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

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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

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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

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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

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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

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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