

האגודה הישראלית לפיזיולוגיה ופרמקולוגיה
ISRAEL SOCIETY FOR PHYSIOLOGY AND PHARMACOLOGY

THE 2019 ION CHANNELS MEETING

ABSTRACT BOOK

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February 13th, 2019, Mishkenot Sha'ananim, Jerusalem



MEETING PROGRAM

Throughout the meeting, each session will consist of three long (25 minutes) lectures presented by PIs and one short lecture (15 minutes) presented by research students and postdocs.

8:00-8:45 – Registration

8:45-9:00 – Introduction: Prof. Nathan Dascal, ISPP president

9:00-10:30 – *Session 1*

From fly to man: structure to function of channels

Chairs: Prof. Arie Moran (Ben-Gurion University), Prof. Baruch Minke (The Hebrew University)

Shai Berlin (Technion): NMDA-receptors dysfunction and epilepsy

Yoni Haitin (Tel-Aviv University): Molecular insights into KCNH1 channels inhibition by Ca²⁺-calmodulin – implications for cellular proliferation

Raz Zarivach (Ben-Gurion University): Cation Diffusion Facilitator: Structural studies using iron transporters of magnetotactic bacteria shed light on the activation and regulation of cation

Rita Gutorov (The Hebrew University): Cholesterol and drosophila TRP channels.

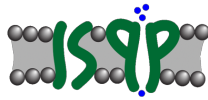
10:30-10:45 – Coffee break

10:45-12:15 – *Session 2*

Regulation of ligand-gated ion channels

Chair: Prof. Yael Stern-Bach (The Hebrew University)

Millet Treinin (The Hebrew University): Regulation of nACh and GABAA receptors by RIC-3



Rami Yaka (The Hebrew University): Targeting AMPA receptors for memory eraser

Yael Stern-Bach (The Hebrew University): Regulation of AMPA receptors by TARPs

Matan Geron (The Hebrew University): TRPV1 pore turret dictates distinct gating by different channel activators

12:15-13:00 – Lunch

13:00-14:30 – *Session 3*

Ca²⁺ channels

Chairs: Daphne Atlas (The Hebrew University) and Nathan Dascal (Tel-Aviv University)

Joel Hirsch (Tel-Aviv University): TBA

Raz Palti (Technion): Regulation of Ca²⁺ release-activated Ca²⁺ channels

Daphne Atlas (The Hebrew University): The β -subunit of the voltage-gated Ca²⁺ channel Ca_v1.2 drives signaling to the nucleus via H-Ras

Moshe Katz (Tel-Aviv University): A potential novel mechanism of regulation of cardiac L-type Ca²⁺ channel by PKA.

14:30-14:45 – Coffee break

14:45-16:15 – *Session 4*

Pumps and transporters

Chairs: Prof. Daniel Khananshvili (Tel-Aviv University), Dr. Moshe Giladi (Tel-Aviv University)

Etana Padan (The Hebrew University): The NhaA Na⁺/H⁺ antiporter, new insights into structural and functional dynamics

Steven Karlish (Weizmann Institute of Science): Specific lipid-protein interactions of Na/K-ATPase



Ehud Ohana (Ben-Gurion University): Pathologies of impaired metabolite transport

Bosmat Refaeli (Tel-Aviv University): Structure-dynamic and functional relationships in a Li⁺-transporting sodium-calcium exchanger mutant

16:15-16:30 – Coffee break

16:30-18:00 – Session 5

Arrhythmia and ion channels

Chairs: Prof. Bernard Attali (Tel-Aviv University), Prof. Yoram Etzion (Ben-Gurion University)

Shimrit Oz (Sheba Tel Hashomer): Ca²⁺-dependent mechanisms of cardiac sodium currents regulation

Lior Gepstein (Technion): Studying channelopathies with IPSCs

Bernard Attali (Tel-Aviv University): SK4 K⁺ channel blockers: a new treatment for cardiac arrhythmias

Max Drabkin (Ben-Gurion University): Nocturnal atrial fibrillation caused by mutation in KCND2, encoding pore-forming (α) subunit of the cardiac K_v4.2 potassium channel



SESSION 1

From fly to man: structure to function of channels

Chairs: Prof. Arie Moran (Ben-Gurion University), Prof. Baruch Minke (The Hebrew University)

TWO-PHOTON COMPATIBLE LIGHT-GATED NMDARS FOR STUDYING DISEASE

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The intricate connections between neurons in the brain (i.e., connectome) is the substrate that allows us to sense the outside world, coordinate activity, store and retrieve memories, as well as instigate disease. Understanding the connectome requires knowledge from the system's level, through cellular, all the way down to the molecular architecture of synapses. Addressing these questions requires methodologies that allow selective and reversible modulation of diverse classes of synaptic functions *in vivo*. **Synthetic optogenetics** is precisely suited for this task. This approach employs genetically-modified receptors to which synthetic light-sensitive molecules—or photoswitches—are appended. Photon-absorption by the molecules causes change in their geometry, which is then leveraged to modulate the activity of the protein they are immobilize onto. The change in geometry is completely reversible, therefore providing the user with the ability to toggle between the active and inactive states of the photoswitches, correspondingly triggering or stopping the protein's activity. This approach provides remote and reversible means to control select receptors of the synapse, as well as their signaling mechanisms, by light. Using this technology, we have previously created a family of light-gated NMDARs (LiGluNRs), explicitly light-blocked Glu1a and -2A; as well as light-activated GluN2A and -B. We now expanded this palette and introduce light-gated GluN2C and -D. To explore the role of receptors' activities in disease requires means to control these using better tissue-penetrating wavelengths, namely two-photon excitation (2PE). Whereas 2PE can activate various optogenetic tools, as well as photoswitches, so far LiGluNs could not be amended by 2PE. With the use of second generation red-shifted photoswitches, and new LiGluN variants, we succeeded in achieving this in hippocampal neurons. We now present new 2PE-compatible LiGluNs with which we intend to explore whether select spatiotemporal activation or block of GluN2A and -B can modulate disease phenotypes.



MOLECULAR INSIGHTS INTO KCNH1 CHANNELS INHIBITION BY Ca^{2+} -CALMODULIN: IMPLICATIONS FOR CELLULAR PROLIFERATION

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The KCNH voltage dependent potassium channels are key regulators of cellular excitability, involved in cardiac long QT syndrome, epilepsy, schizophrenia and cancer. The intracellular domains of KCNH channels are structurally distinct from other voltage-gated channels. The amino-terminal region contains an eag domain, which includes a Per-Arnt-Sim (PAS) module and a PAS-cap region, while the carboxy-terminal region encompasses a cyclic nucleotide-binding homology domain (CNBHD), connected to the pore domain through a C-linker domain. These specialized intracellular domains are the site of many disease-causing mutations and bestow most of the unique gating and regulation functionalities on KCNH channels. Moreover, KCNH1 channels are known to play an important role in cancer biology. Indeed, many cancer-associated mutations localize to the intracellular domains of these channels, highlighting their significance for both channel and cellular function. Here, we have used fluorescence approaches to determine that the eag domain and the CNBHD of KCNH1 form a complex in solution. Furthermore, co-crystals of the eag-CNBHD complex resolve a high-resolution structure of the intracellular complex of KCNH1. This structure demonstrates that the interface between the intracellular domains of KCNH1 serves a pathophysiological hotspot, harboring many LQTS and cancer-associated mutations. Finally, we have recently obtained novel insights into the involvement of these specialized regions in the mechanism of KCNH1 inhibition by Ca^{2+} -calmodulin.



CATION DIFFUSION FACILITATOR: STRUCTURAL STUDIES USING IRON TRANSPORTERS OF MAGNETOTACTIC BACTERIA SHED LIGHT ON THE ACTIVATION AND REGULATION OF CATION TRANSPORTERS

Raz Zarivach

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Divalent transition metal cations (DTMCs), such as Fe, Cu and Mn, participate in many biological processes. They are co-factors in thousands of enzymes, involved in hormone secretion and neuronal activities under physiological and pathological conditions and are involved in photosynthetic processes. Understanding what controls, the transport and its selectivity towards DTMCs is crucial for the study of metal homeostasis through the phylogenetic tree. In this work, we elucidated the structure-function relationships of MamM and MamB, which are involved in iron transport during magnetite biomineralization and constitute a prototypical member of the ubiquitous class of cation diffusion facilitators (CDFs). We used an interdisciplinary approach combining structural determination, biophysical characterizations, *in vivo* genetics and cellular imaging, to uncover the function(s) of MamM and MamB, and to elucidate the structural effects of mutations. This combined use of microbiological, biochemical, and microscopic techniques helped to determine the functional importance of key residues in CDFs, mapped onto the C-terminal domain. These residues are needed for MamM and MamB overall function *in vivo*. Furthermore, it allows us to provide the first mechanistic details for the CDF activation via its CTD. The results of our interdisciplinary and collaborative approach not only improved the understanding of magnetite biomineralization but also yielded broader insights into ion discrimination by CDF proteins and their regulation.

DIFFERENTIAL EFFECT OF CHOLESTEROL DEPLETION BY M β CD ON THE ACTIVATION OF *DROSOPHILA* TRP AND TRPL CHANNELS

Rita Gutorov, Ben Katz, Maximilian Peters, Tal Brandwine and Baruch Minke

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Light activation of *Drosophila* photoreceptors leads to the opening of two classes of Ca²⁺ permeable channels, the transient receptor potential (TRP) and the TRP-like (TRPL). These channels are activated by a G-protein-coupled PLC signaling cascade, while the gating mechanism of the channels following PLC activation is still under debate. The TRP and TRPL have similar structural features with high sequence homology and are thought to be activated by similar mechanism. However, TRP and TRPL differ in their ion selectivity, single channel conductance, their ability to translocate between the cell body and the signaling compartment and their ability to bind to the scaffold protein, INAD. Cholesterol is an essential component of the cell membrane, which modulates the activity of many ion channels. It has been well established that application of the cholesterol sequestering agent, methyl- β -cyclodextrin (M β CD) causes cholesterol depletion and modulates ion channel activity (i.e. Kir, BK, TRPV) by a direct or/and indirect effect. Therefore, we investigated whether the *Drosophila* TRP and TRPL channels activity is affected by modulating the plasma membrane levels of cholesterol. Using whole-cell voltage-clamp measurements from photoreceptor cells, we examined the effect of cholesterol depletion on the response to light. Incubating photoreceptors with extracellular solution containing M β CD (10 mM) reduced the amplitude and kinetics of the macroscopic light response in both TRP and TRPL channels. Further analysis of the TRP channels revealed that the change in the macroscopic response is a consequence of a reduction in the amplitude and frequency of the single-photon responses (quantum bumps). To localize the stage of M β CD operation (i.e. upstream or at the channels level), we analyzed the response of the photoreceptors to light-independent channel activation using application of PUFA (linoleic acid). Interestingly, in the presence of M β CD, PUFA-activation of the TRPL channels was abolished, while PUFA activation of the TRP channels remained intact. Our results suggest that cholesterol depletion has differential effect on the activation of *Drosophila* TRP and TRPL channels: direct inhibition of the TRPL channel but indirect effect on the TRP channel, suggesting different activation mechanism of TRP and TRPL channels.

SESSION 2

Regulation of ligand-gated ion channels

Chair: Prof. Yael Stern-Bach (The Hebrew University)

REGULATION OF NACH AND GABA_A RECEPTORS BY RIC-3

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Nicotinic acetylcholine receptors (nAChRs) are ligand gated ion channels having many functions, including muscle excitation and modulation of neuronal excitability. Coordinated locomotion in the nematode *C. elegans* depends on several nAChRs, including muscle expressed nAChRs needed for muscle excitation, and motor neuron expressed nAChRs affecting excitatory or inhibitory drives onto muscle. RIC-3, first identified and characterized in *C. elegans*, is an evolutionarily conserved ER-resident chaperone of nAChRs. Mechanisms regulating RIC-3 activity enable regulation of nAChR functional expression. One such mechanism is phosphorylation. Two RIC-3 phosphorylation sites have been investigated for their effects on the *C. elegans* motor system. Our results demonstrate complex regulation of locomotion via RIC-3 phosphorylation. Phosphorylation of one site leads to reduced functional expression of muscle expressed GABA_A receptors as part of what is likely to be a homeostatic mechanism within muscle. While, phosphorylation of the other site affects synaptic transmission in the motor system via effects on motor neuron excitability and on nAChR-dependent signaling coordinating activity of inhibitory and excitatory motor neurons.



TARGETING AMPA RECEPTOR FOR MEMORY ERASER

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Upregulation of the glutamate type AMPA receptors (AMPA receptors) is one of the important factors that plays role in the mechanisms of formation and maintenance of long-term memory. Until recently, constitutively active protein kinase M-Zeta (PKMZ) believed to be essential and sufficient factor that drives AMPARs to the synaptic membrane and prevents their endocytosis. Zeta Inhibitory Peptide (ZIP) is a short peptide that mimics the regulatory site of PKMZ and originally synthesized as PKMZ inhibitor. Recent findings show that the activity of ZIP preserved even in the absence of PKMZ. Surprisingly, scrambled ZIP (scrZIP), which contains the same amino acids as ZIP, but in a random sequence, has shown to act similar to ZIP. We hypothesized that mechanism of action of ZIP is independent of PKMZ and may involve direct effects on AMPARs that are independent of PKMZ.

Here we show that application of ZIP and scrZIP onto HEK cell culture expressing GluA1-containing AMPARs causes receptors' relocalization, while GluA2-containing receptors seems to be irresponsible to ZIP and scrZIP. Electrophysiological recordings from acute NAc slices reveal that application of ZIP leads to several effects: depression of AMPAR-mediated EPSCs, inhibition of paired-pulse ratio of AMPAR-mediated EPSCs and late decrease in resting membrane potential (30 to 40 minutes after ZIP-induced decrease in EPSCs). Interestingly, application of scrZIP produced similar synaptic effects, as ZIP. Further, custom-designed peptides, related to ZIP by their structure, but randomized in a different order straightened our hypothesis as demonstrated by electrophysiological recordings from NAc neurons. Finally, behavioral tests shown that custom ZIP-like peptide has the same effect on the cocaine-treated rats, as the parent ZIP compound. Taken together, we conclude that ZIP neuronal activity is partly related to its composition but not to its sequence.



REGULATION OF AMPA RECEPTORS BY TARPS

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AMPA receptors are ionotropic glutamate receptors that mediate the majority of fast excitatory transmission in the brain and critically contribute to synaptic plasticity and pathology. AMPA receptor trafficking and gating are tightly controlled by an array of auxiliary proteins, including members of the transmembrane AMPAR regulatory proteins (TARPs). The prototypical TARP, Stargazin/ γ 2, identified in a mutant mouse line, has been shown to act as a positive allosteric modulator of the AMPA receptors, enhancing both trafficking and gating properties. Understanding the regulatory and mechanistic roles TARPs play in the modulation of AMPA receptors is critical to the elucidation of synaptic function. Using systematic domain swaps and site-directed mutagenesis we identified the critical AMPA receptor-TARP binding interfaces, with the membrane domains of both proteins playing a primary role in receptor regulation. Our study allowed us to propose a two-step action mode in which, the mere binding of TARP to the AMPA receptor membrane domains destabilizes the channel closed state, followed by stabilization the open state via secondary interactions within the extracellular domains. In addition, we could hypothesis on the stoichiometry and spatial organization of the AMPA receptor-TARP complex.



TRPV1 PORE TURRET DICTATES DISTINCT DKTX- AND CAPSAICIN-GATING

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Many neurotoxins inflict pain by targeting receptors expressed on nociceptors, such as the polymodal cationic channel TRPV1. The tarantula double-knot toxin (DkTx) is a peptide with an atypical bivalent structure, providing it with the unique capability to lock TRPV1 in its open state and evoke an irreversible channel activation. Interestingly, DkTx evokes significantly smaller TRPV1 macroscopic currents than capsaicin, with a significantly lower unitary conductance. Accordingly, while capsaicin evokes aversive behaviors in TRPV1-transgenic *Caenorhabditis elegans*, DkTx fails to evoke such response at physiological concentrations. To determine the structural feature(s) responsible for this phenomenon, we engineered and evaluated the function of a series of mutated toxins and TRPV1 channels. We found that elongating the DkTx's linker, which connects its two knots, increases channel conductance when compared to currents elicited by the native toxin. Importantly, deletion of the TRPV1 pore turret, a stretch of amino acids protruding out of the channel's outer pore region, is sufficient to produce both full conductance and aversive behavior in response to DkTx. Interestingly, this deletion decreases the capsaicin-evoked channel activation. Together, our results demonstrate that the TRPV1 pore turret restricts DkTx-mediated pore opening, potentially through steric hindrance, limiting the currents size and mitigating the evoked downstream physiological response. Overall, our findings reveal that DkTx and capsaicin elicit distinct TRPV1 gating mechanisms and subsequent pain responses. Our results further point to the TRPV1 pore turret as an allosteric regulator of the channel gating mechanism.



SESSION 3

Ca²⁺ channels

Chairs: Daphne Atlas (The Hebrew University) and Nathan Dascal (Tel-Aviv University)

Joel Hirsch



MOLECULAR REGULATION OF CRAC CHANNEL ACTIVITY

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Store operated calcium entry (SOCE) represents a key mechanism by which cells generate Ca^{2+} signals and maintain Ca^{2+} homeostasis by replacing Ca^{2+} lost from endoplasmic reticulum (ER) with Ca^{2+} that enters the cytoplasm through plasma membrane channels. SOCE was characterized biophysically over a 20-year period and the field exploded recently with the identification of the genes that encode its essential proteins. The primary components are STIM1, the Ca^{2+} sensor of the ER, which is activated when the ER is depleted of Ca^{2+} and then activates the plasma membrane Ca^{2+} release activated Ca^{2+} (CRAC) channel, and Orai1, the CRAC channel pore forming subunit. Abnormal SOCE due to aberrant expression or function of STIM1 and Orai1 is implicated as a leading cause of several diseases including chronic inflammation, muscle weakness, and a severe combined immunodeficiency syndrome. Yet, although the process of Orai1 channel activation by STIM1 has been intensely investigated the molecular and structural basis of how STIM1 regulates the opening of the Orai1 channel pore remains poorly understood. Here, I will discuss recent work in our laboratory that seeks to understand how coupling with STIM1 leads to molecular rearrangements in the Orai1 channel protein underlying the opening of the channels pore.



β -SUBUNIT OF THE VOLTAGE-GATED Ca^{2+} CHANNEL CAV1.2 DRIVES SIGNALING TO THE NUCLEUS VIA H-RAS

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Depolarization-induced signaling to the nucleus by the L-type voltage-gated calcium channel Cav1.2 is widely assumed to proceed by elevating intracellular calcium. The apparent lack of quantitative correlation between Ca^{2+} influx and gene activation suggests an alternative activation pathway. Here, we demonstrate that membrane depolarization of HEK293 cells transfected with $\alpha_1.2/\beta_2b/\alpha_2\delta$ subunits (Cav1.2) triggers c-Fos and MeCP2 activation via the Ras/ERK/CREB pathway. Nuclear activity is not triggered in the absence of the intracellular β_2 subunit or by disrupting the interaction between $\alpha_1.2$ and β_2 subunits using the channel mutant $\alpha_1.2^{W440A}/\beta_2b/\alpha_2\delta$. In vitro binding of recombinant H-Ras and β_2 and “pull-down” assays in neuronal SH-SY5Y cells confirmed the importance of the intracellular β_2 subunit for depolarization-induced gene activation. Depolarization-induced c-Fos and MeCP2 activation does not depend on Ca^{2+} transport by the channel. The Ca^{2+} -inflow independency was demonstrated using a Ca^{2+} -impermeable mutant channel $\alpha_1.2^{L745P}/\beta_2b/\alpha_2\delta$ or disrupting Ca^{2+} /calmodulin binding to the channel using the channel mutant $\alpha_1.2^{I1624A}/\beta_2b/\alpha_2\delta$. As opposed to the current paradigm in which intracellular Ca^{2+} drives nuclear signaling, Cav1.2-triggered c-Fos or MeCP2 is dependent on extracellular Ca^{2+} and Ca^{2+} occupancy of the channel selectivity filter, but is Ca^{2+} -influx independent. An indispensable β -subunit interaction with H-Ras, which is triggered by conformational changes at $\alpha_1.2$ independently of Ca^{2+} flux, highlights a master regulatory role of β_2 in transcriptional activation via the ERK/CREB pathway. This mode of H-Ras activation could have broad implications for understanding the coupling of membrane depolarization to the rapid induction of gene transcription.



A POTENTIAL NOVEL MECHANISM OF REGULATION OF CARDIAC L-TYPE Ca^{+2} CHANNEL BY PKA

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L-type voltage dependent Ca^{+2} channels (CaV1.2) are crucial in physiological regulation of cardiac excitation-contraction coupling. Ca^{+2} entry via CaV1.2 is up-regulated by activation of G protein-coupled receptors and protein kinase A (PKA). CaV1.2 regulation by PKA was attributed to phosphorylation of specific residues in the channel. However, hitherto, none of the PKA phosphorylation sites in CaV1.2 could be conclusively linked to the physiological cardiac effect in genetically engineered animals. Here we suggest an alternative mechanism for regulation of cardiac L-type Ca^{+2} channel by PKA. We found that the N-terminal (NT) cytosolic segment, which does not have phosphorylation sites, is involved in PKA-dependent up-regulation of CaV1.2. We demonstrated that mutagenesis of the first 5 amino acids or TYP motif in the NT diminished PKA up-regulation in *Xenopus* oocytes. This implies on non-catalytic regulation. Next, we co-immunoprecipitated PKA catalytic subunit (PKA-CS) with truncated CaV1.2 at position 1821 ($\Delta 1821$) and found that they directly interact. Pull down assays revealed that PKA-CS interacts with segments of the C and N termini. Mapping the interaction of PKA-CS in α_{1c} C-terminus using peptide array revealed two main interaction sites: the proximal C-terminal regulatory domain (PCRD) and the distal C-terminal regulatory domain (DCRD). Point mutations in the PCRD, R1696-1697E and R1696-1697K led to decrease in binding in dot blot (RE and RK mutations, respectively). Two electrode voltage clamp Ba^{+2} current recordings in *Xenopus* oocytes expressing the RE and RK mutated α_{1c} showed analogous results, with small responses to cyclic AMP injection. These results indicate that α_{1c} PKA-dependent up-regulation depends on non-phosphorylated residues, and that PKA-CS directly interacts with both the N- and C-termini. We conclude that at least part of CaV1.2 modulation by PKA-CS is mediated by direct binding to the channel.

SESSION 4

Pumps and transporters

Chairs: Prof. Daniel Khananshvili (Tel-Aviv University), Dr. Moshe Giladi (Tel-Aviv University)

NEW STRUCTURAL AND FUNCTIONAL INSIGHTS INTO NHA^A NA⁺/H⁺ ANTIPORTER

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The NhaA crystal structure has provided key insight into the function and regulatory properties of antiporters, shed new light on the general architecture of transport proteins and opened the way to structure based interdisciplinary studies of the Na⁺/H⁺ antiporters that could not otherwise have been carried out. The NhaA structural fold is unique: two inverted repeats of helices with unwound chain in each repeat cross each other. This assembly creates a very delicately electrostatic balanced area in the middle of the membrane essential for activity. Remarkably, the number of secondary transporters with the NhaA fold is steadily increasing although they are not NhaA homologues. The kinetics and thermodynamics of transporters can be explained by the alternating access conceptual model (Jardetzky, 1966) in which the active site has alternating access to either side of the membrane. Therefore, to understand the NhaA functional mechanism in atomic details, we try to crystallize the NhaA's active conformations. In parallel, many site directed techniques have been employed to identify important residues and conformations. As these results left unknown the global movement of the protein we employed Hydrogen-Deuterium Exchange Mass Spectroscopy (HDX-MS). This technique has long been used for elucidating conformational changes in soluble proteins but has rarely been applied to membrane proteins because of their hydrophobicity and detergents need. Solving the technical problems, we applied HDX-MS to NhaA and revealed a global coordinated conformational change in the architecture of NhaA upon Li⁺ binding allowing to suggest a model for cation exchange mechanism of NhaA. Due to NhaA evolutionary conservation, we have succeeded in modeling the structure of NHE1 and NHA2, human homologues that have long been recognized as drug targets. Comprehensive evolutionary analysis of the present 6,537 representative of Na⁺/H⁺ antiporter sequences revealed a sequence motif that appears to determine the phenotypic characteristics of NhaA.



SPECIFIC LIPID-PROTEIN INTERACTIONS OF NA,K-ATPASE

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The classical framework for thinking about the relationship of membrane proteins and lipids was the Fluid Mosaic Model of Singer and Nicholson (Science.(1972) **175** : 720–731). An annulus (or ring) of lipid molecules is known to surround the membrane embedded segments of membrane proteins and the structure and function can be affected by the physical state of the lipid bilayer mediated by the annular lipids (described nowadays in terms of fluidity, hydrophobic matching or lateral pressure). These concepts were then added to by observations of cholesterol-rich lipid microdomains with associated proteins. More recently, much evidence has accumulated, primarily from molecular structures of membrane proteins, for specific phospholipid-protein and cholesterol-proteins interactions. It is now known that biological membranes are composed of tens of thousands of different lipid molecules. In view of the great complexity of the membrane there is an important general question in membrane biology as to which type of lipid-protein relationship predominates in different conditions and affects cellular physiology (and perhaps pathophysiology). Na,K-ATPase is a prototypical membrane protein. This talk will summarize the evidence for specific interactions of phospholipids and cholesterol with purified detergent-soluble recombinant Na,K-ATPase ($\alpha(1-3)\beta(1-3)$ with or without FX_{YD}(1-7). Lipid-Na,K-ATPase interactions either stabilize or stimulate or inhibit Na,K-ATPase activity and have been characterized by structural specificity of the lipids, kinetic mechanism, location in the protein and stoichiometry of binding, as well as isoform selective effects. The physiological rationale for specific lipid-Na,K-ATPase interactions as well as a possible role in neurological diseases will be discussed. Evidently, observations on specific lipid-protein interactions of the Na,K-ATPase may be relevant to other membrane proteins.



PATHOLOGIES OF IMPAIRED METABOLITE TRANSPORT

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It has long been known that kidney stone formation is associated with hypertension, but the molecular mechanism linking the two diseases remained obscure. Here, we have delineated a novel molecular mechanism that tightly modulates the homeostasis of the metabolites citrate and succinate, in which succinate uptake via the slc13/slc26 transport complex regulates blood pressure, possibly through regulation of the renin-angiotensin system. The same pathway also regulates urinary citrate and oxalate thus protecting against Ca^{2+} -oxalate stone formation. We further delineated the binding site and regulatory mechanism by which the Cl-/oxalate transporter, slc26a6, interacts with and inhibits succinate/citrate transport via the slc13 transporters. Our findings provide a link between hypertension and kidney stones through impaired transport of metabolites and suggest potential treatment approaches.

STRUCTURE-DYNAMIC HALLMARKS OF A LITHIUM TRANSPORTING SODIUM-CALCIUM EXCHANGER MUTANT (NCLX_MJ)

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Purpose – The cell-membrane (NCX) and mitochondrial (NCLX) $\text{Na}^+/\text{Ca}^{2+}$ exchanger proteins belong to the Ca/CA (Ca^{2+} /Cation Antiporter) superfamily, the members of which catalyze Ca^{2+} transport in exchange with different monovalent ions (Na^+ , K^+ , Li^+ , or H^+), thus playing an active role in handling and shaping of Ca^{2+} homeostasis and Ca^{2+} signaling from bacteria to humans. The ion selectivity features of Ca/CA proteins are fascinating since ion-coordinating residues are highly conserved among structurally related proteins sharing common protein folding motifs and topological organization of transmembrane helices. As an exception from this structure-functional union, nine (out of twelve) ion-coordinating residues of mitochondrial NCLX are different from NCX, while in contrast with NCX (exhibiting a high selectivity to Na^+) NCLX can transport either Na^+ or Li^+ in exchange with Ca^{2+} .

Results – Here, we analyze the NCLX_Mj mutant (with nine substituted residues) derived from the archaeal NCX_Mj, where the NCLX_Mj mutant imitate the ion selectivity of mammalian NCLX capable of transporting Na^+ and Li^+ with nearly identical affinity ($K_m=1.3\pm 0.2$ mM). Site-directed fluorescent labeling and ion-flux assays revealed the nearly symmetric accessibility of ions to the extracellular and cytosolic vestibules in NCLX_Mj ($K_{\text{int}}=0.8-1.4$), whereas the parent NCX_Mj preferentially adopts the outward-facing (extracellular) accessibility for ions ($K_{\text{int}}=0.1-0.2$). HDX-MS (hydrogen-deuterium exchange mass-spectrometry) identified symmetrically rigidified core helix segments in NCLX_Mj, whereas the matching structural elements are asymmetrically rigidified in the parent NCX_Mj. The HDX-MS analyses of ion-induced conformational changes and the mutational effects on ion fluxes revealed that the “ Ca^{2+} -site” (S_{Ca}) of NCLX_Mj binds Na^+ , Li^+ , or Ca^{2+} , whereas one or more additional Na^+/Li^+ sites of NCLX_Mj are incompatible with the Na^+ sites (S_{ext} and S_{int}) of NCX_Mj.

Conclusions – The structure-functional assignments of four ion-bindings sites are different in the NCX_Mj and NCLX_Mj proteins, thereby suggesting that mitochondrial NCLX does not bind/transport 3Na^+ or 3Li^+ ions in exchange with Ca^{2+} . These structure-functional differences could be related to differential functional contributions of NCX and NCLX to Ca^{2+} homeostasis and signaling in distinct sub-cellular compartments.

SESSION 5

Arrhythmia and ion channels

Chairs: Prof. Bernard Attali (Tel-Aviv University), Prof. Yoram Etzion (Ben-Gurion University)

CA²⁺-DEPENDENT MECHANISMS OF CARDIAC SODIUM CURRENTS REGULATION

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Sodium currents (I_{Na}) are a hallmark of excitability in cells, and impaired I_{Na} underlie life-threatening cardiac arrhythmias. During the cardiac action potential (AP), the low voltage gated-sodium channels $Nav1.5$ convey large I_{Na} transients of few milliseconds. A small fraction of late I_{Na} sustains while a succeeding inward calcium current, via high voltage-gated calcium channel ($Ca_v1.2$), generate the plateau phase of the AP for few hundreds of milliseconds. Nav_s and Ca_v_s share a similar structural blueprint, as Ca^{2+} and calmodulin (CaM) binding elements in the C-terminus (CT). However, while Ca^{2+}/CaM mediates major regulation of Ca_v inactivation, the details and the physiological relevance of Ca^{2+} -regulation of Nav are controversial. CaM interacts with $Nav1.5$ CT, and the linker between domains DIII-IV (DIII-IV linker), and mutations in CaM-interacting domains are hotspots for arrhythmia. However, the contribution of each CaM-interaction domain to the Ca^{2+} -sensing machinery of $Nav1.5$ is not fully established. We show that CaM interaction with the expressed full-length $Nav1.5$ is Ca^{2+} dependent, and that mutation in DIII-IV linker blunt Ca^{2+}/CaM interaction. These findings highlight the role of $Nav1.5$ DIII-IV linker in CaM binding complex and challenge the prevailing view that CaM regulation is exclusively mediated by $Nav1.5$ CT, where CaM interaction is Ca^{2+} -independent. In addition, mutation in CaM interacting domain in the CT of $Nav1.5$ control $Nav\beta1$ -dependent effects. Studies from Dr. I. Deschênes' lab have demonstrated that $Nav1.5$ interact and gate as dimers, and that mutations can impair I_{Na} gate in a dominant-negative fashion. We report that $Nav1.5$ -DIII-IV linker mutant, which is non-conducting and Ca^{2+}/CaM -deficient, could alter the Ca^{2+} -dependent gating properties of co-expressed conducting $Nav1.5$. In summary, the molecular basis of Ca^{2+} -dependent regulation of $Nav1.5$ may involve CaM, $Nav\beta1$, DIII-IV linker and the CT of multiple $Nav1.5$ channels.



STUDYING INHERITED CHANNELOPATHIES WITH iPSCs

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The ability to reprogram adult somatic cells into human induced pluripotent stem cells (hiPSCs) that could later be coaxed to differentiate into a variety of cell-lineages (including cardiomyocytes) opened new avenues for basic and translational cardiac research. Here we describe efforts from our laboratory in establishing and coaxing the cardiomyocyte differentiation of patient-specific hiPSCs lines derived from patients with a variety of inherited arrhythmogenic syndromes. The implications of this technology for cardiovascular drug discovery, disease modeling, and optimizing patient-specific therapies (precision medicine) will then be discussed. To exemplify the unique value of the hiPSCs approach for modeling inherited cardiac disorders, we will present work from our laboratory in establishing and studying hiPSCs-derived cardiomyocytes from patients with a number of inherited arrhythmogenic disorders. These include the congenital long QT syndrome (LQTS); short QT syndrome (SQTS) catecholaminergic polymorphic ventricular tachycardia (CPVT); and arrhythmogenic right ventricular cardiomyopathy (ARVC). These disease states may lead to the development of malignant ventricular arrhythmias and sudden cardiac death in otherwise healthy individuals. The ability of the hiPSCs approach to recapitulate the *in vivo* disease phenotype in the dish, to provide novel mechanistic insights into disease pathogenesis and to evaluate potential disease aggravators and novel customized treatment options will be described and discussed. Next, recent advances made in the lab in combing these patient-specific hiPSC models with CRISPR-based genome editing, developmental biology-based chamber-specific differentiation systems, unique 2-D and 3-D tissue-engineering approaches and optical mapping strategies to characterize conduction and complex arrhythmias in these models will be described. Finally, the potential applications of the iPSCs derived cardiomyocyte technology for drug toxicity screening, for optimizing patient-specific therapy, and as a novel methodology for drug discovery will be discussed.

SK4 K⁺ CHANNEL BLOCKERS: A NEW TREATMENT FOR CARDIAC ARRHYTHMIAS

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Catecholaminergic Polymorphic Ventricular Tachychardia (CPVT) is a stressed-provoked ventricular arrhythmia, which also manifests sinoatrial node (SAN) dysfunction. We recently showed that SK4 calcium-activated potassium channels are important for automaticity of cardiomyocytes derived from human embryonic stem cells. SK4 channels were identified in human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) from healthy and CPVT2 patients bearing a mutation in calsequestrin 2 (CASQ2-D307H) and in SAN cells from WT and CASQ2-D307H knock-in (KI) mice. TRAM-34, a selective blocker of SK4 channels, prominently reduced delayed-afterdepolarizations and arrhythmic Ca²⁺ transients observed following application of the β -adrenergic agonist isoproterenol in CPVT2-derived hiPS-CMs and in SAN cells from KI mice. Strikingly, *in vivo* ECG recording showed that intraperitoneal injection of the SK4 channel blockers, TRAM-34 or clotrimazole, greatly reduced the arrhythmic features of CASQ2-D307H KI and CASQ2 knockout mice at rest and following exercise. None of the existing SK4 blockers acting on the channel pore is not suitable for clinical development because of poor oral availability and selectivity. We succeeded to design and target new SK4 channel blockers to a different channel region, the intracellular calmodulin binding domain (CaMBD). Though starting from the opener template 1-EBIO, we discovered new SK4 channel blockers and built a molecular docking model of the drugs onto the human SK4 channel cryo-EM structure. We hope to obtain a new class of SK4 channel blockers, able to prevent cardiac arrhythmias in *in vitro* and *in vivo* models.

NOCTURNAL ATRIAL FIBRILLATION CAUSED BY MUTATION IN KCND2, ENCODING PORE-FORMING (α) SUBUNIT OF THE CARDIAC KV4.2 POTASSIUM CHANNEL

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Paroxysmal atrial fibrillation (pAF) can be caused by gain-of-function mutations in genes encoding the cardiac potassium channel subunits *KCNJ2*, *KCNE1* and *KCNH2*, mediating the repolarizing potassium currents I_{K1} , I_{Ks} and I_{Kr} respectively. Through genetic studies, we now show that autosomal dominant early-onset nocturnal pAF is caused by p.S447R mutation in *KCND2*, encoding the pore-forming (α) subunit of the Kv4.2 cardiac potassium channel. Kv4.2, along with Kv4.3, contributes to the cardiac fast transient outward K⁺ current, I_{to} . I_{to} underlies the early phase of repolarization in the cardiac action potential, thereby setting the initial potential of the plateau phase and governing its duration and amplitude. In *Xenopus* oocytes, the mutation increases the channel's inactivation time constant and affects its regulation: p.S447 resides in a PKC phosphorylation site, which normally allows attenuation of Kv4.2 membrane expression. The mutant Kv4.2 exhibits impaired response to PKC; hence, Kv4.2's membrane expression is augmented, enhancing potassium currents. Co-expression of mutant and wild-type channels (recapitulating heterozygosity in affected individuals) showed results similar to the mutant channel alone. Finally, in a hybrid channel composed of Kv4.3 and Kv4.2, simulating the mature endogenous hetero-tetrameric channel underlying I_{to} , the p.S447R Kv4.2 mutation exerts a gain-of-function effect on Kv4.3. Thus, the mutation alters the kinetic properties of the Kv4.2 channel, impairs its inhibitory regulation and exerts gain-of-function effect on both Kv4.2 homo-tetramers as well as Kv4.2-Kv4.3 hetero-tetramers. These effects presumably increase the repolarizing potassium current I_{to} , thereby abbreviating action potential duration, creating arrhythmogenic substrate for the nocturnal atrial fibrillation evident in the affected kindred. Interestingly, Kv4.2 expression was previously shown to demonstrate circadian variation, peaking daytime in murine hearts (human nighttime), with possible relevance the nocturnal onset of pAF symptoms in our patients. The atrial-specific phenotype suggests that targeting Kv4.2 might be effective in treatment of nocturnal pAF, avoiding adverse ventricular effects.

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#33

MONOTERPENES REGULATES THE ACTIVITY OF K_{2P} CHANNELS BY TWO DISTINCT MECHANISMS

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Potassium K_{2P} ('leak') channels conduct current across the entire physiological voltage range and carry leak or 'background' currents that are, in part, time- and voltage-independent. The activity of K_{2P} channels affects numerous physiological processes such as cardiac function, pain perception, depression, neuroprotection and cancer development. We establish that the mechano-gated channels $K_{2P2.1}$, $K_{2P4.1}$ and $K_{2P10.1}$ (TREK-1, TRAAK and TREK-2) are opened by monoterpenes (up to 6-fold increase in current) within few minutes of exposure. Cyclic, aromatic monoterpenes containing a phenol moiety, such as carvacrol and thymol, had the most profound effect on the channels. By performing sequential truncation of the carboxyl-terminal domain of $K_{2P2.1}$, we identified two distinct regulatory domains within this part of the protein. One domain, as previously reported, was needed for regulation by arachidonic acid, anionic phospholipids and temperature changes. Within a second domain, a triple arginine residue motif (R344-346), possibly representing a PIP_2 -binding site, was found to be essential for regulation by holding potential changes and important for regulation by monoterpenes. On the other hand, the activity of members of the, acid sensitive, TASK channel family ($K_{2P3.1}$ and $K_{2P9.1}$) was rapidly decreasing by terpenes. Furthermore, only the voltage-dependent portion of the current was eliminated. The current inhibition was reduced with elevation of external K^+ concentration, indicating the participation of the selectivity-filter gate. Our findings imply that the two channel types are regulated by terpenes by two distinct mechanisms. Penetration of monoterpenes into the outer leaflet of the membrane perturb the membrane structure and results in immediate changes at the selectivity filter of members of the TASK channel family. On the other hand, following binding of a defined domain at the carboxyl-terminal of mechano-gated K_{2P} channels to the membrane, the addition of monoterpenes promotes an extensive channel opening.

#34

RECRUITMENT AND RE-RECRUITMENT EVENTS SHAPE THE NEUROTOXIC ARSENAL OF THE SCORPION BUTHACUS LEPTOCHELYS LEPTOCHELYS

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Scorpion venoms are a rich source of bioactive peptides. Most of these peptides are small (23-78 amino acids-long), well packed by several disulfide bridges and affect ion channel function in excitable and non-excitable cells. The venom of each scorpion is a complex mixture of dozens or even hundreds of mainly neurotoxic peptides, interacting specifically with ion channels. The majority of the neurotoxic peptides of scorpion from the *Buthidae* family share a common cystine-stabilized α -helix-loop- β -sheet (CS- α/β) fold motif. It is believed that all toxins sharing this fold originated from defensins, anti-microbial peptides that are found in most of the multicellular organisms. Our goal was to learn about the evolutionary pathway that led to the formation of the scorpion's arsenal. We established a comprehensive dataset of all putative neurotoxins in the venom of the Israeli scorpion *Buthacus leptochelys leptochelys* using next generation sequencing (RNA-seq). Two cDNA libraries were constructed for analysis, one comprising RNA extracted from the venom glands and one comprising RNA extracted from the abdomen segments of the same scorpions. This way, we were able to distinguish between putative toxins, that are venom selective, and other, non-toxic, homologous peptides. Seventy six out of 134 identified putative peptides having the CS- α/β fold motif are expected to be toxic. By performing a network analysis, we were able to show that while indeed all toxins appears to stem from defensins ancestors ('recruitment'), many of the non-toxic peptides are much more related to toxic peptides than to defensins. This led us to assume that some toxin genes also appear capable of 'reverse recruitment', whereby a venom-expressed gene ultimately becomes expressed back in the abdomen. Thus, we hypothesize that toxins evolved by a dynamic process of neofunctionalization and 'recruitment' to the venom, 'reverse recruitment' and probably also re-recruitment.

#35

**SynDIG4/PrrT1 IS REQUIRED FOR EXCITATORY SYNAPSE DEVELOPMENT AND PLASTICITY
UNDERLYING COGNITIVE FUNCTION**

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AMPA receptors (AMPArs) are the primary mediators of fast transmission at glutamatergic synapses and tightly contribute to synaptic plasticity. A complex network of interacting proteins controls AMPAR assembly, trafficking, and function. Of these, the transmembrane AMPAR-associated protein SynDIG1 (Synapse Differentiation Induced Gene 1) that regulates excitatory synapse strength and number as showed in previous work. In this study, we reveal the interaction of the AMPARs with the SynDIG protein family and the mechanisms of SynDIG4 (also known as Prrt1) that modifies the AMPAR gating properties in a subunit dependent manner. Juvenile SynDIG4 knockout (KO) mice have weaker excitatory synapses as evaluated by immunocytochemistry and electrophysiology. Remarkably, adult SynDIG4 KO mice show complete loss of tetanus-induced long-term potentiation (LTP) without a striking reduction in synaptic transmission. Moreover, SynDIG4 KO mice exhibit deficits in two independent cognitive assays. SynDIG4 co-localizes with the AMPAR subunit GluA1 at non-synaptic sites in neurons and brain sections; thus, we propose that SynDIG4 maintains a pool of extrasynaptic AMPARs critical for synaptic potentiation that manifests as altered circuit function in the mature brain.