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Ceramide-1-phosphate (Cer-1-P), one of the simplest of sphingophospholipids, occurs in minor amounts in biological membranes. Yet recent evidence suggests this lipid is a second messenger with crucial roles in cell survival and inflammatory responses. In particular, during inflammation, PLC-α binds to cer-1-p in the presence of Ca^{2+} to facilitate the hydrolysis of structural membrane lipids. To get insight into the mechanism of cer-1-p function in cellular membranes and specifically its interaction with Ca^{2+}, we present a detailed structure of monomolecular layers of cer-1-p at the air-water interface. Combining surface-tension versus molecular area isotherms, Brewster angle microscopy and most importantly in-situ surface sensitive synchrotron diffraction techniques, we determined the morphology and structure at molecular length scales of cer-1-p in the monolayer on four different subphases. On water, 1mM Ca^{2+} and pH 7.2 buffer containing 1 mM Ca^{2+}, we find that cer-1-p forms solid like monolayers. Interestingly, considerable more Ca^{2+} is bound to cer-1-p on buffer containing 1 mM Ca^{2+} than pure water containing 1mM Ca^{2+}. Compared to water and the Ca^{2+} containing subphases, cer-1-p has a much more fluid like behavior on a buffered subphase at pH 7.2 without Ca^{2+}. These results can be qualitatively understood based on the molecular structure of cer-1-p and, in particular, the electrostatic/hydrogen-bond interactions of its phosphomonoester headgroup.

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2912-Pos Membrane Organization And Ionization Behavior Of The Minor But Crucial Lipid Ceramide-1-Phosphate

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Ceramide-1-phosphate (Cer-1-P), one of the simplest of sphingophospholipids, occurs in minor amounts in biological membranes. Yet recent evidence suggests an important role of this lipid as a novel second messenger with crucial roles in cell survival and inflammatory responses. Here we present a detailed description of the physical chemistry of this hitherto little explored membrane lipid. At full hydration Cer-1-P forms a highly organized subgel (crystalline) bilayer phase (L_c) at low temperature, which transforms into a regular gel phase (Lβ) at ~45 °C, with the gel to fluid phase transition (L_β-L_α) occurring at ~65 °C. When incorporated at 5 mol % in a phosphatidylcholine bilayer, the pKa_2 of Cer-1-P, 7.39 ± 0.03, lies within the physiological pH range. Inclusion of phosphatidylethanolamine in the phosphatidylcholine bilayer, at equimolar ratio, dramatically reduces the pKa_2 to 6.64 ± 0.03. We explain these results in light of the novel electrostatic/hydrogen bond switch model recently described for phosphatidic acid. In mixtures with dielaidoylphosphatidylethanolamine, small concentrations of Cer-1-P cause a large reduction in the lamellar-to-inverted hexagonal phase transition temperature, suggesting that Cer-1-P induces, like PA, negative membrane curvature. These properties place Cer-1-P in a class more akin to certain glycerophospholipids (phosphatidylethanolamine, phosphatidic acid) than to any other sphingolipid. In particular, the similarities and differences between ceramide and Cer-1-P may be relevant in explaining some of their physiological roles.

2913-Pos Using Neutron Reflectometry to Study the Adhesion of Living Cells

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The adhesion behavior of mouse fibroblast cells to a substrate has been examined using the surface sensitive technique of neutron reflectometry. Neutron reflectometry can provide density profiles in the range of 5 to 3000 angstroms from the substrate, yielding a better understanding of the length scales involved in the cell adherence. With this technique we are able to see adherence proteins at the interface, the first phospholipid membrane and its environment. Additionally, the separation of the cells from the substrate was seen upon introduction of both trypsin and distilled water.

Nucleocytoplasmic Transport

2914-Pos Conformational Changes in the Transport of Cargo through the Nuclear Pore Complex

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Board B217

Cargo that must pass through the nuclear pore complex (NPC) to complete biological processes within the cell are broad, varied, and of a large range of sizes. Experimental observations have revealed that the NPC can maintain high rates of transport throughput for most cargo, regardless of the actual shape or, to within a limit, size of the cargo as long as the cargo is bound to an NPC Importin molecule.

The exact mechanism that describes molecular transit through the NPC, however, remains unknown. Proposed hypotheses have ranged from simple Brownian diffusion on a larger scale to intricate hydrogel models of the NPC's core granule, yet many of these models break down at various points. In this paper, we examine how the biomechanical properties of the NPC facilitate transport of macromolecular cargo complexes.

2915-Pos Nuclear Pore Complex mediated export of single mRNA transcripts in living cells

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The genetic information of DNA is transcribed into mRNA (messenger RNA) and then exported into the cytoplasm. The control of mRNA availability is a central regulatory mechanism for cellular metabolism. During transcription RNA gets modified and packaged into mRNP (messenger Ribonucleoprotein). We used fluorescence microscopy to study nucleocytoplasmic transport at the single-molecule level. The time for one export event, the orientation of the mRNA during export, how the mRNA unfolds and how transport is terminated are important questions to elucidate our understanding of the RNA pathway. Using a viral system (MS2 phage), it was possible to modify a gene so its mRNA contained a sequence (MBS) that specifically binds with high affinity to the MS2 capsid protein (MCP). MCP is not present in mammalian cells and can be conjugated to a fluorescent protein to be used as mRNA marker. Tracking MS2-tagged mRNAs in living cells with millisecond time resolution will answer some of the above questions concerning the export of mRNPs in vivo. The diffraction-limited signal of individual mRNPs will be located in each frame and the position of each mRNP will be determined with a localization precision of approximately 20 nm. Individual signals in subsequent frames can then be connected to individual traces and should reveal fundamental properties of the mobility of mRNPs transported through the NPC. A fusion of Pom121 with a tdTomato-Fluorescent Protein (TFP) is used to localize the NPC. To measure the interaction time of mRNPs with the pore, the number of frames for each mRNP observed at the nuclear pore is counted and translated into a dwell time. The temporal and spatial details describe the events surrounding the RNA access to the pore, its transit and its release time.

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2916-Pos New Insights into Nuclear Transport Mechanisms: Intracellular Mobility of Import Receptors and Cargoes

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Board B219

The nucleocytoplasmic trafficking of proteins and RNAs is fundamental to the function of eukaryotic cells. Classical nuclear protein import involves the recognition of a nuclear localization signal on the import cargo by the nuclear import receptor importin-α in the cytoplasm and facilitated translocation through nuclear pores by another import receptor, importin-β. Current models for the import process are postulated largely based on protein-protein interactions measured with purified molecules, but a detailed characterization of nuclear import requires investigation of the import process within living cells. We have used two-photon fluorescence correlation spectroscopy to investigate the intracellular dynamics of these nuclear import receptors and NLS cargoes, both in the cytoplasm and the nucleus of HEK 293 cells. We show that the mobility of different import factors can be robustly characterized, and that the mobility of the importins is surprisingly slow in the absence of NLS cargoes. Together with measurements of receptor localization, our measurements lead us to new insights into the biophysical mechanisms involved in the nuclear import pathway.

2917-Pos In Vivo Frap Analysis Of Hiv-1 Tat Peptide Intracellular Targeting Properties

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A large body of work is currently devoted to the rational design of new molecular carriers for the controlled delivery of cargoes (e.g., proteins or nucleic acids) to relevant sub-cellular domains, particularly the nucleus. This latter case is particularly significant, as it is one of the necessary steps for gene-therapy approaches and can be exploited to probe and modify cellular processes of utmost importance. In this article we show that rational mutagenesis of HIV-1 Tat-derived peptide (YGRKKRRQRRR) affords variants with finely tuned inter-compartmental dynamics and controllable transport mechanisms. Our findings are made possible by the demonstration that the Tat peptide possesses two competing functionalities capable of active nuclear targeting and additional binding to intracellular moieties. By altering the competition between these two functions we show how to control cargo localization of Tat-peptide chimeras. The construct subcellular localization was analyzed by confocal imaging while their intracellular dynamics was investigated by Fluorescence Recovery After Photobleaching (FRAP) real-time imaging.

Our investigation provides a unified, coherent description of previous conflicting in vivo and in vitro results and lets the true nature of the Tat peptide emerge.

2918-Pos Cell Cycle Dependence Of Nucleocytoplasmic Transport In Yeast

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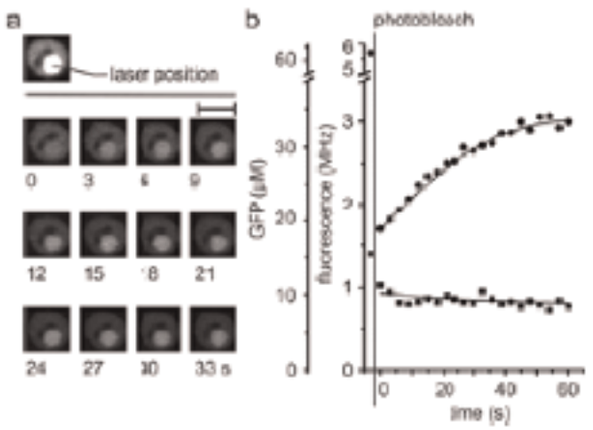
Proteins translocate between the nucleus and the cytoplasm through the nuclear pore complex (NPC), a protein complex of 50 MDa that forms a selective pore with an inner diameter of ~10 nm. Cargo proteins are recognized via a small protein domain, called the nuclear localization signal (NLS). We studied the nucleocytoplasmic transport of NLS-tagged green fluorescent protein (GFP) using a fluorescence recovery after photo-bleaching (FRAP) based assay: selective-FRAP. For a typical selective-FRAP measurement, a confocal image of a yeast cell was recorded and the laser was focussed for ~10 s in the nucleus. This resulted in the photo-bleaching of part of the GFP located in the nucleus (Fig. a). The import of intact GFP was then followed by the subsequent recording of a time series of images. From the fluorescence intensities, the import and the efflux rates were calculated (Fig. b). Selective-FRAP allowed to quantitatively measure nucleocytoplasmic transport in exponential growing yeast cells. New insights on the targeting of mRNAs will be discussed.



2919-Pos Dissociation of Importin α/ Cargo Complexes at the Nuclear Pore Requires CAS and RanGTP

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Macromolecules are transported between the cytoplasm and nucleoplasm of eukaryotic cells through nuclear pore complexes (NPCs). Large (> ~40 kDa) transport cargos that are imported into the nucleus typically form a complex with a least one soluble transport cofactor of the importin β (Imp β) superfamily. Many cargos require an accessory cofactor, importin α (Imp α), which binds to Imp β and to the nuclear localization sequence on the cargo. At least three mechanisms of Imp α/cargo complex dissociation have been proposed: 1) spontaneous dissociation; 2) dissociation induced by binding of a CAS/RanGTP complex to Imp α; and 3) dissociation induced by binding of the Imp α/cargo complex to NUP50, a nuclear pore protein. Single-molecule fluorescence resonance energy transfer (FRET) was used to directly monitor the disassembly of a model Imp α/cargo complex in permeabilized cells. We show here that CAS, the recycling cofactor for Imp α, is essential for dissociation of Imp α/cargo complexes at the NPC. Although about half of Imp α/cargo complexes did not dissociate at the NPC in the presence of CAS, the majority of undissociated complexes returned to the cytoplasm. The dissociation events required RanGTP and appeared to occur near the location of NUP50 on the nuclear basket. FRET was directly observed between Imp α and NUP50. Thus, the data are consistent with a model in which Imp α/cargo complexes exiting the nucleoplasmic side of the NPC are dissociated at or near NUP50, and this dissociation requires both CAS and RanGTP.

2920-Pos Capacitative Ca entry (CCE) is required to activate Nuclear Factor of activated T-Cells (NFAT) in endothelial cells

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Endothelial cells respond to vasoactive agonists (ATP, bradykinin) with a transient elevation of intracellular Ca ($[Ca]_i$). Two Ca sources contribute to this elevation: IP_3-dependent Ca release from the endoplasmic reticulum (ER), which activates Ca influx from the extracellular space (due to CCE). CCE causes a sustained elevation of $[Ca]_i$. NFAT proteins are Ca-sensitive transcription factors which display cytoplasmic localization in their inactive state. Dephosphorylation by the Ca-sensitive phosphatase Calcineurin (CaN) is required to activate NFAT and to mediate its translocation to the nucleus. The present study identified the intracellular Ca signals that activate NFAT in calf pulmonary artery endothelial (CPAE) cells. Cellular distribution of NFAT was analyzed using confocal imaging of NFAT-GFP fusion proteins after adenoviral gene transfer (isoforms NFATc1 and NFATc3). $[Ca]_i$ was monitored simultaneously with the Ca-sensitive dye Rhod-2. Application of ATP for 5 min (5 µM, $[Ca]_o$ = 2 mM), resulted in activation of CCE and robust translocation of NFAT to the nucleus, as quantified by a significant increase in the $NFAT_{NUC}/NFAT_{CYT}$ ratio. This effect was sensitive to the CaN inhibitor Cyclosporin A (CsA, 1 µM). CCE was the underlying Ca source, since NFAT translocation was absent when ATP was applied in the presence of La^{3+} (100 µM, block of CCE) or in the absence of extracellular Ca (both procedures resulted in a comparable ER Ca release without CCE). Furthermore, CCE itself, activated by Ca containing solutions after store-depletion using thapsigargin (10 µM), was capable to activate NFAT. In addition,

Ca-influx alone, induced by hypo-osmotic solutions (presumably via stretch-activated ion channels), was sufficient to induce NFAT translocation. In conclusion, sustained activity of CCE, rather than ER Ca release, is required to induce nuclear translocation of NFAT in endothelial cells.

2921-Pos Structure of the brush-like mesh formed by the FG-repeat Nucleoporin Nsp1

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Nuclear Pore Complexes (NPCs), composed of multiple copies of about 30 distinct proteins collectively termed nucleoporins (nups), form gateways in the nuclear envelope for import and export of macromolecules, such as proteins and RNAs. Inside the NPC channel are a large number of unstructured, 'finger-like' nups, each containing numerous phenylalanine-glycine (FG) repeats. These FG-repeat nups are known to play an important role in controlling the transport of large molecules between the nucleus and the cytosol. However, it remains unclear how these nups aggregate in the NPC and how they selectively permit the passage of transport receptors carrying their cargoes. To examine the structure and dynamics of FG-repeat nup assemblies, we simulated an array of twenty five 100-residue long FG repeat segments taken from Nsp1 in the yeast Saccharomyces cerevisiae, using a residue-based coarse-grained method. During our 1.7 microsecond simulation, the nups which were fixed at one end, shortened from an initially fully extended conformation into a brush-like mesh, due to both entropic effects and specific segment-segment interactions. Following previous experiments demonstrating lethality of the mutant in yeast, we repeated the simulation with most phenylalanines mutated to serines. A brush-like mesh structure was observed also in the mutant simulation, however with larger mesh holes and a longer brush length compared to that of the wild type Nsp1 segments. We propose that while the mesh structure is maintained in the mutant simulation, the larger mesh holes decrease the selectivity, explaining the mutant's lethality to the cell.

2922-Pos The Vehicle and the Gate: Molecular Dynamics Studies on the Permeability Barrier of Nuclear Pore Complexes

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Transport of large proteins through nuclear pore complexes is characterized mainly by two events: binding of the cargo protein to a transport receptor and passage of the cargo-transporter complex through the permeability barrier of the pore. The permeability barrier is formed by largely unordered peptides containing a number of characteristic phenylalanine-glycine (FG) sequence motifs [1].

In order to gain insight into the structure of the barrier and the molecular basis underlying its formation, we have carried out simulations on small model FG-repeat peptides. Our simulations show spontaneous aggregation of the monomers, driven by a combination of hydrogen-bonding and aromatic-aromatic interactions. In contrast, Phe to Ser mutants, lacking the aromatic side chains, fail to aggregate. These results indicate that hydrophobic clusters may play a role in the formation of the permeability barrier in nuclear pore complexes and support the hypothesis that cross-linking between FG-repeat creates a sieve-like structure [2].

In addition, we show that an importin-beta:IBB complex is able to bind the FG-aggregates, in spite of their compact structure. Hereby, the FG-clusters are weakened. These simulations may thus give first insight into the disruption of the permeability barrier by a transport receptor.

As the number and size of the FG-repeat peptides in the nuclear pore are too large to be treated entirely by all-atom models, we have developed a coarse-grained model, which enables us to study the three-dimensional structure of large assemblies of FG-repeat peptides.

References

[1]. Strawn et al. Nat. Cell Biol. *6*, 197–206 (2004).
[2]. Frey et al., Science *314*, 815–817 (2006).

2923-Pos Probing Nucleocytoplasmic Transport With Fluorescence Fluctuation Spectroscopy And Two-photon Activation Of Photoactivable GFP

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Large proteins and macromolecular complexes have to enter and leave the nucleus in an efficient and selective manner. Macromolecules that are greater than 40 kD are transported actively across the nuclear envelope through nuclear pore complexes using soluble transport factors or carrier molecules that cycle between the cytoplasm and nucleus. Interestingly, the carrier proteins themselves interact with each other in order to transport cargo proteins across the nuclear pore complexes. In this work, we apply dual-color time-integrated fluorescence cumulant analysis (TIFCA), a fluorescence fluctuation spectroscopy technique, to investigate the protein interactions of the carrier proteins directly in cells. In addition, we apply two-photon activation to directly examine the nucleocytoplasmic transport of photoactivable GFP tagged carrier proteins. With these two approaches, we are able to probe the nucleocytoplasmic transport process directly inside cells and under equilibrium conditions. We will illustrate our approach and discuss our findings for several model protein systems.

2924-Pos Pharmacological Characterization Of Nuclear NFAT Translocation In Cardiac Myocytes

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Nuclear localization of NFAT transcription factors activates pre-hypertrophic genes related to cardiac hypertrophic remodeling. Dephosphorylation of cytoplasmic (inactive) NFAT by Calcineurin activates Ca-dependent translocation of NFAT to the nucleus. Although this mechanism is well defined in many cell types, little is known about the pathways that control nuclear translocation of NFAT in cardiac tissue. We analyzed subcellular localizations of NFAT in cat atrial and rabbit ventricular myocytes in response to experimental interventions that interfere with nuclear import and export pathways for NFAT proteins. Myocytes were infected with recombinant adenoviruses encoding for NFAT-GFP fusion proteins (isoforms NFATc1 and NFATc3) and kept in culture (up to 48 h) to allow detectable protein expression. Ca signals were measured in the presence of NFAT-GFP using the Ca-sensitive dye Rhod-2. Myocytes expressing NFAT-GFP showed normal cellular morphology and Ca transients in response to electrical field stimulation on day 2 in culture. We observed isoform-specific differences in non-stimulated myocytes: While NFATc3 was predominantly localized in the cytoplasm, NFATc1 displayed nuclear localization in most of the cells. This localization was sensitive to the level of intracellular Ca ($[Ca]_i$), since incubation in Ca-free extracellular solution or lowering $[Ca]_i$ using BAPTA- AM induced a re-distribution of NFATc1 to the cytoplasm. Nuclear accumulation of NFATc1 could be enhanced by blocking the nuclear export protein Crm1 using Leptomycin B (40 nM). In case of NFATc3 stimulation with neurohumoral stimuli Endothelin-1 (100 nM) and Angiotensin II (1 μM) resulted in activation of NFAT import and enhanced nuclear localization. These data suggest that nuclear localization of NFAT in cardiac myocytes is not only determined by the import-activating intracellular Ca signals, but also controlled by nuclear export pathways. Furthermore, NFATc1 and c3 isoforms have differential activation pathways in the same cell.

2925-Pos Nanomechanical Basis of Selective Gating in the Nuclear Pore Complex

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The intrigue surrounding the nuclear pore complex (NPC) lies in its ability to restrict or promote cargo translocation between the cytoplasm and nucleus i.e. selective gating. A mechanistic understanding of the NPC *modus operandi* necessitates a detailed knowledge of the physical responses of the natively unfolded phenylalanine-glycine (FG)-rich domains (FG-domains) to the biochemical interactions that promote nucleocytoplasmic transport (NCT). Hence, conventional approaches may be powerful in identifying such protein-protein interactions, but provide only marginal assessments of its biophysical foundations.

In our lab, we have developed an interdisciplinary approach to reconcile the dualistic nature of selective gating within the context of FG-domain behavior by correlating the nanomechanical responses of the Nup153 FG-domain (cNup153) to the biochemical interactions that govern NCT. Thus, an important consideration is to replicate more closely the nanoscopic, contextual details of the NPC, instead of inferring their behavior from "top-down" macroscopic views. Specifically, cNup153 tethered to gold nanostructures (designed to mimic the NPC geometry) gave rise to a polymer brush-like entropic barrier. We found that binding interactions with the transport receptor, karyopherin-β1, caused the FG-domains to collapse into compact molecular conformations. This effect was reversed by the action of RanGTP, which returned the FG-domains into a polymer brush-like, entropic barrier conformation. Immunogold-labeling electron microscopy substantiated these findings *in situ* by showing that the FG-domains were also reversibly collapsed *in vivo*. Based on these results, we conceptualize that selective gating consists of a rapid, stochastic flux of collapsing and distending FG-domains owing to the dynamic nature of NCT. In closing, we will demonstrate how the aforementioned principles of nanomechanical selective gating can be applied to the construction of a *de novo* designed synthetic NPC.

Auditory Systems

2926-Pos The Endocochlear Potential Depends Upon Two K^+-diffusion Potentials And An Electrical Barrier In The Stria Vascularis Of The Inner Ear

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The endocochlear potential (EP) of ~+80 mV is essential for audition. The stria vascularis in the cochlear lateral wall is crucial for EP-formation, but its mechanism has remained largely unknown. We used multibarreled electrodes to measure potential, input-resistance and K^+-concentration ($[K^+]$) in each of the compartments of the stria vascularis. The intrastrial space (IS), an extracellular space between the inner layer comprising marginal cells and the outer layer composed of intermediate and basal cells in the stria, had low $[K^+]$, high positive potential and a high input-resistance. It indicates an electrical isolation of IS from neighboring extracellular fluids. Inhibition of either the $Na^+,K^+,2Cl^-$-cotransporter or the Na^+,K^+-ATPase on the basolateral membrane of marginal cells or blocking the K^+ channel on the apical membrane of intermediate cells reduced both the potential of IS (intrastrial potential; ISP) and EP. Inhibiting the K^+-transporters caused $[K^+]$ of IS to increase and intracellular $[K^+]$ of marginal cells to decrease. Blocking the