high values. The spatial heterogeneity of expression permitted comparison of flux and buffer properties evaluated with the same depolarizing pulse simultaneously in regions of widely different [biosensor]. The hump of Ca^{2+} release flux and other features of buffer hysteresis were restored in regions of calsequestrin-null cells where D4cpv-calsequestrin reached above 8 μmol/liter of cytosol. The restoration was partial or nil at [biosensor] below 3 μM. At 10 μM a functional calsequestrin moiety in the biosensor should provide 800 μM binding sites (or 400 μM Ca^{2+} at 50% occupancy), a significant contribution compared with the estimates of amount released (1240 μM in WT, 867 μM in the null). Therefore, in addition to a targeted biosensor D4cpv-calsequestrin is a fluorescently tagged Ca^{2+} buffer. Moreover, the restoration of hysteretic Ca^{2+} buffering features indicates that this biosensor contributes the full buffer functionality of calsequestrin. To our knowledge, this is the first example of a molecule with the functional properties of both a biosensor and a native protein. Funded by NIAMS/NIH.

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Role of Triadin in the Organization of Reticulum Membrane at the Muscle Triad

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Calcium release from the sarcoplasmic reticulum (SR) triggers skeletal muscle cell contraction and takes place in highly organized membrane structures, called triads. A triad is composed of two SR terminal cisternae surrounding a plasma membrane transverse-tubule. This architecture is essential to sustain the activity of the calcium channel RyR1, which is located in the membrane of SR cisternae. However, little is known about the molecular mechanisms allowing the formation and maintenance of SR terminal cisternae. We have previously shown that ablation of triadin, a SR transmembrane protein interacting with RyR1, induces a modification of the shape of the triads in KO mice. Here we explore the intrinsic molecular properties of the triadin Trisk 95. We show that when ectopically expressed, Trisk 95 is able to modulate reticulum membrane morphology. The membrane deformations that are induced by Trisk 95 are accompanied by modifications of the microtubule network organization. We provide evidences that the multimerization of Trisk 95 via disulfide bridges, and an indirect interaction with microtubules, are responsible for the ability of Trisk 95 to structure reticulum membrane. When domains responsible for these molecular properties are deleted from Trisk 95, its anchoring to the triads in muscle cells is strongly decreased. Overall, our data suggest that multimerization of Trisk 95 and binding to microtubules contribute to the organization of membranes of the SR in a triad.

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Interactions Between the Isolated DHPR β_{1A} Subunit and the Type 1 Ryanodine Receptor

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Excitation-contraction (EC) coupling in skeletal muscle proceeds in the absence of an influx of external calcium ions. This suggests that there is a physical interaction between the dihydropyridine receptor (DHPR) voltage sensor in the surface/transverse tubule membrane and the skeletal muscle ryanodine receptor (RyR1) calcium release channel in the membrane of the intracellular sarcoplasmic reticulum. The α_{1s} and β_{1a} subunit of the DHPR, as well as RyR1 are essential for skeletal EC coupling, but the mechanism of their interaction remains undefined. One scenario, that α_{1s} subunit communicates with RyR1 via the β_{1a} subunit, is supported by studies of EC coupling in intact cells as well as by interactions between the three proteins in vitro. We have described interactions between the critical region of the α_{1s} subunit and the SH3 domain of the β_{1a} subunit (Karunsekara et al. Biophys Abstract 2011) and between RyR1 and the full length β_{1a} subunit or the 35 residue C-terminal tail of β_{1a} (V^{490} - M^{524}) (Rebbeck et al. Biophys J. 2011;100:922-30). We have also reported that a hydrophobic heptad repeat implicated by others in β_{1a} interactions with RyR1 is not required for the in vitro interactions (Rebbeck et al., Biophysics abstract 2011). We now focus on a ''hydrophobic triplet'' (L^{496}, L^{500} and W^{503}) in the β_{1a} C-tail that is aligned along one surface of an alpha helix and is separated from the extreme C-terminus by 21 residues. Individual substitution of L^{496}, L^{500} or W^{503} with alanine abolished the ability of the peptide to increase channel activity at +40mV, although residual activation remained −40mV. Substitution of the three residues rendered the peptide inactive at both potentials, indicating that all three residues are required for this protein-protein interaction between the β_{1a} C-tail and RyR1.

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Dual Roles of Extracellular Calcium in Excitation-Contraction Coupling of Mouse Skeletal Muscle

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Ca^{2+} ions have four established roles in EC coupling: trigger for contraction, mediators of release (CICR), controllers of RyR channels from the SR-lumen and agents of Ca^{2+}-dependent inactivation. Additionally, in frog muscle extracellular Ca^{2+} protects the t-tubule voltage sensor (DHPR) from voltage-dependent inactivation (Brum *et al*. J Physiol 1988). This ''priming'' role of Ca^{2+} results in rapid DHPR inactivation in solutions with low [Ca^{2+}], unless Ca^{2+} is replaced by suitable cations (Pizarro *et al*. JGP 1989). In mammalian muscle, zero extracellular [Ca^{2+}] is imposed frequently to assess the contribution of SOCE to Ca^{2+} homeostasis, under the assumption that any effects will be mediated by depletion of Ca^{2+} in the SR. In mouse FDB muscle fibers under voltage clamp we combined measurements of SR [Ca^{2+}] ([Ca^{2+}]$_{SR}$), Ca^{2+} release flux and intramembranous charge movement (Q_m). Upon long-lasting depolarization both the net amount of Ca^{2+} released and the measured decay in [Ca^{2+}]$_{SR}$ (consequence of SR depletion) stabilize after 200-500 ms, reflecting the attainment of balance between release through RyR channels and reuptake by SERCA. In ''low (25 μM) Ca'' external solutions net Ca^{2+} release becomes negative -uptake dominates- and concomitantly [Ca^{2+}]$_{SR}$ starts to recover from depletion *during the depolarizing pulse*. The available Q_m is somewhat reduced at rest, and decays to near 0 during the pulse. These observations demonstrate that the priming role of Ca^{2+} is also present in mammalian muscle. The conclusion calls into question the interpretation of earlier studies on mouse muscle (including our own; Royer *et al*. JGP 2010), which attribute the depressing effects of low extracellular [Ca^{2+}] exclusively to SR depletion. In mice as in frogs, low extracellular [Ca^{2+}] also promotes voltage-dependent inactivation of the t-tubule voltage sensor. Supported by NIAMS/NIH(ER) and the Physiological Society (GB).

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Null Mutations for Triadin and Junctin Reveal Triadin *Anchors* and the Requirement for Triadin-Junctin-CASQ Complexes in Stablizing RyR Resting Leak

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Triadin (Tdn) and Junctin (Jct) are structurally related intramembrane proteins that link the ryanodine receptors (RyRs) and calsequestrin (CASQ) at the luminal side of the junctional sarcoplasmic reticulum (jSR). Mice lacking either Tdn (Tdn-/-), or Jct (Jct-/-), or both demonstrate partially overlapping, but distinct roles for Tdn and Jct in stabilizing CASQ expression. By electron microscopy we identify Tdn as the main component of periodic *anchors*, presumably as cross-linked complexes, that connect CASQ and the jSR membrane. Jct is more diffuse and its presence cannot be directly visualized. CASQ expression level is not affected in Jct-/-, but decreased by ~ 40 % in Tdn -/- and by ~93% in the double mutant, suggesting that Tdn is a strong stabilizer of CASQ, but Jct also contributes. The jSR volume closely follows the CASQ content, being unaltered in Jct-/-, but decreased significantly in Tdn-/- and even further in Tr-/-/Jct -/-. In the single nulls, the remaining CASQ is retained within the jSR, but in the double mutant it is found in large extra-junctional cisternae. Ca^{2+} transients induced by K^+ depolarization and caffeine in Tr-/-/Jct-/- are smaller than WT, in parallel to reduced SR Ca^{2+}, but show no shift in voltage dependence of Ca^{2+} release, indicating that the e-c coupling mechanism is not altered by the absence of the Tdn/Jct/CASQ complex. However, increased levels of resting cytoplasmic Ca^{2+} concentration were found in all mutants indicating a stabilizing role of the complex on RyR leak at rest.

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Modular Contribution of $Ca_v1.1$ Voltage Sensors to Calcium Channel Gating and Excitation-Contraction Coupling

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