in FRET does indeed correlate with an increase in Ca-ATPase activity, potentially leading to identification of a drug that could reverse calcium mishandling, as required for treatment of heart disease. This work is supported by grants NIH R01HL139065 (to DDT/RTR) and T32AR007612 (to DDT).

2225-Pos

Substrate binding and conformational transitions of sodium-glucose transporters

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Sodium-glucose linked transporters (SGLTs) harness the sodium gradient to drive transport of sugar across the cell membrane via an alternating-access mechanism. In humans, hSGLT1 and hSGLT2 are responsible for the reabsorption of sugar in the kidney, and hSGLT2 is a key target in the treatment of type 2 diabetes. Experimentally determined structures of bacterial homologs (vSGLT) and, more recently, hSGLTs allow elucidation of regions involved in the inner and outer gates. However, the exact molecular and energetic details of the transport cycle remain unclear, and a fully outward-open human structure has not yet been determined. We use molecular dynamics to capture the dynamic behaviour of SGLTs, including unbiased simulations in different substrate configurations and weighted ensemble simulations exploring the opening of the outer gate, using the outward-open structure of the related sialic acid transporter (SiaT) as a target. These simulations reveal a sodium entry/exit path between binding site and extracellular bulk, accessible in partially outward-open confirmations (not open to the extent of SiaT) and blocked by inhibitor binding; and demonstrate how substrate binding can affect the conformational energetic landscape, e.g. bound sugar ''locking'' the outer gate closed. Improving our understanding of SGLT function through this work is a key step to better inform the treatment of diabetes and other SGLT-related diseases.

2226-Pos

Quantifying the degree of plasma membrane localization in Na^+/K^+-ATPase and screening for FXYD1 mutants

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In examining regulation of Na/K-ATPase (NKA) by its partner FXYD1, we sought to specifically analyze only the proteins that were correctly expressed in the plasma membrane (PM). We acquired images of cells stained with the PM marker WGA, using WGA images as the ground truth data and brightfield images as source data to train an AI model (Nikon Elements AI). The model was tested on novel images, yielding good colocalization between the AI-predicted membrane and true WGA images. AI-predicted images were thresholded, bandpass filtered, and converted to a binary mask in Fiji. Masks were multiplied by images of Cer-NKA and YFP-FXYD1 to yield PM-specific images. We used a custom macro for automatic cell selection and quantification of intensity of PM fluorescence (masked) and total cell fluorescence (unmasked). The percentage of fluorescence localized to the PM was quantified for different fluorescent species. We observed high %PM values for NKA (97%) but low values for negative controls such as the nuclear stain Hoechst (7%) and ER-localized proteins PLB (10%) and SERCA (8%). We used this scoring method to quantify localization of G301R-NKAα, a variant associated with renal hypomagnesemia. We observed a decrease in PM localization of the G301R mutant compared to the wild type. This mislocalization of G301R-NKA may contribute to the observed loss of function of this variant. The new automated scoring method compared favorably to manual selection and quantification of PM localization in confocal image data. We also used this PM selection method to selectively quantify FRET from PM-localized NKA-FXYD1 complex, excluding mislocalized proteins in the cell interior. We observed higher PM-specific FRET compared to whole-cell FRET. The results suggest automated PM scoring can reduce selection bias, improve throughput, and enhance quantification of correctly localized proteins.

2227-Pos

Computational analyses of SERCA, phospholamban, and sarcolipin complexes

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We have performed computational analyses of the sarcoplasmic reticulum Ca^{2+}-transporting ATPase (SERCA) and its regulatory peptides sarcolipin (SLN) and phospholamban (PLB). Recently we developed an improved protocol for isolation of SR vesicles from horse gluteal muscle, demonstrating that (i) horse SERCA exhibits a high specific activity of Ca^{2+}-activated ATP hydrolysis, and (ii) horse SR vesicles exhibit a high rate of Ca^{2+} transport and maximal Ca^{2+} loading. To assess the mechanistic basis of higher enzyme activity by SERCA in horse SR, we compared horse SERCA and SLN with rabbit, mouse, and human orthologs. Based on homology structural modeling of horse SERCA, via correlation with crystal structures and extensive site-directed mutagenesis studies of orthologous SERCA Ca^{2+} pumps, we propose that the large luminal loop between transmembrane helices 7 and 8 of horse SERCA is a structural element that governs the increased V_{max} and transport coupling ratio (Ca^{2+} ions per ATP molecule hydrolyzed). For horse SLN, sequence analysis and isoelectric point calculation show that horse SLN is the most basic of the orthologs analyzed, perhaps contributing to the unique functional modes for SLN regulation of SERCA activity, muscle contractility, and non-shivering thermogenesis. We propose that horse SERCA and SLN amino acid sequences represent evolutionary adaptations that contribute to the high muscular performance in this species. To further understand the availability of the peptide to regulate SERCA, we have also characterized PLB and SLN self-assembly using experimental and computational assays of homo-oligomerization. We have investigated structural determinants involved in stabilization of higher-order oligomers by the leucine/isoleucine zippers heptad-repeat motifs. These results allow us to capitalize on the enhanced knowledge of SERCA regulation for therapeutic use. The University of Minnesota Supercomputing Institute provided computational resources. Funding was provided by NIH R01HL139065 (to D.D.T./R.T.R.).

2228-Pos

A new ATP1A1 variant associated with a novel disease phenotype

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Pathological variants of *ATP1A1*, coding for the Na^+, K^+-ATPase (NKA)-α1 subunit (an αβheterodimer), are known to cause hyperaldosteronism, Charcot-Marie-Tooth neuropathies, hypomagnesemia with seizures and cognitive delay, as well as a group of phenotypes characterized by seizures and developmental delay. We found an *ATP1A1* variant (''variant X'') in a patient with hypothyroidism, congenital heart defects, obstructive hydrocephaly, conductive hearing loss, upper motor neuron symptoms, autism, and global developmental delay. NKA hydrolyzes 1ATP molecule to export $3Na^+$ out of the cell while importing $2K^+$, establishing electrochemical gradients that power excitability and secondary active transport in most cells. Previous studies have reported functional characteristics of the NKA-α1 variants that may contribute to their particular phenotypes, invariably showing loss of NKA function and, in some cases, aberrant ion-channel-like functional characteristics. To identify plausible mechanisms for the novel phenotypes observed in the patient, we expressed mutant and human wild type and mutant NKA-α1 with β1 in Xenopus oocytes, as well as in HEK293 cells for ATPase assays and structural analysis following protein purification. Compared to wild type, Variant X causes loss of NKA function, as indicated by a ∼90% reduced K^+-induced NKA current in two-electrode voltage clamp (TEVC, $n \geq 11$) and reduced maximal ATPase activity in purified protein. Despite similar protein levels in western blots from plasmalemma enriched preparations ($n = 2$), the ouabain-sensitive partial reactions (charge movement elicited by voltage pulses in the absence of external K^+ show a ∼90% reduction of the total charge moved ($n \geq 8$), indicating reduced numbers of functional pumps at the plasmalemma. To evaluate variant X-NKA localization, functional CFP- and YFP-tagged α1 were expressed in HEK293 cells for fluorescent microscopy. Variant X appears to localize in vesicles near the plasma membrane. Evaluation of plausible functional or trafficking dominant-negative effects is underway. NSF-MCB 2003251.

2229-Pos

Nanodisc diameter affects the ATP hydrolysis rate of ABC transporter

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ATP-binding cassette (ABC) transporters are ubiquitous, clinically important, and critical for most aspects of cell physiology. These proteins use ATP as their energy source to move substrates across biological membranes. Purified ABC transporters present a basal ATPase activity that increases in the presence