a heterodimeric bb'from a cyanobacterial ATP synthase exist as traditional left-handed coiled coils for most of their lengths. Upon binding of the soluble E. coli b2 to soluble F1, the tether region of the b-dimer disengages to form a more loosely packed arrangement that then seems to repack closely before entering the membrane-phase.

Using the completely assembled F1Fo-ATPase, we recently observed changes in the packing interactions of b2 during the formation of ATP-hydrolysis transition states. These transitions were observed through the release of spin label catalyzed by non-modified cysteine on the adjacent b-subunit. Only those spin-labels were released that were predicted by us to be close to or at the left-handed coiled coil b-dimer interface, while no release of label from positions that were predicted to be at the outer surface of the helices was observed. The results suggest that while modest conformational changes of the b2-dimer occur during catalysis, the dynamics of these changes do not appear large enough to support a left-handed to right-handed coiled coil conversion that has previously been suggested.

3818-Pos
Partial Reactions of the ATP Synthesis Reaction in the E. Coli βD380C Mutant F1Fo-ATP Synthase
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The initial stage of ATP synthesis by the *E. coli* wild type (WT) and mutant βD380C F_1F_o-ATPase was compared at saturating substrate concentrations and a proton-motive force (*pmf*) of ~315 mV. The enzymes catalyze both ATP hydrolysis (Baylis Scanlon et al., 2008, *J. Biol. Chem.* 283, 26228-26240) and ATP synthesis with similar steady-state parameters. ATP synthesis by the WT enzyme proceeded with neither burst nor lag-phase while ATP synthesis by βD380C (+DTT) showed a burst phase with a stoichiometry of ATP/F_1F_o equal to 1. The burst was simulated using a kinetic model in which the βD380C mutant has a less than 10-fold increase in Pi binding rate in combination with a less than 10-fold decrease in ATP release rate compared to WT. Resolution of the burst allows us to distinguish the partial reactions of the ATP synthesis pathway.

Comparison of the ATP release rates in ATP hydrolysis (Baylis Scanlon et al., 2007, *Biochemistry* 46, 8785-8797) and ATP synthesis suggest differences in the cooperative behavior in the two directions of the reaction. In ATP hydrolysis, positive cooperativity in catalysis is induced by nucleotide (ATP) only, while in ATP synthesis it is induced by both nucleotide (ADP) and the *pmf*. Fast steady-state ATP hydrolysis proceeds through a trisite mechanism, while ATP synthesis uses a bisite mechanism.

We further analyzed the effects of the mutation by forming a stator-rotor disulfide cross-link, βD380C-γC87 induced by DTNB. The cross-linked enzyme catalyzed absolutely no ATP synthesis in the millisecond time domain after inducing a *pmf*. This result suggests that γ subunit mobility is required for the ATP synthetic reaction to occur and is consistent with the model that high affinity Pi binding cannot be achieved without $\Delta\mu_H^+$-dependent γ-subunit rotation.

3819-Pos
Electric Field Driven Torque in ATP Synthase
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Dysfunction of mitochondrial enzymes, including ATP synthase, has been implicated in type-2 diabetes, cancer, heart disease, and neurodegenerative diseases. Our electric field driven torque (EFT) model of ATP synthase predicts a scaling law relating torque to the number of proton binding sites in the rotor (*c*-ring) and the proton motive force (pmf) across the mitochondrial inner membrane. When the F_O complex of ATP synthase is coupled to F_1, the model predicts a critical pmf to drive ATP production. In order to fully understand how the electric field resulting from the pmf drives the *c*-ring to rotate, it is also important to examine the charge distribution on the protonated *c*-ring and in the *a*-subunit, which contains the proton half-channels and acts as a stator. A self-consistent field approach is used in our calculations, based on a refinement of reported ac_{12} structural data. The calculations reveal changes in pKa for key residues on the *a*-subunit and *c*-ring, as well as titration curves and protonation state energy diagrams. Implications for the EFT model will be discussed.

3820-Pos
Cardiac Pacemaker Cells Uniquely Match ATP Supply to Demand
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Sinoatrial node cells (SANC) within the heart's pacemaker govern the heart rhythm and contraction due to constitutively active, Ca^{2+}-activated adenylyl-cyclases (AC) that generate a high level of cAMP/protein kinase A (PKA) dependent, localized, submembrane-compartmentalized Ca^{2+}- and AC-cycling. This rhythmic Ca^{2+} cycling generates rhythmic action potentials (APs), which initiate Ca^{2+} release and SANC contraction. To drive the heart beat SANC must tightly regulate ATP production to supply sufficient ATP for cAMP production, Ca^{2+}-cycling and contraction. To explore how ATP supply-demand is tightly balanced in isolated SANC we measured ATP levels: in control; during PKA-inhibition by specific inhibitor peptide PKI (15μM) or by H-89 (6μM); during intracellular Ca^{2+}-buffering by BAPTA (25μM); during muscarinic-receptors (MR) activation by carbacol 1μM ± MR-inhibition by atropine (10μM). PKA-inhibition by PKI or H-89, which blocked the major ATP-consumption processes, depleted ATP by 45 ± 6% and 44 ± 5%, respectively. Thus, complete inhibition of these major ATP consumption processes in SANC significantly reduces ATP levels **even though** ATP consumption is reduced. Buffering intracellular Ca^{2+} depleted ATP by 54 ± 8%. Hence, Ca^{2+} not only has direct effects on the surface membrane potential to ignite APs, and to activate myofilament-displacement/force production, but also regulates ATP production. In contrast to SANC, in either stimulated (3Hz) or quiescent rabbit ventricular cells the same experimental perturbations had only minor (<6%) effects on ATP levels. These control mechanisms identified by pharmacological-perturbations are apparently utilized in nature, because MR-stimulation of SANC also depleted the ATP by 45 ± 10% (by blocking the ATP-consumption processes). Notably, atropine, a MR-antagonist, substantially reversed the MR-stimulation effect, resulting in only 18 ± 3% ATP depletion. These data suggest that in contrast to ventricular cells, the same signals that drive, and are derived from, the utilization of ATP in SANC also tightly couple the production of ATP to match energy demand.

3821-Pos
Analysis of Intracellular ADP Compartmentation Reveals Functional Coupling between Pyruvate Kinase and ATPases in Rat Cardiomyocytes
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Cardiomyocytes have intracellular diffusion restrictions, which spatially compartmentalize ADP and ATP. According to mathematical models, diffusion restrictions are localized in certain areas of the cell. However, the models so far have used data sets generated on rat heart permeabilized fibers, where diffusion distances may be heterogeneous. This is avoided when using isolated, permeabilized cardiomyocytes. The aim of this work was to analyze the intracellular diffusion of ATP and ADP in rat permeabilized cardiomyocytes. Intracellular energetic communication between mitochondria and AT-Pases was approached from several angles, where respiration rate, ATPase rate and ADP concentration in surrounding solution were determined under several conditions. The data was analyzed by mathematical models reflecting different levels of cell compartmentation. In agreement with previous studies, we found significant diffusion restriction by the outer mitochondrial membrane and confirmed a functional coupling between mitochondria and a fraction of ATPases in the cell. In addition, our experimental data shows that a considerable activity of endogenous pyruvate kinase (PK) remains in the cardiomyocytes after permeabilization. Intriguingly, a fraction of ATPases was inactive without ATP-feedback by this endogenous PK. When analyzing the data, we were able to reproduce the measurements only with the mathematical models that include a tight coupling between fraction of endogenous PK and ATPases. To our knowledge, this is the first time such a strong coupling of PK to ATPases has been demonstrated in permeabilized cardiomyocytes indicating the importance of glycolysis in energy production for cardiac function.

3822-Pos
Diffusion Restrictions in Cardiomyocytes from Low-Performance Heart
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In adult mammalian cardiomyocytes, intracellular diffusion restrictions affect metabolic regulation. Despite extensive studies on rat cardiomyocytes, their cause and role in vivo is still unclear. Intracellular membrane structures may play a role. Previous studies suggest that rainbow trout permeabilized