compartments. To better understand the physical parameters governing coat assembly and coat-induced membrane deformation, we have reconstituted the Arf1-dependent assembly of the COPI coat on giant unilamellar vesicles using fluorescently labelled Arf1 and coatomer. Membrane recruitment of Arf1-GTP occurs exclusively on disordered lipid domains and does not induce optically visible membrane deformation. In the presence of Arf1-GTP, coatomer self-assembles into weakly curved coats on membranes under high tension, while it induces extensive membrane deformation at low membrane tension. These deformations appear to have a composition different from the parental membrane since they are protected from phase transition. These findings suggest that the COPI coat is adapted to liquid disordered membrane domains where it could promote lipid sorting and that its mechanical effects can be tuned by membrane tension.

2824-Plat

The Origin of Antimicrobial Resistance and Fluidity Dependent Membrane Structural Transformation by Antimicrobial Peptide Protegrin-1

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In order to kill bacteria, antimicrobial peptides (AMPs) need to intercalate into bacterial membranes, diffuse laterally, and forms pore. Membrane fluidity is thus a critical aspect in AMP-mediated killing. We studied the proposition in this work by systematically examining the effect of membrane fluidity on the disruption of lipid bilayer by AMP protegrin-1 (PG-1). In a fluid supported bilayer patch, PG-1 induces edge instability, then pore-like surface defects at low concentration, and finally wormlike micelles at higher concentrations. We show that the lipid in fluid phase is more susceptible to the formation of pore and wormlike micelle. The progress of destabilizing gel phase to structural transformations occurred along with crack formation and disordering of the gel phase into fluid phase. In addition, we found that, even with the same phase, longer chain length contributed to the PG-1 resistance. Lastly, we provide clear evidence that altering the fluidity of the bilayer can give rise to AMP resistance. These results agree with other studies in which membrane lysis by antimicrobial peptide occurs preferentially at temperatures above the liquid crystal-gel phase transition of the lipid bilayers. Our works provide possible explanation to one of the physical mechanisms of AMP resistance developed by bacteria in the nature.

Platform AW: Ryanodine Receptors

2825-Plat

A Mutation Associated with Catecholaminergic Polymorphic Ventricular Tachycardia in the Cardiac Ryanodine Receptor (RyR2-V2475F) Yields a Highly Arrhythmogenic Channel

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Mutations in *RYR2*, the gene encoding for the cardiac ryanodine receptor (RyR2), are associated with Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), an arrhythmogenic syndrome characterized by the development of adrenergically-mediated ventricular tachycardia in individuals with an apparently normal heart. CPVT-associated mutations are proposed to result in hyperactive RyR2 channels that "leak" Ca^{2+} excessively during diastole, thus creating a favorable substrate for the development of tachyarrhythmias; however, the vast majority of *RYR2* mutations (~70) fall within three domains of the RyR2 protein that control several aspects of channel function, including Ca^{2+} regulation, phosphorylation, e-c coupling, and FKBP12.6 interaction, among others, thus, a single molecular mechanism of arrhythmogenesis appears unlikely. We expressed CPVT-associated RyR2 mutations, R176Q, R414C, V2475F and G4671R in HEK293 cells and performed [^{3}H]ryanodine binding and single channel experiments to determine the Ca^{2+} dependence of wild type (WT) and CPVT-associated RyR2 mutants. Surprisingly, R176Q, R414C and G4671R yielded no apparent phenotype and displayed Ca^{2+}-activation profiles identical to WT, with threshold for activation at ~*p*Ca7 and maximal activation at ~*p*Ca5. Conversely, V2475F departed from the above mutants and displayed a significantly higher activity at *p*Ca7 (*diastolic* [Ca^{2+}]), suggesting a more severe phenotype for this mutation. Insertion of the V2475F mutation in the murine *RYR2* gene yielded mice with moderate cardiac hypertrophy. Furthermore, β-adrenergic stimulation of isolated hearts with isoproterenol induced tachyarrhythmias and fibrillation to a significantly higher extent than in WT mice. Thus, the V2475F mutation yields a highly arrhythmogenic RyR2 channel that induces some structural remodeling. These findings demonstrate that there is heterogeneity of channel dysfunction among CPVT mutants, and that the severity of the dysfunction probably stems from the location of the mutation.

2826-Plat

Mutant Ryanodine Receptor-dependent Calcium Leak, RyR2 Open Probability, Calcium Sparks And Cardiac Arrhythmogenesis

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Ca^{2+} leak from the sarcoplasmic reticulum (SR) represents a major mechanism underlying arrhythmic disease in the heart. Nevertheless the links between cardiac ryanodine receptor (RyR2) single channel behavior, Ca^{2+} sparks, and Ca^{2+} waves remains surprisingly enigmatic. Here we investigate the relationship between a known missense mutation in RyR2 (R2474S) and Ca^{2+} sparks. R2474S has been shown to cause catecholaminergic polymorphic ventricular tachycardia (CPVT) in humans and knockin mice under conditions of stress and catecholamine exposure. Heterozygous CPVT-mutant RyR2-R2474S/WT (RS/WT) or wild-type (WT) channels were isolated from the hearts of 1) resting, control mice or 2) mice which underwent exercise stress testing ('stressed'). Using the lipid bilayer method (cytosolic $[Ca^{2+}]_{cis} = 150$ nM to approximate diastolic concentrations), RyR2-RS/WT from 'stressed' mice showed a significant gain-of-function defect as evidenced by increased open probability ($Po = 0.134 \pm 0.008$, n=7) versus WT (0.018 ± 0.008, n=7; $P<0.05$). When isolated cardiac myocytes were pre-treated with isoproterenol (1 μM) to mimic catecholaminergic stress and following 1 Hz field pacing, fluo-4-AM loaded RS/WT cells showed a significantly increased spark rate (341.8 ± 115.7% vs. 108.1 ± 44.6%) when compared to WT cells. These findings suggest that changes in RyR2 Po, Ca^{2+} sparks and arrhythmogenesis are linked mechanistically. How the ensemble of findings are interrelated dynamically, however, is model-dependent and this modeling will be presented. These findings suggest that our approach to the investigation of Ca^{2+} dependent arrhythmogenesis broadens understanding of molecular cardiac defects in disease and lays the foundation for the development and testing of novel therapeutic agents.

2827-Plat

Reperfusion after Ischemia Causes Cytosolic Calcium Overload Due to Rapid Calcium Release from the Sarcoplasmic Reticulum

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After a brief ischemic insult, a sustained contractile dysfunction occurs manifested as a sluggish recovery of pump function (myocardial stunning). Substantial evidence supports that myocardial dysfunction is triggered by Ca^{2+} overload during reperfusion (R). Previous results from different laboratories including our own, describe a cascade of events triggered by R that involves the activation of Na^+/H^+ and Na^+/Ca^{2+} (NCX) exchangers, with enhanced Ca^{2+} influx. Whether this Ca^{2+} influx directly produces the increase in cytosolic Ca^{2+} or this increase occurs as a consequence of sarcoplasmic reticulum (SR) Ca^{2+} release triggered in turn by the Ca^{2+} influx, is not known. To address this issue, we performed 12 min of global no-flow ischemia followed by R in the isovolumic Langendorff perfused mouse heart positioned on a Pulsed Local Field Fluorescence microscope and loaded with fluorescent dyes (Rhod-2 or Mag-Fluo-4 to assess cytosolic or SR Ca^{2+}, respectively). The results indicated an initial increase in diastolic Ca^{2+} during early R that gradually returned to pre-ischemic levels. This increase was associated with a decrease in SR Ca^{2+} content that recovered within 10 min, as a mirror image of the diastolic Ca^{2+} profile. Additional experiments in which caffeine pulses (20 mM) were applied, confirmed that SR Ca^{2+} content was greatly diminished at the onset of R and gradually recovered within 10 min of R. The present findings indicate that the increase in diastolic Ca^{2+} that occurs upon R is due to a SR Ca^{2+} release and not just because of the Ca^{2+} entry through the reverse NCX mode, as has been previously thought.