of detergent micelle resonances, a problem overcome by the selective excitation approach.

3507-Pos Board B554

An Important Double Bond: Effects of 22:5n-6 vs. 22:6n-3 on Visual Signal Transduction

Drake C. Mitchell[1], Shui-Lin Niu[2].
[1]Portland State University, Portland, OR, USA, [2]NIH/NIAAA, Bethesda, MD, USA.

In a normal, healthy retinal rod outer segment 40% to 50% of the phospholipid acyl chains consist of docosahexaenoic acid (DHA, 22:6n-3). Diets that are deficient in n-3, or ω-3, fatty acids lead to the replacement of 22:6n-3 with 22:5n6. Dietary n-3 deficiency leads to a spectrum of developmental disorders associated with learning, memory, intelligence, and visual function. We examined rhodopsin, transducin (G_t) and phosphodiesterase (PDE) function and acyl chain packing in large unilamellar proteoliposomes consisting of phosphatidylcholines with *sn-1* = 18:0, and *sn-2* = 22:6n-3, 22:5n-6 or 22:5n-3. Rhodopsin activation and binding to G_t was assayed with steady-state and time-resolved UV/vis spectroscopy, acyl chain packing was assessed via time-resolved fluorescence of diphenylhexatriene (DPH) and PDE activity was determined from the change in pH due to hydrolysis of cyclic GMP. The motion of DPH in the membrane was slower in 22:5n-6 than in 22:6n-3 and overall acyl chain packing was more constrained. The most significant structural difference between the 22:5n-6 containing bilayer and bilayers containing both n-3 polyunsaturates was in the bilayer mid plane where 22:5n-6 produced much higher DPH orientational order. At physiological temperature the formation of both the active metarhodopsin II conformation (MII) and the MII-G_t complex was much slower in 18:0,22:5n-6 PC than in 18:0,22:6n-3 PC and the equilibrium amount of MII formed was 50% higher in 18:0,22:6n-3 PC. In 18:0,22:5n-6 PC PDE activity at a physiologically relevant level of rhodopsin activation is only about 60% of that observed in either 18:0,22:6n-3 PC or 18:0,22:5n-3 PC. Taken together, these results demonstrate that the subtle change in bond configuration from 22:6n-3 to 22:5n-6 produces more structured acyl chain packing in the bilayer midplane, leading to delayed and reduced MII-G_t interaction and PDE function.

3508-Pos Board B555

Dynamics of the Internal Water Molecules in Squid Rhodopsin

Eduardo Jardón-Valadez, Ana-Nicoleta Bondar, Douglas J. Tobias.
UCI-Irvine, Irvine, CA, USA.

G-protein coupled receptors (GPCRs) are major parmaceutical targets because of their key role in a diverse array of physiological functions. The visual rhodopsin is the prototype for the family A of GPCRs. Upon photoisomerization of the covalently-bound retinal chromophore, visual rhodopsins undergo a large-scale conformational change that prepares the receptor for a productive interaction with the G protein. The mechanism by which the local perturbation of the retinal cis-trans isomerization is transmitted throughout the protein is not well understood. The recently reported crystal structure of squid rhodopsin (M. Murakami and T. Kouyama, Nature 453, 363, 2008) displays new features that may provide additional insight into the mechanism of the signal transduction in GPCRs. It has been suggested, based on the location of water molecules in the interhelical region extending from the retinal towards the cytoplasmic side, that a water-mediated hydrogen-bond network may play a role in the activation process. As a first step towards understanding the role of water in rhodopsin function, we have performed a molecular dynamics simulation of squid rhodopsin embedded in a hydrated bilayer of polyunsaturated lipid molecules. Here we report results from the simulation that show that the water molecules present in the crystal structure participate in favorable interactions with side chains in the interhelical region, and form a persistent hydrogen-bond network in connecting Tyr315 to Trp274 via Asp80. We also present preliminary results from a simulation study of the changes in the structure and dynamics of the hydrogen-bond network that accompany the photoisomerization of the retinal chromophore.

3509-Pos Board B556

Single-Molecule Photoactivation and Localization of Signaling Complexes in T cells

Chih-Jung Hsu.
University of Pennsylvania, Philadelphia, PA, USA.

In T cells, membrane receptor and ligand engagement initiates a signaling cascade. Ligand binding is relayed through specific membrane receptors, protein tyrosine kinases, and critical adaptors to regulate downstream activation of transcription factors, cytokine production and cell proliferation. The membrane segregation and relocalization of these signaling components display highly dynamic protein networks in response to distinct stimulations. However, the membrane-protein and protein-protein interactions involved in the formation of signaling complexes are still poorly understood. We are currently using a high-resolution imaging technique to visualize the early stage of signal transduction events at the single-molecule level with photoactivatable or photoconvertable fluorescent markers. The photoactivation and localization of T-cell signaling components allow measurements of molecular spatial and temporal correlation in response to different stimulations. With dual-color application, molecular correlation functions will enhance the understanding of the protein-protein interactions in signaling complexes.

3510-Pos Board B557

Molecular un-stability of peptide-MHC complex and activation of T cell

Naoki Ogawa[1], Yuji C. Sasaki[2], Osami Kanagawa[1].
[1]Research Center for Allergy and Immunology, RIKEN, Yokohama, Japan, [2]Biomedical Group, SPring-8/JASRI (JST/CREST Sasaki-team), Sayou-gun, Japan.

CD4+ T cell responses require the recognition of specific peptide-MHC complexes displayed by APC. It is important to determine how Ag presentations affect the ensuing T cell response. Imunization of B10.BR mice with immunodominant peptide 48-61 of Hen egg lysozyme elicit two different types of T cell responses. First type of T cell (type A termed by Unanue et. al) respond to APC pulsed with either peptide or whole HEL protein. Second type of T cell (termed type B) respond to APC incubated with peptide but showed no response to APC with whole protein. Some of the type B T cell clone exhibit unusual response to the variant of 48-61 peptide and responded better to poor MHC binding peptide that to strong MHC binding peptide. In contrast, reactivity of the type A of T cell clones correlate well with the affinity of the peptide to the MHC molecules. Since weak MHC binding peptides form unstable complex, we hypothesize that T cells, like type B T cell, respond well to unstable MHC peptide complex by interacting with one of multiple transitional conformations. To test this hypothesis, we observed the movement of peptide/MHC complex at the single molecular level by using diffracted X-ray tracking (DXT) method. It was found that movement of the low affinity peptide MHC complex was greater than that of high affinity peptide MHC complex. However, the difference between two complexes was mainly due to the overall movement of the molecule rather than the different movement of the peptides in the MHC groove. Thus, peptide bound to the MHC greatly influence the movement of the whole MHC peptide complex and this movement strongly affect the recognition by T cell.

3511-Pos Board B558

The Influence Of Plasma Membrane Order On TCR Signalling

Ben de Wet, Brid Cronin, Mark Wallace, Thomas Harder.
University of Oxford, Oxford, United Kingdom.

The signalling events that follow T-cell receptor triggering are mediated by multi-molecular complexes consisting of both membrane-associated and cytosolic proteins. Formation of these complexes is driven by a network of protein-protein and protein-lipid interactions. We study how the selective partitioning of signalling components into separate ordered/raft or disordered phases of the plasma membrane controls the assembly and activity of these signalling complexes.

Previous work from our laboratory shows that T cell plasma membrane domains engaged in TCR signalling specifically undergo condensation, and that treatment of Jurkat T-cells with polyunsaturated fatty acids specifically disrupts condensed membrane rafts at TCR activation sites. We have also shown that disruption of membrane order by 7-ketocholesterol leads to reduced recruitment of key TCR signalling components as measured biochemically and by TIRF microscopy of fixed cells. We aim to understand the influence of this biophysical phenomenon on the formation and composition of TCR signalling protein/lipid complexes.

To this end, we are currently studying the effects of raft disruption by 7-ketocholesterol and polyunsaturated fatty acids on the kinetic behaviour of TCR signalling components. We employ single molecule real time TIRF microscopy of fluorescently labelled TCR signalling proteins as well as biochemical and proteomic analysis of TCR activation domains upon raft disruption.

3512-Pos Board B559

Dissecting T cell receptor nanocluster signaling

Boryana N. Manz[1,2], Bryan L. Jackson[1], Rebecca S. Petit[1], Michael L. Dustin[3], Jay T. Groves[1,4].
[1]Department of Chemistry, UC Berkeley, Berkeley, CA, USA, [2]Biophysics Graduate Group, UC Berkeley, Berkeley, CA, USA, [3]Molecular Pathogenesis Program, Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, NY, USA, [4]Howard Hughes Medical Institute, Chevy Chase, MD, USA.

The early steps of T cell activation involve the spatial rearrangement of T cell receptors (TCR) and associated molecules into small nanoclusters at the

interface with the antigen presenting cell. Recent evidence suggests they are actively signaling, however their role in signal initiation and propagation is still unclear. To mechanically control the agonist MCC-MHC per TCR cluster we use microcorrals of fluid supported lipid bilayer with a confined number of MCC-MHC and correspondingly bound TCR. T cell activation (measured by calcium flux) on these microcorrals is significantly reduced at low MCC-MHC concentrations, compared to cells off them. T cell activation depends not on the overall number of agonist-MHC present, but on their number per microcorral. At least two MCC-MHCs per corral are required to trigger T cell calcium flux. Cells that can cluster even more MCC-MHC per TCR cluster do not exhibit higher signaling. This result provides an extension to the heterodimer hypothesis, by which a direct complex between agonist-MHC-TCR and co-agonist-MHC-TCR is the functional signaling unit. It confirms that two MCC-MHC-TCR are required and suggests that they are sufficient for TCR nanocluster signaling.

3513-Pos Board B560

Spatial Mutation of The T Cell Immunological Synapse with Hybrid Protein and Membrane Patterned Surfaces

Cheng-han Yu[1], Niña C. Hartman[1], Jay T. Groves[1,2].
[1]Department of Chemistry, UC Berkeley, Berkeley, CA, USA, [2]Howard Hughes Medical Institute, Chevy Chase, MD, USA.

Reorganization of membrane components, such as membrane receptors and adhesion proteins, plays an important role in signal transduction *in vivo*. Patterned hybrid live cell-supported membrane junctions provide spatial controls over the lateral transport of signaling molecules inside the cell. Moreover, immobile protein patterns within hybrid cell junctions can serve as diffusion hindrances with selectivity to their specific ligand on cellular membranes. Here we combine protein and membrane patterning to study molecular assembly during immunological synapse formation. Specifically, we explore the effects of certain fixed obstacles on the overall intracellular actin flow and receptor transport processes. The micro-patterned membranes with the abilities to selectively retain signaling molecules in position enable us to explore dynamical sorting mechanisms in cellular membranes.

3514-Pos Board B561

NMR Footprinting Of Activating And Non-activating Monoclonal Antibodies On CD3 Indicate Dynamic Quaternary Structure Changes In The TCR Complex

Koh Takeuchi[1], Sun Taek Kim[2], Zhen-Yu J. Sun[1], Maki Touma[2], Amr Fahmy[1], Ellis L. Reinherz[2], Gerhard Wagner[1].
[1]Harvard Medical School, Boston, MA, USA, [2]Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA.

The T cell receptor (TCR) mediates antigen recognition and T cell activation via its dimeric αβ, CD3εγ, CD3εδ and CD3ζζ subunits, however, a structural mechanism relating both functions has remained elusive. Here, we determine the NMR footprints on CD3εγ of one non-agonist and two agonist anti-CD3 monoclonal antibodies (mAbs). The data indicate changes of the site-specific binding topology and the TCR quaternary structure upon activation. NMR cross-saturation and chemical shift mapping showed that agonist and non-agonistic mAbs have distinct binding sites on the CD3εγ heterodimer. Agonistic mAbs bind to the membrane distal CD3ε lobe, whereas a non-agonist mAb targets the cleft between CD3ε and CD3γ causing a non-native quaternary structure in TCRβ-CD3εγ module. Subsequent biological experiments confirmed that the difference in cell triggering is not linked to mAb affinity or CD3ε binding stoichiometry per TCR but to the difference in the binding epitope on CD3εγ. More importantly, an Fab that stabilizes an intact TCRβ-CD3εγ module inhibits antigen-dependent activation. These findings indicate that a dynamic but coordinated receptor quaternary structure change in T cell receptor is important for T cell activation, which offer new insights into functional integration within multi-subunit receptors and may guide design of immunosuppressive mAbs devoid of agonist activity.

3515-Pos Board B562

Pharmacological Properties of a Pore Induced by Rising in Intracellular Ca^{2+}

Robson X. Faria[1], Ricardo Augusto de Melo Reis[2], Cynthia Machado Cascabulho[1], Fernando Pires de Farias[1], Anael Viana Pinto Alberto[1], Andrea Henriques Pons[1], Luiz Anastacio Alves[1].
[1]FIOCRUZ, Rio de Janeiro, Brazil, [2]UFRJ, Rio de Janeiro, Brazil.

Abstract

Recent studies on the $P2X_7$ receptor in 2BH4 cells and peritoneal macrophages have demonstrated that a rise in intracellular Ca^{2+} concentration induces a pore opening similar to $P2X_7$ receptor pore. Herein, we have investigated whether the pore activated by rising in intracellular Ca^{2+} concentration is associated to $P2X_7$ receptor. Using patch clamp in cell attached, whole cell configuration and dye uptake, we measured the pore opening in cell types that express the $P2X_7$ receptor (2BH4 cells and peritoneal macrophages), and in cells that do not express this receptor (HEK-293 and IT45-RI cells). In 2BH4 cells, the stimulation with ionomycin (5-10μM) increased intracellular free Ca^{2+} concentration and induced pore formation with conductance of 421 ± 14 pS, $t_{1/2}$ for ethidium bromide (EB) uptake of 118 ± 17 s, and $t_{1/2}$ for Lucifer yellow (LY) of 122 ± 11 s. $P2X_7$ receptor antagonists did not block this effect. Stimulation of HEK-293 and IT45-RI cells resulted in pore formation with properties similar to those found for 2BH4 cells. Connexin hemichannels inhibitors (carbenoxolone and heptanol) also did not inhibit the pore induced effect following rise in intracellular Ca^{2+} concentration. However, 5-(N,N-hexamethylene)- amiloride (HMA), a $P2X_7$ receptor pore blocker, inhibited the induced pore. Moreover, intracellular signalling enzymes, such as calmodulin, phospholipase-C (PLC), mitogen-activated protein kinase (MAPK), and cytoskeleton components were important for the pore formation. Additionally, we confirmed the results obtained for electrophysiology by using the flow cytometry, and we discarded the possibility of cellular death induced by rise of intracellular Ca^{2+}, at the doses used by using lactate dehydrogenase (LDH) release assay. In conclusion, increased mobilization of intracellular Ca^{+2} induces a novel membrane pore pharmacologically different from the $P2X_7$ associated pore and hemichannel pore.

3516-Pos Board B563

Exercise training during diabetes minimizes loss of Rap2 and Rrad

Caronda Moore[1], Deniz U. Dincer[2], Chun-Hong Shao[1], Keshore R. Bidasee[1].
[1]University of Nebraska Medical Center, Department of Pharmacology and Experimental Neuroscience, Omaha, NE, USA, [2]Indiana University School of Medicine, Well's Center for Pediatric Research, Indianapolis, IN, USA.

Diabetes causes failure of multiple organs. However, molecular mechanisms underlying these defects remain incompletely characterized. Recent studies suggest that alterations in expression/function of GTPases maybe involved. This study was designed to determine whether expression of two of these GTPases, Rap2 and Rrad are altered during diabetes and whether exercise training could blunt these changes. Type 1 diabetes was induced in male Sprague-Dawley rats using streptozotocin (STZ). Three weeks after STZ injection, diabetic rats were divided into two groups. One group underwent exercise training (ExT) for four weeks while the other group remained sedentary. After seven weeks of diabetes, steady state levels of Rap2 protein decreased by 50% in heart, 40% reduction in brain, 30% reduction in liver, but there was no detectable change in kidneys. Steady state levels of Rrad protein was also reduced by 60% in heart, 20% in brain and 25% reduction in liver as well as by 50% in kidney during diabetes. Four weeks of ExT initiated three weeks after the onset of diabetes, attenuated the reduction in Rap2 and Rrad expression. Since Rap2 and Rrad are down stream effectors of the guanine nucleotide exchange protein, EPAC which is activated during diabetes, these data suggest that reduction in steady state levels of Rap2 and Rrad may serve to blunt the effect of persistent sympathetic activation. (Supported in part by minority supplement, NIH).

3517-Pos Board B564

Cardiac Hypertrophy In Diabetic Mice Is Prevented By Ablation Of The G-protein gα11

Sabine Groenke[1], Dieter Hoyer[1], Yüksel Korkmaz[1], Nina Wettschureck[2], Stefan Offermanns[2], Thomas M. Wilkie[3], Klaus Addicks[1], **Hannes Reuter**[1].
[1]University of Cologne, Cologne, Germany, [2]University of Heidelberg, Heidelberg, Germany, [3]UT Southwestern Medical Center, Dallas, TX, USA.

Large clinical studies provided evidence of a lower incidence of a reduced cardiac morbidity in diabetic patients treated with angiotensin type 1 (AT1) receptor blockers. The AT1 receptor is coupled to the Gq class of G-proteins, which stimulate proteinkinase C (PKC) via activation of phospholipase Cβ. To study the role of the Gq protein Gα11 and its signaling through PKC in diabetic heart disease, we induced diabetes in wildtype and Gα11 knockout mice using streptozotocin.

After eight weeks of stable hyperglycemia, cardiac morphology and function were assessed by echocardiography, myocardial expression and translocation of PKC isoforms by immunohistochemistry and immunoblot after tissue fractionation.

Wildtype mice (n=8) but not Gα11 knockout animals (n=8) developed ventricular hypertrophy upon induction of hyperglycemia. PKC isoforms α, βII, δ, ϵ, ζ and θ were all detected in myocardium of wildtype animals. Compared to normoglycemic control animals, PKC isoforms α and ζ showed increased expression levels in diabetic wildtype mice. In addition, PKC ζ was phosphorylated at Thr410/403 and showed strong translocation to nuclear membranes in cardiomyocytes of diabetic but not of control animals. Hearts from normoglycemic Gα11-knockout mice showed lower expression levels of PKC α and δ compared