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performed in HeLa cells expressing MCU-V5 and Mfrn2-GFP. **Results**: In RLM, $Ca^{2+}$ and $Fe^{2+}$ (250 μM) each stimulated respiration to a nearly identical degree, an effect completely blocked by Ru360. In UMSCC22A cells, mRNA and protein expression of Mfrn2 was 2-3-times that observed in UMSCC1 cells. High Mfrn2-expressing UMSCC22 cells also had 3-fold greater rates of mitochondrial $Ca^{2+}$ and $Fe^{2+}$ uptake. After Mfrn2 knockdown (55% decrease), rates of mitochondrial uptake of both $Ca^{2+}$ and $Fe^{2+}$ decreased by ~75%. All uptakes were blocked by Ru360. In HeLa cells co-transfected with MCU-V5 and Mfrn2-GFP, anti-GFP beads pulled down MCU-V5, whereas anti-V5 beads pulled down Mfrn2-GFP. COX-IV was not pulled down by beads, indicating that the interaction between MCU and Mfrn2 was specific. **Conclusions**: 1) Mfrn2 positively modulates Ru360-sensitive respiration-driven mitochondrial uptake of both $Ca^{2+}$ and $Fe^{2+}$. 2) Mfrn2 physically interacts with MCU and appears to be a component/regulator of the MCU complex. 3) The mitochondrial calcium uniporter should more appropriately be called the mitochondrial $Ca^{2+}$,$Fe^{2+}$ uniporter.

**2940-Pos Board B632**

**$K^+$ Translocation by the Giant Axon of the Humboldt Squid $Na^+$/K ATPase**

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The $Na^+$/$K^+$ pump is a membrane protein which plays a fundamental role in maintaining the $Na^+$ and $K^+$ electrochemical gradients in animal cells. When internal and external $Na^+$ is absent the pump can only undergo $K^+$ translocation reactions. At equilibrium, the distribution of the different protein conformations depends on the rate constants of each step leading to $K^+$ binding and unbinding. If some of these rate constants are voltage-dependent, sudden changes in membrane electric potential will shift the binding-unbinding equilibrium. In those translocation reactions, $K^+$ has to travel a fraction of the membrane electric field generating a transient current signal. Here, $K^+$ pump currents were measured under voltage clamp conditions using the giant axon of the Humboldt squid, which due to its large diameter (1 -1.5 mm) allows the detection of these charge movements. By using H2DTG, a reversible inhibitor of the squid $Na^+$/$K^+$ pump, we were able to obtain H2DTG-sensitive transient currents in response to voltage jumps in $K^+$/$K^+$ conditions. Kinetics of these transient currents shows two main components, that in contrast to their $Na^+$ counterpart, appeared to be uncoupled. The origin of the fast component appears to be the movement of ions along an access channel that it is always open, suggesting that the gate that occlude K ions is deep in the permeation pathway. On the other hand, charge displacement distribution and rate constants of the slow component show a clear dependence on the $K^+$ external concentration revealing that the entrance of the $K^+$ to the $Na^+$/$K^+$ pump from the external side is a voltage-dependent step. Supported by FIRCA grant R03 TW008351 and U54GM087519, GM030376, NS64259, HL36783 and the Intramural Program of the NINDS/NIH and FONDECYT 1110430.

**2941-Pos Board B633**

**Conformational Rearrangements of the $Na^+$/$K^+$ ATPase During $Na^+$ Occlusion/Deocclusion Transitions Assessed by Site-Directed Fluorescence**

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The $Na^+$/$K^+$ ATPase is a $E_1$-$E_2$-type pump formed mainly by two subunits: α and β, and is responsible for $Na^+$ and $K^+$ homeostasis, thereby fundamental for cellular life. Most of the voltage dependence of the pump cycle originates from the steps associated with extracellular $Na^+$ binding and release, namely occlusion and deocclusion ($E_1P$ and $PE_2$). To gain further insights into the conformational rearrangements of the squid $Na^+$/$K^+$ pump during the $E_1P$ and $PE_2$ transitions, we have used site-directed flourometry under voltage-clamp. We engineered single cysteine mutants facing the outside of the α and β subunits of the squid Na/K pump, as targets of a cysteine-reactive fluorescent reporter (6-TMR). These constructs were found functional when expressed in *Xenopus* oocytes under voltage-clamp conditions and we obtained simultaneous electrical and fluorescence recordings. We have identified two positions on the α subunit: N894C (linker M7-M8) and D802C (linker M5-M6), and two on the β subunit: D74C (top of the TM helix) and D116C (external cap-helix), that produce voltage dependence fluorescence changes during the $E_1P/PE_2$ transitions. Fluorescence intensities produced by N894C, D74C and D116C constructs were correlated with its respective voltage dependent $Na^+$ translocation curves following a Boltzmann distribution. Interestingly, kinetics of the fluorescence and electrical signals from N894C showed remarkably similar voltage dependence, suggesting functional and conformational correlation during $E_1P/PE_2$ transitions. Surprisingly, fluorescence signal from D116C produced a biphasic kinetics behavior, indicating a complex movement of the β and/or the α with respect to the β subunit. These findings begin to delineate a region in the α-subunit that is moving during the $E_1P/PE_2$ transitions. Supported by U54GM087519 and GM030376.

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**State-Dependent Movement between the First and Last External Loops of the Na/K Pump α Subunit**

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Previously we reported that conserved Na/K pump α-subunit residues D128 (loop L1-2, shark-α1 numbering) and R979 (L9-10) reach atomic proximity in E2P (Artigas, 2009. *Biophys J.* 96(3):145a). L1-2 is expected to move throughout the pump cycle. We used double cysteine substitutions to address the relative displacement of L1-2 with respect to R979. We mutated R979C and concomitantly introduced a cysteine at each of the residues within L1-2, from Q118 (external end of TM1) to L132 (within TM2), and analyzed the functional effect of reducing and oxidizing reagents on these double cysteine mutants heterologously expressed in *Xenopus* oocytes. The pump current ($I_P$) induced by 3 mM K under two-electrode voltage clamp in the absence of Na was measured; first without redox treatment, then after 15 min in 10 mM TCEP (a reducing agent) and subsequently, following oxidation with cupper phenanthroline (100 μM Cu: 300 μM Phe, applied in N-Methyl D-glucamine). TCEP increased the $I_P$ of E122C/R979C (20%), E124C/R979C (20%), P125C/R979C (38%), Q126C/R979C (52%) and D128C/R979C (130%), whilst Cu:Phe induced similar effects, in the opposite direction than TCEP, with a maximum reduction of $I_P$ (>80%) in D128C/R981C. The effect of Cu:Phe on the charge movement without K in the presence of Na was also studied. Cu:Phe abolished charge movement of D128C/R981C and modified the characteristics of the transients of the other double cysteine mutants that showed effects of crosslinking in $I_P$. Our results indicate that residue 128 (at the end of a rigid TM2-helix) must separate from R979 in order for TM2 to perform its required motions, while crosslinking residues further in the flexible loop does not block $I_P$, but modifies the E1-E2 conformational equilibrium in the presence of Na. Supported by R15NS081570-01A1.

**2943-Pos Board B635**

**Single-Molecule Measurements to Investigate the Negative Cooperativity in $Na^+$/$K^+$ -ATPase**

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The $Na^+$/$K^+$-ATPase, a cell membrane ion motive ATPase, uses energy from the hydrolysis of ATP to move $Na^+$ out of and $K^+$ into cells, thus maintaining the membrane resting potential and cellular volume. To investigate how this pump functions, we isolated ATPase from duck supraorbital salt glands and labeled it with Cy3-maleimide (Cy3-ATPase). In bulk experiments, we found that the fluorescence of Cy3-ATPase decreases in the presence of ATP (*Biochim Biophys Acta* 2009; 1794:1549-1557). The kinetics of this ATP-induced fluorescence decrease exhibited negative cooperativity and could be explained in terms of protein aggregation. To further explore the phenomenon of negative cooperativity on the level of individual monomers, we used single-molecule total internal reflection fluorescence (SM-TIRF) microscopy. Protein monomers were solubilized and reconstituted into lipid vesicles to investigate the effect of varying ATP concentration on the fluorescence.

Data from SM-TIRF experiments, analyzed using a hidden Markov model (HMM), suggest that the Cy3-ATPase exists in dynamic equilibrium between a high fluorescence state (unquenched) and a low fluorescence state (partially quenched). These kinetics are characterized by either rapid or slow transitions between these states. Two subpopulations are observed, one where the transitions between the states occur rapidly and the other where the kinetics are slower. Preliminary analysis of the data suggests that ATP shifts the population distribution from those exhibiting rapid transitions to those exhibiting slow transitions. Here, we report on the analysis of these effects and the implications of the above observations on the working of the pump.

**2944-Pos Board B636**

**The Molecular Mechanism of $Na^+$, $K^+$-ATPase Malfunction in Mutations Characteristic for Adrenal Hypertension**

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Mutations within ion transporting proteins may severely affect their ability to properly traffic ions and thus perturb the delicate balance of ion gradients.