The $Ca_V1.1$ calcium channel is the voltage sensor of skeletal muscle excitation-contraction (EC) coupling. The current of the adult $Ca_V1.1a$ splice variant is slowly activating, small and has poor voltage-sensitivity, whereas that of the embryonic $Ca_V1.1e$ splice variant, lacking exon 29 in the IVS3-S4 linker, has an 8-fold higher amplitude, activates fast and at 30mV less depolarizing potentials (Tuluc *et al*.,2009). Here we created intra-molecular chimeras to test the hypothesis that the voltage sensors of homologous repeats I to IV differentially control current kinetics, voltage-dependence, and EC-coupling. Inserting the IVS3-S4 linker (mainly coded by exon 29) plus IVS4 into the corresponding region of repeat I fully restored $Ca_V1.1a$ amplitude and voltage-sensitivity to $Ca_V1.1e$. Transferring the IVS3-S4 linker plus IVS4 into the homologous region of repeat II enhanced the voltage-sensitivity of the calcium current but fully abolished skeletal muscle EC-coupling. Any substitution in IS3-S4 resulted in accelerated activation-kinetics. However, slow activation-kinetics could not be transferred from the I^{st} to the IV^{th} repeat. Interestingly, inserting IS3 plus the IS3-S4 linker into repeat IV fully restored the poor $Ca_V1.1a$ voltage-dependence and small amplitude in the absence of exon 29. Secondary structure prediction revealed a beta-sheet in exon 29 that is missing in $Ca_V1.1e$. Point mutations which abolish this beta-sheet partially recapitulate the effects of deleting exon 29. Together these findings suggest a model according to which the voltage sensor of repeat IV controls the voltage-dependence and amplitude and its properties can be transferred to repeat I; the voltage sensor of repeat I determines the current kinetics exclusively in the context of this repeat; and the voltage sensor of repeat II controls EC-coupling. *Funded by the Austrian Science Fund P20059-B05, P23479-B19 and MFI-2007-417.*

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Calsequestrin-1 is Required for Association but not for Targeting of Triadin to the Junctional Sarcoplasmic Reticulum Membranes

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In striated muscles two membrane systems, the sarcolemma and the sarcoplasmic reticulum (SR) are typically arranged in contiguous junctional membrane complexes (JMCs), called triads. Triads are key components in the excitation-contraction coupling mechanism, as they supply the structural elements necessary for efficient crosstalk between voltage-dependent calcium channels (DHPR) on the T-tubules and the ryanodine receptors calcium channels (RyRs), on the SR. Other proteins including triadin, junctin, calsequestrin and junctophillins are selectively localized to JMCs and assemble into regularly arranged complexes. Mechanisms explaining membrane compartmentalization include selective targeting and ''trapping'' machineries, which may prevent the spread of molecules throughout the membrane surface. However, no specific mechanisms have been yet associated with either assembly or maintenance of JMCs in striated muscles. In previous studies we showed that the mobile fraction of triadin progressively reduced during differentiation, suggesting that a trapping mechanism may be efficiently active in striated muscle and indicate triadin as a good candidate to study protein localization and association to the JMCs.

Expression of GFP-tagged triadin proteins and deletion mutants in rat myotubes showed that three regions (named 1, 2 and 3) were required for protein targeting to the JMCs. On the contrary, analysis of protein mobility revealed that association of triadin to the JMC was mediated by region 3 only. GST-pull down and FRET experiments showed that region 3 was able to interact with calsequestrin-1. Interestingly, experiments performed on myotubes from calsequestrin-1 knockout mice revealed that in the absence of calsequestrin, triadin was properly localized to the JMC, although its mobile fraction was dramatically increased. These results suggest that calsequestrin-1 is directly involved in trapping triadin to the JMCs, whereas selective targeting is likely to be mediated by interactions with other proteins.

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Upregulation of Calcium Influx via Cav1.1 in Skeletal Muscle Fibers from JP-45 Calsequestrin 1 Double Knock Out Mice

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JP-45, a 45 kDa transmembrane protein of skeletal muscle sarcoplasmic reticulum, interacts with the alfa1.1 subunit of the dihydropyridine receptor (Cav1.1) and calsequestrin (CASQ), two key proteins of the EC-coupling machinery. The JP-45 protein may be a component of a signaling pathway connecting the SR lumen (CASQ) to the Cav1.1. We generated JP-45 and CASQ1 double knockout (dKO) mice and compared their phenotype with age-matched wild-type (WT) littermates. Analysis of spontaneous motor activity revealed no difference in the running distance between dKO and WT mice. The intracellular peak Ca2+ signal evoked by a 0.5 ms pulse at supramaximal field stimulation was lower in FDB fibers from JP-45 null, CASQ1 null and dKO mice compared to WT. The global Ca2+ signal evoked by a train of pulses at 100 Hz for 300ms was negligibly affected by La3+ in WT, JP-45 null and CASQ1 null fibers, but strongly inhibited in fibers from dKO mice. Mn2+ quenching experiments in fura-2 loaded FDB fibers indicate that Ca2+ influx accounts for most of the myoplasmic [Ca2+]i increase during tetanic stimulation in fibers from dKO mice. The Mn2+ influx was blocked by 50 μM nifedipine an inhibitor of Ca2+ currents via Cav1.1. These results demonstrate that lack of JP-45 and CASQ-1 upregulate Ca2+ influx via Cav1.1.

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The Changes of $[Ca^{2+}]$ in Sr and Ca^{2+} Release Flux during Fatiguing Activation of Mouse Skeletal Muscle Fibers

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Sustained activity leads to muscle fatigue, cellular correlates of which include depression in Ca^{2+} released per action potential (Györke, JPhysiol, 1993; Westerblad, JAppPhysiol, 1993). This decrease is also observed in cells activated with voltage clamp pulse trains (Royer, JGP, 2010), which points at effectors downstream from the voltage sensor, including depletion of SR Ca^{2+} (Allen, JAppPhysiol, 2011). In mouse FDB cells expressing the biosensor D4cpv-calsequestrin loaded with X-rhod1-AM or in mice constitutively expressing the troponin-based FRET biosensor TnXX (Mank, NatureM, 2008) we measure $[Ca^{2+}]_{SR}$, $[Ca^{2+}]_{cyto}$ and release flux. Fatiguing activation is produced by 0.5s trains of action potentials at 2s intervals. Concentrations were measured before the tetanus (*rest*) or at its end (*tet*) in the rested condition (t0), upon 1 or 3 min of stimulation (t1,t3) and after 10 min recovery (A10). Flux was calculated as amount released/tetanus duration. Further analysis of this quantitative picture of Ca^{2+} handling during fatigue indicates that the reduction in Ca^{2+} release is the outcome of interconnected decreases in SR load, Ca^{2+} release permeability and SR Ca^{2+} buffering power.

t0	t1	t3	A10	
$[Ca^{2+}]_{cyto,\ rest}$, nM	80	127	162	117
$[Ca^{2+}]_{cyto,\ tet}$, nM	250	254	234	187
flux, mM/s	1.32	1.09	0.58	0.72
Δ amount, mM	0.53	0.46	0.27	0.30
$[Ca^{2+}]_{SR,rest}$,μM	290	245	240	280
$[Ca^{2+}]_{SR,tet}$, μM	210	195	195	205

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Endurance Exercise Increases Force Production in Mouse Fast-Twitch Muscles

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Endurance exercise is known to induce mitochondrial biogenesis and improve the ability to utilize oxygen. Here we show that endurance training also can improve force production in mouse fast-twitch extensor digitorium longus (EDL) muscles under non stressed conditions. Wild-type mice had unlimited access to running wheels for 7 weeks. Mice ran in average a distance of 3.500 meters per 24-hour. Control group were mice with non-rotating running wheels in their cages. After 7 weeks mice were sacrificed and force and fatigue was measured in isolated, intact EDL muscles. No difference was observed in the rate of fatigue between exercised and non-exercised mice, which might be due to deficient oxygen diffusion into deeper parts of the isolated muscles. However, the maximal force production in EDL was increased by ~ 30% in exercised compared with non-exercised mice. Reduced FKBP12 binding to the major intracellular Ca^{2+}-release channel (ryanodine receptor type 1 (RyR1)) has been associated with muscle weakness induced by high-intensive exercise. We have indications that the exercised muscles showing increased force have decreased FKBP12 binding compared to the control group. This suggests that decrease in FKBP12 binding to RyR1 might not only be deteriorating for the muscle function. Instead our results indicate a more complex modulation where decreased FKBP12 binding to RyR1 in response to exercise could be either beneficial (training) or detrimental (overtraining) for muscle function.