Assembly of the Adenoviral IVa2 and L4-22K Proteins on the Viral DNA Packaging Sequence

ARTICLE *in* BIOPHYSICAL JOURNAL · FEBRUARY 2009

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2008.12.2670

1 AUTHOR:

Teng-Chieh Yang

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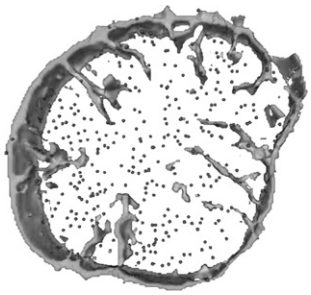
to the cell long axis. This improved the separation of RyR clusters and the classification based on their relationship to the sarcolemma. These data confirmed our initial finding obtained on cells lying flat, that most of RyRs are non-junctional (see figure). It is unclear whether they contribute to the Ca transient. If they do, the transient would reflect the combined activity of locally controlled couplons with the activation of non-junctional RyRs, which are not locally controlled.



2660-Pos Board B630

Stochastic Dynamics of Release Unit in a Cardiac Cell in Electron-Conformational Model

Alexander S. Moskvin[1], **Alexander M. Ryvkin[1,2]**, Olga E. Solovyova[1,2], Vladimir S. Markhasin[2].
[1]Ural State University, Ekaterinburg, Russian Federation, [2]Institute of Immunol & Physiol of the RAS, Ekaterinburg, Russian Federation.

To further understand the role of stochastic dynamics of ryanodine receptor (RyR) channels on spark generating process we studied the stochastic RyR's cluster gating in calcium release unit (RU) in cardiomyocytes under steady-state conditions. We apply a simple biophysically-reasonable electron-conformational (EC) model [Moskvin ea, PBMB, 2006] for the RyR channel. Single RyR channels are characterized by fast electronic and slow classical conformational degrees of freedom. The RyR gating implies calcium induced electronic transitions between two branches of a conformational potential, a conformational Langevin dynamics, thermoactivated transitions and quantum tunneling. The sarcoplasmic (SR) load is incorporated into the model through the effective conformational strain.

We examined different model dependencies of the electronic transition probability on the calcium ion concentration and effective temperature in dyadic space to reproduce all the features observed in lipid bilayer experiments. The 11×11 RyR cluster was build into a simple RU dynamic unit. Model simulations performed in frames of a diabatic approximation with a conformational inter-RyR coupling have revealed different gating regimes with a single RyR channel openings generating a Ca^{2+} synapse (quark) and a cooperative cluster mode, due to a step-by-step opening of a fraction of coupled RyR channels. We have found and analyzed the spark generating openings of groups of channels, providing for a sufficient release. The SR overload was shown to lead to the autooscillation regime with nearly periodical openings-closings of RyR-channels. The RU functioning was examined under different rates of SR load and different strength of the inter-RyR coupling. The EC model was shown to provide an adequate description of the cardiomyocite RU dynamics with valid prediction abilities.

Supported by RFBR Grants 05-04-48352, 07-04-96126.

2661-Pos Board B631

A Local Control Model for Cardiac Excitation-Contraction Coupling in Rat Ventricular Myocytes: Insights into Dynamic Phenomena involving Calcium Release

George S.B. Williams[1], Marco A. Huertas[2], Eric A. Sobie[3], Greg D. Smith[2], M. Saleet Jafri[1].
[1]George Mason University, Manassas, VA, USA, [2]College of William and Mary, Williamsburg, VA, USA, [3]Mount Sinai School of Medicine, New York, NY, USA.

In our prior work, we introduced a computationally efficient moment closure approach to modeling local control of calcium-induced calcium release (CICR) in cardiac myocytes. This approach utilizes ordinary differential equations (ODEs) describing the time-evolution of the first and second moments of probability density functions for local calcium (Ca) concentrations jointly distributed with Ca release unit (CaRU) state coupled to ODEs for the bulk myoplasmic and network SR [Ca]. We have shown that this approach allows a deterministic simulation to capture important aspects of local [Ca] in simulated voltage-clamp protocols while dramatically improving computational efficiency over stochastic Monte Carlo simulations. However, previous results were limited to simulated voltage clamp protocols and incorporated only a minimal representation of the L-type channel. Here we present an expanded formulation that incorporates more realistic CICR dynamics coupled to a dynamic model of the rat action potential. The new model includes biophysically accurate models of the ryanodine receptor and L-type Ca channel which have been shown by previous modeling to be important for governing interval-force relations. We investigate how local control of EC coupling in cardiac myocytes influences phenomena depending on the dynamics of pacing and calcium release properties such as the formation of Ca alternans or RyR "autoregulation" that occurs during changes to RyR Ca sensitivity caused by agonists such as caffeine. Results are validated and benchmarked for computational efficiency by comparison to traditional Monte Carlo simulations.

2662-Pos Board B632

Modeling Nitric Oxide Regulation Of Ec Coupling In Cardiac Myocytes

Lulu Chu, Sa Ra Park, Mayank Tandon, William Guilford, Jeffrey J. Saucerman.
University of Virginia, Charlottesville, VA, USA.

Nitric oxide (NO) signaling is a potent modulator of cardiac contractility in conditions of increased heart rate or ß-adrenergic signaling. Changes in nitric oxide synthase (NOS), the enzyme responsible for NO production, play a significant role in EC coupling observed in heart failure following myocardial infarction. NO signaling is thought to modulate cardiac function by targeting a range of EC coupling proteins including ryanodine receptor, phospholamban, L-type Ca2+ channel and myosin. However, the mechanisms underlying NO signaling and the relative importance of NO targets are unclear. Previous computational models of ß-adrenergic signaling and EC coupling have not accounted for NO regulation. We propose a new model that incorporates NO metabolism and effects of eNOS and nNOS activity on EC coupling. This integrated model provides a consistent framework to quantitatively predict the combined effects of NO on EC coupling and explain discrepancies in prior experimental results.

Cell & Bacterial Mechanics, Motility, & Signal Transduction

2663-Pos Board B633

Assembly of the Adenoviral IVa2 and L4-22K Proteins on the Viral DNA Packaging Sequence

Teng-Chieh Yang.
University of Colorado Health Science Center, Aurora, CO, USA.

Human adenovirus is a non-enveloped virus containing double-stranded DNA. It can cause infection of the respiratory tract, urinary tract, and GI tract, especially in immunocompromised patients. Adenoviral genome packaging requires a *cis*-acting packaging sequence, which is composed of seven repeated elements, called A repeats, which are located at the left end of the genome, as well as *trans*-acting proteins. Previous genetic studies revealed that one of the *trans*-acting proteins, IVa2, interacts with specific sequences in the A repeats. Another *trans*-acting protein, L4-22K, also interacts with A repeats, but this interaction requires the IVa2 protein. In order to elucidate the molecular events that are responsible for adenoviral genome packaging, the binding properties of IVa2 and L4-22K to the packaging sequence were studied *quantitatively* by analytical ultracentrifugation (AUC). In our previous studies, we found that the IVa2 protein binds specifically to a truncated packaging sequence, which contains A repeats I and II, A-I-II, to form a 1:1 IVa2/A-I-II complex. Purified L4-22K binds to the IVa2/A-I-II complex, and requires IVa2 for this interaction. We have begun AUC studies to determine the assembly state of the L4-22K/IVa2/A-I-II ternary complex. Finally, purified L4-22K self associates in a concentration dependent manner. The implication of these results with respect to viral DNA packaging will be discussed.

2664-Pos Board B634

Suppressor Analysis of the MotB(D33E) Mutation, a Putative Proton-Binding Residue of the Flagellar Motor in *Salmonella*

Yong-Suk Che[1,2], Shuichi Nakamura[1,2], Yusuke Morimoto[1,2], Nobunori Kami-ike[2], Keiichi Namba[1,2], Tohru Minamino[1,2].
[1]Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan, [2]Dynamic NanoMachine Project, ICORP, JST, Osaka, Japan.

MotA and MotB form the stator of the proton-driven bacterial flagellar motor, which conducts protons and couples proton flow to motor rotation. Asp-33 of *Salmonella Typhimurium* MotB, which is a putative proton-binding site, is critical for torque generation. However, how does the protonation of Asp could drive the conformational changes requiring for torque generation is largely unknown.

Here, we carried out genetic and motility analysis of a slow motile *motB(D33E)* mutant and its pseudorevertants. We first confirmed that the poor motility of the *motB(D33E)* mutant is neither due to protein instability, mislocalization nor impaired interaction with MotA. We isolated 17 pseudorevertants and identified the suppressor mutations in the transmembrane helices TM2 and TM3 of MotA and in TM and the periplasmic domain of MotB. The stall torque