1899-Plat
Probing Protein Motions and Function in Ci-VSP
Susy C. Kohout, Sarah C. Bell, Ehud Y. Isacoff.
University of California, Berkeley, Berkeley, CA, USA.
With the discovery of the Ciona intestinalis voltage sensor-containing phosphatase (Ci-VSP), voltage sensing domains (VSDs) have moved beyond the exclusive realm of ion channels. By combining a VSD with a lipid phosphatase domain, Ci-VSP is the first enzyme known to be directly regulated by voltage. Interestingly, Ci-VSP is also the first protein with a monomeric VSD. Yet, a monomeric VSD still exhibits complex protein motions indicating that other factors beyond the oligomerization state of the domain play a part. We have applied two electrode voltage clamp electrophysiology to probe phosphatase function as well as voltage clamp fluorometry to probe the conformational changes involved in voltage sensing and the propagation of that signal to the phosphatase. The observed changes in fluorescence correlate with protein motions allowing any factors affecting the protein to be teased apart. These motions are influenced by the catalytic states of the phosphatase consistent with the expected coupling between the two domains. The linker between the voltage sensing domain and the phosphatase domain also influences these motions suggesting the linker may play an active role in protein regulation. By investigating Ci-VSP, we hope to gain a greater understanding of how the VSD functions.

1900-Plat
Uncoupling Of The Phosphatase Produces A Deeper Relaxation Of Ci-VSP
Carlos A. Villalba-Galea[1], Francesco Miceli[2], Francisco Bezanilla[1].
[1]Dept. of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA, [2]Dept. of Neuroscience, University of Naples "Federico II", Naples, Italy.
The coupling of the Voltage sensing domain (VSD) and the phosphatase domain (PD) of the Ciona intestinalis voltage sensing phosphatase (Ci-VSP) is mediated by a putative Phospholipid Biding Motif (PBM), located between the two domains. During depolarization, the movement of the S4 favors the binding of the PBM to the membrane, placing the PD in position to carry out its function. We have shown that upon prolonged depolarization, the VSD of Ci-VSP evolves to a relaxed state, as shown by a shift of the Q-V curve (Villalba-Galea et al., 2008). As a consequence of the relaxation of the VSD, fluorescence changes of a probe attached to the extracellular end of the Ci-VSP S4 were observed. However, we have now found that the relaxation produces changes along the entire S4 segment. We report here that mutations in the PBM, R253A-R254A, uncouple the sensing currents from the phosphatase activity, suggesting that the mutations abolish the coupling by disrupting the binding of the Arginine of the PBM to the phospholipid of the membrane. Interestingly, these mutations also increase the rate of the sensing currents during repolarization. A simple interpretation is that the S4 movement is restricted by the binding of the PBM to the membrane. If this is the case, it may also restrict the relaxation such that during a long depolarization the S4 could undergo a larger movement and reach a deeper relaxed state. Consistent with this interpretation we found that in the PBM mutant, the shift of the Q-V curve during prolonged depolarization is enhanced while the recovery time from the relaxed state is increased. Supported by NIHGM30376.

1901-Plat
Hv1: How A Voltage-sensor May Form A Channel
Younes Mokrab, Mark S.P. Sansom.
University of Oxford, Oxford, United Kingdom.
Unlike canonical voltage sensor domains, which are associated with other functional domains such as channel pore domains, or more rarely enzymes, Hv1 is a voltage sensor that acts as a proton channel per se. We used modelling and simulation tools in order to investigate the molecular mechanisms of function for this recently discovered voltage-gated proton channel.
As no three-dimensional structure has yet been determined for this protein, we built homology models based on the available structures of voltage-gated potassium channels. We performed coarse grain (CG) simulations of the interactions of these models with a phospholipid bilayer, enabling analysis of protein-lipid interactions. The equilibrium orientation from the CG simulations was used to aid setup of full-atom (AT) simulations with the protein embedded in a POPC bilayer. Analysis of the central pore radius and hydrogen bond interactions with water throughout the trajectories suggest that the models may capture different open conformations of Hv1. The models and simulations provide insights into a potential novel mechanism of H+ permeation involving hydronium coordination by Hv1 residues. Site-directed mutagenesis is being used to test the role of specific residues in H+ permeation through Hv1.

1902-Plat
Crystal Structure Of Full-length Kcsa Trapped In Open Conformation Reveals That C-terminal Domain Fine Tunes Activation And Coupled Inactivation
Serdar Uysal, Luis G. Cuello, Vishwanath Jogini, D. Marien Cortes, Shohei Koide, Eduardo Perozo, Anthony Kossiakoff.
University of Chicago, Chicago, IL, USA.
Using chaperone-assisted crystallography, we have recently determined the crystal structure of full-length (FL) KcsA in its closed conformation. The FL structure reveals that the C-terminal domain (CTD) extends ~70 Å towards the cytoplasm as a canonical four helix bundle stabilizing the closed state. Electrophysiological analysis of KcsA/Fab4 complex demonstrates that CTD remains as a bundle during activation gating. This motivated us to crystallize open conformation (OC) of FL KcsA using the same antibody fragments and a recently developed constitutively open mutant, which was crystallized in its truncated form. We solved the FL KcsA/Fab2 complex structure in the open state at 3.9 Å resolution. FL open-structure bends at G104 and exhibits ~10 Å displacement at the V115 creating four side windows large enough to accommodate hydrated K+ ions right below the gate. The CTD remains as four helix bundle, exerting strain on the bulge helices which connects the bundle and the transmembrane domain.
Based on the current structure and an earlier set of truncated KcsA structures displaying different degree of openings, we suggest that the cytoplasmic domain not only stabilizes the closed state but also fine tunes the level of opening at the activation gate and thus determine the level of inactivation occurring at the selectivity filter. Brownian Dynamics, simulation, electrophysiological studies and electrostatic calculation are ongoing efforts to dissect the ion permeation pathway.

1903-Plat
High Resolution AFM of KcsA Structure and Clustering in a Lipid Bilayer
Joanna A. Sobek[1], Sonia Antoranz Contera[1], Sonia Trigueros[1], Constantina Fotinou[2], Frances M. Ashcroft[2], J.F. Ryan[1].
[1]Department of Physics University of Oxford, Oxford, United Kingdom, [2]Department of Physiology, Anatomy and Genetics University of Oxford, Oxford, United Kingdom.
KcsA, a potassium channel found in *Streptomyces lividans*, is an important prototype for all other K^+ channels which share the highly conserved signature sequence TVGYG. It oligomerizes to form a tetrameric channel which is intracellular pH-activated in the range pH $<$ 5.5. The crystal structure of the transmembrane section in the closed state has been resolved at 2.0 Å which has provided an insight into the mechanisms of ion selectivity and gating. Its biological function remains poorly understood, but protein clustering observed *in-vivo* and *in-vitro* may be significant.
Amplitude Modulation Atomic Force Microscopy (AM-AFM) has been used to achieve sub nm single molecule structural information of the topology of membrane protruding segments of KcsA reconstituted in lipid bilayers under conditions approaching physiological ideal and to monitor changes induced by different pH conditions. The spatial resolution of AFM also allowed investigation of the supramolecular organization of KcsA within the membrane.
We have imaged KcsA reconstituted in 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] supported bilayers under neutral and acidic conditions (pH 3-7). We obtained high resolution images showing well-defined subunit structure with marked heterogeneity at low pH. Under neutral conditions, a change in channel height occurs, which is likely to be due to a conformation change and is accompanied by a loss of subunit detail. We also found the distribution of tetrameric KcsA in the lipid bilayer changed with pH. We infer that protein-protein interactions are responsible for lowering the 4-fold symmetry of the tetramer, and that pH-mediated clustering of KcsA and conformational change are governed by protein-protein and lipid-protein interactions. The observed dependence of KcsA spatial organization on pH has possible implications for channel activation (cooperative gating) and biological function.

1904-Plat
Using Electron Spin Echo Envelope Modulation (ESEEM) to Probe the Local Environment of Residues in the KcsA Potassium Channel
John A. Cieslak, Adrian Gross.
Northwestern University, Chicago, IL, USA.
Abstract: Recent studies on the voltage-dependent potassium channel KvAP have demonstrated the importance of the lipid bilayer in maintaining the correct orientation and packing of the voltage-sensor domain with respect to the pore domain [1, 2]. It has also been suggested that lipid may play an integral role in voltage-dependent gating [3, 4]. To date, the precise environment surrounding

the four important S4 arginine residues on the voltage-sensing paddle is still unknown, with some models placing them in an aqueous crevice, and others a lipid environment. To learn more about the intricate role of lipid in the structure and function of potassium channels we have studied deuterium and phosphate ESEEM on spin-labeled, liposome reconstituted KcsA. By scanning the trans-membrane helices of KcsA, we show that deuterium coupling can be used to determine residue depth within a lipid bilayer. In addition, residues that interact with the phosphate head-groups of the lipid can be determined by phosphate coupling, and their precise location modeled.

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Platform AF: Cardiac Muscle II

1905-Plat

Direct Evidence In Man For Haploinsufficiency As The Mechanism Of Action Of Myosin-binding Protein C Mutations That Cause Hypertrophic Cardiomyopathy

Steven Marston[1], O'Neal Copeland[1], Adam Jacques[1], William McKenna[2], Victor Tsang[2], Karen Livesey[3], Sebastian Carballo[3], Charles Redwood[3], Hugh Watkins[3].
[1]Imperial College London, London, United Kingdom, [2]University College London, London, United Kingdom, [3]University of Oxford, Oxford, United Kingdom.

Sarcomeric mutations in MyBPC that cause hypertrophic cardiomyopathy (HCM) may act as dominant negative alleles by encoding 'poison polypeptides'or as null alleles resulting in haploinsufficiency. To resolve this we have studied left ventricular muscle samples from patients undergoing surgical myectomy for obstructive HCM and compared these with samples from non-failing (donor) heart muscle. Seven out of 27 myectomy samples were found to contain mutations in MyBP-C: two previously described missense alleles (Glu258Lys, Arg502Trp) and five premature terminations (truncating in domains C3, C5, C7 [x2], C10). Western blots were performed using an antibody shown to recognise specifically the N-terminal region (C0-C2) of MyBPC. MyBPC content was quantified by ELC and densitometry and normalised to staining with an anti-actin antibody.

No truncated peptides were detected in whole muscle homogenates, or the myofibrillar fraction, of HCM tissue (including in overloaded gels). However, the overall level of MyBP-C in myofibrils was reduced by 24 ± 4% in myofibrils from tissue containing a MyBP-C mutation: 0.76+/-0.04 (n=39) vs 1.00+/-0.05 in non-failing (n=19)* and 1.01+/-0.05 (n=24) in non-MyBPC mutant myectomies. *p=0.0005. Four of the myectomy samples individually showed statistically significant differences from the non-failing group; these included both truncation and missense samples.

The absence of detectable lower molecular weight protein suggests that the truncated MyBPC proteins are degraded, arguing against their incorporation in the myofibre and any dominant negative effect. In contrast, the lowered relative level of full length MyBPC in the myofibre argues strongly for haploinsufficiency as the disease mechanism (potentially for missense as well as truncation alleles). Previous work on partial extraction of MyBPC suggests that lowered MyBPC stoichiometry would be expected to alter muscle function.

1906-Plat

The Effects of Troponin T Heterogeneity on Reducing Myocardial Efficiency

Hanzhong Feng, J.-P. Jin.
Evanston northshore University Healthsystem and Northwestern University Feinberg School of Medicine, Evanston, IL, USA.

Cardiac TnT variants with abnormal splicing in the N-terminal region have been found in avian and mammalian cases of dilated cardiomyopathy. Similar abnormality also occurs in myopathic and failing human hearts. These cardiac TnT variants may play a role in the pathogenesis and pathophysiology of cardiomyopathy and heart failure. Without losses of function, the cardiac TnT variants result in only minor differences in thin filament Ca^{2+} sensitivity. Therefore, we hypothesize that the heterogeneity resulting from the presence of two or more functionally distinct cardiac TnT variants in the normally uniform adult cardiac muscle thin filaments desynchronizes myofilament activation and decreases the contractile efficiency. We studied transgenic mouse hearts expressing one or two of the myopathy-related cardiac TnT variants together with the wild type adult cardiac TnT. The function of isolated working hearts was examined for pumping efficiency in the absence of neurohumoral influence. The results showed that at heart rate of 480 beat per minute and pressure load of 90 mmHg, contractile and relaxation velocities were lower in the transgenic mouse hearts than that in the wild type hearts. Left ventricular pumping efficiency calculated by the ratio of ejection integral to total systolic integral was also lower in the transgenic mouse hearts than that in wild type controls. When stressed by pacing at 600 beats per minute and giving 10 nM isoproterenol, the transgenic mouse hearts exhibited shorter ejection time and decreased cardiac efficiency than that of wild type hearts. These results indicate a chronic pathogenic mechanism that TnT heterogeneity leads to decreased myocardial efficiency due to desynchronized responses to intracellular Ca^{2+} transient.

1907-Plat

The N-terminus of Cardiac Myosin Binding Protein-C Contains Multiple Binding Sites for F-actin

Justin F. Shaffer[1,2], Robert W. Kensler[3], Samantha P. Harris[2].
[1]University of Washington, Seattle, WA, USA, [2]University of California, Davis, Davis, CA, USA, [3]University of Puerto Rico, San Juan, PR, USA.

Cardiac myosin binding protein-C (cMyBP-C), long known to interact with thick filaments, also interacts with thin filaments (actin) through its N-terminus. However, a single actin binding site has not been identified and it is unclear whether one or more N-terminal domains of cMyBP-C interact with actin. In this study we aimed to characterize the interaction of the N-terminus of cMyBP-C with actin using recombinant proteins consisting of various cMyBP-C N-terminal domains. Results from high speed cosedimentation binding assays showed that recombinant proteins containing the C1 domain and the MyBP-C motif bound to F-actin at a 1:1 molar ratio with a dissociation constant (K_d) ~ 10 uM. In contrast, proteins containing either C1 or the motif showed reduced binding at a 1:2 molar ratio. Proteins containing both C1 and the motif also bundled actin filaments, suggesting multiple actin interaction sites. Binding of recombinant proteins to Ca^{2+} regulated thin filaments was similar to binding to F-actin alone. Strongly bound myosin cross-bridges (myosin S1, no ATP) abolished cMyBP-C binding to actin, while weakly bound cross-bridges (myosin S1 plus ATP) diminished, but did not abolish, binding. Recombinant myosin ΔS2, which binds to the MyBP-C motif *in vitro* (~6 uM), did not affect cMyBP-C binding to actin. However, phosphorylation of the motif or alkaline pH both reduced binding. Together, these results suggest that the N-terminus of cMyBP-C contains at least two binding sites for actin and that binding is modulated through electrostatic interactions. Supported by NIH HL080367 to SPH and a NSF Graduate Research Fellowship to JFS.

1908-Plat

Myosin Binding Protein C Mutations and Hypertrophic Cardiomyopathy: Haploinsufficiency, Deranged Phosphorylation and Cardiomyocyte Dysfunction

Sabine J. van Dijk[1], Dennis Dooijes[2], Cris dos Remedios[3], Jos M.J. Lamers[2], Folkert J. ten Cate[2], Ger J.M. Stienen[1], Jolanda van der Velden[1].
[1]VU University Medical Center, Amsterdam, Netherlands, [2]Erasmus Medical Center, Rotterdam, Netherlands, [3]Muscle Research Unit, Sydney, Australia.

Background Mutations in the *MYBPC3* gene, encoding for cardiac myosin binding protein C (cMyBP-C), are a frequent cause of familial hypertrophic cardiomyopathy (FHCM). In the present study we investigated if protein composition and function of the sarcomere are altered in a homogenous FHCM patient group with truncating mutations in *MYBPC3* ($MYBPC3_{mut}$).

Methods and Results Comparisons were made between cardiac samples from *MYBPC3* mutant carriers (c.2373dupG, n=7; c.2864_2865delCT, n=4) and non-failing donors (n=8). Western Immunoblotting using antibodies directed against different parts of cMyBP-C did not reveal truncated cMyBP-C in $MYBPC3_{mut}$. Protein expression of cMyBP-C was significantly reduced in $MYBPC3_{mut}$ by 33 ± 5%. Cardiac MyBP-C phosphorylation in $MYBPC3_{mut}$ samples was similar to the values in donor samples, whereas the phosphorylation status of troponin I (cTnI) was reduced by 84 ± 5%, indicating divergent phosphorylation of the two main contractile target proteins of the beta-adrenergic pathway. Force measurements in mechanically isolated Triton-permeabilized cardiomyocytes demonstrated a decrease in maximal force per cross-sectional area of the myocytes in $MYBPC3_{mut}$ (21.4 ± 3.9 kN/m^2) compared to donor (34.5 ± 1.7 kN/m^2). Moreover, Ca^{2+}-sensititivy was higher in $MYBPC3_{mut}$ (pCa_{50}=5.60 ± 0.04) than in donor (pCa_{50}=5.52 ± 0.03), consistent with reduced cTnI phosphorylation. Treatment with exogenous protein kinase A, to mimic beta-adrenergic stimulation, did not correct reduced