2586-Pos

Adrenergic Regulation of the Human Ether-A-Go-Go-Related Gene Channel Protein Abundance Occurs at the Surface of the Endoplasmic Reticulum

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Long QT syndrome (LQTS) is a disorder of ion channels that can lead to the potentially lethal ventricular arrhythmias. Human *ether-a-go-go*-related gene product (HERG), a potassium channel responsible for the rapidly inactivating delayed rectifier current, is a genetic target in hereditary LQTS (specifically, LQTS2). Furthermore, pharmacological compounds of multiple classes can result in abnormal synthesis and/or function of HERG. Because arrhythmic events are often triggered in patients with LQTS2 following various stressors, it has been postulated that adrenergic regulation plays a key role in acute HERG regulation and LQTS pathogenesis. Recently, we have shown that chronic cAMP treatment (a surrogate for β-adrenergic signaling) significantly augments HERG protein abundance in HEK293 cells. Here, using velocity gradient centrifugations, we show that upon elevation of cAMP, the channel accumulates in the endoplasmic reticulum (ER) disproportionately more than at the plasma membrane. We also show that localized inhibition of protein kinase A (PKA) signaling at the surface of the ER by a targeted PKA inhibitory peptide (PKI) completely abolishes the effect; channels on the plasma membrane are unaffected by this inhibitor, as shown by patch-clamp experiments. Next, we targeted specific cAMP and PKA activity FRET-based biosensors (ICUE2 and AKAR3, respectively) to the ER surface; cell-permeable cAMP as well as the β-agonist isoproterenol elicited major FRET signal in the case of either biosensor, indicating that χΑΜΠ/ΠΚΑ σιγ ναλινγ ισ ινταχτ ιν τηισ χομπαρτμεντ. Τηε χΑΜΠ-δεπενδεντ αυγμεν τατιον ρεμαινσ πρεσεντ εππεν αφτερ τηε ΗΕΡΓ χοδινγ σενενχε ισ χοδον-οπτιμιζεδ (38% διφφερεντ φρομ τηε οριγιναλ ατ τηε πριμαρψ σενενχε λεππελ), ινδιχατινγ τηατ χΑΜΠ-δεπενδεντ αυγμεντατιον ισ νοτ μΡΝΑ δεπενδεντ. Τηερεφορε, ωε χονχλυδε τηατ β-adrenergic regulation of HERG occurs during the early stages of protein synthesis or folding at the level of the ER.

2587-Pos

Identification of the Escherichia Coli SecA Solution State Dimer Orientation using Forster Resonance Energy Transfer

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Over the past 8 years four different SecA dimer crystal structures have been solved. While the subunits of the dimer remain essentially the same, the orientation of the subunits is drastically different for each of the crystal structures. Some data has been published (Ding, H., Hunt, J.F., Mukerji, I., Oliver, D.B. 2003 *Biochemistry* 42, 8729-38) that suggest one of the anti-parallel dimers (Hunt, J.F., Weinkauf, S., Henry, L., Fak, J.J., McNicholas, P., Oliver, D.B., Deisenhofer, J. 2002 *Science* 297, 2018-26) is the solution state orientation, while other data suggests that it is the parallel dimer (Vassylyev, D.G., Mori, H., Vassylyeva, M.N., Tsukazaki, T., Kimura, Y., Tahirov, T.H., Ito, K. 2006 *J Mol Biol* 364, 248-58). Thus, the solution state orientation of the SecA dimer is currently unknown. The aim of the present study is to identify the dominant dimer orientation of *E. coli* SecA in solution. Förster Resonance Energy Transfer (FRET) was used to measure distances between dye-labeled monocysteine residues on SecA dimer subunits. Three fluorescent dye pairs with R_0 values of 34 Å, 62 Å, and 82 Å were used to measure distances from ~11 Å to 140 Å, which corresponds to the possible distances expected within any of the 4 proposed dimer orientations. The FRET measured distances were then compared to those measured in the SecA dimer crystal structures to determine if one of the structures represents the dominant solution state dimer orientation or if a mixed population of dimer states appears likely.

Funding provided by National Institute of Health

2588-Pos

Progesterone Impairs the Trafficking and Maturation of HERG Potassium Channels

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The prolongation of QT intervals in both mothers and fetuses during the later period of pregnancy implies that higher level of progesterone may have impact on the function of cardiac ion channels. In this study, we investigated the effect of progesterone on the expression, trafficking and function of human *ether-a-go-go-related* gene (HERG) potassium channel, a key ion channel responsible for controlling the length of QT intervals. We found that progesterone decreased fully-glycosylated form of HERG channels in both concentration- and time-dependent manners. Progesterone also significantly decreased HERG current density. Immunofluoresence microscopy showed that progesterone preferentially decreased HERG channel protein in the plasma membrane. Neither blockade of progesterone receptor with RU486 nor inhibition of protein synthesis with cycloheximide reversed the effect. The effect of progesterone was rescued by both lower temperature culture (27°C) and application of HERG channel blocker (E4031), but not by blockade of protein kinases including ERK1/2, JUNK, PI3K/Akt and PKA. Application of a sterol binding agent rescued the effect of progesterone. Moreover, disturbance of intracellular cholesterol homeostasis with simvastatin and imipramine mimicked the effect of progesterone. In conclusion, progesterone impairs HERG channel trafficking and maturation via disturbing intracellular cholesterol homeostasis. Our findings uncover the mechanism for the QT prolongation and high risk of arrhythmias during pregnancy.

2589-Pos

Overexpression and Biophysical Characterization of Human Interleukin-1 Alpha

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Interleukin-1 alpha (IL-1) regulates a wide range of important cellular processes. Structure- function data has been limited for IL-1α because of the difficulty in its overexpression in mammalian cells. In this study we propose the cloning, expression, biophysical and biological characterization of the human IL-1. Human IL-1 has been expressed and purified from *Escherichia coli* in high yields (~ 4 mg per liter of the bacterial culture). IL-1 was purified to homogeneity (~ 98% purity) using affinity chromatography and size exclusion chromatography. Results of the steady state fluorescence and differential scanning calorimetry experiments show that the recombinant IL-1 is in a folded conformation. Far-UV circular dichroism (CD) data suggest that IL-1 is an all -sheet protein with a -barrel architecture. IL-1 is a unique protein that affects nearly every cell type and for the many proteins that lack the N-terminal signal sequence, IL-1 is believed to be a model for understanding their endoplasmic reticulum- Golgi independent secretion. By characterizing and establishing the non-classical secretion route of IL-1, a more complete understanding can be accomplished for this special class of proteins. With the expected completion of the research outlined in this proposal, greater knowledge of other molecules with non-classical releases can be attained.

Exocytosis & Endocytosis I

2590-Pos

Elastic, Electrostatic and Electrokinetic Processes Contributing to Membrane Curvature - Membrane Shape as a Memory Element

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The curvature of cellular membranes is controlled by a variety of physical mechanisms, whose relative importance and interplay is still to be worked out. Moreover the notion that the membrane curvature controls a variety of biological processes, and that it also may be an important factor in regulating vesicular secretion, is gaining ground. In this study we evaluate what mechanical forces, such as may be generated by the cytoskeleton, are needed to generate required membrane deflection and deformation. We also consider the role the electrical forces play in the membrane deformation, and which may be present under physiological conditions. Membrane deformation is evaluated using a coupled system of linear elastic equations and electrostatic-electrokinetic (Poisson-Nernst-Planck) equations. If the fixed charges on the membrane are asymmetrical the electrostatic forces generated can produce significant bending of the membrane. Even when the distribution of fixed charges on both sides of the membrane is symmetrical the membrane bending occurs, if the intracellular and extracellular ion concentrations are different. Finally, upon removal of the forces (mechanical or electrical) that induce the membrane curvature the membrane relaxes toward the original configuration with a time course that depends on the membrane properties. The shape of the membrane can thus serve as a memory element regulating various biological processes including those of vesicular secretion.

2591-Pos

Molecular Mechanism of Membrane Constriction and Tubulation Mediated by the F-BAR Protein Pacsin

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Peripheral membrane proteins of the Bin/amphiphysin/Rvs (BAR) and FerCIP4 homology-BAR (F-BAR) family participate in cellular membrane trafficking and have been shown to generate membrane tubules. The degree of membrane bending appears to be encoded in the structure and immanent curvature of the particular protein domains, with BAR and F-BAR domains inducing high- and low-curvature tubules, respectively. In addition, oligomerization and the formation of ordered arrays influences tubule stabilization. Here, the F-BAR domain-containing protein Pacsin was found to possess a unique activity, creating small tubules and tubule constrictions, in addition to the wide tubules characteristic for this subfamily. Based on crystal structures of the F-BAR domain of Pacsin and mutagenesis studies, vesiculation could be linked to the presence of unique structural features distinguishing it from other F-BAR proteins. Tubulation was suppressed in the context of the full-length protein, suggesting that Pacsin is autoinhibited in solution. The regulated deformation of membranes and promotion of tubule constrictions by Pacsin suggests a more versatile function of these proteins in vesiculation and endocytosis beyond their role as scaffold proteins.

2592-Pos

Membrane Properties Influence the Membrane Deformation Activity Mediated by BAR Domain Proteins

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The ability of cells to maintain and change the shapes of their membranes is vital for many cellular processes. Peripheral membrane proteins in the BAR (Bin/amphiphysin/Rvs) superfamily have been identified as membrane remodelers involved in cellular trafficking. Their membrane deformation abilities can be attributed to their intrinsically curved molecular shape. In addition, formation of oligomers in ordered arrays also aid in stabilization of curved membranes. However, changes in membrane topology also depend on mechanical properties of the bilayer, which in turn are influenced by factors such as temperature and lipid compositions. Using negative stain electron microscopy, we show that by altering these not-so-subtle membrane properties, we observe differences in the membrane deformation activities of several BAR domain proteins. Our results provide a systematic and unbiased approach towards understanding the general mechanisms underlying membrane deformation mediated by BAR superfamily proteins, and in particular the role of membrane properties in this process.

2593-Pos

Dimeric Endophilin Stimulates Self-Assembly and GTPase Activity of Dynamin

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Endophilin, which participates in membrane vesiculation during receptor mediated endocytosis, is a 40-kDa SH3 domain-containing protein that binds to the proline/arginine-rich domain of dynamin, a 100kDa GTPase essential for endocytic membrane scission. The N-terminal BAR domain of endophilin contains an amphipathic helix, which has been shown to penetrate the hydrophobic core of the membrane bilayer and initiate membrane bending which is subsequently stabilized by the remainder of the protein. When BAR domains dimerize, they present a concave, positively-charged surface that could interact with, and thereby deform, membranes containing negatively charged lipids. Since the oligomerization state of endophilin is important for its biological function we studied its dimer-monomer equilibrium using analytical ultracentrifugation and fluorescence polarization/anisotropy, which yielded Kd values of ~5 micromolar and 15 micromolar, respectively. We also demonstrated that endophilin significantly enhances the self-assembly of dynamins 1 and 2 and that this enhancement is proportional to the fraction of dimeric endophilin present. Moreover, there is a close correlation between the concentrations of endophilin that promote dynamin self-assembly and those that stimulate dynamin GTPase activity. Finally, we used two-photon FCS to study the interaction of EGFP-endophilin with polymerizing dynamin.
This work was supported by National Institutes of Health grant RO1GM076665 (DMJ).

2594-Pos

Variability of Dynamin and Clathrin Dynamics in Clathrin Mediated Endocytosis

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Clathrin mediated endocytosis (CME) is a pathway which internalizes receptors from the cell surface. The scission of clathrin coated vesicles from the plasma membrane requires dynamin. However, there are multiple models of dynamin mechanism without a consensus on the exact nature of its role. Total internal reflection fluorescence (TIRF) microscopy allows the visualization of fluorescently tagged proteins during individual endocytic events. TIRF provides better sensitivity than other techniques, however the analysis of this data remains challenging due to several factors including a low signal-to-noise ratio and an abundance of clathrin on the membrane. To overcome this problem, it is common to impose rules on the data and group intensity traces from individual clathrin spots, aligning them to a common event. We have examined the basis of these criteria, and in the experiments presented here we employed a very broad selection criteria. Using TIRF we imaged dynamin and clathrin in Cos7 cells, and characterized many individual vesicles. We observe a variety of dynamic behaviors at the cell membrane, including major differences in the time of loss of clathrin and dynamin fluorescence in individual traces. We found that grouping and aligning to a common event masked relevant differences and dynamics of the molecules with respect to each other. The time differences between clathrin and dynamin leaving the plasma membrane are not tightly correlated; these different behaviors could represent different sub-populations of membrane events, or heterogeneity within a single class of event. Our data indicates that another marker for endocytic events must be used. This will be especially important in the search for the mechanism of dynamin, to ensure that conclusions drawn from in vivo imaging studies pertain to a genuine biological action.

2595-Pos

Dephosphorylation of Dynamin1 is a Ca^{2+} Sensor that Triggers Clathrin-Independent Vesicle Recycling Processes in Pancreatic Beta Cells

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By tagging fluorphores to different endocytic proteins such as clathrin and dynamin, clathrin-mediated receptor internalization process has been visualized in a number of non-excitable cells *in vivo* using total internal reflection fluorescence microscopy. In contrast, systematic examination of the tempo-spatial relationship between different exocytotic and endocytic proteins has not been done in excitable cells. The clathrin-dependent receptor endocytosis in non-excitable cells has a long life time. In contrast, the clathrin-dependent vesicle recycling process is faster and subjected to further acceleration by increase in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$), as proved by our previous data in pancreatic beta cells and also by recently papers on Calyx neurons. Moreover, elevated $[Ca^{2+}]_i$ triggers and accelerates a type of clathrin-independent but dynamin-dependent endocytosis in beta cells . As dynamin1 is a neuronal specific subtype of dynamin that can be dephorsphorylated at S774 and S778 positions upon stimulation-induced Ca^{2+} influx , we test whether it acts as a signal molecule to sensor increase in $[Ca^{2+}]_i$ and act to trigger vesicle recycling processes in our insulin-secreting cells. By mutating the S774 and S778 into A and E, we can mimic dephorsphorylated and phorsphorylated status of dynamin1. We show that dephorsphorylated dynamin1 has a statistically shortened life time as compared to the life times of dynamin1 in cells expressed wide-type dynamin1 or dynamin1 S774ES778E mutant. Moreover, dynamin1 S774AS778A is more likely to be recruited to the vesicle fusion sites, which represent clathrin-independent but dynamin-dependent endoytic events in beta cells. In the end, the fast capacitance decay evoked by homogenous elevation in $[Ca^{2+}]_i$ induced by flash-photolysis is selective inhibited by expressing either dynamin1 mutants in INS-1 cells, further reinforce the important role of dynamin1 phorsphorylation-dephorphosphorylation cycle in clathrin-independent vesicle retrieval process.

2596-Pos

Analysis of Clathrin Self-Assembly by Infrared Spectroscopy

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The clathrin protein self-assembles into a lattice that coats intracellular vesicles involved in sorting and transport of membrane-associated proteins. Inside a cell, clathrin self-assembly is initiated by interaction with adaptor proteins, but the basic self-assembly reaction can be recapitulated in vitro with recombinant fragments of clathrin that represent the C-terminal third of the