methods, including the presence of 40 and 80 degree physical substeps (Yasuda et al. 2001) and the order and kinetics of chemical substeps. We are interested in using single molecule techniques to observe the effects of *mgi* mutations on enzyme kinetics and torque production in F_1 from the yeast *Saccharomyces cerevisiae*.

Using a high speed imaging camera, we have captured the rotation of wild-type and mutant forms of yeast F_1-ATPase. Rotation data for the wild-type and preliminary data for some *mgi* strains will be presented. We show for the first time that at saturating ATP, wild-type yeast F_1 rotates approximately four times faster than the thermophilic F_1. Kinetic and substepping behaviour in yeast appears to be similar to that observed in bacterial F_1.

742-Pos Board B621

Structure Analysis of F_1-ATPase via Molecular Dynamics

Yuko Ito, Mitsunori Ikeguchi.
Yokohama-city univ., Yokohama, Japan.

F1-ATPase is comprised of five different subunits (α to ϵ). The $\alpha 3\beta 3$ hexamer contains nucleotide binding sites and γ rotates sequentially by a cooperative binding change mechanism for ATP synthesis and hydrolysis. The structures of β subunits, undergoing large conformational changes during the binding change mechanism, can be classified as tight (β_{DP}), loose (β_{TP}) or empty (β_E). To elucidate the relationship between intrinsic dynamics of F1-ATPase and its function, we have carried out a equilibrium molecular dynamics simulation for a F1-ATPase crystal structure (PDB cord: 2JDI) for 30 ns. The structural features of each subunit and their inter-subunit interactions were analyzed by the residue fluctuations and correlation. Previous studies revealed that the catalytically active β_{DP} subunit interacts strongly with α_{DP}. However, we found that the non-catalytic pair, $\beta_{DP}\alpha_E$ also interacts strongly. This suggests that sandwiched β_{DP} can efficiently transmit some structural change caused by the chemical reaction to the adjacent subunits. Furthermore, structural fluctuation of the γ subunit was correlated mainly with β_{DP}. This result suggests that the chemical reaction on β_{DP} can affect not only the conformational change for the other α, β subunits but also the γ-subunit rotation.

743-Pos Board B622

Interplay of Ligand Binding, Domain Interaction and Chaperone Mediated Cu^+ Delivery to Cu^+ Transport ATPases

Deli Hong, Manuel Gonzalez-Guerrero, Jose M. Arguello.
Worcester Polytechnic Institute, Worcester, MA, USA.

Cu^+-ATPases receive Cu^+ from specific chaperones via ligand exchange and subsequently drive the metal efflux from the cell cytoplasms. Cu^+-ATPases have two transmembrane metal binding/transport sites (TM-MBS) and various cytoplasmic domains: the actuator (A-domain) and ATP binding domains (ATPBD), and regulatory N-terminal metal binding domains (N-MBD). *Archaeoglobus fulgidus* CopA, the Cu^+-ATPase used in these studies, contains a single N-MBD and an apparently non-functional C-terminal MBD. The Cu^+ dependent interaction of N-MBD and ATPBD was postulated as a possible mechanism for enzyme regulation (Tsivkovskii et al. JBC, 2001, 276: 2234). Similarly, we hypothesized that ligand (Cu^+ or nucleotide) binding to cytoplasmic domains might be required for chaperone-Cu^+-ATPase interaction. Testing these ideas the interactions among isolated cytoplasmic domains and the chaperone-Cu^+ transfer to the TM-MBS in the full length ATPase were characterized. Studies using isolated domains showed that while the N-MBD interacts with ATPBD, the presence of Cu^+ or nucleotide (ADP) prevents this interaction. The N-MBD does not interact with the A domain. Alternatively, the C-MBD interacts with both ATPBD and A-domains in a ligand independent fashion. The Cu^+ transfer form the chaperone to CopA is independent of the N-MBD capability to bind Cu^+. However, only one Cu^+ is transferred to CopA in absence of nucleotides, while the presence of ADP allows full loading of TM-MBS. Since this effect of ADP was observed even when N-MBD was loaded with Cu^+, the nucleotide role in TM-MBS Cu^+ loading seems unrelated to the N-MBD-ATPBD interaction. Different from the well-described alkali metal transport by P-type ATPases, the requirement of nucleotide binding for Cu^+ loading along with the practically irreversible binding of metal to the transport sites, appear as significant mechanistic elements necessary for Cu^+ transport by these ATPases.

744-Pos Board B623

Structural Dynamics Of The Phospholamban-SERCA Complex By Site-Directed EPR Spectroscopy

Zachary M. James, Kurt D. Torgersen, Christine Karim, David D. Thomas.
University of Minnesota, Minneapolis, MN, USA.

We are using site-directed spin-labeling (SDSL) and EPR spectroscopy to study the structural dynamics of phospholamban (PLB), a 52-residue integral membrane protein that regulates the SR calcium ATPase (SERCA). PLB binds and inhibits SERCA at sub-micromolar calcium concentrations, while phosphorylation of PLB at Ser16 relieves this inhibition without dissociating the two proteins (Mueller et al., 2004). Employing solid-state peptide synthesis, we have created PLB analogs in which the spin-labeled amino acid TOAC is substituted for residues along the backbone. Doubly-labeled proteins were studied by DEER, a pulsed EPR experiment that can measure inter-spin-label distances from 2 to 7 nm. Our results agree with previously published EPR dynamics data showing that PLB exists in both a compact, ordered (T) state and an extended, dynamically disordered (R) state (Karim et al., 2006). Alone, PLB primarily occupies the (T) state, while this equilibrium shifts in favor of the (R) state upon SERCA binding or PLB phosphorylation. However, SERCA-bound PLB becomes more ordered and compact upon phosphorylation. We are also using relaxation enhancement to study the movement of PLB's single transmembrane (TM) helix relative to the membrane plane. In these experiments, the spin-lattice relaxation rate of excited spins is enhanced by the presence of paramagnetic relaxation agents (PRAs), which collide with these spins and cause them to relax faster. For spin-labels incorporated into the TM domain, PLB motions that reposition this helix will make the spin-label more or less accessible to water-soluble PRAs, while having the opposite effect for lipid-soluble PRAs. The magnitude of change in the relaxation rate can be used to gauge the movement of the TM helix upon SERCA binding and following phosphorylation. With these experiments, we are constructing a more complete model of PLB dynamics during its interaction with SERCA.

745-Pos Board B624

Structural Dynamics Of Sarcoplasmic Reticulum Ca2+-ATPase (SERCA) Studied By Molecular Simulations Of Site-specific Labeled Protein

Bengt Svensson[1], L. Michel Espinoza-Fonseca[1,2], David D. Thomas[1].
[1]Univ. of Minnesota, Minneapolis, MN, USA, [2]Instituto Politécnico Nacional, Mexico City, Mexico.

Structural dynamics of the proteins involved in Ca^{2+} transport and its regulation is studied in our laboratory by EPR and fluorescence spectroscopy. To interpret these experimental results and to generate new structural and mechanistic models, we have performed computational simulations of SERCA labeled with spectroscopic probes. Our approach provides information on the conformational landscape sampled by SERCA during its catalytic cycle. X-ray crystal structures suggest that the nucleotide-binding and actuator domains of SERCA move apart by about 3 nm upon Ca^{2+} binding, undergoing a transition from open to closed conformations. To test this hypothesis, we constructed a fusion protein containing CFP linked to the N-terminus (the A-domain) of SERCA. CFP-SERCA was then specifically labeled with FITC in the N-domain. FRET was then used to monitor the A to N interdomain distance (Winters, Autry, Svensson and Thomas, 2008, *Biochemistry* 47, 4246-56). To interpret the FRET data, simulations of the CFP-SERCA fusion protein were conducted to generate a representative ensemble of conformations. FRET parameters were calculated using both distance and orientation information. Based on FRET data and simulations, we conclude that (a) the cytoplasmic headpiece maintains a compact structure throughout its catalytic cycle, rather than the open E1.Ca crystal structure, and/or (b) the Ca-bound E1 state is dynamically disordered and samples both open and closed conformations, with an average structure that is only slightly different from the closed E2 structure. To extend the simulations, we have developed force-field parameters for the fluorescence labels AEDANS and FITC. This will enable direct comparisons of results from molecular dynamics simulation and fluorescence spectroscopy experiments. This work was supported by NIH (GM27906, AR007612) and the Minnesota Supercomputing Institute.

746-Pos Board B625

Molecular Dynamics Simulations Reveal Intrinsic Features of SERCA Dynamics

L. Michel Espinoza-Fonseca, Bengt Svensson, David D. Thomas.
University of Minnesota, Minneapolis, MN, USA.

X-ray crystallography of SERCA (an integral membrane calcium pump in muscle) suggests that upon Ca^{2+} binding (transition from E2 to E1), the nucleotide-binding (N) and actuator (A) domains increase their separation by 3 nm. However, FRET data shows that Ca^{2+} produces only a slight distance increase between these domains (Winters et al., 2008, *Biochemistry* 47:4246-56). To understand this discrepancy, we have performed all-atom molecular dynamics (MD) simulations on the crystal structures of the E1 (PDB: 1SU4) and E2 (PDB: 1IWO) states, in explicit lipid bilayer and water at constant temperature (310K) and pressure (1 atm). Trajectories (40 ns) revealed that both domains display significant flexibility, with N more flexible than A. Principal component analysis showed that these domains move toward each other in the E1 state and apart in the E2 state; making these structure converge toward each other, in

agreement with published FRET data. Cross-correlation matrices revealed correlated motions between the A and phosphorylation (P) domains in both E1 and E2 states. In contrast, anticorrelated motions were observed between N and A/P domains, with slight differences between E1 and E2, suggesting a Ca^{2+} effect. Solvent-accessible surface area around the ATP site increased in the simulated E2 state. Conclusions: (a) Crystal structures of E1 and E2 states of SERCA are not representative of the populations under physiological conditions, where the two structures differ much less than in the crystal. (b) The simulation indicates a much more accessible ATP-binding site than observed in the E2 crystal structure. (c) Calcium-induced modulation of interdomain anticorrelated motions involving the N domain may be important for ATP binding, catalysis and gating. This work was supported by NIH (GM27906, AR007612) and the Minnesota Supercomputing Institute.

747-Pos Board B626

Investigation Of Electrogenic Partial Reactions In Detergent-solubilized Na,K-ATPase

Michael Habeck, **Hans-Juergen Apell**.
University of Konstanz, Konstanz, Germany.

Electrochromic styryl dyes are in use now for almost two decades to detect ion movements in various P-type ATPases. The extremely hydrophobic dye molecules have a high partition coefficient in favor of the hydrophobic core of lipid phase of membrane preparations. Fluorescence changes are obtained by modification of local electric fields in the membrane dielectric produced by ions bound to or released from binding sites of the ion pumps. To obtain significant signals a prerequisite is a high density of active proteins in the membranes. This limitation could be overcome by solubilization of the Na,K-ATPase in mixed micelles of protein/lipid/detergent obtained by incubation of microsomal membranes from rabbit kidney with dodecyl maltoside. In this preparation the specific enzyme activity of the Na,K-ATPase was reduced compared to that in native membranes. This effect was assigned to the highly affected lipid environment of the singularized proteins which may be depleted of specific lipid components and the content of dodecyl maltoside. The fluorescence changes which were detected with the styryl dye RH421 showed smaller amplitudes than in the case of purified membrane preparations, however, the responses on Na^+ binding, Na^+ release upon enzyme phosphorylation and conformation transition, and subsequent K^+ binding in the E_2P conformation were clearly detectable. Na^+ binding affinity and its dependence on Mg^{2+} concentration and buffer pH, as well as K^+ affinity were comparable to the results obtained with native preparations. The transfer of the method to solubilized ion pumps will allow investigations of mutants isolated from cell in which they were expressed in low density. In addition, this approach will possibly extend method also to the investigation of ion channel molecules by electrochromic styryl dyes.

748-Pos Board B627

Influence of Phosphate Analogs on Palytoxin-opened Na,K-ATPase Pump-channel

Ayako Takeuchi, David C. Gadsby.
Laboratory of Cardiac/Membrane Physiology, Rockefeller University, New York, NY, USA.

Na,K-ATPase pumps generate steep transmembrane Na and K gradients by behaving like ion channels with gates constrained to open and close alternately. Palytoxin binding disrupts this coupling between the gates, allowing both to sometimes be open, transforming pumps into ion channels. The gates of these pump-channels still respond to extracellular Na and K ions and to cytoplasmic nucleotides. Here we test phosphate mimics. Saturating, 50 nM, palytoxin induced large Na,K pump-channel currents in outside-out patches from *Xenopus* oocytes with 5 mM ATP in the pipette, and smaller currents with no ATP, but failed to induce any currents when pipettes contained BeF_x (200 μM $BeSO_4$ plus 5 mM NaF). In inside-out patches, with 100 nM palytoxin in the pipette, BeF_x or AlF_x (200 μM $AlCl_3$ plus 5 mM NaF) strongly decreased (by at least 70%) the small pump-channel currents seen without ATP and severely impaired (by ~90%) the large current increase by 1 mM ATP that reflects internal gate opening. Be ions alone (200 μM $BeSO_4$) somewhat diminished pump-channel currents without ATP, but did not affect activation by ATP; however, gate closure upon ATP removal was markedly slowed (>100-fold). As subsequent BeF_x application almost abolished activation by ATP, the effects of BeF_x and of Be ions are distinct. Al ions alone (200 μM $AlCl_3$) had similar effects to Be ions, though weaker. In contrast, MgF_x (5 mM $MgCl_2$ plus 5 mM NaF), or even just $MgCl_2$, simply reversibly decreased pump-channel currents in the absence of ATP, without affecting activation by ATP. These results suggest that, despite the presence of palytoxin, phosphate mimics BeF_x and AlF_x stabilize an Na,K pump-channel conformation with the internal gate firmly shut, like that in X-ray crystal structures of E2-BeF_3^- SERCA ATPase. [HL36783]

749-Pos Board B628

Truncation Of The Na/K Pump's C-terminus Attenuates Voltage-dependent Binding Of External Na^+ By Destabilizing Na^+ Occlusion

Natascia Vedovato, David C. Gadsby.
The Rockefeller University, New York, NY, USA.

In the recent Na/K pump crystal structure the C-terminus directly contacts the transmembrane region, and deletion of its last five residues (KETYY in pig Na/K pumps) strongly lowered (26-fold) apparent affinity for Na^+-dependent phosphorylation, which reflects cytoplasmic-side Na^+ binding (Morth et al., 2007). Here we use two-microelectrode voltage-clamp recordings in *Xenopus* oocytes to investigate the consequence of this C-terminal truncation on the external Na^+ dependence of steady-state, and transient, Na/K pump-mediated currents. The corresponding deletion (ΔKESYY in *Xenopus α1*) was introduced in *Xenopus α1β3* pumps made ouabain resistant by either Q120R-N131D (RD) or C113Y (C-Y) mutations. All extracellular solutions then contained 1 μM ouabain to silence the endogenous *Xenopus* Na/K pumps, and the mutant pump currents were determined by addition of 10 mM ouabain. Inhibition of steady outward Na/K pump current (at 15 mM external K^+) by 125 mM extracellular Na^+ at negative voltages was weaker in both RD and C-Y pumps than in wild-type *Xenopus* Na/K pumps, but was almost completely absent (at -120 mV) in RD-ΔKESYY or C-Y-ΔKESYY pumps. Transient, external Na^+-dependent, pump currents were similarly measured as 10 mM ouabain-sensitive currents, but in K^+-free solution. The voltage dependence of these transient charge movements was shifted to more negative potentials for RD-ΔKESYY and C-Y-ΔKESYY pumps compared to RD or C-Y pumps, similar to the shift caused in wild-type pumps by decreasing the extracellular Na^+ concentration. Together, these results suggest that the C-terminal deletion lowers the apparent affinity with which extracellular Na^+ ions bind to the Na/K pump to reverse Na^+-ion deocclusion. The C-terminus therefore seems important for stabilizing the occluded conformation of the phosphorylated Na/K pump containing 3 bound Na^+ ions. [NIH HL36783]

750-Pos Board B629

Temporal and Steric Analysis Of Ionic Permeation and Binding in Na+,K+-ATPase via Molecular Dynamic Simulations

James E. Fonseca[1,2], Savas Kaya[2].
[1]Rush University Medical Center, Chicago, IL, USA, [2]Ohio University, Athens, OH, USA.

Abstract: The Na^+, K^+-ATPase is ubiquitous in animal cells. Yet, many atomic-level details of the structure/function relationship of its electrogenic translocation process remain unanswered. This work employs computational methods to investigate the specific amino acid residues that constitute the two K^+ and three Na^+ ion binding sites. Putative lumenal and cytoplasmic ion permeation pathways are also determined. Homology models of the human α1 isoform of the Na^+, K^+-ATPase based on X-ray structures of the SERCA Ca^{2+}-ATPase in several conformations (E1, E2, and E2P) were created using the Modeller homology modeling software. The sequence alignment incorporated an array of experimental results and consensus with similar proteins (e.g. H^+, K^+-ATPase). The E2P homology model presented here agrees well with the recent X-ray crystallographic structure of the Na^+, K^+-ATPase. The three models were simulated in a water/lipid environment with the GROMACS molecular dynamics package. Established equilibration techniques were followed by several nanoseconds of analyzable production run trajectories for each conformation. Atomic trajectories were analyzed with the steric pathway tool, CAVER, to provide putative ion permeation pathways. These pathways were consistent with regions of negative potential determined via time-averaged electrostatic calculations of the same trajectories. The electrostatic calculations provide a 3D view of the potential landscape encountered by cations. Amino acid residues (as suggested by SERCA structures and mutagenesis studies) involved in the creation of putative binding sites were investigated by placing Na^+ and K^+ ions at these locations and evaluating protein-ion interactions during simulation trajectories. In addition to the results of the Na^+, K^+-ATPase simulations, the homology models and methodology presented here provide a blueprint for the study of the larger class of P-type ATPases.

751-Pos Board B630

Relative Movement Of The α-Subunit's First And Last External Loops Throughout Na/K-Pump Cycle

Pablo Artigas.
Department of Cell and Molecular Pysiology Texas Tech University Health Sciences Center, Lubbock, TX, USA.

Structural models of the Na/K-pump α-subunit show that conserved residues D121 (loop 1-2, pig-α1 numbering) and R972 (loop 9-10) are 5-7 Å apart in E2, and more than 12 Å away in E1. To check whether these residues reach