE

All eukaryotic cells must maintain high ATP-to-ADP ratios. During skeletal muscle contractions, ATP is hydrolyzed and free ADP is produced by the myosin ATPase reaction (Fig. 2). Depending on the extent of ATP demand and the capacity of the mitochondria to regenerate ATP, energy homeostasis may be modestly (ATP demand can be matched by mitochondrial ATP synthesis) or more robustly (ATP demand exceeds mitochondrial ATP synthesis) altered. A lack of balance between ATP demand and mitochondrial ATP synthesis, if great enough, will increase lactate concentration and further increase the free ADP-to-ATP ratio, which will then be amplified into a much larger increase in the AMP-to-ATP ratio via adenylyl kinase.

The idea that variations in cellular energy status may contribute to the regulation of nuclear gene expression is not new, but the recent identification of several molecules capable of sensing and transducing changes in cellular energy status into altered nuclear gene expression has provided researchers with a basis for understanding this process in greater detail.

AMP-activated protein kinase. β-Guanidinopropionic acid (β-GPA) is a creatine analog that depletes high-energy phosphate compounds, including phosphocreatine and ATP, from skeletal muscle (43). Chronic feeding with β-GPA reportedly stimulates mitochondrial biogenesis in rat skeletal muscle, particularly in fast-twitch skeletal muscle (44, 137). However, transgenic mice expressing a dominant-negative mutant of the AMP-activated protein kinase (AMPK) α2-subunit do not show mitochondrial biogenesis in response to β-GPA feeding (166), suggesting that AMPK is capable of sensing and transducing changes in cellular energy status (i.e., depletion of...
high-energy phosphates) into nuclear gene expression change-
s. AMPK, an energy-sensing enzyme activated by 5′-AMP, exists as heterotrimeric complexes containing a catalytic α-subunit and two regulatory subunits, β and γ. Binding of AMP to AMPK stimulates phosphorylation and activation of AMPK by the protein kinase, LKB1 (also known as STK11) (56, 133). AMPK activation also occurs directly as a result of Ca²⁺ signaling in myotubes (45). In tissues other than skeletal muscle, AMPK may also be phosphorylated and activated by CaMK kinase (57, 67, 161). The β-subunits of AMPK contain a conserved central domain that binds glycogen (66) and would be involved in negative regulation of AMPK activity. Since AMPK has been extensively reviewed elsewhere (22, 54, 55, 76, 80, 157), we will herein focus on a small subset of target proteins that are particularly relevant to skeletal muscle gene expression.

Fig. 1. Diagram summarizing the theoretical framework used as the basis of this review. Listed are messengers and energy sensing molecules that have been shown to be responsive to variations in energy homeostasis. Even if not represented, mitochondria are key contributors in the overall response both by modulating the response of energy sensing molecules and by generating their own signaling. AMPK, AMP-activated protein kinase; cGC, cytosolic guanylyl cyclase; HIF-1, hypoxia-inducible factor-1; MondoA, basic helix loop helix-leucine zipper partner for the Max-like protein, Mlx; PPAR, peroxisome proliferator-activated receptors; Sirt1, sirtuin 1; Sirt3, sirtuin 3.

Fig. 2. Simplified overview of muscle bioenergetic highlighting messengers involved in signal transduction. Energy is provided by the oxidation of glucose, glycogen, and triglycerides. The relative contribution of these substrates depends on exercise intensity and training status. ADP is formed by the myosin ATPase reaction. Cytosolic creatine kinase (CK), in conjunction with their mitochondrial isoenzymes (mt-CK), shuttle ADP from cytosol into the mitochondrial intermembrane space. Direct diffusion of ADP also participates in the transport of adenylate. In fast-twitch muscles, AMP can be metabolized to inosine monophosphate (IMP) and ammonia (NH₃) by AMP deaminase (AMPd). In slow-twitch muscles, AMP is predominantly metabolized to adenosine by the 5′-nucleotidase (5′-Nase). G6P, glucose-6-phosphate. LDH, lactate dehydrogenase; Cr, creatine; CrP, creatine phosphate; PPi, inorganic pyrophosphate.
AMPK is activated in skeletal muscle during exercise (25, 107, 158) and electrically stimulated contraction (4, 69, 107, 151). In skeletal muscle, AMPK activates expression of peroxisome proliferator-activated receptor (PPAR) α (89), PPARγ coactivator-1α (PGC-1α) (89, 143, 146), and nuclear respiratory factor 1 (NRF-1) (10). Consistent with its positive effect on PGC-1α and NRF-1 expression, AMPK has also been shown to stimulate mitochondrial biogenesis in skeletal muscle fibers (10, 115, 143, 159). The mechanisms by which AMPK regulates PGC-1α expression are currently unknown, but one possibility is that myocyte enhancer factor-2 and the transcriptional coactivator, p300 histone acetyl transferase, which are both regulated by AMPK (162, 165), promote transactivation of the PGC-1α gene promoter (53, 114). It is also important to note that AMPK may not be essential for adaptations of skeletal muscle to endurance exercise training (62, 77) and that additional signaling pathways are involved in triggering muscular adaptations to endurance training (recently reviewed in Ref. 84). Another recently identified target for AMPK in skeletal muscle is the protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway, which is involved in protein synthesis and muscle hypertrophy (reviewed in Refs. 49, 75).

AMPK activation has been found to reduce protein synthesis by 45% in muscle homogenates, in association with decreased activation of Akt, mTOR, ribosomal protein S6 kinase, and eukaryotic initiation factor 4E-binding protein (16). In addition, AMPK is believed to promote phosphorylation of tuberous sclerosis complex 2 (TSC2) (72, 145), which forms a complex with TSC1 to allow conversion of the small G-protein Ras homolog enriched in brain (Rheb) to a GDP-bound form that no longer activates mTOR (55). This AMP-mediated cascade may account for a number of specific muscular adaptations to endurance training (4), including the observation that endurance training is generally not associated with a gain in muscle mass. A number of other downstream targets of AMPK are potentially relevant for skeletal muscle physiology, including the HuR mRNA binding protein, which is involved in protein stabilization during cytokine-induced skeletal muscle cachexia (34), as well as the forkhead box O (Foxo) 1a transcription factor (9) and hypoxia inducible factor-1 (HIF-1) (70, 88). However, no studies to date have definitively shown that AMPK activates these target proteins in skeletal muscle.

HIF-1. All mammalian cells have the ability to sense and respond to changes in O2 concentration; this ability is based on the action of HIF-1, which functions as a master transcriptional factor in response to changes in tissue oxygenation. HIF-1 is a heterodimeric protein composed of HIF-1α and HIF-1β subunits (154). Under normoxic conditions, the HIF-1α protein is subject to hydroxylation by prolyl hydroxylases (for review, see Refs. 8, 128), which triggers binding of von Hippel Lindau protein to HIF-1α and targets it for proteasomal degradation. In contrast, the prolyl hydroxylases do not function under low O2 concentrations, leading to accumulation of HIF-1α, heterodimerization of HIF-1α with HIF-1β, and subsequent activation of HIF-1-dependent target gene expression. These targets include the gene encoding vascular endothelial growth factor, as well as genes that enable cells to survive O2 deprivation by providing an O2-independent means of ATP production (glucose transporters and glycolytic enzymes) or by inhibiting hypoxia-induced apoptosis (129, 130).

As exercise intensity increases, muscle cells are subject to substantial decreases in intracellular PO2 (iPo2) (28, 119). Although recent evidence suggests that iPo2 does not decrease linearly with increasing metabolic rate (118), iPo2 falls to a level of ~4 Torr at a maximum work rate (118). It is therefore possible that HIF-1α protein accumulates in response to reduced PO2 in skeletal muscles undergoing high-intensity exercise, suggesting that this oxygen-sensitive pathway could be involved in muscular adaptation to exercise. Consistent with this hypothesis, HIF-1α protein levels and HIF-1α DNA binding activity increases markedly in human skeletal muscle immediately after a one-legged knee-extension exercise, along with a concurrent decrease in von Hippel Lindau protein levels (1), suggesting that the observed increase in HIF-1α protein level is due to protein stabilization. Functional study in HIF-1α knockout mice demonstrates that the increased expression of HIF-1α-dependent target genes (e.g., vascular endothelial growth factor, glucose transporter 4) normally observed in wild-type animals in response to skeletal muscle exercise is strongly diminished in HIF-1α knockout mice, demonstrating that HIF-1α plays an important role in muscle adaptation to exercise (97).

These data collectively suggest that HIF-1α plays a significant role in mediating muscle adaptations to muscle contraction. However, some questions remain unanswered. In particular, the regulation of HIF-1α prolyl hydroxylases in response to endurance exercise has not yet been investigated. It is also unknown whether O2 is evenly distributed throughout muscle fibers during exercise or whether O2 microdomains exist for heterogeneous regulation of HIF-1α activity. The regulation of HIF-1α expression in response to acute bouts of exercise is also poorly understood (94).

PPARs. The PPARs constitute a class of metabolic sensors within the nuclear receptor family, with three isotypes (PPARα, PPARβ/δ, and PPARγ) cloned to date from humans and rodent (reviewed in Refs. 32, 33). In skeletal muscle, the relative expression levels of PPARs are, in descending order, PPARβ/δ > PPARα > PPARγ (48). The PPARs bind DNA at the PPAR response element as heterodimers with the retinoid X receptor (48). Either partner can regulate the transcriptional activity of the DNA-bound complex by interacting with its cognate ligand either singly or in concert. A large number of genes encoding proteins involved in mitochondrial metabolism have been shown to be PPAR responsive in skeletal muscle, including those encoding proteins responsible for fatty acid transport and activation, mitochondrial β-oxidation, and energy uncoupling (37, 96, 106, 144, 155).

Studies have shown that numerous fatty acids, especially unsaturated fatty acids, can bind to PPARα, PPARβ/δ, and PPARγ with varying affinities to control target gene transcription (reviewed in Refs. 32, 33), indicating that the PPARs act as important metabolic sensors. Since oxidation of triglycerides from extramuscular sources and intramuscular stores is used by skeletal muscle fibers to provide energy during endurance exercise (81), PPARs may regulate gene expression in response to variations in the levels of intramuscular fatty acids during endurance exercise. This notion is strongly supported by a gain-loss of function study in mice, showing that targeted expression of an activated form of PPARδ in skeletal muscle increased the number of type I muscle fiber, the expression of genes involved in
mitochondrial biogenesis, and mitochondrial metabolism, and endurance performance in these mice (155). Similarly, the mRNA levels of the slow isof orm of troponin I, cytochrome c, and subunit IV of cytochrome oxidase are all upregulated in wild-type mice receiving the PPARγ-specific agonist, GW501516. Conversely, PPARγ-null mice can only sustain 30–40% of the running time and distance of their wild-type controls (155). Although this study does not show direct activation of PPARγ by fatty acids in response to endurance exercise or endurance training, the data strongly support the notion that PPARγ activation is an important mediator of muscular adaptation to endurance exercise.

Sirtuin 1. Chung et al. (27) originally suggested that the ratio NAD+/NADH might be involved in the regulation of nuclear gene expression following their observation that the binding of proteins on an 8-bp response element (called the REBOX) present in the promoters of the genes encoding adenine nucleotide translocase and ATP synthase-β was sensitive to variations in NADH concentration. However, the redox status of NADH was definitively shown to be involved in nuclear gene expression with the subsequent discovery that NAD+ is an essential substrate for a family of class III histone deacetylases, collectively called the sirtuins. The sirtuins, which are enzymes capable of coupling the cleavage of NAD+ to nuclear gene expression (reviewed in Refs. 15, 31), were originally identified in yeast as NAD-dependent proteins that deacetylate histones and participate in the regulation of longevity (71). In mammals, Sirtuin 1 (Sirt1) deacetylates histone proteins as well as some non-histone proteins, including the TAF68 TATA box-binding protein (108) and p53 (95, 152). This unique property implies that Sirt1 may function as an energy sensor linking energy metabolism to transcriptional regulation.

Decreases in the NAD+/NADH ratio have been observed during muscle differentiation (47), suggesting that Sirt1 may participate in the regulation of myogenesis. Consistent with this notion, inhibition of Sirt1 induces premature differentiation of C2C12 myoblasts, whereas activation of Sirt1 via its upstream activators. These hypotheses are supported by experimental evidences (68, 111, 164), collectively showing that preservation of free ADP lowers the rate of AMPK activation (35) and the availability of free fatty acids. Although these results should be interpreted within the context of tissue-specific Sirt1 effects, the studies collectively suggest that Sirt1 may establish regulatory cross-talk among various energy-sensing molecules. Clearly, a complete characterization of the role of Sirt1 in skeletal muscle, as well as the identification of its molecular partners, will be important to draw precise conclusions on its physiological relevance in skeletal muscle.

REGULATION OF ENERGY-SENSING MOLECULES IN RESPONSE TO ENDURANCE TRAINING

The concept of the preservation of energy homeostasis in endurance-trained skeletal muscle, initially explored by Holloszy and Booth (60) and later developed by others (50, 61, 64), is of critical importance when considering the energy dependence of nuclear gene expression in skeletal muscle. Central to this concept is the observation that the mitochondrial content of skeletal muscle increases in response to endurance training (for review, see Ref. 64). Thus the same cellular rate of mitochondrial oxidative phosphorylation is attained with a lower rate of oxidative phosphorylation “per mitochondrion” for any absolute workload in muscle fibers with elevated mitochondrial content. ATP and creatine phosphate concentrations decrease to a smaller degree, and the free ADP concentration increases to a lesser extent in cells with elevated mitochondrial contents at the same absolute work rate (30, 38). Better preservation of free ADP lowers the rate of AMP formation, ultimately decreasing the activation of AMPK and its upstream activators. These hypotheses are supported by experimental evidences (68, 111, 164), collectively showing that preservation of energy homeostasis in response to endurance training contributes to the regulation of AMPK activity. Other well-described metabolic adaptations of skeletal muscle to endurance training may also participate in the regulation of energy-sensing molecules. A considerably lower increase in lactate concentration is observed after endurance training when exercise is performed at the same submaximal workload (30).
Table 1. Examples of genes that are altered in response to variations in mitochondrial respiratory chain activity

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<thead>
<tr>
<th>Gene</th>
<th>Cell Type (Ref. No.)</th>
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<tr>
<td>Ca(^{2+}) homeostasis</td>
<td>C2C12 (11)</td>
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<tr>
<td>Calcineurin (↑)</td>
<td>C2C12 (2, 14)</td>
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<tr>
<td>Calreticulin (↑)</td>
<td>C2C12 (11, 13, 14)</td>
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<tr>
<td>Calsequestrin (↑)</td>
<td>C2C12 (11, 13, 14)</td>
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<tr>
<td>RyR 1 (↑)</td>
<td>C2C12 (2, 11, 13, 14)</td>
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<th>Tumor invasion and cell cycle</th>
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<tr>
<td>Cathepsin L (↑)</td>
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<td>p21 (↑)</td>
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<td>PCNA (↑)</td>
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<td>TGF-β (↑)</td>
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<tr>
<th>Apoptosis</th>
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<tr>
<td>Bad (↑)</td>
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<td>Bcl2 (↑)</td>
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<td>Bid (↑)</td>
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<table>
<thead>
<tr>
<th>Gene expression</th>
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<tr>
<td>ATF-2 (↑)</td>
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<td>cRel (↑)</td>
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<tr>
<td>IkBa (↑)</td>
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<tr>
<td>Myc (↑)</td>
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<td>Myogenin (↓)</td>
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<td>NFAT (↑)</td>
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<td>p50 (↑)</td>
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<td>p53 (↑)</td>
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All studies were performed in vitro. Arrows indicate whether gene expression was increased (↑) or decreased (↓). Decrease in mitochondrial respiratory chain activity was induced by ethidium bromide (2, 11–14), chloramphenicol (121, 132), and respiratory chain-interfering drugs (2, 11–13). Increase in mitochondrial respiratory chain activity was induced by pyruvate (40). C2C12, mouse muscle cells; L6E9, rat muscle cells; QM7, quail muscle cells; RyR 1, ryanodine receptor 1; TGF-β, transforming growth factor-β; ATF-2, activating transcription factor-2; PCNA, proliferating cell nuclear antigen; NFAT, nuclear factor of activated T cells.

Preservation of the pyruvate-to-lactate ratio blunts the decrease in the NAD\(^{+}\)-to-NADH ratio, thus potentially contributing to the regulation of Sirt1 activity. Muscle glycogen stores are depleted less rapidly during standardized exercise in the trained state. This glycogen-sparing effect may contribute to reduce AMPK activation in trained muscles. Indeed, AMPK activity and high glycogen levels show an inverse relationship in skeletal muscle (160), but low muscular glycogen levels at the onset of exercise were shown to enhance AMPK activation vs. muscles with high glycogen contents (140). Finally, the decrease in the oxidation of carbohydrate during submaximal exercise in response to endurance training is compensated for by a proportional increase in fat oxidation (61). This may alter intramuscular free fatty acid levels during submaximal exercise, suggesting that PPAR transcriptional activity may also be modified in response to endurance training.

Another important possibility in considering the energy dependence of nuclear gene expression is that endurance training could alter the expression of energy-sensing molecules. Increased expression of AMPK isozymes γ3 (41), α1, β2, and γ1 (46), and α1 (111) and decreased expression of AMPK γ3 (46), have been reported in response to exercise training in rats (41) and human skeletal muscles (46, 111). Changes in PPARα (124), HIF-1α, and HIF-1β mRNA levels (94) have also been reported in response to endurance training. Such changes may alter the sensitivity of energy-sensing molecules to variations in energy homeostasis.

In 1956, Otto Warburg observed that experimentally induced carcinogenesis of rodent cells was accompanied by reduced respiration-coupled oxidative metabolism and increased glycolysis (156), raising the intriguing possibility that the original noncarcinogenic phenotype of these mammalian cells was regulated by mitochondrial oxidative phosphorylation. The existence of mitochondrial regulation of nuclear gene expression was later demonstrated directly in C2C12 skeletal myoblast, wherein depletion of mitochondrial DNA (mtDNA) and subsequent disruption of mitochondrial membrane potential results in enhanced levels of several Ca\(^{2+}\)-responsive proteins (calcineurin, nuclear factor of activated T cells) and Ca\(^{2+}\) handling proteins (ryanodine receptor 1, calreticulin) (11). When the partial depletion of mtDNA is reverted in

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**Fig. 3.** Schematic overview of mitochondrial signaling. Under conditions where cell energy demand is challenged, mitochondria produce ATP to meet the energy demand but also initiate and modulate signaling cascades participating in nuclear gene expression. This function is referred to as mitochondrial signaling or mitochondrial retrograde signaling. The identity of some messengers and downstream effectors is discussed in the text.
C2C12 myoblasts, the expression of the nuclear genes encoding these proteins reverts to near control levels, inferring the direct link between changes in mitochondrial membrane potential and nuclear gene expression. Table 1 shows several examples of genes that are altered in response to variations in mitochondrial respiratory chain activity in skeletal muscle cells. Taken together, these data unambiguously demonstrate that in addition to generating ATP for energy tasks, mitochondria also initiate and modulate signaling cascades that participate in nuclear gene expression. This function, referred to as mitochondrial signaling or mitochondrial retrograde signaling (21), is defined as mitochondrial respiration-dependent pathway of communication between mitochondrial and nucleus leading to changes in nuclear gene (Fig. 3). Mitochondria may therefore be viewed as energy-sensing organelles that adapt nuclear gene expression under conditions where the cell energy demand is challenged. The cascade of events connecting the mitochondria to nuclear gene expression is not yet fully understood, but several potential signaling molecules and triggers have been identified.

**Reactive oxygen species.** Within the cell, the main sources of reactive oxygen species (ROS) are the mitochondria, which produce superoxide anions mainly at complexes I and III of the respiratory chain (Fig. 4). In skeletal muscle, isolated mitochondria respiring in absence of ADP (state IV of respiration) direct 0.15–0.5% of the total O2 consumption to ROS generation (3). Mitochondrially derived ROS function in numerous signaling processes in a variety of cell types, where they regulate the redox status of protein kinases (85, 110, 117, 142) and transcription factors (24, 63, 82, 91, 117). In cardiomyocytes, mitochondrial ROS production is associated with p38 MAPK phosphorylation (85) and JNK activation (36). Myotubes treated with TNF-α show increased ROS release from mitochondria (91), leading to NF-κB activation and myofibrillar protein loss (92). ROS production may also be involved in the regulation of myogenic differentiation. Exposure of differentiating myoblasts to hydrogen peroxide almost totally abolished muscle-specific protein expression and myogenic differentiation, and this effect is reversed when cells are incubated with the ROS scavenger, N-acetyl cysteine (86). The contribution of mitochondrially derived ROS in this situation remains to be determined.

Contractile activity in skeletal muscle increases the concentration of superoxide anions in both the extracellular (98, 99) and intracellular compartments (100, 138), suggesting that muscle exercise may be accompanied by increased mitochondrial ROS production. This raises an important question concerning the relationship between oxidant formation and mitochondrial oxidative phosphorylation rate (debated in Refs. 5, 65, 79, 90). Mitochondria isolated from adult mouse skeletal

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**Fig. 4. Mechanisms for mitochondrial reactive oxygen species (ROS) generation.** Complexes I and III are responsible for most of superoxide anion (O2•−) production. Release of O2•− in the intermembrane space appears to mainly occur at complex III (5, 18, 149). O2•− is rapidly converted to hydrogen peroxide (H2O2) by manganese superoxide dismutase (MnSOD) on the inner side and copper/zinc superoxide dismutase (CuZnSOD) on the outer side. A number of regulatory influences may profoundly affect mitochondrial ROS production. An increase in mitochondrial matrix Ca2+ concentration activates pyruvate dehydrogenase and enzymes of tricarboxylic acid (TCA) cycle, isocitrate dehydrogenase (ICDH) and α-ketoglutarate dehydrogenase (α-KGDH) (101, 102), as well as complex IV of the respiratory chain (79, 93), further contributing to increase mitochondrial ROS production (79, 87, 93). Mitochondrial Ca2+ is also a potent activator of nitric oxide (NO) production by the mitochondrial nitric oxide synthase (mtNOS) (29, 58, 148). NO can bind iron and copper atoms at the active site of cytochrome c oxidase (135), thus reversibly inhibiting cytochrome c oxidase activity by reducing the affinity of the enzyme for molecular oxygen (O2). This ultimately increases the formation of ROS by mitochondria (23, 103). Recent evidences also suggest that α-KGDH directly participates in the mitochondrial production of ROS (139). CoQ, coenzyme Q; Cyt c, cytochrome c; ΔΨ, mitochondrial membrane potential; FAD, flavine adenine dinucleotide; i.m., inner membrane; mt-Uni, mitochondrial Ca2+ uniporter; NAD+, nicotinamide adenine dinucleotide; o.m., outer membrane; I, II, III, and IV refer to the protein complexes of the electron transport chain.
muscle show significant increases in hydrogen peroxide release after contractile activity compared with precontraction values (150), supporting the notion that mitochondria significantly contribute to exercise-induced ROS production (79). A recent study further showed that contraction-induced AMPK activity and phosphorylation were blocked with the ROS scavenger N-acetylcysteine, suggesting that exercise-induced mitochondrial ROS production may be involved in the regulation of AMPK-dependent pathway (126). One may also ask the question about the physiological relevance of mitochondrial ROS in the response of skeletal muscle to endurance training. This must be critically examined in light of the variations in antioxidant capacity that may occur in response to endurance training, some reports describing an increase in antioxidant capacity (74, 131, 147, 153), whereas others report unchanged antioxidative defense (147, 153). The extent of endurance training-induced mitochondrial biogenesis has also to be taken into account. All these parameters will combine to determine the importance of mitochondrial ROS signaling for a given exercise intensity after endurance training. Clearly, additional experiments will be required to determine if and to what degree mitochondrial ROS production is involved in the adaptive response of skeletal muscle to endurance exercise and training.

**Calcium ion.** Recent studies have identified a mitochondrial component to the regulation of Ca\(^{2+}\)-dependent signaling events (2, 11, 13, 14). For example, disruption of the mitochondrial membrane potential triggers a two- to threefold increase in the cytosolic Ca\(^{2+}\) concentration of C2C12 myoblasts, accompanied by changes in the expression levels of Ca\(^{2+}\)-responsive proteins (e.g., calcineurin, nuclear factor of activated T cells) and genes of the NF-κB pathway (11) (see also Table 1). In contrast, removal of free Ca\(^{2+}\) by chelating agents reverses the effect of mitochondrial signaling on nuclear gene expression (2, 13, 14). Furthermore, the activation of Ca\(^{2+}\)-responsive factors is abolished in calcineurin A β−/− cells, as well as in cells treated with the calcineurin inhibitor FK506, showing that calcineurin may be a downstream effector of mitochondrial signaling (13).

In skeletal muscle fibers, mitochondria actively participate in the regulation of Ca\(^{2+}\) homeostasis by rapidly and efficiently accumulating Ca\(^{2+}\) in the mitochondrial matrix (19). The Ca\(^{2+}\)-buffering capacity of mitochondria is tightly linked to the rate of mitochondrial oxidative phosphorylation. Addition of respiratory substrates to permeabilized skeletal muscle fibers greatly diminishes frequency of Ca\(^{2+}\) sparks from the sarcoplasmic reticulum (73), which is directly proportional to the mitochondrial content and to the ability of mitochondria to accumulate Ca\(^{2+}\) in the matrix, due to the close proximity of some mitochondria to Ca\(^{2+}\) release sites (73, 136). In contrast, addition of a nonmetabolizable substrate or a mitochondrial inhibitor promotes Ca\(^{2+}\) sparks from the sarcoplasmic reticulum (73), illustrating a decrease in the Ca\(^{2+}\)-buffering capacity of mitochondria. Clearly, the buffering capacity of mitochondria may be particularly relevant in spatiotemporal regulation of cytosolic Ca\(^{2+}\) concentrations in contracting muscles fibers. This may contribute to modulating the activity of Ca\(^{2+}\)-responsive proteins involved in gene expression, such as CaMK, calcineurin, or Ca\(^{2+}\)-sensitive protein kinase C (PKC) isoforms (reviewed in Refs. 26, 84). Additional work will be required to determine the extent to which the mitochondrial Ca\(^{2+}\) buffer-

**SPECIFICITY OF MITOCHONDRIAL SIGNALING DURING SKELETAL MUSCLE REGENERATION**

The signaling function of mitochondria appears to be particularly relevant to the regulation of myogenic differentiation. For example, respiratory-deficient primary myoblasts depleted of mitochondrial DNA or myoblasts treated with chloramphenicol and tetracycline fail to differentiate (52, 59, 83, 121), whereas stimulation of mitochondrial metabolism induces cell cycle arrest in L6 muscle cells (40), and stimulation of mitochondrial biogenesis by the exogenous expression of p43, a triiodothyronine-dependent mitochondrial transcription factor, increases muscle cell differentiation (121, 132). Consistent with these observations, myogenic differentiation is accompanied by strong stimulation of mitochondrial biogenesis both in vitro (105) and in vivo during skeletal muscle regeneration (39). The existence of distinct and opposite metabolic profiles as a function of cellular state is also illustrated by the observation that quiescent satellite cells have a strong mitochondrial oxidative potential and a low glycolytic potential when compared with proliferating myoblasts (6, 7). Taken together, these findings support the notion that mitochondrial metabolism and mitochondrial content may exert a myogenic influence, and stimulation of mitochondrial biogenesis is necessary for the commitment of myoblasts into myotubes. Seyer and colleagues (132) recently provided new insights into the myogenic influence of mitochondria by showing that stable overexpression of p43 decreases the mRNA and protein levels of c-myc. Notably, this is true even in serum-rich medium, further illustrating that p43 potently blocks proliferation. Overexpression of p43 stimulates expression of the myogenic transcription factor myogenin (121, 132), whereas c-myc overexpression abrogates the ability of myogenin and MyoD to induce terminal differentiation (132). Therefore, mitochondria appear to exert their myogenic influence by regulating both cell cycle withdrawal and the expression of myogenic proteins, possibly by regulating c-myc expression. Future studies will be required to determine the pathways and target genes involved in the myogenic influence of mitochondria, as well as to explore the relationship between the pathways regulating mitochondrial biogenesis and myogenic differentiation.

**FUTURE DIRECTIONS AND CONCLUDING REMARKS**

The concept of the energy dependence of nuclear gene expression in skeletal muscle is still taking shape, but our current understanding provides a comprehensive and synthetic view of muscle plasticity and the relationships among various metabolic and genetic aspects. Additional studies aimed at identifying the various messengers, energy-sensing molecules, downstream effector molecules (kinases, phosphatases, transcription and translation factors) and target genes will benefit our understanding of energy sensing in signaling. For instance, messengers whose activities are regulated by changes in energy homeostasis, such as acetyl-CoA (36) and cytochrome c (42), could be studied in further detail. Additional knowledge regarding the role of amino acids in regulating protein translation and gene expression will also be useful in this context (for review, see Ref. 78). Several of the recently identified energy-
sensing molecules, such as Sirt1 (discussed above), Sirt3, cytosolic guanylyl cyclases (cGCs) and MondoA, could be studied in greater detail. Localized inside the mitochondrion, Sirt3, like Sirt1, is a NAD+-dependent protein deacetylase. Expression of Sirt3 in HB1B adipocytes enhances expression of PGC-1α and uncoupling protein 1 (134). This effect involves the phosphorylation of cAMP response element binding protein (CREB) in the cytosol, suggesting that Sirt3 expression triggers a mitochondrial signaling response. This is strengthened by the observation that Sirt3 regulates the amount of acetyl-CoA available for the tricarboxylic acid cycle (51), thus contributing to the regulation of mitochondrial respiration. The GCs are nitric oxide (NO+) “receptors” that convert GTP to cGMP, a second messenger that activates G family kinases. Activation of cGMP-dependent pathways by NO+ activates Sirt1 expression (113) and stimulates mitochondrial biogenesis in skeletal muscle (112). cGC is inhibited by the binding of ATP on an allosteric purinergic site of the enzyme (123). Notably, this inhibitory effect tightly depends on the production of ATP by mitochondrial oxidative phosphorylation, showing that cGC has the capability to modulate nuclear gene expression by sensing local variations in ATP concentration. Another molecule whose activity is presumably regulated by energy homeostasis is MondoA, a bHLH-leucine zipper partner for the Max-like protein, Mix. MondoA is a nuclear transcription factor that shuttles between mitochondria and the nucleus in skeletal muscle to regulate expression of glycolytic enzymes, such as lactate dehydrogenase, hexokinase, and phosphofructokinase (127). Given the role of mitochondria in intracellular bioenergetics, it has been proposed that MondoA communicates information about the energy state between the mitochondria and the nucleus to activate the transcription of glycolytic genes (127). Overall these data are providing multiple avenues for further study of the regulation of muscle gene expression by energy-sensing molecules. The development of knockout mice for energy-sensing molecules, as well as the use of drugs or diets that markedly alter the energetic status of skeletal muscle, will also be helpful for testing the responses of various energy sensing molecules. Finally, the role of acute and chronic muscle contractile activity in triggering and modifying the process of energy sensing should be evaluated. Clarifying the exact mechanisms and regulatory influences among energy-sensing molecules will be essential for a comprehensive analysis of skeletal muscle cell plasticity in response to altered functional demand.

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