

Corrections

Mitragotri, Samir, Mark E. Johnson, Daniel Blankschtein, and Robert Langer. 1999. *Biophys. J.* 77:1268–1283.

On page 1277, second paragraph, 19th line, “. . . so deduced is 1.1 Å for trans-bilayer. . .” the sentence should read, “. . . so deduced is 0.85 Å for trans-bilayer. . .”.

McGrath, James L., John H. Hartwig, and Scot C. Kuo. 2000. *Biophys. J.* 79:3258–3266.

The figures originally published were missing data curves. Below are the revised figures containing the missing data.

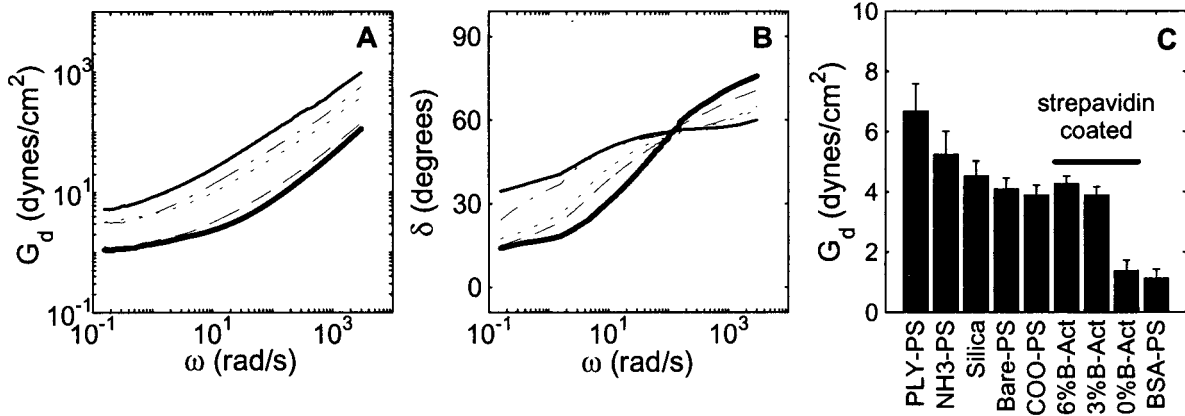


FIGURE 3

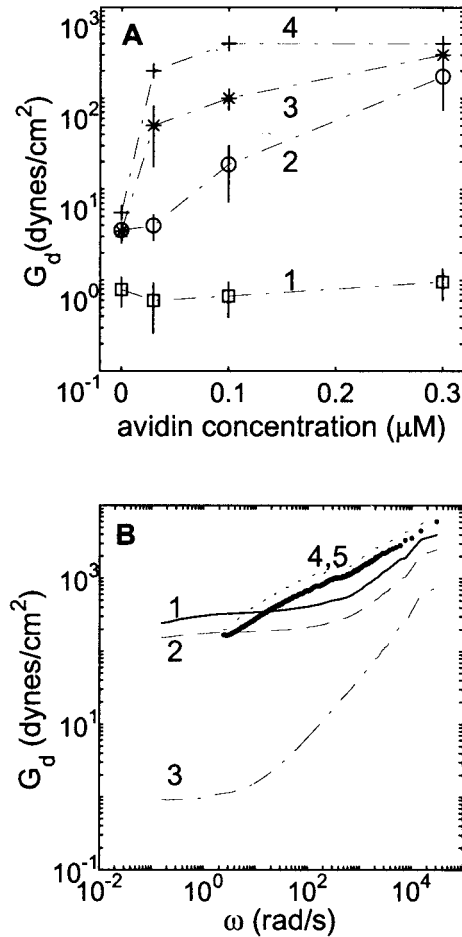


FIGURE 5

The following Abstracts either did not appear in the 2001 Abstracts Issue, or were printed with incomplete or incorrect information. The correct versions are printed here in their entirety.

585-Pos

EFFECT OF METABOLIC INHIBITION AND OXIDATIVE STRESS ON THE EXTRACELLULAR DIVALENT CATION-INHIBITED NONSELECTIVE CONDUCTANCE IN CARDIAC CELLS. Regina Macianskiene, Peter Matejovic, Karin Sipido, **Kanigula Mubagwa**; University of Leuven, Campus Gasthuisberg, Leuven, B-3000 Belgium.

In many cells removing Ca_o and Mg_o activates a cation nonselective current (I_{NS}). I_{NS} was studied in ventricular cells at 22°C, under whole-cell voltage-clamp, with Ca^{2+} and K^+ channels blocked. I_{NS} in mouse myocytes did not differ between cells dialyzed with or without ATP (mean±SEM at -80 mV: $-9.8±2.3$ pA/pF, n=14; vs. $-9.0±2.4$ pA/pF, n=12). Glucose removal reversibly increased I_{NS} in 0 Ca_o and 0 Mg_o (to $-17.5±2.5$ pA/pF, n=12). However, glucose removal and additional inhibition of glycolysis by 2-deoxyglucose or of mitochondrial function by rotenone or CN⁻ failed to induce I_{NS} in the presence of Ca_o and Mg_o . Similarly, I_{NS} preactivated in 0 Ca_o and 0 Mg_o was increased by the oxidants thimerosal (50 $μ$ M; from $-22.7±5.9$ to $-28.6±7.8$ pA/pF, n=6) and diamide (500 $μ$ M; from $-6.2±1.8$ to $-9.3±1.3$ pA/pF, n=10), but none of these drugs elicited I_{NS} in the presence of Ca_o and Mg_o . In rat myocytes 50 nM Ag^+ induced I_{NS} ($-15.4±2.0$ pA/pF, n=12; reversing at -5 mV; blocked by 100 $μ$ M Gd^{3+}) even in the presence of divalent cations. We conclude that metabolic inhibition regulates preactivated I_{NS} but does not induce the opening of closed channels, and that small oxidants like Ag^+ may activate I_{NS} by accessing at sites unavailable for larger molecules.

703.10-SYMP

CHROMOSOME DYNAMICS AND KINETOCHORE FUNCTION. Xiangwei He, Peter deWulf, Frederic Sallman, Chris Espelin, Kim Simmons, Dan Rines and **Peter Sorger**, MIT

Key questions in the study of chromosome segregation include (i) what restricts centromeres to one and only one per chromosome (ii) what are the relative roles of microtubule dynamics and microtubule-based motors in force generation (iii) how do cells sense chromosome-microtubule attachment? We will describe our analysis of kinetochore assembly in budding yeast, emphasizing the architecture of the CBF3-centromere complex that forms a scaffold onto which a microtubule attachment site assembles. We will also report the use of transient sister chromatid separation as an *in vivo* method to identify kinetochore proteins involved in force generation. Yeast centromeres undergo elastic deformation and transient separation during metaphase in a microtubule and kinetochore-dependent process. Among 8-10 proteins isolated as defective in transient separation are yeast orthologs of mammalian microtubule binding proteins. We will summarize this data in a molecular model for chromosome-microtubule attachment. Classic experiments with micro-manipulated chromosomes suggest that tension plays a key role in regulating the mitotic checkpoint. The existence of transient sister separation during budding yeast metaphase is consistent with the idea that the yeast kinetochore is a molecular tensiometer. We are currently attempting to dissect this tensiometer genetically.

836.10-Pos Board B45.10

PIP AQUAPORINS IN THE MOTOR CELLS OF SAMANEA SAMAN: DIURNAL AND CIRCADIAN REGULATION. Menachem Moshelion¹, Dirk Becker², Rainer Hedrich², Alexander Biela², Beate Otto², Hadas Levi¹, **Nava Moran**¹ and Ralf

Kaldenhoff². ¹Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel and ²Julius-von-Sachs-Institute, Department of Molecular Plant Physiology and Biophysics, Julius-von-Sachs-Platz², D-97082 Wuerzburg, Germany.

Leaf-moving organs, remarkable for the rhythmic volume changes of their motor cells, served as a model system to study the regulation of membrane water fluxes. Two aquaporins, SsAQP1 and SsAQP2, cloned from this organ, conducted water when expressed functionally in *Xenopus* oocytes. SsAQP2 imparted a 10-fold higher permeability to water than SsAQP1. SsAQP1 alone was glycerol-permeable and only SsAQP2 was inhibited by Hg^{2+} . Plasma-membrane water permeability of motor-cell protoplasts was also inhibited by Hg^{2+} and was diurnally regulated. Both aquaporin transcript levels differed in their spatial distribution in the leaf and were diurnally regulated, in phase with leaflet angle changes. Notably, the mRNA level of SsAQP2, but not of SsAQP1, fluctuated also under constant conditions in continuous darkness, again, in phase with leaflet angle, demonstrating - for the first time - a circadian control of an aquaporin. Taken together, these results suggest that aquaporins, SsAQP2 in particular, participate in the mechanism of leaf movement, by directly regulating membrane water permeability.

1215.10-Pos Board B423.10

LIGHT-INDUCED CHANGES IN THE STRUCTURE AND ACCESSIBILITY OF THE CYTOPLASMIC LOOPS OF RHODOPSIN IN THE ACTIVATED MII-STATE. T.

Mielke, U. Alexiev, M. Gläsel, **Harald Otto**, M. P. Heyn. Freie Universität Berlin, Physics Dept., D- 14195 Berlin, Germany.

Bovine rhodopsin was specifically labeled on the cytoplasmic surface at C140 or at C316 with the fluorescent labels fluorescein and texas red. These loops are involved in activation and signal transduction. The time-resolved fluorescence depolarization was measured at these positions in the dark-state and in the MII-state, with samples consisting of rhodopsin- OG micelles and ROS membranes. The Rhodopsin MII was trapped for the duration of the fluorescence experiments (20' at pH6, 6°C). For both types of samples and at both positions the dynamics of the label and loop motion as expressed by the time constants of the depolarization were not significantly different in the two states of the receptor. The end-anisotropy increased however from 0.071 in the dark to 0.172 in the MII for ROS samples labeled at C140 and from 0.074 to 0.147 at C316. Light-induced activation in MII is thus associated with a large increase in the loop steric hindrance due to a changed loop domain structure on the cytoplasmic surface. These results are supported by experiments with the collisional quencher I-, which indicate a significant decrease in the quenching constant k_q and in accessibility in the MII-state at both positions. The rotational correlation time of the rhodopsin micelles increased from 48 ns in the dark state to 60 ns in MII. This increase is caused by a change in volume and/or shape and is also consistent with a structural change.

1355-Pos Board B563

AMPHIPHILIC PEPTIDES AT MEMBRANE INTERFACES. A COMPARATIVE MOLECULAR DYNAMICS STUDY. Tuck C. Wong¹, Troy Wymore²;

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One of the difficulties encountered when simulating proteins/peptides in explicit lipid bilayers using molecular dynamics (MD) methods is the slow relaxation times of the lipid molecules and hence the convergence of time averaged properties from the simulation. We have performed MD simulations of substance P, a neuropeptide, in three different membrane mimic environments for over 2 ns each. The different membrane mimics are i) a water/ hexane biphasic cell; ii) a solvated sodium dodecylsulfate (SDS) micelle; and iii) a solvated dimyristoylphosphatidylcholine (DMPC) bilayer. All simulations were performed with CHARMM using periodic boundary conditions and particle mesh Ewald for long-range electrostatic interactions. The results were analyzed by comparing radial distribution functions between peptide-peptide atoms and peptide-environment atoms and autocorrelation functions of N-H vectors of the peptide across all three systems. The effects of the head group and the curvature of the water-hydrophobic interface on the mode of binding and the dynamics of the peptide were examined. These results will help identify how well simulations from the simpler interfaces can be extrapolated to the more detailed and realistic lipid bilayers.

1744-Pos Board #B240

MOTION MAKER SOFTWARE TO ENHANCE TIME LAPSE FLUORESCENCE IMAGE ANALYSIS. Michael P. Heldebrant, Eugenia Trushina, Cynthia T. McMurray, Mayo Clinic and Foundation

Time lapse fluorescence imaging has significantly enhanced our ability to monitor the dynamic properties of subcellular particles in living cells. However, quantitative analysis of moving particles is difficult because of the simultaneous motion of many particles in each image frame. Additionally, without constantly referring to previous image frames it is impossible to determine if a particle is stationary or in motion from a single image frame. To perform this analysis we have developed Motion Maker, a software program designed to specifically aid in the analysis of data sets consisting of multiple moving particles. By determining whether a particle is located in the same position over consecutive time points, the program is able to differentiate between moving and stationary particles within a user defined time window. Motion Maker reads motion information from this window and encodes it back into a single frame. Analyze (Biomedical Imaging Resource, Mayo Clinic) provides the image analysis toolkit which allows the processing and pseudocoloring of particles into three discrete classes (particles currently in motion, particles currently paused, and stationary particles that have not moved during data acquisition) without otherwise altering the data. Color coding of these three classes of particles dramatically improves both time required for analysis and accuracy. Motion Maker can assist in many particle tracking tasks such as velocity, net distance traveled, and processivity of motion.

1993-Pos Board # B490

ORGANIC OSMOLYTES PERMEATE CLC-3 CHLORIDE CHANNELS. Marcela A. Hermoso¹, D Duan², M Nagasaki², L Ye², B Horowitz², J R Hume²; ¹ICBM, Facultad de Medicina, Universidad de Chile, Casilla 70058, Correo 7, Santiago, Chile, ²Center of Biomedical Research Excellence, University of Nevada/MS352, Reno, NV 89557

In response to acute exposure to hypotonic external solutions, cells swell and then recover to normal size through a regulatory volume decrease (RVD) process. In addition to Cl⁻ and K⁺ ions, efflux of organic osmolytes such as taurine, glutamate, and aspartate also make a significant contribution to RVD. The aim of this work was to study if these amino acids permeate through the same conduction pathway as Cl⁻ in volume-sensitive ClC-3 channels. Whole-cell currents were recorded from NIH/3T3 cells transfected with gpClC-3/GFP when extracellular and/or intracellular Cl⁻ ions were replaced by various

amino acids under hypotonic conditions and permeability ratios (Paa/PCl) were calculated from measured reversal potentials. Substitution of extracellular Cl⁻ by aspartate, glutamate, and isethionate (a surrogate for taurine) all reduced Paa/PCl. When isethionate was the only permeant anion in extra- and intra-cellular solutions, significant volume-sensitive currents were still observed. Partial replacement of external isethionate by Cl⁻ increased current amplitudes and Paa/PCl. The relationship between Paa/PCl and the mole fraction ratio of permeant anions indicates that the permeation of isethionate and that of Cl⁻ ions through ClC-3 channels is not independent. (NIH P20RR15581 & Fondecyt 3980043).

2105.10

MECHANISM OF PEPTIDE SYNTHESIS FROM THE COMPLETE ATOMIC STRUCTURE OF THE LARGE RIBOSOMAL SUBUNIT AND SUBSTRATE COMPLEXES.

Thomas Steitz, Yale Univ/HHMI, Steitz T.A.^{1,2,3}, Nissen P.¹, Ban, N.¹, Hansen J.¹ and Moore P.B.^{2,1}, ¹Dept. of Molecular Biophysics & Biochemistry and ²Dept. of Chemistry, Yale University and ³Howard Hughes Medical Institute, New Haven, CT 06520-8114

The crystal structure of the large ribosomal subunit from *Haloarcula marismortui* at 2.4 Å resolution includes 2833 of the subunit's 3045 nucleotides and 27 of its 31 proteins. The RNA domains have irregular shapes and fit together like the pieces of a three-dimensional jigsaw puzzle to form a large, monolithic structure. Proteins are abundant everywhere on its surface except in the active site where peptide bond formation occurs and the surface that contacts the small subunit. Most of the proteins stabilize the structure by interacting with several RNA domains, often using idiosyncratically folded extensions that reach into the subunit's interior. Abundant examples of two previously unrecognized RNA motifs are seen; one stabilizes RNA helix-helix interaction and the other produces a bend and protein binding site. Using the structures of complexes with two substrate analogs, we have established that the ribosome is a ribozyme. Both analogs contact exclusively conserved ribosomal RNA (rRNA) residues from domain V of 23S rRNA; there are no protein side-chain atoms closer than about 18 Å to the nascent peptide bond. The mechanism of peptide bond synthesis appears to resemble the reverse of the acylation step in serine proteases, with the base of A2486 (A2451) stabilizing the transition state and possibly playing the same role as His57 in chymotrypsin. Several antibiotics are observed to bind in the polypeptide exit tunnel which is largely formed by RNA but with contributions from proteins L4, L22, and L39e.

2748-Pos Board # B435

A NOVAL CALCIUM INFLUX FACTOR RELEASED BY TRANSFORMED FIBROBLASTS. Alexander Pieter Theuvsen¹; University of Nijmegen, Toernooiveld¹, Nijmegen, Gelderland 6525 ED Netherlands

Normal rat kidney fibroblasts are used by us as a model system for studying cellular alterations upon transformation. When cultured in the presence of epidermal growth factor (EGF) as the sole polypeptide growth factor, these cells undergo contact inhibition of growth and become quiescent at a critical cell density. Subsequent exposure of these growth-arrested cells to for instance transforming growth factor (TGF)-beta restimulates their growth, thereby inducing phenotypic characteristics of transformation. These transformed NRK fibroblasts release a lipid in their growth medium, that exhibits the ability to depolarize their normal counterparts. The depolarisation is caused by the opening of Ca²⁺-activated Cl⁻ channels. This indicates that the factor released by the transformed NRK fibroblasts primarily acts as an elevator of intracellular Ca²⁺. Preliminary spectrometric analysis of the purified lipid indicates that it is a novel prostaglandin-like mole-

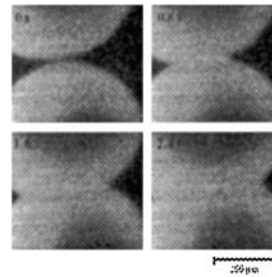
cule, distinct from others that have previously been shown to act primarily by inducing a release of intracellularly stored Ca^{2+} . The factor does not cause inositol lipid degradation with subsequent mobilization of intracellularly stored Ca^{2+} , but uniquely induces entry of extracellular Ca^{2+} . The factor is therefore provisionally named fibroblast-derived Ca^{2+} influx factor (FCIF). Current investigations are aimed to characterize the calcium channel(s) activated by FCIF, and the localization of its binding site on the channel. Results of these studies will be presented.

2750-Pos Board # B437

CALCIUM WAVESPEED AND DIFFUSION COEFFICIENT IN AGAROSE GEL WITH SUSPENDED SR VESICLES. INFLUENCE OF MITOCHONDRIA. Kirsten Krannich, Manfred Hans-Peter Wussling; Martin Luther University Halle-Wittenberg

We recently established a novel excitable medium of vesicles of the sarcoplasmic reticulum (SR) in agar gel showing calcium waves which propagate slower than those in cardiac myocytes but faster than those observed in *Xenopus* oocytes (Wussling et al., 1999). To further char-

acterize the in vitro system we investigated colliding circular calcium waves (see Fig.) by CLSM and determined diffusion coefficient and



speed of plane waves by means of the velocity curvature relationship, $N = c(0) - 2DK$, where N and $c(0)$ velocity of curved and plane waves, respectively, D apparent diffusion coefficient, and K curvature of wavefront. Results: $D = 215.4 \pm 75.4 \mu\text{m}^2/\text{s}$, $c(0) = 45.7 \mu\text{m}/\text{s}$ in the absence, and $D = 149.1 \pm 42.1 \mu\text{m}^2/\text{s}$, $c(0) = 43 \pm 13.3 \mu\text{m}/\text{s}$ after addition of mitochondria. The

present results suggest that the spreading of Ca^{2+} waves in the novel excitable medium is rather determined by the kinetics of ryanodine receptors than by diffusion between the calcium release sites. Ref.: Wussling, M. H. P., K. Krannich, G. Landgraf, A. Herrmann-Frank, D. Wiedenmann, F. N. Gellerich, and H. Podhaisky. FEBS Letters 463: 103-109 (1999).