lifting inhibition. The force-sensing ability of titin kinase was demonstrated in AFM experiments and simulations [Puchner et al., 2008, PNAS 105, 13385], which showed that mechanical forces can remove the autoinhibitory tail of titin kinase. We report here steered molecular dynamics simulations (SMD) of the very recently resolved crystal structure of twitchin kinase, containing the kinase region and flanking fibronectin and immunoglobulin domains, that show a variant mechanism. Despite the significant structural and sequence similarity to titin kinase, the autoinhibitory tail of twitchin kinase remains in place upon stretching, while the N-terminal lobe of the kinase unfolds. The SMD simulations also show that the detachment and stretching of the linker between fibronectin and kinase regions, and the partial extension of the autoinhibitory tail, are the primary force-response. We postulate that this stretched state, where all structural elements are still intact, may represent the physiologically active state.

Excitation Contraction Coupling I

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FRET Reveals Substantial Reorientation of the Cytoplasmic Interface of the Skeletal Muscle DHPR in the Presence of RyR1
Alexander Polster1, Joshua Ohtman1, Kurt G. Beam1, Symeon Papadopoulos2.
1University of Colorado - Denver, Aurora-Denver, CO, USA, 2University of Cologne, Cologne, Germany.
In skeletal muscle, the dihydropyridine receptor (DHPR) in the t-tubular membrane serves as Ca2+-channel and as voltage sensor for excitation-contraction (EC) coupling, triggering Ca2+-release via a physical/conformational coupling to the type I ryanodine receptor (RyR1) in the sarcoplasmic reticulum (SR) membrane. The particulars of the structural and functional links between these two proteins are widely unknown. The putative intracellular portions of the DHPR a1S subunit, the N-terminus, C-terminus, and the loops connecting the four homologous repeats (I-IV), play important roles in the communication with the RyR1. Examples are the β-subunit recruiting function of the I-II loop, the bi-directional signaling function of the II-III loop with the RyR1 during EC-coupling, the influence of the III-IV loop on RyR1 mediated Ca2+-delivery, and the a1S C-terminus. These channel parts are believed to either directly or indirectly interact with the RyR1, and the close spatial proximity between the two channels at t-tube/SR ‘junctions’ constitutes the structural prerequisite for this linkage. The present work provides for the first time a structural insight into the arrangement of the crucial molecular components of the DHPR-RyR1 interaction, by using measurements of fluorescence resonance energy transfer (FRET), conducted within the cellular environment of living myotubes. Upon expression, the degree of FRET was determined for different combinations of labeled cytoplasmic a1S domains, using a sensitized emission FRET variant. Confocal fluorescence microscopy was applied to check for correct expression and function of the constructs upon expression in dyspedic (RyR1 null) and dysgenic (a1S null) myotubes. The presence of RyR1 significantly altered the intramolecular energy transfer for almost every double tagged a1S variant. Confocal fluorescence microscopy was applied to check for correct expression of labeled cytoplasmic a1S domains, using a sensitized emission FRET under patch clamp we measured luminal [Ca2+]lum with the novel sensor D4cpv-calsequestrin (Sztretye et al. JGP 2011a) in parallel with SR Ca2+-release flux (derived from the fluorescence of cytosolic X-rhod 1). These simultaneous measurements allowed the dynamic monitoring of Ca2+-buffering power of the SR (BP = Δ[Ca2+]lum, Sr/Δ[Ca2+]lum). BP started at 180 (SEM 30, 27 cells) and decreased to 89 (SEM 14) as the SR lost Ca2+ upon SR-depleting membrane depolarization (400 ms, >30 mV). The stage of high BP is also characterized by a ‘hump’ in the release flux waveform. Recovery of SR Ca2+-content after the end of depolarization proceeded at the lower BP value (a mismatch that we call ‘buffer hysteresis’). BP regained slowly its initial value, as demonstrated by persistent low power and absence of hump in the second depolarizing pulse of pairs separated by 600 ms. Full restoration of hump and BP was observed after 2 min rest. In calsequestrin1-null cells BP was a constant 40 during the pulse (3.6, 29 cells), there was neither buffer hysteresis nor flux hump. Therefore, calsequestrin 1 is the time-dependent, hysteretic buffer, and contributes approximately 75% of the SR buffering power. Total releasable Ca2+, however, only decayed by 30% in CSQ-null cells, a paradox explained by a more thorough description of calsequestrin-dependence proposed to accompany Ca2+-binding (e.g. Park et al. JBC 2004). In this view, the hysteresis reflects the time required for the Ca2+-association–induced structural changes in calsequestrin to reverse upon Ca2+ reuptake into the SR. Funded by NIAMS/NIH and MDA.

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Role of STIM1 in Skeletal Excitation-Contraction Coupling
Jin Seok Woo, Keon Jin Lee, Eun Hui Lee.
Sch of Med, The Catholic Univ of Korea, Seoul, Korea, Republic of.
STIM1, a Ca2+-sensing protein on ER/SR membrane, mediates a store-operated Ca2+-entry (SOCE) by activating Orai1 Ca2+-entry channel on plasma membrane. E136X mutant of STIM1, truncated STIM1 missing binding abilities to Orai1, has been found in patients with immunodeficiency accompanying muscular hypotonia. To identify causes of the muscular hypotonia in patients with E136X is due to changes in excitation-contraction coupling. Additionally, we suggest that C-terminus of STIM1 that is missing in E136X participates in the regulation of skeletal EC coupling. Additionally, we suggest that C-terminus of STIM1 that is missing in E136X participates in the regulation of skeletal EC coupling.