(SOCE), depends on the cooperation of several proteins as STIM1, Orai1 and TRPC1. The role of STIM1 as the calcium sensor of the ER and Orai1 as the Ca^{2+} influx channel is well-known from the recent publications, but the function of TRPC1 as a store-operated channel remains elusive.
Here TRPC1 was overexpressed by liposome-mediated transfection in C2C12 mouse skeletal muscle cell line. Overexpression was confirmed at mRNA level by RT-PCR and at protein level by immunostaining and Western-blot. The SOCE mechanism was studied by measuring the changes in $[Ca^{2+}]_i$ evoked by the re-addition of 1.8 mM $[Ca^{2+}]_e$ following the SERCA-inhibition by thapsigargin. As a result of TRPC1 overexpression, the amplitude and the maximum of the derivative of SOCE was significantly increased. When YM-58483, the antagonist of TRPC1 was used, these differences were eliminated, moreover in TRPC1-overexpressing myotubes the SOCE was slightly but not significantly lower, suggesting the downregulation of the STIM1-Orai1 system. This decrease in the expression level of STIM1 was confirmed by Western-blot together with the downregulation of SERCA. As a consequence a reduction in maximal Ca^{2+} uptake, and a higher resting $[Ca^{2+}]_i$ following the transients evoked by 120 mM KCl were detected. Morphological changes caused by the overexpression of TRPC1 were also observed. The differentiation of the myoblasts started later, and the myotubes were thinner in TRPC1-overexpressing cultures.
Our results suggest that enhancing the expression level of TRPC1 increases SOCE and has a negative feedback effect on the STIM1-Orai1 system, suggesting a cooperation between these proteins.

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Leucine-Zipper Mediated Intermolecular Interaction between MG53 is Essential for Cellular Membrane Repair

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We recently found that MG53, a muscle-specific TRIM family protein, functions as a sensor of oxidation to nucleate the assembly of cell membrane repair machinery (Cai et al, 2009 Nature Cell Biology). Our data showed that disulfide formation mediated by Cys242 is critical for MG53-mediated translocation of intracellular vesicles toward the injury sites. Here we test the hypothesis that leucine-zipper motifs in the coiled-coil domain of MG53 constitute an additional mechanism that can facilitate oligomeric formation of MG53 for assembly of the cell membrane repair machinery. Chemical cross-linking studies show that the coiled-coil domain of MG53, which contains the two putative leucine zipper motifs LZ1 (L176/L183/L190/V197) and LZ2 (L205/L212/L219/L226), is involved in formation of MG53 dimers. While mutation of LZ2 does not affect oligomeric interaction of MG53, replacement of 3 leucine residues to alanines in LZ1 leads to compromised oligomeric formation of MG53. Moreover, double mutation of LZ1 and LZ2 completely disrupts MG53 oligomeric formation, even under non-reducing conditions. Live cell imaging revealed that the movement of GFP-tagged MG53 mutants, GFP-LZ1 and GFP-LZ2, in response to mechanical damage of the cell membrane is significantly reduced relative to the wild type GFP-MG53 construct. Furthermore, the GFP-LZ12 double mutant is completely ineffective in translocation toward the injury sites, and cannot repair acute damage to cell membranes. Our data show that leucine-zipper mediated oligomer formation is essential for cell membrane repair. LZ1 likely constitutes a critical motif for disulfide cross-link between Cys242, during assembly of the membrane repair machinery.

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Fatigue in Mouse Muscle Fibers: Role of Mitochondrial ATP-Sensitive Potassium Channels

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Aim. The role of mitochondrial K_{ATP} (mitoK_{ATP}) channels on muscle fatigue was assessed in adult mouse skeletal muscle fibers.
Methods. Muscle fatigue was produced by eliciting short repetitive tetani. Isometric tension and the rate of production of reactive oxygen species (ROS) were measured at room temperature (20-22°C) using a force transducer and the fluorescent indicator CM-H_2DCFDA.
Results. We found that opening mitoK_{ATP} channels with diazoxide (100 ∈ μM) significantly reduced muscle fatigue. Fatigue tension was 34% higher in diazoxide-treated fibers relative to controls. This effect was blocked by the mito-K_{ATP} channel blocker 5-Hydroxydecanoate (5-HD), by the protein kinase C (PKC) inhibitor chelerythrine, and by the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME). We found that the rate of ROS production in muscle fibers incubated with diazoxide under non-fatigue and fatigue conditions was similar to control experiments, but that the increase in the rate of ROS production during recovery from fatigue was greatly reduced.
Conclusions. A physiological role of mitoK_{ATP} channels on muscle fatigue is proposed.
Diazoxide, an opener of mitoK_{ATP} channels, reduces muscle fatigue possibly through a preservation of mitochondrial volume and function.
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Inducible Activation of Akt Increases Skeletal Muscle Mass and Force Without Satellite Cell Activation

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A better understanding of the signaling pathways that control muscle growth is required to identify appropriate countermeasures to prevent or reverse the loss of muscle mass and force induced by aging, disuse or neuromuscular diseases. However, two major issues in this field have not yet been fully addressed. The first concerns the pathways involved in leading to physiological changes in muscle size. Muscle hypertrophy based on perturbations of specific signaling pathways are either characterized by impaired force generation, e.g. myostatin knockout, or incompletely studied from the physiological point of view, e.g. IGF-1 over-expression. A second issue is whether satellite cell proliferation and incorporation into growing muscle fibers is required for a functional hypertrophy. To address these issues, we used an inducible transgenic model of muscle hypertrophy by short-term Akt activation in adult skeletal muscle. In this model, Akt activation for three weeks was followed by marked hypertrophy (approximately +50% of muscle mass) and by increased force generation, as determined in vivo by ankle plantar flexor stimulation, ex vivo in intact isolated diaphragm strips and in single skinned muscle fibers. No changes in fiber type distribution and resistance to fatigue were detectable. BrdU incorporation experiments showed that Akt-dependent muscle hypertrophy was accompanied by proliferation of interstitial cells but not by satellite cells activation and new myonuclei incorporation, pointing to an increase in myonuclear domain size. We can conclude that during a fast hypertrophic growth myonuclear domain can increase without compromising muscle performance.

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Heat-Shock Treatment Induces Hypertrophy in C2C12 Muscle Cells

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Heat shock proteins (Hsp's) are molecular chaperones that are critical for the maintenance of cellular homeostasis and have been shown to protect cells and tissues from a large variety of damaging insults. Conflicting reports have been published on the effects of HS on whole animal models of hypertrophy. Some reports suggest that HS inhibits skeletal muscle growth, while others show an increase in the size and proliferative potential of intact skeletal muscles. However recent studies have suggested that heat shock can induce hypertrophy in cardiac muscle cell lines. The cellular response of skeletal muscle cells exposed to heat shock (HS) is to our knowledge an untested treatment method for studying muscle hypertrophy. Therefore, our main goal was to examine the hypertrophic reactions to a mild HS treatment (43°C for 20 min) in C2C12 muscle cells. C2C12's are widely considered to be very representative of skeletal muscle cells in vivo, and can therefore serve as a useful model of physiological hypertrophy. We found that our mild HS treatment induced significant hypertrophy (> 30% of cell area growth) of differentiated C2C12 myotubes, indicating that HS is an effective treatment for induction of hypertrophy in C2C12 muscle cells. We are now investigating the mechanisms underlying HS-induced hypertrophy in C2C12 muscle cells. We believe that these studies will be useful in establishing the foundation for a better and in-depth understanding of HS-induced stress in skeletal muscles. *(Support:American Heart Association 0535355N, Missouri Life Sciences Research Board & NIH Opportunities Grant/Recovery Act (GO Grant) to MB*.