inhibition relief. To further elucidate this mechanism, we have used site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy to study the interaction between the transmembrane (TM) domains of SERCA and PLB, where regulation is mediated. Spin labels were attached along PLB's TM helix for accessibility EPR measurements as well as double electron-electron resonance (DEER) distance measurements to spin-labeled SERCA. Our results suggest that Ser16 phosphorylation induces a change in the topology of PLB's TM helix that results in disruption of SERCA inhibition without dissociating the two proteins. Acknowledgments: spectroscopy was performed in the Biophysical Spectroscopy Center at the University of Minnesota, and funded by NIH grants to DDT (R01 GM27906, P30 AR507220).

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Regulatory Mechanism of SERCA-PLB Complex Determined by FRET

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We have used time-resolved fluorescenceresonance energy transfer (TR-FRET) to study the structural basis of regulation of sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) by a single-pass transmembrane protein, phospholamban (PLB). SERCA actively sequesters Ca^{2+} back into the sarcoplasmic reticulum to cause muscle relaxation. In the heart, SERCA is inhibited by unphosphorylated PLB. Physiologically, this inhibition is relieved by either micromolar Ca^{2+} or phosphorylation of PLB. Dysregulation of SERCA-PLB complex causes Ca^{2+} mishandling in cardiomyocyte, which is a prominent feature in heart failure. Our goal is to elucidate the structural basis for the regulation of the complex, with an emphasis on the structural rearrangement during relief of inhibition. We have used time-resolved fluorescence resonance energy transfer (TR-FRET) to probe the complex directly by measuring the distances between SERCA (C674) and a series of positions on PLB in co-reconstituted vesicles. Our data supports that the cytoplasmic domain of bound PLB exists in two states. The relief of inhibition is achieved by combined effect of dissociation of the complex and shifting the equilibrium of the two states of bound PLB. Spectroscopy was performed in the Biophysical Spectroscopy Center at the University of Minnesota. This work was funded by NIH grants to DDT (R01 GM27906 and P30 AR0507220).

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Polyamines Depolarize the Membrane and Initiate a Cross-Talk Between Plasma Membrane Ca^{2+} and H^+ Pumps

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There are multiple studies, exploring effects of polyamines on cation and K^+ channels in animal and plant cells, but much less is known on their potential effects on cation-transporting ATPases. In this work non-invasive MIFE and conventional microelectrode techniques were applied to study the effects of polyamines (PAs) on Ca^{2+} and H^+ transport and membrane potential in living pea roots. Putrescine (Put^{2+}) or spermine (Spm^{4+}) in 1 mM concentration caused a large increase of Ca^{2+} efflux across plasma membrane (PM), sensitive to Ca^{2+} pump inhibitor eosine yellow (EY) but insensitive to amiloride. Put^{2+} and Spm^{4+} caused contrasting effects on H^+ fluxes: induced net H^+ efflux (due to activity of the PM H^+ pump) and influx, respectively. Use of EY and vanadate, non-specific inhibitor of P-type pumps, converted Spm^{4+}-induced H^+ influx to transient H^+ efflux and suppressed H^+ fluxes, respectively. The former result was consistent with Ca^{2+}/H^+ antiport activity of the PM Ca^{2+} pump. However, EY also reduced Put^{2+}-induced H^+ efflux. The latter result may be explained by down-regulation of the H^+ pump activity by increased intracellular Ca^{2+}. When it comes to direct effect of PAs on the H^+ pump in isolated PM vesicles, Put^{2+} did not produce any effect and Spm^{4+} partly suppressed H^+ pumping. All PAs, $Spm^{4+}>Spd^{3+}>Put^{2+}$ caused substantial (up to 70 mV) membrane depolarization, abolished by Gd^{3+} and strongly decelerated by removal of external Ca^{2+}. Analysis of net ion fluxes, induced by different PAs, led us to a conclusion that transport of PAs themselves directly contributes to membrane potential changes. Therefore, PAs induce Ca^{2+} pump activity and differentially affect H^+ fluxes and electric potential difference across the PM. Supported by CONACyT and ARC grants and by University of Tasmania visiting professorship to IP.

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Structure/Function Analysis of the Ubiquitous Secretory Pathway Ca^{2+} Pump SPCA1a

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Ca^{2+} is a universal intracellular messenger controlling a host of diverse physiological processes. Two housekeeping P-type Ca^{2+}-transport ATPases, SERCA2b in the endoplasmic reticulum and SPCA1a in the Golgi, play a crucial role in cellular Ca^{2+} homeostasis by accumulating Ca^{2+} into their respective stores. SPCA1a also supplies the Golgi with Mn^{2+}, a property that might be important for Mn^{2+}removal/detoxification. Whereas SERCA2b contains two Ca^{2+}-transport sites, SPCA1a possess only 1 Ca^{2+}/Mn^{2+} site. No specific SPCA1a inhibitors are known, although SPCA1a inhibition might have therapeutic potential suppressing certain types of breast cancer. Some specific SERCA2b inhibitors have the ability to quench SPCA1a activity, although at higher IC_{50}.
Since endogenous expression levels of the ubiquitous SPCA1a are too low to perform extensive structure/function analysis, overexpression and purification strategies are inevitable. We successfully overexpressed and affinity purified recombinant SPCA1a from yeast. Purified pumps are then reconstituted in Golgi-like liposomes for detailed functional analysis. Reconstituted SPCA1a remains active displaying similar properties as expressed in mammalian fractions. The reconstituted system was now deployed to compare the potential of various compounds to inhibit SERCA and SPCA1a. Remarkably, the putative SPCA1a inhibitor Bisphenol displays equal affinity for SPCA1a and SERCA1a. Given the µM affinity of Thapsigargin (Tg) for SPCA1a and the fact that it can be chemically modified, Tg is used as a lead compound for generating more specific SPCA1a inhibitors. Several analogues were already tested. Furthermore, the purified and reconstituted SPCA1a is currently used to investigate the role of its N- and C-terminus, since the N- and C-termini of the related SPCA2 isoform interact with Orai1, a process known as store-independent Ca^{2+}-entry that is activated in lactation and breast cancer.

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Oriental Plant Alkanna Orientalis Extracts Effects on Enterococcal Membrane-Associated Properties

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The oriental plant Alkanna orientalis tissues extracts have been shown to have antimicrobial activity against a number of gram-positive bacteria, particularly Enterococcus hirae, however mechanisms of this activity is not clear enough. A. orientalis intact root and callus cells extracts influence on E. hirae ATCC9790 whole cells H^+/K^+ exchange, membrane vesicles accessible SH-groups number and ATPase activity was investigated. It was shown that 50 and 100 µl root extract inhibited H^+ release ~1.27 and ~1.47 fold and K^+ accumulation ~1.25 and ~1.36 fold, respectively. Whereas, callus cells extract had a stronger effect on ions fluxes rates. The inhibitor of proton translocating F0F1-ATPase, N,N'-dicyclohexylcarbodiimide (DCCD) - sensitive H^+ efflux were observed upon addition of both extracts. The number of SH-groups was lowered ~1.7 and 2 fold compared with the control in the presence of root and callus cells extracts, respectively. The strongest effect was in the case when these substances were added in the assay medium together with 0.2 mM DCCD. The ATPase activity was also decreased ~3.1 and 3.5 fold compared with the control in the presence of 100 µl intact root and callus cells extracts, respectively. Maximum inhibition of ATPase activity was detected again when intact root and callus cells extracts were added in the assay medium with 0.2 mM DCCD simultaneously.
The results point out that A. orientalis intact root and callus cells extracts can directly affect enterococcal membrane-associated FoF1-ATPase, which can be considered as a target for antimicrobial activity.

Electron and Proton Transfer

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Bridging a Gap Between Cytochrome Bc1 Complex Structure and Function

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Cytochrome (cyt) bc1 complex has two active sites where energy-sustaining electron/proton transfer reactions occur. To determine the exact transfer steps