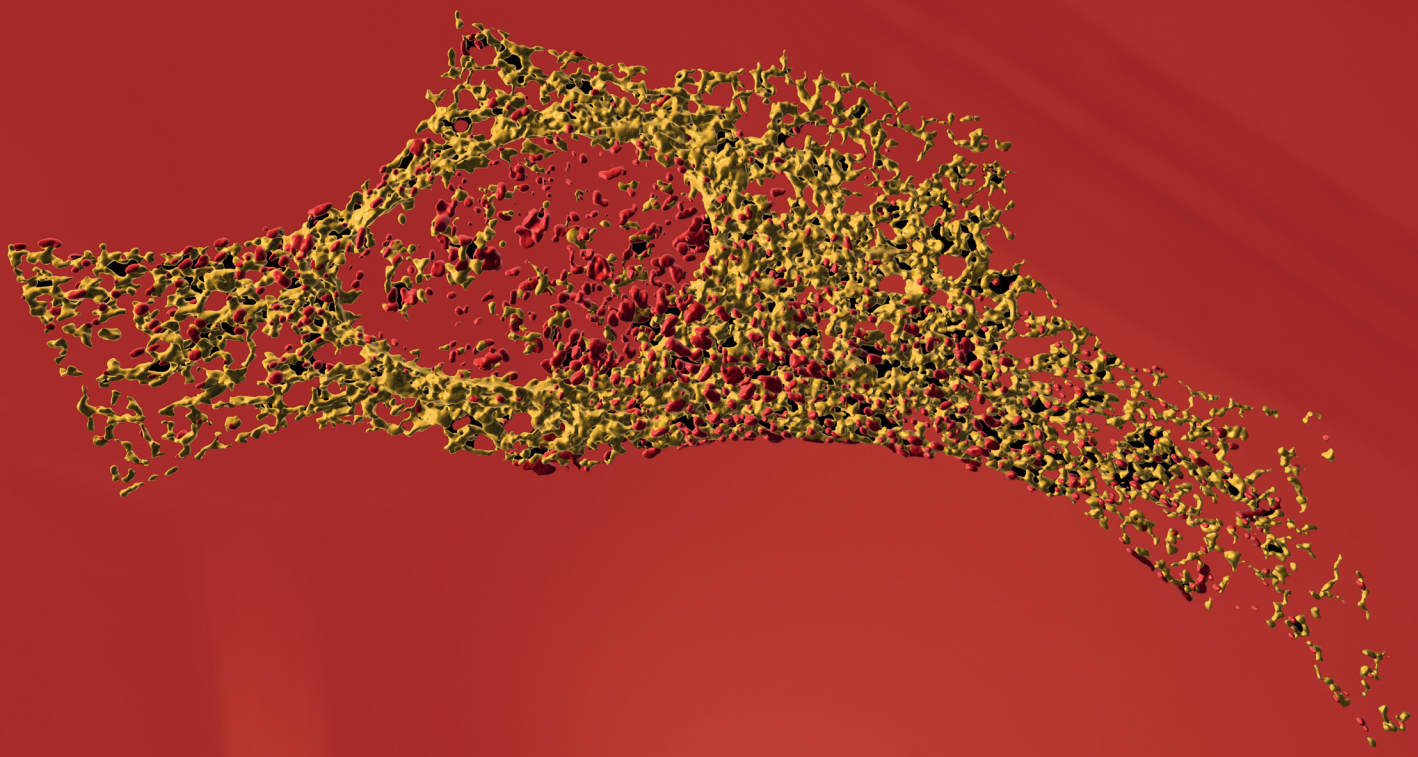
Weill Cornell Medicine-Qatar

european calcium society

18th International Meeting of the European Calcium Society

FEBRUARY 1-4, 2026



SUNDAY FEBRUARY 1, 2026

8:30 – 10:00 | Registration

Time	Title/Topic	Speaker
Opening Session		
10:00 - 10:15	Welcome and Overview	Dr. Khaled Machaca
10:15 - 11:15	Berridge Keynote Lecture	Dr. Rosario Rizzuto Chair: Dr. Sandip Patel
11:15 - 11:45	**Coffee Break**	
Session 1: Ca^{2+} Signaling in Rare Genetic Diseases & Aging		
Chair: Dr. Geert Bultynck		
11:45 - 12:15	Linking ER Calcium Dysregulation to Mitochondrial Dysfunction in Wolfram Syndrome	Dr. Allen Kaasik
12:15 - 12:45	Linking IP3 Receptor 3 to Peripheral Nerve Dysfunction and Other Disease Conditions	Dr. Henna Tyyinisma
12:45 - 13:00	Mutation-Induced Rewiring of the Conformational Dynamics of the N-Terminal of Human Inositol 1,4,5–Trisphosphate Receptor Type	Yu Zhu
13:00 - 13:15	SPCA1-Dependent Golgi Regulation Controls Coronavirus Spike Processing and Fusion Activity	Dr. Melanie Robitaille
13:15 - 14:30	**Lunch**	
Session 2: Calcium Signaling in Metabolism		
Chair: Dr. Khaled Machaca		
14:30 - 15:00	SERCA2 Regulates Plasma Cell Development and Antibody Mediated Immunity	Dr. Stefan Feske
15:00 - 15:30	Tracking and Function of alternative STIM variants	Dr. Barabara Niemeyer
15:30 - 15:45	Metabolic consequences of the reduction of Store-Operated Ca^{2+} Entry	Dr. Dana Al Ansari
15:45 - 16:00	Glucose-Dependent Modulation of Cholinergic Ca^{2+} Signaling in Pancreatic β-Cells	Dr. Leonardo Gallegos
16:00 - 16:30	**Coffee Break**	

Time	Title/Topic	Speaker
Session 3: Calcium Signaling in Neurovascular Mechanosensitivity, Inflammation & Disease		
Chair: Dr. Mohamed Trebak		
16:30 - 17:00	Amyloid Beta Contributes to Cerebral Hypoperfusion in Alzheimer's Disease via Novel Vascular Calcium Signals	Dr. Harry Pritchard
17:00 - 17:30	Piezo1 Integrates Hemodynamic Forces to Tune Neurovascular Coupling	Dr. Osama Harraz
17:30 - 17:45	Atrial shear-mechanotransduction and local Ca^{2+} adaptation	Dr. Sun-Hee Woo
17:45 - 18:00	Calmodulin G133V: Disrupted Calcium Signaling and Potential Drug Interactions in Arrhythmia	Dr. Helene H Jensen & Dr. Malene Brohus
19:00 - 21:00	**Dinner and Posters**	

MONDAY FEBRUARY 2, 2026

Time	Title/Topic	Speaker
Session 4: Inositol 1,4,5 Trisphosphate Receptors		
Chair: Dr. David Yule		
9:00 - 9:30	Synergism of IP3R and Parkin Mutants Identifies Mitochondrial Stress as an Early Feature of Parkinson's Disease	Dr. Gaiti Hasan
9:30 - 10:00	Decoding Type 2 IP3R: Cryo-EM Insights into Isoform-Specific Properties	Dr. Irina I.Serysheva
10:00 - 10:15	Characterization of Ca^{2+} oscillations in glioma cells using computational modelling	Mehrosh Ahmed
10:15 - 10:30	IP_3Rs critically contribute to ferroptosis-induced cell death	Dr. Ophélie Champion
10:30 - 11:00	**Coffee Break**	

Session 5: Calcium Signaling in Cell Differentiation

Chair: Dr. Khaled Machaca

11:00 - 11:30	Membrane Contact Sites and Calcium Signaling	Dr. Paola Pizzo
11:30 - 12:00	Calcium at the Intersection of Metabolism and Embryo Development	Dr. Carmen J. Williams
12:00 - 12:15	Calcium oscillations and early embryogenesis -- novel paradigms for phospholipase C zeta	Dr. Junaid Kashir
12:15 - 12:30	Molecular partnerships of CRISPs in calcium expulsion pathways	Dr. Valdehi Miya
12:30 - 14:00	**Lunch**	

Session 6: Signaling by Mitochondrial Ca^{2+} Binding Proteins

Chairs: Dr. Gary Shaw & Dr. Peter Stathopulos

14:00 - 14:30	Excessive Ca^{2+} -dependent ER-mitochondrial Contact Stabilization Drives Metabolic Liver Injury	Dr. Dipayan Chaudhuri
14:30 - 15:00	The Miro-1 GTPase and Calcium Sensor in the Regulation of Mitochondrial Homeostasis	Dr. Josef Kittler
15:00 - 15:15	Novel Insights into Calcium-Dependent Mitochondrial Regulation of Neuroinflammation in Early Parkinson's Disease Models	Dr. Dharmendra Kumar Khatri
15:15 - 15:30	The architecture of the cortical endoplasmic reticulum supports Ca^{2+} tunneling	Dr. Raphael Courjaret
15:30 - 16:00	**Coffee Break**	
16:00 - 17:00	Claude Klee Keynote Lecture | Long-Distance Calcium Signaling in Neurons	Dr. Jennifer Lippincott -Schwartz **Chair: Dr. Martha Cyert**
17:15 – 18:15	ECS General Assembly	
19:00 - 21:00	Dinner and Posters	

TUESDAY FEBRUARY 3, 2026

Time	Title/Topic	Speaker

Session 7: Ryanodine Receptors and Other ER Channels

Chair: Dr. Jan B. Parys

09:00 - 09:30	Structural and functional insights into disease mechanisms and pharmacology of Ryanodine Receptors	Dr. Filip Van Petegem
9:30 - 10:00	Resting Ca^{2+} Fluxes through ER and Plasma Membrane: Implications for Cell Health and Excitability	Dr. Robert Blum
10:00 - 10:15	Novel ryanodine receptor isoform (RyR1alt) Expressed in the Liver and its role in Ca^{2+} Signaling	Dr. Juliana Corrêa-Velloso
10:15 - 10:30	S-Acylation by zDHHC20 targets STIM1 to cholesterol-rich ER-PM contact sites to sustain Ca^{2+} signalling at the immune synapse	Raphaël Nere
10:30 - 11:00	**Coffee Break**	

Session 8: Probing the Frontiers in Lysosomal Ca^{2+} Signalling

Chair: Dr. Christian Grimm

11:00 - 11:30	Biased Signalling through Lysosomal Two-Pore Channels	Dr. Sandip Patel
11:30 - 12:00	Regulation and Function of Lysosomal Two-Pore Channels	Dr. Michael H. Zhu
12:00 - 12:15	Investigating the Interplay between Lysosomal and ER Ca^{2+} channels	Dr. Siddhi Shetty
12:15 - 12:30	NAADP-dependent Ca^{2+} Signaling in Natural killer Cell Activitym	Dr. Björn-Philipp Diercks

Session 9: Flash Talks

Moderator: Dr. Malene Brohus

12:30 - 13:00	Flash Talks | 12 talks 2 min each	
13:00 - 14:30	**Lunch**	
14:30 -	**Free Afternoon**	
20:00 - 23:00	Gala Dinner | Al-Dana Garden, Sharq Village & Spa Hotel	

WEDNESDAY FEBRUARY 4, 2026

Time	Title/Topic	Speaker
Opening Session		
Chair: Dr. Gaiti Hassan		
09:00 - 10:00	James Putney Keynote | IP3R: defining Ca^{2+} signals in time and space	Dr. David Yule
10:00 - 10:30	**Coffee Break**	
Session 10: NAADP Signaling		
Chair: Dr. Andreas H. Guse		
10:30 - 11:00	NAADP in the Early Phase of T Cell Activation	Dr. Feng Gu
11:00 - 11:30	Two-Pore Channels as Regulators of Inter-Organellar Calcium Homeostasis and Immune Cell Signalling	Dr. Susanna Zierler
11:30 - 12:00	Organellar Ca^{2+} Regulators (OCaRs) Encoded by TMEM63 Proteins	Dr. Marc Freichel
12:00 - 13:30	**Lunch**	
Session 11: Late Breaking		
Chair: Dr. Barabara Niemeyer		
13:30 - 14:00	Calcium Signaling at the Interface between Microglia, Neuroinflammation, and Inflammation-induced Depression Behaviors	Dr. Murali Prakriya
14:00 - 14:30	How Does the Energetically Expensive Mammalian Brain Adapt in Times of Food Scarcity	Dr. Zahid Padamsey
14:30 - 14:45	Two-pore channel-2 controls calmodulin-dependent STIM1 inactivation	Dr. Nicolas Demaurex
14:45 – 15:00	Orai2-dependent alteration of Ca^{2+} profile and suppressive capacity of human regulatory CD4T cells	Dr. Dalia Alansary
15:00 - 15:15	Ryanodine receptors dynamically control lysosomal trafficking in neurons	Dr. Geert Bultynck
15:15 – 15:30	**Coffee Break**	
Session 12: jECS Talks and Award Presentation		
15:30 - 16:00	jECS talks winners	
16:00 – 17:00	Awards Presentation and Closing	

Index

10:15 - 11:15 | Berridge Keynote Lecture | Dr. Rosario Rizzuto

Chaired by: Dr. Sandip Patel

Dr. Rosario Rizzuto
Dept. Biomedical Sciences and National Center for Gene Therapy and RNA Therapeutics, University of Padua, Italy
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Biography:
Rosario Rizzuto is Professor of General Pathology and Chairman of the Department of Biomedical Sciences at the University of Padua. His research activity has been focused on the study of intracellular calcium homeostasis in physiological and pathological conditions. He first proposed the use of recombinant luminescent (aequorin, luciferase) or fluorescent (GFP) proteins as organelle-targeted probes (Nature, 1992). With these probes, he demonstrated the occurrence of calcium microdomains at contact sites between ER and mitochondria (Science 1993, 1998). He recently identified the mitochondrial channels allowing Ca2+ and K+ fluxes, i.e. the Mitochondrial Calcium Uniporter, MCU, (Nature 2011) and the ATP-sensitive mitochondrial K+ channel, mitoKATP (Nature 2019). Overall, Prof. Rizzuto authored more than 340 publications in international journals listed by Pubmed (59,000 citations; h-index 112, from Scopus).

The Mitochondrial Calcium Uniporter in the control of inflammation

Gaia Gherardi, Agnese De Mario, Denis Vecellio Reane, Francesca Spinelli, Pampa Pain, Samuele Amadori, Antonia Esposito, Anna Raffaello, Rosario Rizzuto

Dept. Biomedical Sciences and National Center for Gene Therapy and RNA Therapeutics, University of Padua, Italy

Abstract:
The mitochondrial Ca^{2+} channel exhibits high molecular diversity in different cell types and pathophysiological conditions: two pore subunits (MCU, MCUb), three regulatory elements (MICU1, MICU2, MICU3) and a scaffolding factor (EMRE), that underlies different functional properties. In muscle regeneration, MCUb is overexpressed after tissue damage and promotes regeneration by controlling the acquisition of anti-inflammatory, pro-regenerative phenotype of macrophages. MCUb-deficient mice maintain a pro-inflammatory phenotype, exhaustion of the satellite cell pool and delayed regeneration (1). We thus investigated the role of the MCU complex in the different phases of inflammation. By utilizing MCU-specific drugs and transgenic models, we demonstrate the effect of MCU-mediated Ca^{2+} uptake on mitochondrial morphological remodelling, mtDNA release, inflammasome activation and IL-1 secretion. Overall, the data highlight MCU as a promising target for RNA therapies of inflammatory conditions. The Italian National Center on Gene Therapy and RNA-based therapeutics aims to develop new nucleic-acid encoded drugs based on the elucidation of the molecular understanding of disease pathogenesis. In this context, we are developing MCU siRNAs as novel anti-inflammatory drugs, testing a variety of cellular models, organoids and animal models of pathophysiological interest (inflammatory bowel and lung diseases, arthritis, systemic inflammation).

1. Feno et al, Sci. Signal. (2021) https://doi.org/10.1126/scisignal.abf3838

Session 1: Ca^{2+} Signaling in Rare Genetic Diseases & Aging

Chaired by: Dr. Geert Bultynck

11:45 - 12:15 | Linking ER Calcium Dysregulation to Mitochondrial Dysfunction in Wolfram Syndrome | Dr. Allen Kaasik

Allen Kaasik
Department of Pharmacology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
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Biography:
Allen Kaasik performed his PhD studies in 1998 at the University of Tartu and the University of Helsinki, focusing on the regulation of cardiac function by thyroid hormones. He subsequently carried out postdoctoral research at INSERM in Châtenay-Malabry, France, in the field of muscle bioenergetics. He was appointed full professor in 2008 and, from 2019, has served as Head of the Department of Pharmacology. From 2025, he will also lead Estonian Bioimaging, a national network of high-end imaging facilities. His research focuses on organellar biology in neurodegenerative diseases, with recent discoveries relating to mitochondrial function and dynamics and the role of calcium in Wolfram syndrome.

Abstract:
Wolfram syndrome is a rare inherited disorder caused by pathogenic variants in WFS1 or CISD2, characterized by early neurodegeneration. Although defective endoplasmic reticulum (ER) Ca^{2+} homeostasis is a recognized hallmark of the disease, the downstream mechanisms linking altered Ca^{2+} handling to neuronal dysfunction remain poorly understood. In our study, we investigated primary neurons, the cell type most vulnerable in Wolfram syndrome, to define how disruptions in ER Ca^{2+} signaling impact mitochondrial function and neuronal viability. Neurons lacking WFS1 or CISD2 exhibited reduced ER Ca^{2+} stores and compromised ER–mitochondria contact sites, resulting in attenuated IP_3 receptor–dependent Ca^{2+} transfer to mitochondria and diminished mitochondrial Ca^{2+} uptake. This impairment led to suppressed oxidative phosphorylation, elevated $NADH/NAD^+$ ratios, and the development of reductive stress. Together, these metabolic alterations undermined neuronal health. Importantly, we identify pharmacological strategies that correct Ca^{2+} imbalance, improve mitochondrial performance, and rescue neuronal function, highlighting Ca^{2+} signaling at ER–mitochondria interfaces as a therapeutic target in Wolfram syndrome.

12:15 - 12:45 | Linking IP3 Receptor 3 to Peripheral Nerve Dysfunction and Other Disease Conditions | Dr. Henna Tyynismaa

Dr. Henna Tyynismaa
Faculty of Medicine, University of Helsinki
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Biography:
Henna Tyynismaa is a Professor of Medical Molecular Genetics at the Faculty of Medicine, University of Helsinki. With MSc in human genetics, she earned her PhD in mitochondrial medicine in 2007 at the University of Helsinki, completed a postdoc at University College London, and founded her research group in Helsinki in 2011. She has served as Director of the Faculty's Research Programs Unit (2015–2024) and as Vice Dean for Research since 2024. Her research focuses on the genetics and molecular mechanisms of neuromuscular diseases, especially those affecting peripheral nerves, and on the regulation of mitochondrial metabolism in disease, using human iPSC-derived models.

Abstract:
Charcot-Marie-Tooth (CMT) disease is a group of inherited peripheral neuropathies that cause progressive weakness and sensory loss, typically starting in the feet and legs. Common features include high arches (pes cavus), hammertoes, distal muscle atrophy, foot drop, areflexia, and reduced vibration/position sense, with variable age of onset from childhood to late in life. CMT is genetically heterogeneous, as it can be caused by monogenic disease variants in more than 100 different genes. In 2020, our group identified variants in ITPR3 gene (encoding IP3 receptor 3, IP3R3) as a new cause of dominant demyelinating CMT, subsequently named CMT1J. Other groups have since described more patients with ITPR3 variants, with a surprising range of phenotypes, varying from severe early-onset to mild late-onset neuropathy, or immunodeficiency, and/or tooth deformities. We also identified the first spontaneous ITPR3 disease in pet dogs, Lancashire Heelers, caused by a recessive nonsense variant. The affected dogs present with a severe developmental enamel defect and reduced nerve conduction velocity. To model the human cell type specific effects of IP3 receptors, we have used CRISPR/Cas9 genome editing to generate single, double and triple knockout lines of IP3 receptor genes ITPR1, ITPR2, and ITPR3, and CMT1J disease variant lines in human induced pluripotent stem cells (iPSC). These iPSC lines will be used to identify the primary cell target of the disease variants.

Oral Presentations-Sunday, February 1, 2026

12:45 – 13:00 | Poster 57: Mutation-Induced Rewiring of the Conformational Dynamics of the N-Terminal of Human Inositol 14,5–Trisphosphate Receptor Type I | Yu Zhu

Yu Zhu, Taufiq Rahman
Department of Pharmacology, University of Cambridge

Abstract:
Inositol 14,5–trisphosphate receptors (IP_3Rs) represent a major family of intracellular Ca^{2+} channels, residing mainly within the membrane of the endoplasmic reticulum (ER). These ion channels play critical role in the initiation as well as subsequent propagation of intracellular Ca^{2+} signals when cell surface receptors are stimulated by various agonists that activate phospholipase C enzymes. A functional IP_3R is a tetramer of large subunits, each of which has modular architecture comprising of the N terminus, the intermediary regulatory domain and the distal C terminus harbouring the channel pore. Their N-terminal region plays a pivotal role in ligand recognition, inter-domain communication, and allosteric regulation of channel gating. A growing number of disease-associated mutations, together with variants predicted to be functionally disruptive, have been identified within this region. However, the molecular mechanisms by which these sequence perturbations alter protein dynamics and lead to dysfunctional Ca^{2+} signalling remain incompletely understood. To address this challenge, an integrative ensemble-based computational strategy was employed to characterise the conformational landscapes of the wild-type IP_3R1 N-terminus and a curated set of pathogenic and predicted mutations. Amongst the three mammalian IP_3R isoforms, IP_3R1 was chosen, given this being the most heavily mutated isoform so far with clear pathophysiological relevance. Conformational ensembles were first generated using the generative modelling framework BioEmu, enabling efficient sampling of equilibrium-like structural heterogeneity beyond static structural representations. Joint projection of all ensembles into a common low-dimensional embedding space revealed mutation-specific redistribution of conformational populations, as well as altered dynamical pathways connecting functionally relevant states when compared with the wild type. Representative conformations from distinct regions of the embedding space were subsequently subjected to extensive atomistic molecular dynamics simulations. By combining equilibrium and non-equilibrium sampling approaches, Markov state models were constructed to quantify both the thermodynamic stability and kinetic connectivity of key conformational states. This multi-scale analysis reveals that mutations exert their effects primarily by reshaping the underlying conformational energy landscape and modulating collective motions within the N-terminal domains, rather than by inducing isolated local structural changes. These dynamical perturbations provide a mechanistic basis for altered allosteric communication and impaired IP_3-dependent regulation. Overall, this work highlights the importance of an ensemble-centric perspective for understanding mutation-driven dysfunction in IP_3R1 and offers a dynamic framework for linking sequence variation in the N-terminal region to altered regulatory behaviour and aberrant Ca^{2+} signalling.

13:00 – 13:15 | Poster 30: SPCA1-Dependent Golgi Regulation Controls Coronavirus Spike Processing and Fusion Activity | Dr. Mélanie Robitaille

Mélanie Robitaille, Farzaneh Fourouz[1], Chintan Bhavsar[1], Sarah Roberts-Thomson[1,2], Larisa Labzin[3] and Gregory Monteith[1].
[1]School of Pharmacy and Pharmaceutical Sciences, The University of Queensland, Brisbane, QLD, Australia; [2]Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia; [3]Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia.

Abstract:
Secretory pathway calcium-ATPase 1 (SPCA1) actively transports cytosolic calcium (Ca^{2+}) and manganese (Mn^{2+}) ions into the Golgi lumen, playing a crucial role in maintaining Golgi ion homeostasis and supporting Golgi-dependent functions. Several Golgi-resident enzymes, including glycosyltransferases and proprotein convertases, have activity that is sensitive to Ca^{2+} and/or Mn^{2+} ions. Ca^{2+}/Mn^{2+} homeostasis in the Golgi is therefore essential for correct protein folding, post-translational modification and efficient trafficking through the secretory pathway. This may be especially important during viral infection. Indeed, SPCA1 is required for proper processing of viral glycoproteins by supporting the maturation of a variety of RNA viruses, including members of the Paramyxoviridae, Flaviviridae, and Togaviridae families. The SARS-CoV-2 spike protein undergoes extensive post-translational modification in the Golgi, including proteolytic cleavage and glycosylation. We hypothesized that SPCA1-mediated Golgi Ca^{2+}/Mn^{2+} homeostasis is required for the proper processing of SARS-CoV-2 spike protein. Using CRISPR/Cas9-mediated SPCA1 depletion and rescue experiments, together with cell-cell fusion assays, mass spectrometry-based glycoproteomics, and proteolytic cleavage analyses, we found that SPCA1 is a regulator of SARS-CoV-2 spike protein maturation and function. Loss of SPCA1 reduced spike-mediated cell fusion, altered glycosylation patterns and impaired spike cleavage. A Ca^{2+}/Mn^{2+} transport-deficient SPCA1 mutant failed to rescue spike cleavage, suggesting that SPCA1's ion transport activity is essential for proper SARS-CoV-2 spike processing. These findings support the role of SPCA1 as a viral host factor, offering a novel therapeutic approach to limit coronavirus infection

Session 2: Calcium Signaling in Metabolism

Chaired by: Dr. Khaled Machaca

14:30 - 15:00 | SERCA2 Regulates Plasma Cell Development and Antibody Mediated Immunity | Dr. Stefan Feske

Dr. Stefan Feske
New York University Grossman School of Medicine, New York, NY, USA
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Biography:
Stefan Feske is the J. Bergstein Professor of Medicine and Director of the Ion Channels & Transporters in Immunity (ICTI) program at New York University. Trained at the Max Planck Institute for Immunobiology and Harvard Medical School, he made fundamental contributions to the discovery ORAI1 as the CRAC channel pore subunit and identified the first patients with ORAI1/STIM1 mutations, defining CRAC channelopathy. His lab revealed how CRAC channels regulate lymphocyte function and thus, control immune responses to infection, cancer, autoimmunity, and allergy. Beyond CRAC channels, his lab focuses on characterizing novel ion channels, transporters, and ionic signaling pathways that regulate lymphocyte function and immune responses. He is a scientific co founder of CalciMedica.

Abstract:
B cells play critical roles in protective immunity to infection but also drive autoimmune pathologies. Plasma cells (PCs) are terminally differentiated B cells that produce large amounts of IgG, IgA, or IgE antibodies. Defects in B cell and PC development or function cause inborn errors of immunity and increased, often fatal, susceptibility to infections. The role of Ca^{2+} signaling in B cells is complex. Abolishing SOCE through CRAC channels encoded by ORAI and STIM genes does not affect B cell development or antibody production upon immunization despite defects in proliferation and metabolic function. Interfering with Ca^{2+} release from ER stores by deleting the three IP3 receptor genes (ITPR1, ITPR2, ITPR3) reduces B cell numbers, impairs proliferation and survival, and alters antibody responses. Conversely, preventing ER refilling by deleting SERCA2 and SERCA3 also reduces mature B cell numbers by suppressing expression of recombination-activating genes RAG1 and RAG2and thus V(D)J recombination of the BCR. We identified SERCA2 (ATP2A2) in an ICT-focused CRISPR screen in B cells and demonstrate it is essential for PC development and antibody production after immunization and infection. We delineated the mechanisms by which reduced ER Ca^{2+} and increased cytosolic Ca^{2+} affect B cell signaling and gene expression to impede PC development.
Our findings emphasize that tight regulation of ER and cytosolic Ca^{2+} is essential for PC development and antibody-mediated immunity.

15:00 - 15:30 | Tracking and Function of alternative STIM variants | Dr. Barbara Niemeyer

Dr. Barbara A. Niemeyer
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Biography:
B. A. Niemeyer obtained her PhD from the University of California, San Diego (UCSD), working on the mechanisms of phototransduction and the founding members of the TRP channel family (with C. Zuker, HHMI). After a postdoc with T.L. Schwarz at Stanford (SNARE proteins), she became a group leader in the Department of Pharmacology at the Saarland University, Germany, working on mammalian TRP channels. She holds a tenured professorship in Molecular Biophysics at Saarland University where the lab is interested in the regulation of Ca^{2+} selective ion channels on a molecular, cellular and also systemic physiological, but also pathophysiological level. A recent focus also includes the role of immune cells mediating a critical crosstalk between chronic kidney and heart disease.

Abstract:
Ca^{2+} homeostasis is essential for cellular functions, with regulation by Store-Operated Ca^{2+}Entry (SOCE) omnipresent. Due to a reduced ER-luminal EF hand Ca^{2+} affinity, STIM2 can regulate basal cytosolic Ca^{2+} but also increases interaction and activation of ORAI proteins at ER/plasma-membrane junctions after stimulation, while STIM1 requires stronger store depletion. With alternative splicing taking place in about 95% of human genes resulting in alternative protein variants being able to shape both the physiology and pathophysiology of organisms, we have set out to identify and characterize a number of alternate STIM1 and STIM2 variants. While RNA-Sequencing allows for a more rapid screening of variants, due to low abundance and short read sequences, a clear discrepancy exists between available information on isoform and junction-level quantification. To close this gap and to check for the abundance of alternatively spliced proteins and their interactomes, we devised a splice-specific endogenous tagging strategy using Homology-Independent Target Integration (HITI) in a mouse model expressing Cas9. To gain insights into the physiological function, opticaland systemic recordings investigating synaptic responsiveness in a splice-deficient mouse model will be presented.

15:30 – 15:45 | Poster 10: Metabolic consequences of the reduction of Store-Operated Ca^{2+} Entry | Dr. Dana E. Al-Ansari

Al-Ansari D. E., Courjaret R., Yu F., Zarif L., Mohamed H., Nader N. and Machaca K.
Weill Cornell Medicine – Qatar, Doha, Qatar

Abstract:
Obesity is a major global health burden and is associated with severe comorbidities, including hypertension, cardiovascular disease, and diabetes. Store-Operated-Ca2+Entry (SOCE) is a mechanism responsible for refilling ER Ca2+ stores and regulating several cellular functions. Emerging evidence links SOCE to various metabolic processes including glucose intolerance, insulin resistance and lipid accumulation. However, direct evidence linking SOCE to obesity and adipose tissue function remains limited, due to the lethality and severe complications associated with complete knockouts in mice of the SOCE genes, including STIM1 and Orai1. To overcome this limitation, we utilized a novel STIM1 hypomorphic mouse model, exhibiting partial reduction of SOCE activity, and AdipoQ-Cre STIM1 knockout model, allowing the investigation of both systemic and adipose-specific SOCE function. The aim of this study is to investigate the physiological role of STIM1 in white and brown adipose tissue (WAT and BAT) homeostasis during diet-induced obesity. STIM1 hypomorphic knock-in (Hypo-KI) mice, AdipoQ-Cre STIM1 knockout mice, and their respective controls were subjected to a high-fat diet (HFD; 60% fat) to induce obesity. Body weight, body composition, and metabolic parameters were assessed using TD-NMR, metabolic cages (CLAMS), glucose and insulin tolerance tests (GTT, ITT), and whole-blood lipid profiling. Thermogenic capacity was evaluated using infrared thermal imaging and continuous measurement of interscapular temperature via implanted RFID sensors, followed by histological analyses. Ex-vivo measurements of lipolysis and lipogenesis were performed on iWAT explants. Tissue-specific RNA sequencing and proteomic analyses were performed on BAT, inguinal WAT (iWAT), visceral WAT (eWAT) and Liver to identify molecular pathways altered by reduced STIM1-dependent calcium signaling. Hypo-KI mice fed a high-fat diet (HFD) exhibited significantly increased weight gain ($p<0.01$), elevated fat-to-lean mass ratio, and adipocyte hypertrophy compared with controls. Both Hypo-KI and control mice developed diabetic phenotypes under HFD conditions; however, no significant differences were observed between groups in glucose or insulin tolerance tests (GTT, ITT). Hypo-KI mice displayed significantly reduced circulating LDL and total cholesterol levels. Notably, thermogenic capacity was impaired in Hypo-KI mice, as evidenced by reduced interscapular BAT temperature ($p<0.001$). AdipoQ-Cre STIM1 KO mice phenocopied several metabolic features observed in Hypo-KI mice, including increased adiposity and adipocyte hypertrophy under HFD conditions, without significant differences in GTT or ITT profiles. In addition, AdipoQ-Cre mice exhibited lower interscapular temperatures, reduced locomotor activity as measured by CLAMS and enlarged BAT mass. Ex vivo functional analyses of iWAT revealed enhanced lipogenic capacity and reduced lipolytic responses. Tissue-specific proteomic analysis demonstrated enrichment of lipid synthesis pathways, including activation of transcriptional factors associated with lipogenesis, including SREBP and C/EBP pathways. Together, these findings demonstrate that reduced SOCE activity promotes diet-induced obesity, impairs thermogenesis, and disrupts adipose tissue metabolism. Using in vitro approaches, ongoing work will focus on defining the molecular pathways downstream of STIM1 that link calcium signaling to adipose tissue function and metabolic homeostasis.

15:45 – 16:00 | Poster 21: Glucose-Dependent Modulation of Cholinergic Ca^{2+} Signaling in Pancreatic β-Cells | Dr. Leonardo Gallegos

M.L. Gallegos-Gómez et al[1,2], Ana Paola Salgado Álvarez[1], Jonatan Rojo Ruiz[1], María Teresa Alonso Alonso[1]

[1]Institute of Biomedicine and Molecular Genetics of Valladolid (IBGM), University of Valladolid and Spanish National Research Council (CSIC), Valladolid, Spain. [2]Center for Research and Advanced Studies of the National Polytechnic Institute (Cinvestav), City of Mexico, Mexico.

Abstract:

Calcium (Ca^{2+}) signaling is a central regulator of insulin secretion in pancreatic β-cells, acting as the principal messenger that links glucose metabolism to insulin exocytosis. However, β-cell secretory function does not rely solely on changes in extracellular glucose concentration; it is also shaped by neuronal and paracrine inputs that modulate β-cell excitability. Among these, cholinergic stimulation mediated by acetylcholine (ACh) plays a key role in enhancing insulin release in synchrony with autonomic nervous system activity. Despite its importance, the interplay between cholinergic signaling and glucose metabolism in regulating subcellular Ca^{2+} dynamics remains not fully understood. We have studied the role of glucose in Ach-induced intracellular Ca^{2+} responses of cytosolic ([Ca^{2+}]c) and endoplasmic reticulum ([Ca^{2+}]ER) in pancreatic β-cells. To this end, Fura-2 was loaded into islets from an in-house-generated transgenic mouse model (Ins-erGAP3) that expresses the ER-targeted Ca^{2+} sensor erGAP3 exclusively in pancreatic β-cells. This approach enables simultaneous monitoring of [Ca^{2+}]c and [Ca^{2+}]ER dynamics without disrupting the islet's native architecture. Both resting [Ca^{2+}]c and [Ca^{2+}]ER were elevated in response to increasing extracellular glucose concentration. Cholinergic Ca^{2+} responses in β-cells were modulated by cytosolic Ca^{2+} levels in a biphasic manner, such that ACh elicited maximal Ca^{2+} release at intermediate glucose concentrations. Ach-induced Ca^{2+} responses were dampened at low and high glucose levels because the former had a low [Ca^{2+}]ER, while the latter involved a rapid Ca^{2+}-induced inactivation of IP_3R. These findings show that glucose finely tunes cholinergic Ca^{2+} signaling in pancreatic β-cells. Moreover, the Ins-erGAP3 mouse model represents a novel tool to study the integration of metabolic and neuronal Ca^{2+}-mediated signals that govern β-cell secretory function. This project was funded by the Spanish Ministry of Science and Innovation (PID2023-146434NB-I00), and the IBGM Strategic Programme, Escalera de Excelencia, Junta de Castilla y Leon (CLU2025-02-01).

Session 3: Calcium Signaling in Neurovascular Mechanosensitivity, Inflammation & Disease

Chaired by: Dr. Mohamed Trebak

16:30 - 17:00 | Amyloid Beta Contributes to Cerebral Hypoperfusion in Alzheimer's Disease via Novel Vascular Calcium Signals | Dr. Harry Pritchard

Dr. Harry Pritchard

University of Manchester & the Geoffrey Jefferson Brain Research Centre

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Biography:

Harry is an Alzheimer's Society Dementia Research Leader fellow, in the division of Cardiovascular Sciences at the University of Manchester. His research is focused on the biophysical regulation of blood vessel diameter in the cerebral circulation, and how modifiable risk factors impact cerebral blood flow and therefore contribute to dementia pathology. To study this, his lab uses techniques such as electrophysiology, calcium imaging and ex vivo arterial contraction studies. Recently this has developed to include more in vivoapproached including different modalities of measuring cerebral blood flow and mouse cognition.

Abstract:

In Alzheimer's disease (AD), amyloid beta 1-40 (Aβ1-40) is known to deposit within the wall of the of cerebral arteries of AD patients, damaging vascular function, driving a reduction in cerebral blood flow that contributes to cognitive decline. We recently showed that in a mouse model of Alzheimer's disease (APP23) at 18 months of age, a loss of large-conductance Ca^{2+} activated potassium (BK) channels function in vascular smooth muscle cells, due to impaired Ca^{2+} release events (Ca^{2+} sparks) from the sarcoplasmic reticulum (SR) that activate the channel. This led to hypercontractile vessels that contributes to the reduced cerebral blood flow phenotype. Conversely, direct application of Aβ1-40 to a cerebral artery isolated from a young healthy wild-type mouse did not lead to a reduction in Ca^{2+} sparks, but instead, it induced a Ca^{2+} wave events and vasocontraction via a different pathway.

Interestingly, at 9 months of age there is a reduction in cerebral blood flow in the APP23 mouse, that predates cognitive impairment. At this age, we now see the presence of the Ca^{2+} waves in pressurised cerebral arteries, in addition to the reduction in Ca^{2+} sparks. We also see that there is a reduced store load in vascular smooth muscle cells that may contribute to the reduced Ca^{2+} spark frequency. This work supports the hypothesis that vascular dysfunction occurs early in AD progression and understanding mechanisms to intervene may prevent or delay cognitive decline.

17:00 - 17:30 | Piezo1 Integrates Hemodynamic Forces to Tune Neurovascular Coupling | Dr. Osama Harraz

Dr. Osama Harraz
University of Vermont
Osama.Harraz@uvm.edu

Biography:
Osama Harraz, PhD, Bloomfield Professor in Cardiovascular Research and Assistant Professor of Pharmacology at the University of Vermont, studies how brain vascular ion channels regulate blood flow and contribute to brain health. He earned his BSc and MSc from Alexandria University (Egypt) and his PhD from the University of Calgary (Canada). His doctoral studies investigated voltage-gated calcium channels, and he was supported by the prestigious Vanier Scholarship. During postdoctoral training in Vermont, he discovered a phospholipid regulator of calcium and electrical signaling in brain capillaries. Since founding his lab in 2021, his research has focused on mechanosensitive Piezo1 channels and neurovascular coupling, supported by the NIH and Bloomfield Professorship.

Abstract:
Endothelial cells (ECs) are continuously exposed to mechanical forces generated by blood flow, yet how these forces shape cerebral blood flow (CBF) regulation has remained unclear. We identify the mechanosensitive ion channel Piezo1 as a critical "mechano feedback" regulator within brain capillaries. Using electrophysiology, Ca^{2+} imaging, in vivo two photon microscopy, and genetically engineered gain of function (GOF) mouse models, we show that physiological flow fluctuations activate Piezo1, driving EC depolarization and suppressing neurovascular coupling (NVC). Piezo1 engagement reduces hyperemia amplitude, slows the upstroke, and accelerates the downstroke of CBF responses. EC specific Piezo1 GOF mice exhibit impaired functional hyperemia and corresponding deficits in recognition memory, linking endothelial mechanotransduction to cognition. Mechanistically, GqPCR signaling enhances Piezo1 activity through PLC dependent PIP_2 depletion, and exogenous PIP_2 corrects Piezo1 GOF channelopathy and restores NVC. These findings establish Piezo1 as a central integrator of hemodynamic forces and biochemical signals, defining a previously unrecognized endothelial mechanism governing brain blood flow and cognitive function.

17:30 – 17:45 | Poster 47: Atrial Shear-mechanotransduction and local Ca^{2+} Adaptation | Sun-Hee Woo

Phuong Kim Luong, Tran Quoc Dat, Hieu Trong Huynh, Le Nguyen Que Minh, Sun–Hee Woo
College of Pharmacy, Chungnam National University, Daejeon 34134, South Korea

Abstract:
Pathological shear stress induces Ca^{2+} waves in quiescent atrial myocytes via gap junction hemichannel-mediated ATP release. We examined whether and how shear stress alters atrial local Ca^{2+} signaling and its underlying mechanisms. Using two-dimensional confocal Ca^{2+} imaging in combination with a microfluidic jet system, we assessed the effects of shear stress on peripheral junctional and central non-junctional Ca^{2+} transients in field-stimulated rat and mouse atrial myocytes and in the HL-1 mouse atrial cell line. Pharmacological and genetic interventions as well as local mitochondrial Ca^{2+} measurements were adopted. In intact murine atrial cells, shear stress (~16 dyn/cm²) elicited transient enhancements in local Ca^{2+} transients and diastolic Ca^{2+} increases, with larger effects in the center than in the periphery. This stimulatory effect was rapidly reversed to inhibitory effects on Ca^{2+} transients by prolonged shear stress. In cardiac-specific connexin 43 conditional knockout mouse atrial myocytes, this shear effect was maintained. Shear-induced stimulatory effects on Ca^{2+} transients were significantly suppressed by pretreatment with the pannexin blocker probenecid (800 μM), P2 purinoceptor antagonist suramin (30 μM) or the inositol 1,4,5-trisphosphate receptor (IP_3R) inhibitor 2-APB (3–10 μM). To test the role of mitochondrial Ca^{2+} mobilization in the shear-Ca^{2+} response, cytosolic and mitochondrial Ca^{2+} were simultaneously imaged (60–120 Hz) using rhod-2 and CEPIA2mt in monolayered HL-1 cells, respectively. Similar shear-induced local Ca^{2+} changes were observed in highly confluent (>90%) HL-1 cells. Mitochondrial Ca^{2+} increased following cytosolic Ca^{2+} rise, with larger and faster changes in the periphery than in the center. Inhibition of pannexin, P2 purinoceptors (by suramin), or the sarcoplasmic reticulum (SR) Ca^{2+} pump (by thapsigargin) almost completely suppressed the shear-induced Ca^{2+} increases in both the cytosol and mitochondria. Pretreatment with 2-APB suppressed both cytosolic and mitochondrial Ca^{2+} increases under shear stress in a concentration-dependent manner (3–10 μM), with more potent effects in the mitochondria than in the cytosol. Blockade of the mitochondrial Ca^{2+} uniporter (MCU) with Ru360 (10 μM) selectively inhibited mitochondrial Ca^{2+} increase under shear stress. Our data suggest that shear stress enhances Ca^{2+} transients in beating atrial myocytes via pannexin–P2 receptor signaling-mediated SR Ca^{2+} release, resulting in rapid Ca^{2+} loading in peripheral mitochondria via the IP_3R and MCU. This shear-induced local Ca^{2+} signaling pathway may allow atrial myocytes to measure atrial blood volume or regurgitant jets, thereby immediately adapting by enhancing both contractility and Ca^{2+}-dependent mitochondrial function.

17:45 – 18:00 | Poster 16: Calmodulin G133V: Disrupted Calcium Signaling and Potential Drug Interactions in Arrhythmia | Helene H. Jensen and Malene Brohus

***Brohus M[1], *Jensen HH[1], Nyegaard M[2,3], Olsen A[1], Freude K[4], Dick IE[5], Jensen HK[6,7], Overgaard MT[1]**
[1]Department of Chemistry and Bioscience, Aalborg University. [2]Department of Health Science and Technology, Aalborg University, Denmark. [3]Statens Serum Institute, Denmark. [4]Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark. [5]Department of Pharmacology and Physiology, University of Maryland School of Medicine, Baltimore, USA. [6]Department of Clinical Medicine, Aarhus University. [7]Department of Cardiology, Aarhus University Hospital. * equal contribution

Abstract:
Calmodulin is a ubiquitous calcium sensor encoded by three genes (CALM1, CALM2, CALM3). Rare variants in these genes can cause fatal cardiac arrhythmia, particularly when calcium-binding EF-hands are affected. We identified the variant CALM1-G133V in a 5-year-old boy who suffered cardiac arrest during moon-car driving in the kindergarten. He was diagnosed with long QT syndrome and treated with metoprolol and an implantable cardioverter-defibrillator. Notably, ten weeks prior to the arrest, he had initiated methylphenidate therapy for attention deficit-hyperactivity disorder (ADHD). These observations prompted us to investigate how this variant alters calmodulin's calcium-sensing function and whether methylphenidate may contribute to arrhythmia risk. We purified and expressed the G133V calmodulin protein and found a ~10-fold reduction in calcium affinity, severely impairing sensor function. We further examined its regulation of two key calcium channels—voltage-gated calcium channel 1.2 and ryanodine receptor 2—both central to calmodulinopathy mechanisms. The G133V variant markedly reduced calcium-dependent binding to channel domains and impaired channel closure. To explore potential interactions with methylphenidate, we generated patient-derived induced pluripotent stem cells that were differentiated to cardiomyocytes. Further, we have also introduced the variant into Caenorhabditis elegans. These models will allow us to test whether methylphenidate exacerbates arrhythmia phenotypes in the context of calmodulin dysfunction.

Session 4: Inositol 1,4,5 Trisphosphate Receptors

Chaired by Dr. David Yule

9:00 - 9:30 | Synergism of IP3R and Parkin Mutants Identifies Mitochondrial Stress as an Early Feature of Parkinson's Disease | Dr. Gaiti Hasan

Dr. Gaiti Hasan
National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research, Bangalore
Mrudula Dileep, Anamika Sharma, Syed Kavish Nizami, and Nandashree Kasturacharya
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Dr. Gaiti Hasan is a Senior Professor at the National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research, Bangalore, and an internationally recognized expert in Drosophila neurogenetics and intracellular calcium signaling. Her research focuses on how calcium dependent pathways regulate neuronal development, function, and neurodegeneration. She earned her Ph.D. from the University of Cambridge (1983) and has held research positions at TIFR Mumbai and Brandeis University. Dr. Hasan is a Fellow of the Indian National Science Academy (INSA) and the Indian Academy of Sciences, and has received prestigious honors including the Sir M. Visvesvaraya Lifetime Achievement Award.
Her laboratory pioneered reverse genetic approaches to study the IP_3 receptor in Drosophila, contributing foundational insights into neuronal circuit formation and calcium linked neurological disorders.

Abstract:
Our understanding of mechanisms underlying familial Parkinson's disease (PD) have benefitted from studies in Drosophila models of PD. In order to understand genetic predisposition to sporadic PD, we studied Drosophila with single-copy mutation of the recessive IP3R-encoding gene (itpr) in combination with a recessive null mutation of the parkin gene. Surprisingly, mitophagy and mitochondrial Ca^{2+} were barely affected. Instead, flight motor deficits of single-copy itpr/+, parkin/+ mutants correlated with elevated levels of mitochondrial H2O2, and reducing H_2O_2 levels by genetic means restored mitochondrial function and flight to a significant extent. This study underlines the importance of mitochondrial oxidative stress as an early phenotype in PD and suggests that humans with recessive variants in either pathway have a higher chance of developing sporadic PD.

9:30 - 10:00 | Decoding Type 2 IP3R: Cryo-EM Insights into Isoform-Specific Properties | Dr. Irina I. Serysheva

Dr. Irina Serysheva
McGovern Medical School at The University of Texas Health Science Center at Houston
irina.i.serysheva@uth.tmc.edu

Biography:
Irina Serysheva is the Jesse H. Jones Chair in Molecular Biology and Professor in the Department of Molecular Biology and Biochemistry at UTHealth McGovern Medical School in Houston. She earned her B.S. in virology and M.S. in biochemistry from Moscow State University (Russia) and her Ph.D. from the Russian Academy of Sciences, followed by postdoctoral training at Moscow State University, UCLA, and Tufts University School of Medicine. Dr. Serysheva has been a faculty member at UTHealth since 2008, after serving on the faculty of Baylor College of Medicine from 1992 to 2008. She is the Faculty Director of the UTHealth Cryo-EM Core. Her research focuses on structural analysis of calcium signaling complexes, including the first cryo-EM structural determination of the inositol 1,4,5-trisphosphate receptor.

Abstract:
Calcium release via inositol 1,4,5-trisphosphate receptors (IP_3Rs) is a universal signaling process that regulates muscle contraction, neuronal signaling, and many other cellular functions. Among the three isoforms, IP_3R2 is the predominant Ca^{2+} channel in excitable tissues; however, its full-length tetrameric structure remained unknown. We determined high-resolution cryo-EM structures of mammalian IP_3R2 in both the Ca^{2+}/IP3/ATP-bound (activated) and ligand-free (closed) states, revealing the complete channel architecture. While the IP_3-binding pocket is conserved across isoforms, subtype specificity arises from the dynamic ARM2 domain, which contains an autoinhibitory loop that modulates IP_3 access. Structural and functional analyses define the ATP-binding site, explaining IP_3R2's enhanced ATP sensitivity relative to IP_3R1 and IP_3R3. The ion-conduction pathway features two constriction points that dilate upon activation to form a continuous Ca^{2+} route. Notably, the disease-causing G2498S mutation resides within the selectivity filter, underscoring its functional significance. Comparative analysis across the IP_3R family reveals distinct gating motions and allosteric couplings defining IP_3R2 regulation. These structures provide a mechanistic framework for subtype-specific ligand recognition, allosteric modulation, and Ca^{2+} permeation, offering a blueprint for targeting IP_3R-mediated signaling.

10:00 – 10:15 | Poster 29: Characterization of Ca^{2+} oscillations in glioma cells using computational modelling | Mehrosh Ahmed

Mehrosh Ahmed[1], Frank Winkler[2] and Geneviève Dupont[1]
[1]Unit of Theoretical Chronobiology, Université Libre de Bruxelles (ULB), Brussels, Belgium.
[2]Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg, Heidelberg, Germany.

Abstract:
Gliomas are aggressive brain tumors originating from glial cells. During tumor progression, interconnected glioma cells communicate through microtubes and display repetitive Ca^{2+} pulses that contribute to the activation of MAPK and NF-κB pathways, thereby promoting tumor growth. These oscillations depend on classical inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} release and require Ca^{2+}-activated K^+ channels, which are strongly upregulated in glioblastoma [1]. We first analyzed Ca^{2+} traces from human glioma cell lines BG5 and S24 to classify cells on the basis of their activity pattern. The pipeline discriminates the cells based on the width and the shape of the Ca^{2+} spikes, as well as depending on the frequency of oscillations. We then developed a detailed single-cell computational model that incorporates IP_3 synthesis and degradation, Ca^{2+} fluxes through IP_3 receptors, Ca^{2+} ATPases, the Na^+–Ca^{2+} exchanger, ORAI channels, and membrane-voltage dynamics including KCa3.1(KCNN4), K(V), Ca(V), and Na(V) channels [2]. Activation of KCa3.1 by cytosolic Ca^{2+} hyperpolarizes the membrane and enhances Ca^{2+} influx through ORAI1 channels, allowing tight control of IP_3-dependent Ca^{2+} oscillations. Model predictions on the impact of selectively inhibiting Ca^{2+} pathways agree with experimental data. Using transcriptomic analyses, we plan to further validate the model by testing if the different oscillatory behaviors that we have identified can be reproduced when changing the simulated activity of the channels according to the expression data. Extending the framework to a coupled two-cell system demonstrated that electrical and Ca^{2+} coupling can induce oscillations in a non-oscillatory cell when paired with an oscillatory partner. This result captures a potential mechanism for signal propagation within glioma networks. Future work will investigate how Ca^{2+} waves propagate across multicellular networks and hopefully predict how to decrease global oscillatory activity, and thus tumor progression. [1] Hausmann D., Hoffmann, D., Venkataramani, V., … and Winkler, F. (2022) Autonomous rhythmic activity in glioma networks drives brain tumour growth. Nature 613, 179-186. [2] Dupont, G., Falcke, M., Kirk, V. and Sneyd, J. (2016) Models of calcium signalling. Springer.

10:15 – 10:30 | Poster 34: IP_3Rs critically contribute to ferroptosis-induced cell death | Ophélie Champion

Champion Ophélie[1], La Rovere Rita[1], Sassano Maria Livia[2,3], Agostinis Patrizia[2,3], von Karstedt Silvia[5,6], Bultynck Geert[1]

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Abstract:
Ferroptosis is a recently characterized, iron-dependent form of regulated cell death, defined by the accumulation of lipid peroxides within cellular membranes. This process results from iron-driven oxidation of phospholipids, ultimately leading to membrane rupture and finally cell death. Emerging evidence implicates the endoplasmic reticulum-mitochondria contact sites (ER-mitochondria contact sites, EMCSs) as crucial early hotspots for phospholipid peroxidation and as signaling hubs controlling the initiation of ferroptosis. These inter-organelle regions also host essential players of calcium homeostasis and apoptosis regulation, including IP_3Rs and members of the BCL-2 family. However, the crosstalk between ferroptosis, calcium signaling, and BCL-2/IP_3R modulation remains poorly understood. This project aims to elucidate how BCL-2 and IP_3Rs influences ferroptosis-induced cell death. Specifically, it investigates whether BCL-2, known for its anti-apoptotic functions and capacity to inhibit IP_3R-mediated calcium fluxes, can suppress ferroptosis by altering IP_3R activity, calcium transfer, and ER-mitochondria redox signaling. Preliminary data show that HEK IP_3R triple knockout (TKO) cells display markedly higher resistance to erastin-induced ferroptosis compared to wild-type counterparts, suggesting a key role for IP_3Rs in ferroptotic signaling (Dr. Maria Livia Sassano, KU Leuven, Belgium). By recomplementing each IP_3R isoform in HEK TKO cells, IP_3R1 and IP_3R2 appear to mediate susceptibility to ferroptosis, while IP_3R3 does not. Supporting this, diffuse large B-cell lymphoma (DLBCL) cells with high IP_3R2 expression are particularly sensitive to both RSL3 and erastin-induced ferroptosis. Calcium chelation experiments using BAPTA-AM or EGTA-AM further confirm that calcium contribute to ferroptotic cell death, as chelators significantly delay RSL3 and erastin-triggered lethality. This was also confirm in two small lung cancer cell lines (H441 and A549) in Dr. Silvia von Karstedt laboratory (University of Cologne, Germany). To dissect the molecular interplay between BCL-2 and IP_3Rs in ferroptosis regulation, a series of targeted BCL-2 mutants were employed. The BCL-2 K17D mutant disrupts IP_3R binding while retaining Bax-binding capacity, whereas the BCL-2 GR/AA mutant loses Bax interaction but continues to inhibit IP_3Rs. However, our data show that overexpression of either wild-type BCL-2 or these mutants does not significantly alter ferroptosis-induced cell death, indicating that modulation of IP_3R binding by BCL-2 is not sufficient to suppress ferroptotic signaling. This suggests that BCL-2's anti-apoptotic mechanisms may not directly extend to ferroptosis, or that additional regulatory pathways override its inhibitory effects under ferroptotic conditions. In parallel, I will use CySLess-IP_3R1 constructs, in which all cysteine residues are replaced by alanine, preventing receptor oxidation and allowing assessment of redox-sensitive IP_3R modulation to determine whether IP_3R oxidation is a key event linking ferroptosis inducers to calcium dysregulation and ER-mitochondria oxidative stress. Future experiments will quantify the impact of ferroptosis inducers on ER-mitochondria calcium fluxes, redox changes, and contact site dynamics using split-FAST fluorescence reporters. In DLBCL cell lines, genetic modulation of IP_3R2 expression, via knockdown in SUDHL4 or overexpression in OCILY1 cells, will clarify the relationship between IP_3R2 abundance and ferroptotic vulnerability. Comparing responses to erastin and RSL3 will also reveal whether ferroptosis suppression through calcium modulation is universal or pathway-specific, and would open new therapeutic targets in B-cell malignancies.

Session 5: Calcium Signaling in Cell Differentiation

Chaired by: Dr. Khaled Machaca

11:00 - 11:30 | Membrane Contact Sites and Calcium Signaling | Dr. Paola Pizzo

Paolo Pizzo
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Biography:
Paola Pizzo is Full Professor of General Pathology at the Medical School of the University of Padua (UniPD, Italy). She graduated in Biology at UniPD and got her Ph.D. at the University of Bologna (Italy) on extracellular ATP as a new signal in immunity. She did a postdoc at the Weizmann Institute of Science (Israel), on Duchenne muscular dystrophy. Back to UniPD, she worked with Tullio Pozzan on the mechanisms of Ca^{2+} homeostasis in mammalian cells in physio-pathological conditions. As a PI, she studied Ca^{2+} dysregulation and mitochondrial dysfunction in Alzheimer's Disease (AD). Her lab is now focused on understanding how neuroinflammation contributes to AD onset/development and whether an altered interaction between Ca^{2+} handling organelles might contribute to this disease.

Abstract:
Membrane contact sites (MCSs) are regions where organelles come in close proximity and have recently emerged as critical hubs for cell communication, mediating a broad range of physiological processes, including Ca^{2+} signaling. Specific protein and lipid compositions, as well as specialized molecular tethers, characterize each type of MCS, ensuring the performance of their functionalities. The precise molecular composition of these sites can vary between cell types and can be dynamically regulated with high spatiotemporal resolution in response to cellular needs, adding complexity to their molecular architecture. Of note, early disturbances in MCS structure/function have been described in different high-incidence disorders, such as metabolic and neurodegenerative diseases, and several cancers, suggesting that such alterations might underlie their pathogenesis.

I will present some recent data on the development and use of a new set of reversible chemogenetic reporters for MCSs that allows the study of their dynamics in physio-pathological conditions. Moreover, by adding suitable Ca^{2+}-sensing modules to these probes, we generated a series of single reporters capable of simultaneously detecting MCSs and measuring the associated Ca^{2+} signals. I will focus my presentation on endoplasmic reticulum (ER)-mitochondria MCSs, sites of privileged Ca^{2+} transfer which appear to be modulated by ER Ca^{2+} content, defying the molecules involved in this pathway.

11:30 - 12:00 | Calcium at the Intersection of Metabolism and Embryo Development | Dr. Carmen J. Williams

Dr. Carmen J. Williams
National Institute of Environmental Health Sciences, NIH, USA
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Biography:
Carmen Williams, MD, PhD is a Senior Investigator and Deputy Chief of the Reproductive & Developmental Biology Laboratory at the National Institute for Environmental Health Sciences, NIH. She trained and practiced clinically as an Obstetrician/Gynecologist and Reproductive Endocrinology & Infertility physician scientist at the University of Pennsylvania. She then switched her career path away from the clinic to ask basic science questions about the mechanisms underlying the establishment of pregnancy. Her lab focuses on how calcium signaling following fertilization is regulated and its impacts on preimplantation embryo development and long-term offspring health.

Abstract:
Calcium (Ca^{2+}) signals initiate embryo development at fertilization and are frequently disrupted in human assisted reproduction, which is associated with metabolic abnormalities in children. In mice, excess or inadequate Ca^{2+} signals at fertilization impair embryo development and alter adult metabolism, but the mechanisms are unknown. We hypothesize that the impact of cellular Ca^{2+} signals on energy and metabolism may explain this connection. I will describe our use of orthogonal approaches to show that excess Ca^{2+} signaling in one-cell mouse embryos alters the production of epimetabolites required for nuclear reprogramming and zygotic genome activation. Imbalance in these metabolites, particularly a reduction in lactyl coenzyme A, alters how the embryo is epigenetically reprogrammed and impairs embryo development. Our results indicate that Ca^{2+} dynamics drive metabolic regulation of epigenetic reprogramming at fertilization, connecting Ca^{2+} signaling at the initiation of development to long-term metabolic function in adults.

12:00 – 12:15 | Poster 20: Calcium oscillations and early embryogenesis – novel paradigms for phospholipase C zeta | Dr. Junaid Kashir

Junaid Kashir[1], Bhavesh Mistry[2], Mohamed Rajab[2], Lujain BuSaleh[3], Raed Abu-Dawud[4], Sarah Alharbi[5], Michail Nomikos[6], Saad AlHassan[7], Serdar Coskun[8], Abdullah Assiri[9]
[1]Department of Biology, College of Arts and Sciences, Khalifa University, Abu Dhabi 127788, United Arab Emirates. [2]Department of Comparative Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh 11564, Saudi Arabia. [3]College of Medicine, Alfaisal University, Riyadh 11533, Saudi Arabia. [4]Institute for Molecular Medicine, MSH Medical School, 20457 Hamburg, Germany. [5]Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh 11564, Saudi Arabia. [6]College of Medicine, QU Health, Qatar University, Doha 2713, Qatar. [7]Department of Obstetrics and Gynaecology, King Faisal Specialist Hospital and Research Centre, Riyadh 11564, Saudi Arabia.

Abstract:
Cases of male infertility are increasingly associated with the efficacy of oocyte activation. Mammalian oocyte activation occurs in response to specific profiles of calcium (Ca^{2+}) oscillations driven by sperm-specific phospholipase C zeta (PLCζ), abrogation of which in sperm is extensively linked with male infertility where oocyte activation is deficient (OAD). However, less is known whether sperm PLCζ underlies cases of defective embryogenesis and failed pregnancy following fertility treatment. We propose that a significant causative factor underlying poor global ART success are abnormalities/insufficiencies in Ca^{2+} oscillation-driven early embryogenic events, mediated by abnormalities/insufficiencies in sperm PLCζ. Indeed, Ca^{2+} oscillation profiles are necessary for early embryonic competency, mediated via the efficacy of PLCζ-driven oocyte activation. Ultimately, we aim to establish the link(s) between such PLCζ-mediated parameters with fertility treatment outcome. We have been evaluating PLCζ profiles in sperm from human couples undergoing fertility treatment in correlation with time-lapse morphokinetic analysis of resultant embryos, correlating such profiles to pregnancy status. Concurrently, we have also generated two strains of mutant PLCζ mice using CRISPR/Cas, using mutant sperm to generate embryos with wild-type (WT) oocytes via in vitro fertilisation (IVF). We found that minimal levels of PLCζ within a specific range were required for optimal early embryogenesis, correlating with increased pregnancy. Levels of sperm PLCζ below specific thresholds did not correlate to effective embryogenesis and pregnancy, despite eliciting successful fertilisation, indicating that minimal PLCζ levels are required to initiate fertilisation and support effective embryogenesis. Transgenic sperm exhibited severely reduced PLCζ levels, with transgenic mice yielding a drastically reduced litter size and reduced numbers of embryos reaching developmental milestones. Furthermore, embryos generated with transgenic sperm and WT oocytes exhibited high rates of polyspermy, alongside delayed embryonic progression compared to embryos generated using WT sperm and oocytes. Our data suggests that utilisation of PLCζ may benefit the larger population of couples seeking fertility treatment and could be used to enhance the success rates of fertility treatments.

12:15 – 12:30 | Poster 50: Molecular partnerships of CRISPs in calcium expulsion pathways | Dr. Vaidehi Miya

Valdehi Miya, Ananya Breed, Chandan Kumar, Susan Thomas, Bhakti R Pathak
ICMR NIRRCH

Abstract:
CRISP/s (Cysteine Rich Secretary Proteins) show an expression bias in the male reproductive tract where they are sequentially acquired on sperm during epididymal transit. Additionally, CRISPs are reported in the venom secreting ducts of insects and reptiles. CRISPs harbor an ion channel regulatory motif in the C-terminus; however, identity of ion channels regulated by mammalian CRISPs is limited. To address this, an interactome analysis of CRISP4 from rat caudal spermatozoa was carried out. Rat CRISP4 (rCRISP4) was immunoprecipitated from caudal spermatozoa and subjected to LC-MS analysis. Plasma membrane localizing proteins were shortlisted and the interaction of PMCA4b (Plasma membrane calcium ATPase, isoform b), a calcium extrusion pump, was validated by co-immunoprecipitation and co-localization. The effect of CRISPs on PMCA4b mediated intracellular calcium [Ca^{2+}] efflux was determined using ratiometric fluorescent dye Fura-2-AM. Impact of presence or absence of binding partners of CRISPs on calcium expulsion was also compared. Membrane interactome of rCRISP4 from caudal spermatozoa revealed PMCA4b as a novel binding partner and their interaction was validated. Multiple CRISPs interacted with hPMCA4b where association of hPMCA4b with human CRISP1 and rCRISP4 delayed hPMCA4b mediated calcium extrusion but not human CRISP3. Effect of different CRISPs on PMCA4b mediated calcium expulsion was correlated with their secretion propensity and cholesterol binding. Our study unveils a previously unknown mechanism for the role of CRISPs in modulating intracellular calcium levels via PMCA4b.

Session 6: Signaling by Ca^{2+} Mitochondrial C^{2+} Binding Proteins

Chaired by: Dr. Gary Shaw & Dr. Peter Stathopulos

14:00 - 14:30 | Excessive Ca2+-dependent ER-mitochondrial contact stabilization drives metabolic liver injury | Dr. Dipayan Chaudhuri

Dr. Dipayan Chaudhuri
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Biography:
Dr. Chaudhuri is an Associate Professor in the Division of Cardiology, University of Utah, and an investigator at the Nora Eccles Harrison Cardiovascular Research and Training Institute (CVRTI). His research focuses on changes in mitochondrial metabolism during heart and liver failure. One area of focus is how mitochondrial Ca^{2+} signaling, which regulates ATP synthesis, can be leveraged for new therapies in heart failure. A second area of focus is examining how mitochondrial Ca^{2+} signaling is altered in metabolic liver disease, how this alters paracrine signaling between liver and heart, and whether it can also be targeted for therapeutic benefit. Dr. Chaudhuri is an attending physician and the medical director of the inpatient cardiovascular units at the University of Utah Hospital.

Abstract:
That mitochondrial remodeling was associated with ER Ca^{2+} release was established >20 years ago, but its significance and mechanism remain elusive. In fact, extreme mitochondrial remodeling and ER Ca^{2+} dysregulation are prominent features of metabolic-associated steatohepatitis (MASH). To investigate this further, we focused on a gene of uncertain function, EF-Hand Domain Family Member D1 (EFHD1), identified in human genome-wide association studies of MASH. EFHD1 is a little-studied calcium-binding protein found primarily on the mitochondrial outer membrane, including at ER-mitochondria contact sites (ERMCS). We investigated EFHD1 ($Efhd1^{-/-}$) knockout mice fed normal (chow) and MASH-inducing diets. In $Efhd1^{-/-}$ hepatocytes, mitochondria were significantly longer, ERMCS were narrower and unstable, and both Ca^{2+}-induced fission and Ca^{2+}-induced actin bundling wereabrogated. These results suggest a new model of ERMCS contact stabilization, driven by spatiotemporal coincidence detection of inter-organellar proximity and ER Ca^{2+} release. Moreover, during MASH, EFHD1 upregulation drove pathological mitochondrial fragmentation via excessive contact persistence. Consequently, acute or chronic EFHD1 inhibition was protective against metabolic or chemotoxic liver disease by preventing fibrosis and inflammation. These findings identify EFHD1 as a Ca^{2+}-dependent ERMCS stabilizer, reveal a hepatocyte-intrinsic injury pathway, and suggest EFHD1 inhibition as a therapeutic strategy.

14:30 - 15:00 | The Miro-1 GTPase and Calcium Sensor in the Regulation of Mitochondrial Homeostasis | Dr. Josef Kittler

Dr. Josef Kittler
Neuroscience, Physiology and Pharmacology Department, University College London (UCL), United Kingdom
j.kittler@ucl.ac.uk

Biography:
Josef Kittler is a Professor of Neurobiology at UCL. His research focuses on the cytoskeleton and associated motor/adaptor proteins in regulating organelle and receptor trafficking within neurons, particularly to synapses. A major aim is understanding how these mechanisms influence neuronal development, plasticity, and disease. A key area of investigation is how mitochondria are delivered and positioned at critical neuronal sites, and how this affects brain connectivity and synaptic function in both health and neurological disorders.

Abstract:
Neuronal mitochondrial homeostasis requires precise coordination of mitochondrial positioning, dynamics, and calcium handling to support local metabolic and signaling needs. The outer mitochondrial membrane protein Miro1 acts as a calcium sensor that couples intracellular calcium changes to mitochondrial trafficking, positioning, and function, playing a central role in maintaining homeostasis in polarized neurons.The presentation will first discuss how Miro1-dependent mitochondrial positioning influences presynaptic calcium dynamics and neurotransmission. Studies using genetic, optical, and electrophysiological methods show that mitochondria at presynaptic terminals buffer calcium via uptake pathways such as the mitochondrial calcium uniporter (MCU), thereby regulating presynaptic calcium homeostasis and synaptic plasticity in hippocampal circuits.

The talk will then expand to consider how Miro1-related calcium-sensing mechanisms regulate mitochondria beyond the presynapse. Recent evidence indicates that Miro family proteins contribute to compartment-specific control of mitochondrial dynamics and calcium-dependent synaptic function, underscoring a broader role for Miro-mediated calcium sensing in coordinating mitochondrial homeostasis across synapses.

Collectively, this work supports a model in which Miro1 and related mitochondrial calcium sensors integrate activity-dependent calcium signals to direct mitochondrial behaviour, thereby maintaining synaptic stability and plasticity through the regulation of mitochondrial homeostasis.

15:00 – 15:15 | Poster 11: Novel Insights into Calcium-Dependent Mitochondrial Regulation of Neuroinflammation in Early Parkinson's Disease Models | Dr. Dharmendra Kumar Khatri

Dharmendra Kumar Khatri
NIMS Institute of Pharmacy, NIMS University Rajasthan, Jaipur, India.

Abstract:
Aim / Background: Calcium (Ca^{2+}) signaling plays a central role in neuronal homeostasis, linking metabolic activity to synaptic function and survival. Dysregulation of intracellular Ca^{2+} has been implicated in neurodegenerative diseases, particularly Parkinson's disease (PD), where early mitochondrial dysfunction precedes overt cell loss. However, the mechanisms by which aberrant Ca^{2+} dynamics drive neuroinflammation and mitochondrial impairment in PD remain incompletely understood. This study investigates how perturbations in Ca^{2+}-dependent mitochondrial regulatory pathways influence glial activation and neuronal survival in early PD models, with a focus on the interplay between Ca^{2+}-binding effector proteins, mitochondrial bioenergetics, and inflammatory cascades. Methods: We employed an integrative experimental paradigm combining in vitro and in vivo PD models. Primary rat dopaminergic neurons and astrocytes were exposed to sub-toxic concentrations of rotenone to mimic early PD-like stress. Intracellular Ca^{2+} dynamics were quantified using live-cell fluorescence imaging of Ca^{2+} indicators alongside genetically encoded Ca^{2+} sensors targeted to mitochondria. Mitochondrial respiratory function and membrane potential were evaluated using high-resolution respirometry and JC-1 assays, respectively. To dissect Ca^{2+}-dependent signaling, we manipulated expression of key mitochondrial Ca^{2+}-handling proteins -mitochondrial calcium uniporter (MCU), MICU1, and NCLX using viral vectors and siRNA knockdown. Parallel in vivo studies involved unilateral intranigral infusion of MCU modulators in a rat rotenone model, followed by behavioural assessments (rotarod, open field) and post-mortem analysis of inflammatory markers (IL-1β, TNF-α) via ELISA and immunohistochemistry. Transcriptomic profiling of substantia nigra tissues was performed to identify Ca^{2+}-regulated gene networks. Results: We observed that rotenone exposure induced sustained elevations in cytosolic Ca^{2+}, accompanied by excessive mitochondrial Ca^{2+} uptake and compromised bioenergetic function. Overexpression of MICU1 preserved Ca^{2+} homeostasis, maintained mitochondrial respiration, and attenuated rotenone-induced deficits. Conversely, MCU upregulation exacerbated mitochondrial depolarization and reactive oxygen species (ROS) production. In astrocytes, elevated mitochondrial Ca^{2+} was linked to a robust pro-inflammatory transcriptional profile, implicating Ca^{2+}-dependent activation of NF-κB and inflammasome pathways. In in vivo experiments, targeted modulation of MCU/NCLX ratio significantly influenced motor behaviour and nigral dopaminergic neuron survival, correlating with reductions in glial activation and pro-inflammatory cytokines. Transcriptomic data revealed a distinct enrichment of Ca^{2+}-regulated stress response networks in PD models, including components of mitophagy and inflammatory signaling. Conclusion: Our findings demonstrate that Ca^{2+} dysregulation within mitochondria contributes to early neuroinflammatory signalling and neuronal dysfunction in PD. Precise control of mitochondrial Ca^{2+} handling via key regulators such as MICU1 and NCLX preserves mitochondrial bioenergetics and mitigates inflammatory responses, highlighting a mechanistic link between Ca^{2+} signaling and neurodegeneration. These insights offer potential targets for therapeutic modulation in PD and other neurodegenerative conditions where Ca^{2+}-dependent mitochondrial dysfunction is a critical driver. Keywords: Calcium signaling; Parkinson's disease; mitochondrial dysfunction; neuroinflammation; mitochondrial calcium uniporter; MICU1; bioenergetics; neurodegeneration; rotenone model.

15:15 – 15:30 | Poster 37: The architecture of the cortical endoplasmic reticulum supports Ca^{2+} tunneling | Dr. Raphael Courjaret

Courjaret R.[1], Lee L.[2], Mohamed H.[1], Sneyd J.[3], Yule D.[4], and Machaca K.[1]
[1]Department of Physiology and Biophysics, Weill Cornell Medicine--Qatar, Doha, Qatar; [2]Department of Mathematics, University of Auckland, Auckland, New Zealand, [4]Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY, USA.

Abstract:
Ca^{2+} signaling intervenes in virtually all cellular processes and relies on two main sources of Ca^{2+}: the extracellular space and the endoplasmic reticulum (ER). After store depletion, both compartments are coupled through Store-Operated Ca^{2+} Entry (SOCE) to refill the ER stores using the extracellular Ca^{2+} pool. Ca^{2+} entering the cell through SOCE also activates intracellular Ca^{2+} sensitive targets and because of the compactness of the SOCE microdomain those targets need to be localized very near to the entry point of Ca^{2+}. To activate distal effectors using SOCE cells rely on a mechanism termed Ca^{2+} tunneling (Courjaret and Machaca, 2025). Ca^{2+} tunneling develops when both IP_3-receptors and SOCE are active. Extracellular Ca^{2+} enters the cell through SOCE, is transported by SERCA pumps into the ER and channeled towards distant cortical targets using the ER lumen as a tunnel, isolating the signal from the cytosol. Cortical delivery of Ca^{2+} to PM effectors such as ion channels is favored by tunneling while deeper targets receive less Ca^{2+} (Courjaret and Machaca, 2025). We recently outlined the 3D localization of the elements of Ca^{2+} tunneling relative to each other: SOCE cluster, SERCA pump, PMCA, and IP_3 receptors (Courjaret and Machaca, 2025). Here, using 3D electron microscopy we identify a "basket" structure in the cortical ER joining membrane contact sites where SOCE occurs. Mathematical modeling shows that the ER Basket greatly enhances the efficiency of tunneling. To further evaluate the importance of the cortical ER, we manipulated the actin cytoskeleton and ER structure and measured the functional consequences on SOCE and tunneling. Disrupting actin polymerization had little effect on SOCE and tunneling, while stabilizing the cytoskeleton favored tunneling. Brefeldin treatment increased the amount of cortical ER and enhanced Ca^{2+} tunneling. Together those results support a critical role for a stable cortical ER architecture in Ca^{2+} tunneling. Further investigation will be required to identify differences in this architecture depending on the cell type and/or physiological conditions, particularly in secretory cells. Courjaret et al., J. Cell. Biol. 2025 Courjaret and Machaca, Cold Spring Harb. Perspect. Biol. 2025

16:00 - 17:00 | Claude Klee Keynote Lecture | Dr. Jennifer Lippincott-Schwartz

Chaired by: Dr. Martha Cyert

Dr. Jennifer Lippincott-Schwartz
HHMI Janelia Research Campus
lippincottschwartzj@janelia.hhmi.org

Biography:
Dr. Jennifer Lippincott-Schwartz is a Senior Group Leader and Head of 4D Cellular Physiology at the Howard Hughes Medical Institute's Janelia Research Campus. A pioneering cell biologist, she is widely recognized for her groundbreaking work in live-cell imaging, organelle dynamics, and the development of advanced fluorescence-based microscopy techniques. Her research has reshaped the understanding of how cellular organelles behave, communicate, and regenerate within living systems.
Dr. Lippincott-Schwartz earned her B.A. from Swarthmore College, an M.S. in Biology from Stanford University, and a Ph.D. in Biochemistry from Johns Hopkins University. She completed her postdoctoral training at the National Institutes of Health (NIH), where she later became Chief of the Section on Organelle Biology at the Eunice Kennedy Shriver National Institute of Child Health and Human Development.
A leader in her field, she has been instrumental in developing photoactivatable fluorescent proteins and super resolution imaging methods such as PALM, which have revolutionized the visualization of molecular organization within cells. Her contributions have earned her numerous honors, including election to the National Academy of Sciences and the National Academy of Medicine.

Long-Distance Calcium Signaling in Neurons

Abstract:
A major question in neuronal cell biology is how signals from postsynaptic neurotransmission are integrated across the vast expanse of dendrites. Calcium acts as a critical second messenger in this process, with its cytoplasmic transients tightly controlled by buffers, pumps, exchangers, and Ca^{2+}-binding proteins. Early studies suggested that calcium influx triggered by synaptic receptors remained largely confined to the heads of dendritic spines, spreading only minimally into the shaft. Our findings reveal an additional mechanism that supports long-distance calcium signaling: the organization of dendritic endoplasmic reticulum (ER) and plasma membrane (PM) into regularly spaced junctions. We discovered that dendrites arrange their ER and PM into specialized contacts occurring about every 1 μm, where short ER segments cross the plasma membrane at near-perpendicular angles. These structures form a ladder-like pattern in proximal and medial dendrites. The junctions are enriched with ryanodine receptors (RyRs) and voltage-gated calcium channels, and they are stabilized in part by the scaffolding protein junctophilin 3 (JPH3). Structurally and functionally, these ER–PM junctions are reminiscent of contacts seen in muscle cells and in neuronal subsurface cisternae, both of which are known to couple membrane events with intracellular calcium dynamics. Functionally, ER–PM junctions expand dendritic signaling capacity well beyond what is possible through local receptor activation or action potential backpropagation alone. By providing sites for calcium uptake, release, and amplification, these junctions allow activity arising from a single spine to influence distant dendritic regions. This arrangement creates a mechanism for excitatory inputs to communicate across large portions of the dendritic tree, supporting long-range synaptic integration and crosstalk between inputs that would otherwise remain isolated at the level of individual spines.

Session 7: Ryanodine Receptors and Other ER Channels

Chaired by: Dr. Jan B. Parys

09:00 - 09:30 | Structural & Functional Insights into disease mechanisms and pharmacology of Ryanodine Receptors | Dr. Filip Van Petegem

Dr. Filip Van Petegem
Department of Biochemistry and Molecular Biology, University of British Columbia
filip.vanpetegem@ubc.ca

Biography:
Filip Van Petegem completed his PhD in 2002 at Ghent University, Belgium, where he used X-ray crystallography to study extremophilic enzymes. He then moved to UCSF (San Francisco) to perform postdoctoral studies in the lab of Dan Minor, where he solved the first high-resolution structures of voltage-gated calcium channel subunits. In 2007, he started as an Assistant Professor at the University of British Columbia, was promoted to Associate Professor in 2012, and to Full Professor in 2017. His lab studies the structure and function of excitation-contraction coupling components, including the Ryanodine Receptor and its associated proteins. He is also a scientific advisor to the RyR-1 Foundation and co-founder of Novobind.

Abstract:
Ryanodine Receptors (RyRs) are large ion channels that release Ca^{2+} from the ER and SR. Humans express three isoforms, which differ in functional properties and sensitivities to ligands. RyR1 is abundantly expressed in skeletal muscle and sequence variants have been linked to malignant hyperthermia (MH), a fulminant reaction to volatile anesthetics, and central core disease (CCD). RyR2 is highly expressed in cardiac tissue and has been linked to catecholaminergic polymorphic ventricular tachycardia (CPVT). All three isoforms are also expressed in the brain, where they play roles in learning and memory. Despite presenting major pharmaceutical potential, only one RyR inhibitor – dantrolene - has been approved by the FDA. Due to its hepatotoxicity, its use is limited to acute conditions like MH.

We solved cryo-EM structures of pathogenic variants of RyR1 and show how these affect the conformational landscape in different ways: they either destabilize the closed state, or selectively stabilize the open state, leading to gain-of-function. We mapped binding sites for various modulatory compounds, including scorpion-derived peptides that cause distinct subconductance states in planar lipid bilayer recordings. We also show that RyRs are an important off target for commonly prescribed drugs such as statins. We reveal the binding sites for three atorvastatin molecules per subunit (12 per full tetrameric protein), which selectively stabilize the open state, promoting Ca^{2+} leak.

9:30 - 10:00 | Resting Ca^{2+} Fluxes through ER and Plasma Membrane: Implications for Cell Health and Excitability | Dr. Robert Blum

Dr. Robert Blum
University Hospital Würzburg, Department of Neurology, Würzburg, Germany
blum_r@ukw.de

Biography:
Robert Blum studied Biology at the University of Kaiserslautern, Germany, and held academic positions at LMU Munich, where he completed advanced postdoctoral qualification in Physiology. He later became Senior Lecturer and Group Leader at University Hospital Würzburg. Robert Blum currently leads a research group focusing on translational neurobiology, with particular emphasis on Parkinson's disease and pain. His long-standing basic research interests include plasticity of neural cells, calcium homeostasis, excitability, and signalling.

Abstract:
Ca^{2+}-homeostasis is considered a static process that maintains resting intracellular Ca^{2+} levels. However, in neurons, direct ER Ca^{2+} measurements revealed a resting SOCE that counterbalances ongoing ER Ca^{2+} loss to extracellular sites. Therefore, we asked whether resting SOCE serves functions beyond simple Ca^{2+} maintenance. Because of limitations in neurons, we addressed this question in astrocytes, which allow simultaneous imaging of ER and cytosolic Ca^{2+} dynamics. The data show that the ER Ca^{2+} leak critically shapes canonical Ca^{2+} signals, including IP_3- and Ca^{2+}-induced Ca^{2+} release, as well as intracellular Ca^{2+} oscillations. Disruption of ER Ca^{2+} homeostasis caused prolonged Ca^{2+} influx and mitochondrial fragmentation within 10 min, which could be prevented by Ca^{2+}-free conditions, inhibition of SOCE, or attenuation of STIM. At the molecular level, ER Ca^{2+} depletion or inhibition of resting SOCE induced rapid and widespread transcriptional changes within one hour, including strong upregulation of specific transcription factors. Moreover, PERK, an ER stress sensor, was rapidly phosphorylated following Ca^{2+} removal or inhibition of resting SOCE. We propose that resting Ca^{2+} influx is driven by the ER Ca^{2+} leak and signals cell health. Returning to neurons, we asked whether we can measure resting SOCE in the "noise" of Ca^{2+} imaging data. Initial results suggest that noise-like Ca^{2+} dynamics may reflect homeostatic Ca^{2+} signals and report changes in neuronal excitability.

10:00 – 10:15 | Poster 19: Novel ryanodine receptor isoform (RyR1alt) expressed in the liver and its role in Ca^{2+} signaling | Dr. Juliana Corrêa-Velloso

Juliana C. Corrêa-Velloso, Lawrence D. Gaspers, Harsh Bansia, Mitali Bhate, Amedee des Georges and Andrew P. Thomas
Rutgers University

Abstract:
Inositol-1,4,5-trisphosphate (IP_3) dependent Ca^{2+} oscillations evoked by hormones and other agonists play a key role in the regulation of liver physiology. While these Ca^{2+} oscillations are primarily driven by IP_3 receptor (IP_3R) Ca^{2+} channels, we have also identified a role for ryanodine receptor (RyR) Ca^{2+} channels in hepatocytes. RyRs in SR/ER are well known for controlling skeletal and cardiac muscle contraction, and also play important roles in other excitable cells. Despite being widely expressed, the role of RyR in nonexcitable cells is less understood. Our previous study demonstrated the presence in the liver of a novel alternative isoform of RYR type 1, referred to here as RyR1alt. In hepatocytes, the RyR1alt isoform participates in the generation of Ca^{2+} oscillations and waves that are generated through positive feedback on the IP_3R. To further investigate this novel RyR isoform, the two ends of the RyR1alt were identified by Rapid Amplification of cDNA Ends (RACE) and the entire sequence of the RyR1alt clone was obtained. The RyR1alt mRNA is 6445 bp long compared to the predicted 15220 of the full-length rat RyR1. RyR1alt displays complete homology with the 3' half of RyR1 except for a 50 bp sequence spliced out in the 5'-end of exon 61, resulting in a N-terminal truncated protein. Mass spectrometry analysis confirmed the N-terminal open reading frame sequence, and that RyR1alt comprises 1943 amino acids yielding a protein of 218 kDa, about 40% the size of full-length RyR1. In 3D structural predictions, both homology and AlphaFold models show that RyR1alt adopts a tetramer channel-like fold, similar to the full length RYR1. In HEK cells, overexpressed RyR1alt co-localizes with calnexin, confirming that this novel RyR isoform is localized in the ER. RyR1alt is functional and increases intracellular Ca^{2+} mobilization, exhibiting a more robust Ca^{2+} release in response to carbachol in HEK cells. Additionally, the presence of RyR1alt changes the kinetics of Ca^{2+} release in response to thapsigargin, showing a rapid burst phase compared to the control cells. In permeabilized primary hepatocytes and in intact HEK-RyR1alt cells, cADPR elicits intracellular Ca^{2+} increase, suggesting cADPR as modulator, that enhances RyR1alt-mediated Ca^{2+} release.

10:15 – 10:30 | Poster 36: S-Acylation by zDHHC20 targets STIM1 to cholesterol-rich ER-PM contact sites to sustain Ca^{2+} signalling at the immune synapse | Raphaël Néré

Raphaël Néré[1], Sana Kouba[1], Amado Carreras-Sureda[1], Laurence Abrami[2], Gisou Van Der Goot[2], Nicolas Demaurex[1]
[1]Department of Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland;
[2]Ecole Fédérale Polytechnique de Lausanne, Lausanne, Switzerland

Abstract:
Store-operated calcium entry (SOCE) is a fundamental signalling pathway that underlies the ability of immune cells to mount long-lasting effector responses. Mutations in STIM1 and ORAI1 genes that mediate SOCE cause severe immunodeficiencies. Depletion of endoplasmic reticulum (ER) Ca^{2+} stores activates the ER-resident Ca^{2+} sensor STIM1 by triggering its oligomerisation and translocation to ER-plasma membrane (PM) contact sites, where it traps and gates ORAI1 Ca^{2+}-permeable channels. The sequence of molecular steps underlying STIM1 activation is well characterized, but how S-Acylation impact the targeting of STIM1 to ER-PM junctions and its interaction with ORAI1 remains poorly understood. S-Acylation, a reversible post-translational lipid modification, can target proteins to lipid domains enriched in cholesterol. We previously showed that S-Acylation regulates the activity ORAI1 channels in T-cells. Here, we identify a critical role for S-Acylation in promoting the efficient translocation of STIM1 to ER-PM contact sites. Using biochemical assays, mutagenesis, Ca^{2+} imaging, and TIRF microscopy, we show that STIM1 is S-acylated on cysteine 437, and that zDHHC20 (PAT20) catalyses this lipidation. Substitution of Cys-437 with alanine (C437A) markedly impaired SOCE in HEK-293 and Jurkat T cells and prevented the recruitment of STIM1 to the PM upon store depletion. To identify the conformational activation step controlled by S-Acylation, we used a $STIM1^{1-448}$ truncation mutant that binds to PM phosphoinositides (PI) via a stretch of positively charged residues (4K) within its STIM-ORAI activating region (SOAR), independently of distal cytosolic STIM1 domains. The C437A mutation strongly reduced the recruitment of $STIM1^{1-448}$ to ER-PM junctions, indicating that SOAR-PI interactions are not sufficient for efficient PM trapping. We then used a shorter $STIM1^{1-431}$ mutant lacking the critical cysteine that does not translocate to ER-PM contact sites upon store depletion. Remarkably, re-introducing a cysteine at position 432 restored PM translocation, demonstrating that S-Acylation is sufficient to drive STIM1 membrane targeting, likely by promoting its integration into specific lipid microdomains. To assess the implication of membrane lipids, we acutely depleted cholesterol from cells with methyl-β-cyclodextrin (MβCD). MβCD treatment dramatically inhibited the translocation of wild-type STIM1 to ER-PM contact sites upon store depletion, phenocopying the behavior of the non-acylated C437A mutant, which itself was not impacted by MβCD. These findings indicate that S-Acylation targets STIM1 to ER-PM contact sites enriched in cholesterol. In T-cells, these molecular defects impaired immunological synapse formation and diminished downstream signalling. Wild-type STIM1 accumulated robustly at synapses formed on anti-CD3-coated coverslips or with antigen-presenting Raji cells, whereas the C437A mutant displayed significantly reduced enrichment. Consistently, NFAT-driven transcriptional activity was strongly attenuated in cells expressing the S-Acylation-deficient mutant. Our results identify S-Acylation as a regulatory mechanism controlling STIM1 recruitment to PM domains enriched in cholesterol. This reversible change in lipid affinity enables STIM1 to sequentially interact with PM domains enriched in phosphoinositides via its polybasic and SOAR domains and with cholesterol via its S-Acylated C437, facilitating interactions with ORAI1 channels at immune synapses. These insights shed new light on how the membrane environment shapes Ca^{2+} signalling and may inform strategies aiming to modulate immune responses by targeting lipid modification.

Session 8: Probing the Frontiers in Lysosomal Ca^{2+} Signalling

Chaired by: Dr. Christian Grimm

11:00 - 11:30 | Biased Signalling through Lysosomal Two-Pore Channels | Dr. Sandip Patel

Dr. Sandip Patel
University College London
patel.s@ucl.ac.uk

Biography:
Sandip obtained a BSc in Medical Biochemistry from the University of Birmingham and a PhD in Pharmacology from Cambridge University with Colin Taylor.
He was awarded a Wellcome Trust "Prize" International Travel Fellowship allowing post-doctoral work in the USA with Andrew Thomas, initially at Thomas Jefferson University and then at Rutgers University. He spent the last year of his fellowship back in the UK withAntony Galione at Oxford University.
He went on to obtain a Wellcome Trust Career Development Fellowship allowing him to establish his own lab at Oxford. He also held the Hayward Junior Research Fellowship at Oriel College at this time. He re-located to University College London in 2001 where he is now Professor and Head of the Research Department of Cell and Developmental Biology.

Abstract:
Ion channels possess selectivity filters that are thought to be hardwired to ensure the selective passage of ions. Two-pore channels found on lysosomes however are unusual in switching their selectivity to cations in an agonist-specific manner allowing them to differentially regulate organellar activity. Here I present our recent findings showing that this 'biased signaling' proceeds through molecular determinants remote from the selectivity filter that temper Ca^{2+} permeability to maintain endo-lysosomal well-being.

11:30 - 12:00 | Regulation and Function of Lysosomal Two-Pore Channels | Dr. Michael X. Zhu

Dr. Michael X. Zhu
The University of Texas Health Science Center at Houston
michael.x.zhu@uth.tmc.edu

Biography:
Michael X. Zhu is professor in the Department of Integrative Biology and Pharmacology, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, Texas. He received his B.S. degree in Biology from Fudan University, Shanghai, China, in 1984, and his M.S. and Ph.D. degrees from University of Houston, Houston, Texas, USA, in 1988 and 1991, respectively. He had his postdoctoral training in Cellular and Molecular Biology from 1991-1994 at Baylor College of Medicine. He then worked as an Assistant Researcher in the Department of Anesthesiology at UCLA from 1994 to 1997. In autumn 1997, he went to the Ohio State University to establish his own lab and advanced from the rank of Assistant Professor to Full Professor in the Department of Neuroscience. In 2010, he moved to the University of Texas. Dr. Zhu's research interests encompass several aspects of cell signaling, particularly those involving heterotrimeric G proteins and ion channels that regulate Ca^{2+} signaling. He has published over 200 research papers and review articles on these topics and delivered lectures at numerous international conferences and symposia. Dr. Zhu's main contributions include identification and characterization of multiple TRPC channels in mammalian species and determination of the molecular identity of endolysosomal Ca^{2+} release channels activated by the Ca^{2+} mobilizing messenger, NAADP.

Abstract:
The endolysosomal system plays a pivotal role in cellular function. Before reaching lysosomes for degradation, the endocytosed cargoes are sorted at various stages of endosomal trafficking for recycling and/or rerouting. The proper execution of these processes depends on tightly regulated ion fluxes across endolysosomal membranes. Recent studies have demonstrated the importance of two-pore channels (TPCs), including TPC1 and TPC2, in endolysosomal trafficking. These channels are expressed in the membranes of distinct populations of endosomes and lysosomes, where they respond to nicotinic acid adenine dinucleotide phosphate (NAADP) and phosphatidylinositol 3, 5-bisphosphate [PI(3,5)P2] to conduct Ca^{2+} and Na^{+} release from these acidic organelles. Here, I will discuss the potential implications of Ca^{2+} and Na^{+} fluxes mediated by TPCs across endolysosomal membranes in the physiological and pathophysiological functions of these organellar channels.

12:00 – 12:15 | Poster 44: Investigating the interplay between lysosomal and ER Ca^{2+} channels | Dr. Siddhi Shetty

Investigating the interplay between lysosomal and ER Ca^{2+} channels

Siddhi Shetty[1], Vikas Arige[3], Sundeep Malik[1], Franz Bracher[2], Marco Keller[2], Sandip Patel[3], David I. Yule[1]
[1]Department of Pharmacology and Physiology, School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642. [2]Department of Pharmacy-Center for Drug Research, Ludwig-Maximilian University, Munich, Germany. [3]Department of Cell and Developmental Biology, University College London, London, UK.

Abstract:
Calcium (Ca^{2+}) is a ubiquitous signaling messenger that regulates diverse physiological functions including contraction, gene expression, metabolism, and cell proliferation. Moreover, Ca^{2+} is involved in the activation of diametrically opposite cellular responses such as cell proliferation and cell death exemplifying the need for exquisite specificity of Ca^{2+} signaling events. Many of these Ca^{2+} signaling events occur in specific nanodomains where intracellular organelles interact with each other or with the plasma membrane. The endoplasmic reticulum (ER) is the largest Ca^{2+} store in the cell. Inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) are the major Ca^{2+} release channels located in the ER membrane and exhibit subtype specific regulation and properties. In addition, acidic organelles such as lysosomes and endosomes also participate in intracellular Ca^{2+} signaling. Two pore channels (TPCs) are a class of ubiquitously expressed ion channels that reside in this compartment. These channels switch their ion permeability when stimulated with nicotinic acid adenine dinucleotide phosphate (NAADP) or upon direct activation with phosphatidylinositol -3,5-bisphosphate ($PI(3,5)P_3$). The discovery of cell-permeable, specific, TPC2 agonists that mimic NAADP and $PI(3,5)P_2$ has made it possible to study signaling through TPC2 in an endogenous setting. TPC2-A1-N mimics NAADP, promoting Ca^{2+} currents, while TPC2-A1-P is a $PI(3,5)P_2$ mimetic, activating robust Na^+ currents. We hypothesize that local Ca^{2+} signals originating from TPC2 sensitize ER Ca^{2+} release channels resulting in global Ca^{2+} responses. Preliminary data from our lab suggests that TPC2-evoked Ca^{2+} signals sensitize IP_3Rs and RyRs; however, the spatiotemporal constraints and characteristics of this crosstalk remain unresolved. Data will be presented using molecular tools combined with state-of-the-art live-cell Ca^{2+} imaging techniques investigating the functional and structural interactions between endolysoosomal TPC2 and the individual isoforms of ER-resident IP_3Rs and RyRs.

12:15 – 12:30 | Poster 6: NAADP–dependent Ca^{2+} signaling in natural killer cell activity | Dr. Björn-Philipp Diercks

Fynn Gerlach[1], Franziska Möckl[1], Annika Ahrenstorf[2], Benedetta Padoan[2], Marcus Altfeld[2], Björn-Philipp Diercks[1]
[1]The Calcium Signalling Group, Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg–Eppendorf, Hamburg, Germany. [2]Research Department Virus Immunology, Leibniz Institute for Experimental Virology, Hamburg, Germany.

Abstract:
Intracellular Calcium (Ca^{2+}) signaling is crucial for the activation and function of a variety of immune cells, including NK cells. Nicotinic Acid adenine diphosphate (NAADP) is the most potent calcium-releasing second messenger known to date. While extensive data are available on NAADP–dependent Ca^{2+} mobilization in T cells, very little is known about it in NK cells and the possible role during NK cell education. Previously, we showed that educated NK cells exhibit a higher global Ca^{2+} response upon receptor stimulation, indicating stronger signaling events taking place in these educated NK cells. To investigate NAADP–dependent Ca^{2+} signaling in NK cells, we used fluorescence microscopy with a human NK cell line (KHYGI) and primary human NK cells. Stimulation of the NAADP pathway using the recently published synthetic membrane-permeable NAADP analogue MASTER-NAADP increased both global and local Ca^{2+} signals in KHYGI cells. In line, inhibition of NAADP signaling by the NAADP antagonist BZ194 decreased global Ca^{2+} responses and initial Ca^{2+} microdomains in these cells, demonstrating the crucial role of NAADP in Ca^{2+} release in NK cells. Next, we studied the effect of BZ194 on Ca^{2+} signals during target cell contact (K562) in primary human NK cells using a newly developed microscopy-based live-cell killing assay. Here, in parallel, the Ca^{2+} response in NK cells and in target cells was monitored. Blocking the NAADP signaling pathway resulted in significantly reduced peak amplitude, peak number, and overall AUC in NK cells. To understand the downstream function of NAADP-dependent Ca^{2+} signals in NK cells, we evaluated the impact of BZ194 on primary human NK cells 24 hours after co–incubation with K562 cells, using a Lactate Dehydrogenase (LDH) release assay and surface marker analysis (CD107a and TNF–α) to assess degranulation. BZ194 reduced both killing capacity and the expression of degranulation markers and pro-inflammatory cytokines. In summary, our data suggest that NAADP-dependent Ca^{2+} signals are essential for human NK cell cytotoxicity.

Session 9: Flash Talks

Moderator: Dr. Malene Brohus

12:30 - 13:00 Flash Talks | 11 talks 2 min each

Poster 13

Dr. Femke Speelman-Rooms
Unraveling the Ca^{2+}-independent impact of BAPTA–AM on autophagic pathway
KU Leuven, Babraham Institute Cambridge

Poster 28

Dr. Martin Falcke
The Information in IP3-induced Ca^{2+} Spike Sequences
Max Delbrück Center for Molecular Medicine Berlin

Poster 38

Dr. Razan Orfali
Mechanistic Evidence for Dual-Channel Modulation by Eact via TRPV4 in Cystic Fibrosis
Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia

Poster 46

Dr. Subash Chinnathambi
Microglial CX3CR1 receptor undergoes endocytosis and vesicular trafficking upon extracellular Tau exposure
National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, India

Poster 56

Yongsoo Park
Hyperexcitability in TRPC4 knockout hiPSC-derived neurons is reversed by endocannabinoid 2 receptor agonists for potential therapeutics of autism
Hamad Bin Khalifa University (HBKU), Qatar Biomedical Research Institute

Poster 7

Dr. Carlos Villalobos
Transcriptional analysis of calcium remodeling in samples of human glioblastoma and surrounding tissue with high and low probability of tumor recurrence as shown by AI
Institute of Biomedicine and Molecular Genetics of Valladolid (IBGM), University of Valladolid and CSIC, Spain

Poster 14

Dr. Franziska Moeckl
MASTER-NAADP – Membrane permeAble, STabilized, biorEversibly pRotected precursor of the Ca^{2+} mobilizing second messenger NAADP
University Medical Center Hamburg–Eppendorf, Germany

Poster 18

Dr. Joshua Graham
Long-QT syndrome associated calmodulin mutations disrupt key phospho-regulatory proteins involved in calcium cycling
University of Liverpool, UK

Poster 27

Dr. María Teresa Alonso
Mechanism of intracellular Ca^{2+} waves upon mechanical stimulation of retinal epithelium cells
Instituto de Biomedicina y Genética Molecular de Valladolid (IBGM), Universidad de Valladolid - CSIC, Spain

Poster 53

Xiaoxuan Lin
Functional Studies of ATP Binding Sites in Inositol (1,4,5)-Trisphosphate receptors
University of Rochester Medical Center, USA

Poster 40

Dr. Rute Isabel Honorio
Decoding the role of the cyclic GMP – PKG and downstream organelles to control the last step of the intraerythrocytic cycle of the malarial parasite Plasmodium falciparum
Rutgers University and University of São Paulo

09:00 - 10:00 | James Putney Keynote | IP3R: Defining Ca^{2+} Signals in Time and Space | Dr. David Yule

Chaired by: Dr. Gaiti Hasan

Dr. David I. Yule
University of Rochester
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Biography:
I received a BSc. in Pharmacology from Portsmouth Polytechnic in the United Kingdom. (10/1983-6/1986). During my undergraduate studies, I became fascinated by signal-transduction and decided to continue my graduate education by completing a PhD at the University of Liverpool, UK. My research explored intracellular calcium signaling in exocrine cells (09/1986-06/90) working with Dr. David Gallacher. I moved to The University of Michigan to continue studies on Ca2+ signaling in exocrine cells working with Prof. John Williams. In 1998, I moved my laboratory to the Department of Physiology and Physiology at the University of Rochester and was appointed as an Assistant Professor (1998), Associate Professor (2003) and Full Professor (2009).

IP3R: Defining Ca2+ signals in Time and Space

Abstract:
Agonist- stimulated Ca^{2+} signals occur following Gq-coupled receptor activation of phospholipase C and the production of inositol 1,4,5 trisphosphate (IP_3) resulting in Ca^{2+} release from the endoplasmic reticulum via IP_3 receptors (IP_3R). In the pancreas and salivary glands, this canonical pathway results in Ca^{2+} signals with defined spatiotemporal characteristics which are important for activation of the primary effectors important for fluid and protein secretion. Indeed, Ca^{2+} signals and secretion are largely absent when IP_3R are ablated in exocrine glands- confirming the absolute requirement for IP_3R Ca^{2+} release channels for physiological function. The central tenet of our research is that the spatiotemporal patterns of Ca^{2+} signals are controlled by the abundance, localization and regulation of IP_3R in individual cells. Data will be presented using exocrine cells as an exemplar, detailing how physiological Ca^{2+} signals are defined by localization and subtype regulation of IP_3R and how aberrant IP_3R function results in dysregulated Ca^{2+} signals that are often associated with disease.

Session 10: NAADP Signaling

Chaired by: Dr. Andreas H. Guse

10:30 - 11:00 | NAADP in the Early Phase of T Cell Activation | Dr. Feng Gu

Dr. Feng Gu
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Biography:
I completed my Bachelor of Medicine at Southern Medical University in Guangzhou, China. When I was doing my bachelor's degree, I figured out that I was more interested in what is going on behind the symptoms of diseases in our bodies. On top of that, I wanted to experience a different culture. That's why I decided to switch to sciences and started learning German. Then, I completed my Master of Science and PhD at the University of Hamburg in Germany. I'm currently working as a research scientist and junior group leader at the University Medical Center Hamburg-Eppendorf.

Abstract:
NAADP is the most potent Ca^{2+}-mobilizing second messenger to date. It is synthesized within seconds after the stimulation of T cell receptor (TCR) and contributes to the formation of Ca^{2+} microdomains below the plasma membrane, which triggers subsequent global Ca^{2+} signaling. Dual NADPH oxidase 2 (DUOX2) is the NAADP-forming enzyme in T cells. In $Duox2^{-/-}$ T cells, less Ca^{2+} microdomains are formed in the first seconds post TCR/CD28 stimulation, and10 min after stimulation, delayed and reduced global Ca^{2+} signals were observed, whiledeletion of CD38, NOX1, or NOX2 (2 other members of the NADPH oxidase family) do not alter Ca^{2+} signals after T cell activation.

DUOX2 requires several factors for its enzyme activity to produce NAADP: 1) DUOX2 contains an EF-hand motif in the cytosol, and a higher Ca^{2+} concentration increases its enzyme activity. In intact T cells, local Ca^{2+} signals evoked by T cell contact to laminin-1 or collagen type IV, or ATP-mediated Ca^{2+} entry via P2X4 form small Ca^{2+} microdomains independently of TCR stimulation. 2) Phosphorylation increases DUOX2's enzyme activity. It is phosphorylated by two protein kinases in T cells, namely PKC θ and PKA Cβ2. 3) DUOX2 requires O2 as electron acceptor. Accordingly, hypoxia reduces its enzyme activity. Delayed and reduced global Ca^{2+} signals were observed in the T cells in hypoxia.

11:00 - 11:30 | Two-Pore Channels as Regulators of Inter-Organellar Calcium Homeostasis and Immune Cell Signalling | Dr. Susanna Zierler

Susanna Zierler
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Biography:
Susanna Zierler leads the Institute of Pharmacology at JKU Linz and is a group leader at LMU Munich. She holds a PhD in cell biology and completed postdoctoral training in biophysics. Her research focuses on ion channel physiology and pathophysiology, with a special interest in TRPM7 and two-pore channels (TPC1/TPC2), aiming to identify novel pharmacological targets for inflammatory diseases, allergies, and cancer.

Abstract:
The endo-lysosomal (EL) two-pore channels, TPC1 and TPC2, are crucial for intra-cellular and inter-organellar ion homeostasis, vesicle fusion and cycling, viral uptake and secretion. They are activated by $PI(3,5)P_2$ or NAADP together with accessory proteins, respectively. We recently found that TPC1-deficient ($Tpc1^{-/-}$) mice develop enhanced systemic anaphylaxis due to altered inter-organellar calcium (Ca^{2+}) homeostasis in mast cells. Closer structural analysis of the observed EL-endoplasmic reticulum (ER) interactions, using transmission electron microscopy (TEM), revealed large three-dimensional contact areas between these organelles, which depend on TPC activity. Utilizing an atopic dermatitis model, we find enhanced ear swelling, infiltration of immune cells and increased IL-6 release in $Tpc1^{-/-}$ mice upon repeated allergen exposure. To determine the impact of resident macrophages in this model, we utilized pharmacologic and genetic tools ($TPC1^{-/-}$, $TPC2^{-/-}$, $TPC1/2^{-/-}$ J774 macrophages) and performed detailed correlative light and electron microcopy (CLEM), immunogold labeling and Ca^{2+} imaging experiments. Collectively our results demonstrate that in macrophages TPC1 is the key regulator of endocytic trafficking of early and recycling endosomes and contributes to auto-phagolysosome function and inter-organellar Ca^{2+} homeostasis, while TPC2 governs lysosomal homeostasis and autophagic progression, thus highlighting their complementary role in cell signaling in macrophages.

11:30 - 12:00 | Organellar Ca^{2+} Regulators (OCaRs) Encoded by TMEM63 Proteins Determining NAADP-mediated Ca^{2+} Release from Acidic Intracellular Stores | Dr. Marc Freichel

Dr. Marc Freichel
Institute of Pharmacology, Dept. of General Pharmacology, Heidelberg University
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Biography:
After completing medical studies, an MD thesis in Biochemistry, and clinical training in cardiology and endocrinology, Dr. Freichel began his research on TRP channels using transgenic animal models in 1995. Following a Professorship at Saarland University (2004–2011), he joined Heidelberg University as Director of Dept. of General Pharmacology at the Institute of Pharmacology in 2011. His research focuses on ion channels in plasma and endolysosome membranes, and their role in heart disease, metabolism, neuroinflammation and mast cells. He is co-founder of CalTIC, a spin-off company developing TRPC inhibitors. He is involved in several collaborative research networks and coordinates graduate school in CardioScience, teaches pharmacology, and is a member of the Review Board for Medicine of the DFG.

Abstract:
TMEM63 proteins (TMEM63A, TMEM63B, TMEM63C) are the closest homologues of OSCAs, a family of hyperosmolality-gated calcium-permeable channels [1]. TMEM63A is localized in the membrane of lysosomes and secretory granules in pancreatic acinar cells as revealed by microscopic analysis in cells of TMEM63A-YFP knock add-on mice and high-resolution organellar proteomics, indicating its dynamic subcellular (re-)distribution during endo-/exocytosis [2], [3]. Because of its subcellular localization and function, we dubbed TMEM63A Organellar Calcium Regulator protein 1 (OCaR1): Using GCamP6 targeted directly to TPC2-containing vesicles, we showed that TMEM63A/OCaR1 controls Ca^{2+} release from acidic Ca^{2+} stores, and in lysosomal patch-clamp recordings, it functionally antagonizes TPC1 and TPC2 channels. Accordingly, OCaR1 deletion results to extensive Ca^{2+} release from NAADP-responsive, acidic Ca^{2+} stores, thereby exacerbating the disease phenotype in murine models of severe and chronic pancreatitis [3].
In my ESC lecture, I will provide evidence that OCaR proteins also act as central orchestrators of Ca^{2+} release originating from NAADP-sensitive acidic organelles, mediated via TPC channels, in other cell types.

Session 11: Late Breaking

Chaired by: Dr. Barbara Niemeyer

13:30 - 14:00 | Calcium Signaling at the Interface between Microglia, Neuroinflammation, and Inflammation-induced Depression Behaviors | Dr. Murali Prakriya

Dr. Murali Prakriya
Feinberg School of Medicine, Northwestern University
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Biography:
Dr. Murali Prakriya, PhD, is the Magerstadt Professor of Pharmacology and a Professor in the Departments of Pharmacology and Medicine at the Feinberg School of Medicine, Northwestern University. His research focuses on the molecular and cellular mechanisms of intracellular calcium (Ca^{2+}) signaling, with particular emphasis on store operated calcium channels (SOCs), a vital class of plasma membrane Ca^{2+} channels activated by depletion of endoplasmic reticulum calcium stores. He is internationally recognized for his pioneering work on Orai channels, the pore forming subunits of SOCE/CRAC channels. Mutations in these channels lead to severe human immunodeficiency, muscle weakness, and neurological abnormalities, highlighting the clinical significance of Dr. Prakriya's work.
His laboratory integrates electrophysiology, imaging, molecular biology, structural simulations, and genetically engineered mouse models to investigate how Orai channels regulate gene expression, immune cell function, and neuronal physiology. His work extends into understanding how calcium signals influence neuroinflammation, metabolic activity, synaptic communication, and cognition.

Abstract:
Microglia are the primary resident immune cells of the central nervous system that play key roles in brain inflammation in many neurological and psychiatric diseases. Microglial functions are tightly regulated by dynamic transitions between homeostatic and reactive cell states, which can exacerbate or resolve inflammation. However, the molecular mechanisms governing these cell state transitions remain poorly understood. In this talk, I will describe our recent work identifying the Orai1 calcium channel as a key regulator of microglial plasticity and inflammation-induced behavioral dysfunction in mice. Through integrated transcriptomic and metabolomic analyses, we find that conditional deletion of Orai1 in microglia suppresses proinflammatory gene programs linked to immunity, cell cycle progression, and metabolism, while enhancing anti-inflammatory and pro-resolving factors such as BDNF, ARG1, and itaconic acid. In vivo, Orai1 deficiency also attenuates microglial/astrocyte reactivity and reduced cytokine (IL-1β, IL-6) levels in response to systemic lipopolysaccharide (LPS) challenge. In line with these cellular changes, microglial Orai1 cKO mice were protected against LPS-induced changes in motivational behaviors, including impairments in reward-seeking and escape responses. These findings identify Orai1 signaling as a central checkpoint in microglial cell state transitions and neuroimmune responses, highlighting its therapeutic potential for neuroinflammatory disorders.

11:30 - 12:00 | Organellar Ca^{2+} Regulators (OCaRs) Encoded by TMEM63 Proteins Determining NAADP-mediated Ca^{2+} Release from Acidic Intracellular Stores | Dr. Marc Freichel

14:00 - 14:30 | How Does the Energetically Expensive Mammalian Brain Adapt in Times of Food Scarcity?| Dr. Zahid Padamsey

Dr. Zahid Padamsey
Weill Cornell Medicine-Qatar
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Biography:
Dr. Zahid Padamsey is an Assistant Professor of Research in Cell and Developmental Biology at Weill Cornell Medicine-Qatar (WCM-Q). He received his MSc and PhD from the University of Oxford (2014, 2017) and his BSc (Hons) from the University of Toronto (2014). His research explores how brain energy and function is regulated by diet, particularly in the context of metabolic and neurological diseases. His lab uses state-of-the-art calcium imaging, electrophysiology, and oxygen-sensing techniques in vivo and in vitro to monitor neuronal activity and energy use.

Abstract:
Information processing in the mammalian neocortex is energetically expensive. Here, using in vivo two-photon calcium and ATP imaging, we examine how the mouse visual cortex adapts to food scarcity. We find that somatic calcium signalling and neuronal spiking during visual stimulation are largely preserved under food restriction. Nonetheless, neurons expend less ATP owing to a reduction of synaptic and resting membrane conductances. These changes, whilst saving energy, degrade the precision with which visual information is encoded in neuronal spiking. Finally, we show that energy-saving adaptations in the neocortex are driven by a reduction of circulating leptin levels, which are driven by a loss of fat mass. Our findings reveal that metabolic state potently regulates neuronal energy expenditure and information coding in the mammalian neocortex.

4:30 – 14:45 | Poster 32: Two-pore channel-2 controls calmodulin-dependent STIM1 inactivation | Nicolas Demaurex

Nicolas Demaurex
University of Geneva, Switzerland & Yonsei University, Republic of Korea

Abstract:
Lysosomes and the endoplasmic reticulum (ER) are Ca^{2+} stores that interact to generate Ca^{2+} signals regulating fundamental cellular processes. NAADP-sensitive TPC2 channels on lysosomes generate local Ca^{2+} elevations that sensitize ER Ca^{2+} release channels, triggering global Ca^{2+} signals. The ensuing ER Ca^{2+} depletion activates store-operated Ca^{2+} entry (SOCE) operated by STIM1-gated ORAI1 channels to sustain long-lasting Ca^{2+} signals. How TPC2 channels interact with STIM1 to integrate distinct intra and extracellular cues is unclear. Here, we show that TPC2 activation inhibits SOCE by enforcing rapid and persistent Ca^{2+}-CaM-dependent inactivation of the STIM1 channel activating domain (CAD). The TPC2 agonists NAADP and TPC2-A1-N abrogated SOCE in multiple cell lines and enhanced the slow Ca^{2+} dependent inactivation (SCDI) of STIM1-gated ORAI1 channels. TPC2 engagement triggered lysosomal Ca^{2+} release and mobilized ER Ca^{2+} stores independently of inositol trisphosphate receptors but prevented RFP-STIM1 recruitment to the TIRF plane by thapsigargin and disassembled RFP-STIM1 clusters forming after store depletion, preventing and acutely reversing SOCE. These effects persisted in STIM1 mutants truncated after the CAD and were prevented by genetic or pharmacological invalidation of TPC2, Calmodulin (CaM) inhibition, and cytosolic Ca^{2+} chelation. We conclude that Ca^{2+} ions released by TPC2 channels on lysosomes regulate CaM-dependent SOCE deactivation.

14:45 – 15:00 | Poster 9: Orai2-dependent alteration of Ca^{2+} profile and suppressive capacity of human regulatory CD4 T cells| Dalia Alansary

Dalia Alansary
Saarland University, Germany

Abstract:
A tightly balanced immune response by different T cell subpopulations plays an essential role driving disease initiation and/or progression in autoimmune diseases but also tumor development. Moreover, dysregulated Ca^{2+} signals result in a plethora of T cell dependent diseases. The differential role of Ca^{2+} in regulation of subpopulation dependent-functions is yet poorly understood. We found that in vitro polarized regulatory $CD4^+$ T cells (Treg) have higher levels of store operated Ca^{2+} entry (SOCE) than corresponding conventional T cells (Tcon), a phenotype also recapitulated by in vivo differentiated Treg. An mRNA array and subsequent meta-analysis identified candidate genes potentially underlying differential Ca^{2+} signatures. The current work aims to investigate how these identified Ca^{2+} signaling-related genes contribute to the modulation of Treg suppressive function. A key altered candidate is Orai2. We have previously shown that Orai2 acts as a negative regulator of SOCE which together with the altered Ca^{2+} profile of Treg and the new finding that Orai2 is downregulated in Treg suggest a crucial role of Orai2 in modulation of Ca^{2+} dependent functions of Treg. Therefore, using combined approaches of expression analysis, intracellular Ca^{2+} measurements, and suppression assays, we set out to explore if and how Orai2 modulates the suppressive function of Treg. Our data demonstrate that heterologous overexpression of Orai2 in human Tregs reduces both cytosolic Ca^{2+} uptake and their suppressive capacity. To gain insight into the Ca^{2+} dependence of the altered suppressive capacity, we explored the cytokine and transcription factor profiles of Tregs. Furthermore, we are investigating potential Orai2-dependent alterations of metabolic profile and ROS signaling in Treg. Combined, these approaches will provide deeper mechanistic insights underlying Orai2 dependent reduction of Treg suppressive function.

15:00 – 15:15 | Poster 15: Ryanodine Receptors Dynamically Control Lysosomal Trafficking in Neurons | Dr. Geert Bultynck

Dr. Geert Bultynck
KU Leuven, Belgium

Abstract:
Ca^{2+} signaling at membrane contact sites critically controls cell function, while its dysregulation drives pathogenesis. Here, we identify ATP6v0a1 as a novel partner of ryanodine receptors (RyRs), contributing to ER-lysosomal tethering and controlling intracellular availability of lysosomes to participate in autophagic flux. Recent work revealed that ER-resident RyRs suppress autophagosome turnover by the lysosomes. In familial Alzheimer's disease, hallmarked by excessive RyR activity and lysosomal dysfunction, inhibition of RyR activity restored autophagic flux by normalizing lysosomal vacuolar H+-ATPase (vATPase) levels. Yet, the mechanisms by which RyRs control lysosomal function and how it involves the vATPase remain unknown. We found that RyR2, the neuronal RyR isoform, directly interacts with the ATP6v0a1 subunit of the vATPase, contributing to ER-lysosomal contact site formation. Moreover, the RyR2::ATP6v0a1 complex was impaired in cortical samples derived from 3xTG Alzheimer Disease mouse models. ATP6v0a1 itself suppressed RyR2-mediated Ca^{2+} release, which impacts the handling of lysosomal cargo. Pharmacological inhibition of RyR activity was sufficient to mimic these effects on lysosomal cargo handling. In iPSC-derived cortical neurons, endogenous RyRs promoted lysosomal exocytosis, while inhibiting this spontaneous RyR activity limits lysosomal exocytosis, thus retaining lysosomes inside cells. The latter increases ER-lysosomal contact site formation, rendering lysosomes more available for autophagic flux. This places RyRs as dynamic control centers directing lysosomes towards secretion versus autophagy.

POSTER ABSTRACTS

Abstract 1

Abdella M Habib
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MDFIC2 tunes PIEZO channel activity to alleviate mechanical allodynia

Abdella M Habib[1], Shengnan Li[2], Chenjing Zhang[3], Meijun Ji[3], Nancy Osorio[4,5], Virginie Penalba[4,5], Jesus M Torres[2,6], Samuel J Gossage[2], Mehdi A Rezai[2], Amy F Geard[7], Ahad A Rahim[7], Ahmed M M Mahmoud[2,8], Sonia Santana-Varela[2], Jun Zhou[2,9], Jing Zhao[2], John N Wood[2], Andrei L Okorokov[2], Xuelong Zhou[10], James J Cox[2], Bertrand Coste[4,5]

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Abstract:
Mechanical allodynia is a hallmark of neuropathic pain and arises, in part, from aberrant mechanotransduction in sensory neurons. PIEZO1 and PIEZO2 are mechanosensitive, Ca^{2+}-permeable ion channels that contribute to touch sensation and pain; yet, the molecular mechanisms that tune PIEZO gating in native sensory neurons remain incompletely understood. Here, we identify MDFIC2 as a sensory-neuron-enriched modulator of PIEZO channels. Transcriptomic and immunohistochemical analyses show that MDFIC2 is preferentially expressed in $PIEZO2^{+}$ nociceptive dorsal root ganglion neurons, with enrichment in $IB4^{+}$ non-peptidergic populations. Whole-cell mechano-clamp recordings in heterologous expression systems demonstrate that MDFIC2 reshapes PIEZO1- and PIEZO2-mediated mechanically evoked currents by shifting activation thresholds and altering inactivation kinetics, thereby modifying force-response relationships without abolishing mechanosensitivity or inducing constitutive channel activity. In vivo, intrathecal knockdown of Mdfic2 in naïve mice selectively enhances mechanical sensitivity without affecting thermal nociception or motor performance. Conversely, AAV-mediated MDFIC2 overexpression suppresses established mechanical allodynia in a spared nerve injury model. Together, these data identify MDFIC2 as a context-dependent regulator of PIEZO channel gating that controls mechanosensory gain in nociceptors and support the PIEZO-MDFIC2 axis as a tractable therapeutic entry point for the mechanical allodynia present in neuropathic pain.

Abstract 2

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Identifying Brain-Permeable CRAC Channel Inhibitors Targeting CNS Disorders

Aida Ashrafzadeh, Marcos Rubio Alarcon, Graham Ladds, Taufiq Rahman

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Abstract:
Store-operated calcium entry (SOCE), largely mediated by calcium release–activated calcium (CRAC) channels, is triggered by endoplasmic reticulum (ER) calcium depletion and is a key regulator of intracellular calcium dynamics. Beyond replenishing ER stores, CRAC-mediated calcium signals activate the downstream nuclear factor of activated T cells (NFAT) pathway, which controls gene expression programs critical for immune and neuronal function. CRAC channels are valid drug targets, and small-molecule inhibitors have been developed to modulate their activity in peripheral inflammatory and autoimmune diseases. CRAC upregulation has also been linked to CNS disorders, including autoimmune encephalomyelitis, neurodegeneration, neuropathic pain, and brain cancers. Nonetheless, development of brain permeable inhibitors remains limited. This study aims to identify and characterize brain-penetrant SOCE inhibitors using an integrated computational and experimental approach. 731 confirmed brain-permeable compounds were virtually screened using ROCS and EON (OpenEye Scientific Software) and ranked based on their similarities in 3D shape and surface electrostatics with few well-known CRAC inhibitors used as queries. This led to the shortlisting 20 hits, designated C1 to C20, were then evaluated against thapsigargin-evoked SOCE in Jurkat T cells using a FlexStation-based Ca^{2+} flux assay with Calbryte 520. Initial screening identified several compounds reducing Tg-evoked SOCE at 10 µM. Those producing ≥30% inhibition of $[Ca^{2+}]i$ were tested at 3 µM; compounds with ≥20% inhibition were advanced to dose response assays, yielding four compounds of interest (C1–C4) with an IC50 values within nanomolar range (C2) and three compounds in the micromolar range (C1, C3, C4). To further characterize these hits as CRAC inhibitors, I assessed how these molecules might affect NFAT activation, a downstream readout of SOCE-mediated Ca^{2+} signalling, in Jurkat-Lucia™ NFAT-CD28 reporter cells. NFAT activation was induced by TCR (anti-CD3/CD28 Abs) or Tg stimulation. Both Pyr6 and C3 robustly suppressed NFAT activation following TCR and Tg stimulation, even though C3 exhibited only moderate SOCE inhibition in the Ca^{2+} assay. C1 and C2 did not suppress NFAT at all concentrations where they inhibited CRAC, with effects only observed at higher concentrations. NFAT activation is regulated by intracellular Ca^{2+} dynamics and a coordinated signalling cascade initiated by various stimuli, including the TCR, that involves upstream and downstream regulators such as calcineurin. The observation that CRAC inhibition of those hits did not always produce significant reduction in NFAT activation is suggestive of involvement of some compensatory cellular factors that may or may not involve the known targets of these molecules. In CNS disorders, inhibiting SOCE can treat either upregulated CRAC activity or pathological calcium overload where CRAC channels are the primary entry point. Thus, C3, with its strong NFAT suppression, may be advantageous in neuroinflammatory diseases, while C1 and C2, with weaker effects, might be better suited for conditions where immune function should be preserved. This ongoing work and findings so far highlight the value of integrating in silico screening with functional validation to accelerate CNS drug discovery targeting CRAC channels.

Abstract 3

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Functional Consequences of Calcium Binding Loop Position 12 Variants in Human Calmodulin

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Abstract:
Background: Calmodulin (CaM) is a Ca^{2+} sensor protein, and disease-associated CaM mutations cause calmodulinopathy with severe cardiac arrhythmias, including long-QT syndrome. These mutations impair CaM-dependent regulation of cardiac ion channels, particularly the L-type Ca^{2+} channel CaV1.2. CaM binding to the CaV1.2 IQ-domain is required for Ca^{2+}-dependent inactivation, which limits Ca^{2+} entry and helps maintain electrical stability in cardiomyocytes. Accurate Ca^{2+} binding by CaM is therefore essential for proper channel regulation. Several disease-associated CaM mutations target residues directly involved in Ca^{2+} coordination, including the conserved Glu at position 12 of the EF-hand Ca^{2+}-binding loop. This residue is unique because it is the only coordinating amino acid that donates two oxygen atoms directly to Ca^{2+} binding, thereby completing and stabilizing the canonical EF-hand coordination geometry. Despite its clear clinical relevance, the functional consequences of identical substitutions in the position-12 residue in the different EF-hands of CaM have not yet been addressed. Research Question: In this study, we investigate the Ca^{2+}-binding properties of CaM variants carrying position-12 Glu to Ala and Glu to Lys substitutions within each of the four individual EF-hands, four of which corresponds to mutations observed in cardiac arrhythmia patients. We also examined their interaction with the CaV1.2 IQ-domain. Methods: We expressed and purified Ca^{2+}-binding loop position 12 Glu to Ala and Glu to Lys CaM variants, and Ca^{2+} affinity was assessed by intrinsic fluorescence-based Ca^{2+} titrations; Phenylalanine fluorescence for the N-domain and Tyrosine fluorescence for the C-domain. Ca^{2+}-dependent binding to the CaV1.2 IQ-domain was measured using a fluorescence anisotropy-based titration assay, using a TAMRA-labeled CaV1.2 peptide. Results: Not surprisingly, all Ca^{2+}-binding loop position-12 substitutions reduced Ca^{2+} affinity. However, we observed clear EF-hand-specific differences. Some of the substitutions even diminished the ability to bind Ca^{2+} in the entire domain and thus seem to affect the paired neighbor EF-Hand. We also observe some evidence of inter-Lobe cooperativity being affected by C-domain substitutions. In line with this, all variants displayed a severe effect in the Ca^{2+}-dependent interaction with the CaV1.2 IQ-domain, again with different effects between the affected EF-Hands. Conclusion: Identical position-12 substitutions in CaM show clear EF-hand-specific effects on Ca^{2+} binding and CaV1.2-IQ domain interactions, suggesting that mutations in different EF-hands may contribute differently to the phenotypic expression of calmodulinopathies, including CaM-linked cardiac arrhythmias.

Abstract 4

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ITPR3 Variant in an Immunodeficient Patient results in a Leaky Defective IP3R3 Calcium Channel

Asha Elmi[1], Satanay Hubrack[1], Fang Yu[2], Vikas Arige[1], Rafah Mackeh[1], Bernice Lo[1], Amel Hassen[1], David Yule, Khaled Machaca[2]

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Abstract:
Calcium ions function as critical second messengers in immune cells, where precise regulation of store-operated calcium entry (SOCE) is essential for lymphocyte activation, and its disruption can result in severe immunodeficiency. Calcium release from endoplasmic reticulum (ER) stores following T-cell receptor (TCR) stimulation is mediated by inositol 1,4,5-trisphosphate receptors (IP3Rs), which form homo- or hetero-tetramers of three isoforms (IP3R1–3). Here, we report a de novo heterozygous missense variant in ITPR3 (c.7570C>T; p.R2524C), encoding IP3R3, identified by whole-genome sequencing and confirmed by Sanger sequencing in a patient of Egyptian Arab descent with immunodeficiency. Functional analyses demonstrated that the variant did not affect ITPR3 transcript or protein expression, as assessed by quantitative real-time PCR and immunoblotting. However, calcium flux studies revealed significantly reduced ER calcium stores in patient lymphocytes, accompanied by impaired calcium signaling following TCR stimulation. Functionally the IP3R3 R2524 mutant is sensitized to IP3 compared to WT. Consistently, T-cell proliferation assessed by CFSE division assay after anti-CD3/anti-CD28 stimulation was markedly reduced. Immunophenotyping showed a significant decrease in naïve CD^+ and $CD8^+$ T-cell compartments, accompanied by increased in naïve B cells, and reduced memory B cells, correlating with decreased immunoglobulin levels and the clinical need for monthly intravenous immunoglobulin (IVIG) replacement therapy. Collectively, these findings establish a functional link between defective IP3R3-mediated calcium release and immunodeficiency, expanding the spectrum of inborn errors of immunity associated with impaired ER calcium mobilization and underscoring the heightened sensitivity of lymphocytes to disruptions in calcium signaling pathways.

Abstract 5

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ZP2-Mediated Sperm Recognition and Morphological Characterization of Human Sperm Populations

Dania Hamada, Manar Ataa, Maha Abdulla, Maria Esteves, Abbirami Sathappan, Fadi Choucair, Johnny Awwad, & Matteo Avella*

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Abstract:
Successful fertilization in mammals depends on selective interactions between sperm and the egg's extracellular matrix, the zona pellucida, which in humans is composed of four glycoproteins (ZP1- ZP4). Among these, ZP2 is the primary ligand mediating sperm recognition and binding. Only a small, functionally competent subset of sperm within a heterogeneous ejaculate can engage in ZP2-dependent interactions, making this process a critical checkpoint in fertilization. Cleavage of the N-terminal domain of ZP2 following fertilization abolishes sperm binding and prevents polyspermy. ZP2 peptide-beads, generated by immobilizing the N-terminal region of human ZP2 onto Sepharose beads, provide an in vitro platform that mimics sperm-zona binding. Quantitative characterization of sperm interacting with this system may enhance understanding of sperm-egg recognition and inform the identification of sperm populations with favorable morphological features relevant to reproductive biology and assisted reproductive technologies. Human sperm samples were processed and allocated into two experimental conditions: unbound sperm and sperm incubated with Sepharose beads functionalized with the N-terminal domain of human ZP2. Following incubation, samples were fixed and immunostained to visualize key structural components. Nuclear DNA was labeled using Hoechst, acrosomal and membrane glycoproteins using wheat germ agglutinin (WGA), and the flagellum using α-tubulin immunostaining. Samples were imaged using a Zeiss LSM 780 confocal microscope with a 100× water-immersion objective, and z-stacks were acquired under identical acquisition settings within each condition. Three-dimensional image stacks were analyzed using Zeiss ZEN software to quantify sperm head dimensions, acrosome area, and tail length. Statistical comparisons between free and bead-bound sperm were performed within each patient using the Wilcoxon rank-sum test (Mann-Whitney U). All statistical analyses and visualizations were conducted in R. Quantitative analysis demonstrated no significant differences in sperm head or acrosome area between free and ZP2 bead-bound sperm in either patient sample. In contrast, tail length differed significantly between free and bead-bound sperm across patients, indicating selective enrichment of sperm with distinct tail morphology following ZP2-mediated binding. This study establishes a quantitative, imaging-based framework for assessing human sperm populations interacting with ZP2 peptide-beads. The findings support the use of ZP2 peptide-beads as a biologically relevant platform for analyzing morphologically distinct and functionally relevant sperm subpopulations, with potential implications for sperm selection strategies in assisted reproduction.

Abstract 6

Björn-Philipp Diercks
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NAADP–dependent Ca^{2+} signaling in natural killer cell activity

Fynn Gerlach[1], Franziska Möckl[1], Annika Ahrenstorf[2], Benedetta Padoan[2], Marcus Altfeld[2], Björn-Philipp Diercks[1]

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Abstract:
Intracellular Calcium (Ca^{2+}) signaling is crucial for the activation and function of a variety of immune cells, including NK cells. Nicotinic Acid adenine diphosphate (NAADP) is the most potent calcium-releasing second messenger known to date. While extensive data are available on NAADP–dependent Ca^{2+} mobilization in T cells, very little is known about it in NK cells and the possible role during NK cell education. Previously, we showed that educated NK cells exhibit a higher global Ca^{2+} response upon receptor stimulation, indicating stronger signaling events taking place in these educated NK cells. To investigate NAADP–dependent Ca^{2+} signaling in NK cells, we used fluorescence microscopy with a human NK cell line (KHYGI) and primary human NK cells. Stimulation of the NAADP pathway using the recently published synthetic membrane-permeable NAADP analogue MASTER-NAADP increased both global and local Ca^{2+} signals in KHYGI cells. In line, inhibition of NAADP signaling by the NAADP antagonist BZ194 decreased global Ca^{2+} responses and initial Ca^{2+} microdomains in these cells, demonstrating the crucial role of NAADP in Ca^{2+} release in NK cells. Next, we studied the effect of BZ194 on Ca^{2+} signals during target cell contact (K562) in primary human NK cells using a newly developed microscopy-based live-cell killing assay. Here, in parallel, the Ca^{2+} response in NK cells and in target cells was monitored. Blocking the NAADP signaling pathway resulted in significantly reduced peak amplitude, peak number, and overall AUC in NK cells. To understand the downstream function of NAADP-dependent Ca^{2+} signals in NK cells, we evaluated the impact of BZ194 on primary human NK cells 24 hours after co–incubation with K562 cells, using a Lactate Dehydrogenase (LDH) release assay and surface marker analysis (CD107a and TNF–α) to assess degranulation. BZ194 reduced both killing capacity and the expression of degranulation markers and pro-inflammatory cytokines. In summary, our data suggest that NAADP-dependent Ca^{2+} signals are essential for human NK cell cytotoxicity.

Abstract 7

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Transcriptional analysis of calcium remodeling in samples of human glioblastoma and surrounding tissue with high and low probability of tumor recurrence as shown by AI.

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Abstract:
Glioblastoma (GBM) is the most aggressive primary brain tumor characterized by marked molecular heterogeneity and high recurrence rates. Alterations in intracellular calcium (Ca^{2+}) homeostasis have emerged as key contributors to tumor progression, invasion, and therapy resistance in some forms of cancer. In this study, we performed a transcriptomic analysis focused on genes involved in intracellular Ca^{2+} transport and regulation in GBM and peritumoral tissues to identify molecular signatures associated with tumor biology and potential recurrence risk. RNA sequencing (RNA-Seq) was conducted on a total of 33 human paired, blind samples (α, β, and γ) derived from tumor and peritumoral regions with high and low probability of tumor recurrence according to Artificial Intelligence analysis of 8 additional GBM patients. Total RNA was extracted, quality-controlled, and sequenced, and raw data were processed using Illumina BaseSpace to generate gene expression matrices. Differential gene expression analyses were performed in R using established bioinformatic pipelines. Differential expression analysis revealed significant transcriptional differences exclusively between samples α versus γ and GBM versus γ, whereas no significant differences were detected between GBM and α or β samples, nor between α and β. These findings suggest the existence of two molecularly similar phenotypes (GBM/α and β) reflecting tumor cells, and a distinct γ phenotype reflecting normal surrounding tissue devoid of tumor cells. A total of 1,515 and 1,495 differentially expressed genes (DEGs) were identified in the α vs. γ and GBM vs. γ comparisons, respectively, with 673 genes shared between both contrasts. Focusing on Ca^{2+}-related genes, 20 DEGs associated with Ca^{2+} transport and regulation were identified in each of the significant comparisons, representing more than 1% of total DEGs, with 14 genes overlapping between α vs. γ and GBM vs. γ. Voltage-operated calcium channel analysis revealed downregulation of CaV1.4 and upregulation of CaV3.2 in both α and GBM samples compared with γ. Among Ca^{2+} extrusion and sequestration systems, SERCA1 was selectively upregulated in α samples. Purinergic receptor genes were consistently downregulated in α and GBM samples relative to γ. Analysis of transient receptor potential (TRP) channels showed a conserved pattern across comparisons, characterized by downregulation of TRPV3, TRPV5, TRPV6, and TRPM6, and upregulation of TRPC6 and TRPV4. Additionally, components of store-operated calcium entry (SOCE) signaling exhibited altered expression. Finally, genes involved in endoplasmic reticulum and mitochondrial Ca^{2+} handling were predominantly downregulated. In conclusion, this study identifies a distinct Ca^{2+}-related transcriptional signature differentiating GBM-like samples from a molecularly divergent peritumoral phenotype. These alterations highlight the relevance of Ca^{2+} homeostasis pathways in GBM biology and suggest potential biomarkers and therapeutic targets associated with tumor aggressiveness and recurrence.

Abstract 8

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Consequences of a STIM1 hypomorphic mutation on the nervous system.

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Abstract:
Cells generate intracellular Ca^{2+} signals using two main sources of Ca^{2+}: the extracellular space and internal stores in the endoplasmic reticulum (ER). The Ca^{2+} ER stocks are finite and need to be refilled upon depletion to be able to sustain the wide range of cytosolic Ca^{2+} signals generated by the opening of ER channels such as IP3 and ryanodine receptors. Store-Operated Ca^{2+} Entry (SOCE) fulfills this function: ER Ca^{2+} depletion induces the conformational change of the luminal Ca^{2+} sensor STIM1 that then aggregates at ER-plasma membrane contact sites (ERPMCS) and open the Orai channels, allowing an influx of Ca^{2+} that is then pumped into the ER by SERCA pumps. Beyond store refilling, SOCE is a signaling module involved in multiple Ca^{2+}-regulated cellular processes. Its role has been very well characterized in non-excitable cells, particularly within the immune system but the implication of SOCE in the physiology of excitable tissues is still largely unknown. This is partly because it is technically difficult to separate the very small Ca^{2+} signals due to SOCE from the very large Ca^{2+} events that occur in excitable cells. Still, there is increasing evidence that SOCE is involved in brain function in neuronal and non-excitable cell types. The mechanisms are however poorly understood, genetic ablation of SOCE elements do not lead to major behavioral deficits despite measurable changes in excitability and synaptic transmission (Courjaret et al., 2024). Here we used the recently described mouse model carrying a hypomorphic mutation in STIM1, which reduces -but does not eliminate - SOCE thereby limiting the drastic effects of a full body knock-out and better mimicking pharmacological modulation (Yu et al., 2022). Behavioral experiments did not indicate major changes apart from a trend to hyperactivity in the mutant animals. SOCE measurements on isolated primary astrocytes confirmed a ≈50 % reduction in the Ca^{2+} signal. Surprisingly, the animals had a lower threshold and ability to recover from seizures induced by pentylenetetrazole injection, particularly in animals older than 3 months. This was not coupled with any obvious change in the population of interneurons in the cortex or hippocampus. In addition, patch-clamp recordings of hippocampal slices did not reveal changes in basal synaptic activity. Transcriptomics analysis revealed a strong reduction in the alpha2 subunit of GABAA receptors that could be confirmed by western blotting. Together these findings suggest that STIM1 alteration disrupts brain inhibitory pathways by impairing GABA signaling and increasing the sensitivity to seizures of the animals. This mechanism might be a key contributor to an observed sudden death phenotype, with approximately 20% mortality by five months of age, coinciding with the age-dependent failure of seizure recovery.

Abstract 9

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Oral2-dependent alteration of Ca^{2} profile and suppressive capacity of human regulatory CD4 T cells

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Abstract:
A tightly balanced immune response by different T cell subpopulations plays an essential role driving disease initiation and/or progression in autoimmune diseases but also tumor development. Moreover, dysregulated Ca^{2+} signals result in a plethora of T cell dependent diseases. The differential role of Ca^{2+} in regulation of subpopulation dependent-functions is yet poorly understood. We found that in vitro polarized regulatory $CD4^{+}$ T cells (Treg) have higher levels of store operated Ca^{+} entry (SOCE) than corresponding conventional T cells (Tcon), a phenotype also recapitulated by in vivo differentiated Treg. An mRNA array and subsequent meta-analysis identified candidate genes potentially underlying differential Ca^{+} signatures. The current work aims to investigate how these identified Ca^{+} signaling-related genes contribute to the modulation of Treg suppressive function. A key altered candidate is Oral2. We have previously shown that Oral2 acts as a negative regulator of SOCE which together with the altered Ca^{+} profile of Treg and the new finding that Oral2 is downregulated in Treg suggest a crucial role of Oral2 in modulation of Ca^{2+} dependent functions of Treg. Therefore, using combined approaches of expression analysis, intracellular Ca^{2+} measurements, and suppression assays, we set out to explore if and how Oral2 modulates the suppressive function of Treg. Our data demonstrate that heterologous overexpression of Oral2 in human Tregs reduces both cytosolic Ca^{2+} uptake and their suppressive capacity. To gain insight into the Ca^{2+} dependence of the altered suppressive capacity, we explored the cytokine and transcription factor profiles of Tregs. Furthermore, we are investigating potential Oral2-dependent alterations of metabolic profile and ROS signaling in Treg. Combined, these approaches will provide deeper mechanistic insights underlying Oral2 dependent reduction of Treg suppressive function.

Abstract 10

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Metabolic consequences of the reduction of Store-Operated Ca^{2+} Entry.

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Abstract:
Obesity is a major global health burden and is associated with severe comorbidities, including hypertension, cardiovascular disease, and diabetes. Store-Operated-Ca^{2+} Entry (SOCE) is a mechanism responsible for refilling ER Ca^{2+} stores and regulating several cellular functions. Emerging evidence links SOCE to various metabolic processes including glucose intolerance, insulin resistance and lipid accumulation. However, direct evidence linking SOCE to obesity and adipose tissue function remains limited, due to the lethality and severe complications associated with complete knockouts in mice of the SOCE genes, including STIM1 and Orai1. To overcome this limitation, we utilized a novel STIM1 hypomorphic mouse model, exhibiting partial reduction of SOCE activity, and AdipoQ-Cre STIM1 knockout model, allowing the investigation of both systemic and adipose-specific SOCE function. The aim of this study is to investigate the physiological role of STIM1 in white and brown adipose tissue (WAT and BAT) homeostasis during diet-induced obesity. STIM1 hypomorphic knock-in (Hypo-KI) mice, AdipoQ-Cre STIM1 knockout mice, and their respective controls were subjected to a high-fat diet (HFD; 60% fat) to induce obesity. Body weight, body composition, and metabolic parameters were assessed using TD-NMR, metabolic cages (CLAMS), glucose and insulin tolerance tests (GTT, ITT), and whole-blood lipid profiling. Thermogenic capacity was evaluated using infrared thermal imaging and continuous measurement of interscapular temperature via implanted RFID sensors, followed by histological analyses. Ex-vivo measurements of lipolysis and lipogenesis were performed on iWAT explants. Tissue-specific RNA sequencing and proteomic analyses were performed on BAT, inguinal WAT (iWAT), visceral WAT (eWAT) and Liver to identify molecular pathways altered by reduced STIM1-dependent calcium signaling. Hypo-KI mice fed a high-fat diet (HFD) exhibited significantly increased weight gain ($p < 0.01$), elevated fat-to-lean mass ratio, and adipocyte hypertrophy compared with controls. Both Hypo-KI and control mice developed diabetic phenotypes under HFD conditions; however, no significant differences were observed between groups in glucose or insulin tolerance tests (GTT, ITT). Hypo-KI mice displayed significantly reduced circulating LDL and total cholesterol levels. Notably, thermogenic capacity was impaired in Hypo-KI mice, as evidenced by reduced interscapular BAT temperature ($p < 0.001$). AdipoQ-Cre STIM1 KO mice phenocopied several metabolic features observed in Hypo-KI mice, including increased adiposity and adipocyte hypertrophy under HFD conditions, without significant differences in GTT or ITT profiles. In addition, AdipoQ-Cre mice exhibited lower interscapular temperatures, reduced locomotor activity as measured by CLAMS and enlarged BAT mass. Ex vivo functional analyses of iWAT revealed enhanced lipogenic capacity and reduced lipolytic responses. Tissue-specific proteomic analysis demonstrated enrichment of lipid synthesis pathways, including activation of transcriptional factors associated with lipogenesis, including SREBP and C/EBP pathways. Together, these findings demonstrate that reduced SOCE activity promotes diet-induced obesity, impairs thermogenesis, and disrupts adipose tissue metabolism. Using in vitro approaches, ongoing work will focus on defining the molecular pathways downstream of STIM1 that link calcium signaling to adipose tissue function and metabolic homeostasis.

Abstract 11

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Novel Insights into Calcium-Dependent Mitochondrial Regulation of Neuroinflammation in Early Parkinson's Disease Models

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Abstract:
Aim / Background: Calcium (Ca^{2+}) signaling plays a central role in neuronal homeostasis, linking metabolic activity to synaptic function and survival. Dysregulation of intracellular Ca^{2+} has been implicated in neurodegenerative diseases, particularly Parkinson's disease (PD), where early mitochondrial dysfunction precedes overt cell loss. However, the mechanisms by which aberrant Ca^{2+} dynamics drive neuroinflammation and mitochondrial impairment in PD remain incompletely understood. This study investigates how perturbations in Ca^{2+}-dependent mitochondrial regulatory pathways influence glial activation and neuronal survival in early PD models, with a focus on the interplay between Ca^{2+}-binding effector proteins, mitochondrial bioenergetics, and inflammatory cascades. Methods: We employed an integrative experimental paradigm combining in vitro and in vivo PD models. Primary rat dopaminergic neurons and astrocytes were exposed to sub-toxic concentrations of rotenone to mimic early PD-like stress. Intracellular Ca^{2+} dynamics were quantified using live-cell fluorescence imaging of Ca^{2+} indicators alongside genetically encoded Ca^{2+} sensors targeted to mitochondria. Mitochondrial respiratory function and membrane potential were evaluated using high-resolution respirometry and JC-1 assays, respectively. To dissect Ca^{2+}-dependent signaling, we manipulated expression of key mitochondrial Ca^{2+}-handling proteins -mitochondrial calcium uniporter (MCU), MICU1, and NCLX using viral vectors and siRNA knockdown. Parallel in vivo studies involved unilateral intranigral infusion of MCU modulators in a rat rotenone model, followed by behavioural assessments (rotarod, open field) and post-mortem analysis of inflammatory markers (IL-1β, TNF-α) via ELISA and immunohistochemistry. Transcriptomic profiling of substantia nigra tissues was performed to identify Ca^{2+}-regulated gene networks. Results: We observed that rotenone exposure induced sustained elevations in cytosolic Ca^{2+}, accompanied by excessive mitochondrial Ca^{2+} uptake and compromised bioenergetic function. Overexpression of MICU1 preserved $Ca^{2}{}_{3}$ homeostasis, maintained mitochondrial respiration, and attenuated rotenone-induced deficits. Conversely, MCU upregulation exacerbated mitochondrial depolarization and reactive oxygen species (ROS) production. In astrocytes, elevated mitochondrial Ca^{2+} was linked to a robust pro-inflammatory transcriptional profile, implicating Ca^{2+}-dependent activation of NF-κB and inflammasome pathways. In in vivo experiments, targeted modulation of MCU/NCLX ratio significantly influenced motor behaviour and nigral dopaminergic neuron survival, correlating with reductions in glial activation and pro-inflammatory cytokines. Transcriptomic data revealed a distinct enrichment of Ca^{2+}-regulated stress response networks in PD models, including components of mitophagy and inflammatory signaling. Conclusion: Our findings demonstrate that Ca^{2+} dysregulation within mitochondria contributes to early neuroinflammatory signalling and neuronal dysfunction in PD. Precise control of mitochondrial Ca^{2+} handling via key regulators such as MICU1 and NCLX preserves mitochondrial bioenergetics and mitigates inflammatory responses, highlighting a mechanistic link between Ca^{2+} signaling and neurodegeneration. These insights offer potential targets for therapeutic modulation in PD and other neurodegenerative conditions where Ca^{2+}-dependent mitochondrial dysfunction is a critical driver. Keywords: Calcium signaling; Parkinson's disease; mitochondrial dysfunction; neuroinflammation; mitochondrial calcium uniporter; MICU1; bioenergetics; neurodegeneration; rotenone model.

Abstract 12

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Calcium dynamics in iPSC models of ITPR3-linked Charcot-Marie-Tooth disease type 1I

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Abstract:
Charcot-Marie-Tooth disease type 1J (CMT1J) is a recently identified demyelinating peripheral neuropathy. It is caused by pathogenic variants in the ITPR3 gene encoding the type 3 inositol 1,4,5-trisphosphate receptor (IP3R3). IP3 receptors are intracellular Ca^{2+}-release channels that mediate Ca^{2+} flux from the endoplasmic reticulum, controlling multiple cellular processes such as cell signaling, apoptosis, and metabolism. While extensively studied in various cell types, the specific roles of IP3R3 channels in human peripheral nerve cells remain underexplored. Previous studies performed in HEK293 cells lacking all three IP3R types and overexpressing the pathogenic ITPR3 variants showed constitutively leaky IP3R3 channel pores with altered gating by IP3. Our research aims to elucidate the significance of IP3Rs in nerve cells derived from human induced pluripotent stem cells (iPSC), and the molecular mechanisms by which ITPR3 disease variants contribute to CMT1J. To investigate the roles of IP3Rs and the pathogenic CMT1J variants in IP3R3, we have used gene editing to develop two kinds of iPSC models: (1) iPSC with targeted deletions of one, two or all three IP3R genes, and (2) iPSC harboring pathogenic ITPR3 missense variants (p.V615M, p.T1424M, and p.R2524C). We performed single live-cell Ca^{2+} imaging on the iPSC models with dysfunctional IP3R3 to explore the molecular implications of the disease and their impact at physiological level. Following the differentiation of these iPSCs into motor neurons and Schwann cells, we will use omics techniques and fluxomics studies to explore the consequences of IP3R loss or IP3R3 mutations in disease-relevant models. These studies aim to uncover the cell-type specific molecular mechanisms driving CMT1J and advance the development of therapeutic strategies for this disease.

Abstract 13

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Unraveling the Ca^{2+}-independent impact of BAPTA–AM on autophagic pathway

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Abstract:
Introduction: Ca^{2+} signaling controls cell homeostasis and function, while its dysregulation is linked to various diseases, including neurodegeneration, and cancer. A common tool to probe the role of Ca^{2+} signaling in cell biology is BAPTA, a fast, high-affinity Ca^{2+} chelator that can be loaded in cells as an AM ester. When a cellular process is affected by intracellular BAPTA (BAPTAic), it is usually attributed to its Ca^{2+}-chelating properties. Yet, recent work indicates that BAPTAic directly affects cellular targets, independently of Ca^{2+} chelation. The role of Ca^{2+} in autophagy, a lysosomal turn-over process, has been subject of debate for several years. This could be explained by the frequent use of BAPTAic, often without complementary approaches or appropriate controls. Revealing Ca^{2+}-independent actions of BAPTAic is key to clarifying the Ca^{2+}-dependence of autophagy. Aim: Here, we investigate the Ca^{2+}-independent impact of BAPTAic on autophagy. Methods: We monitored early and late autophagy markers in cells exposed to BAPTA-AM, TF-BAPTA-AM and EGTA-AM. TF-BAPTA, a low Ca^{2+} affinity variant of BAPTA (Kd = 65 µM), is used as a control for BAPTA's Ca^{2+}-independent effects. Results: BAPTAic suppresses mTORC1 activity (Sneyers et al., 2023), a negative regulator of autophagy. Therefore, BAPTAic is expected to stimulate autophagic flux and thus promote LC3-II formation. Yet, we observed that BAPTAic suppressed LC3-II formation upon mTORC1 inhibition by PP242. Moreover, BAPTAic and TF-BAPTAic were equally potent in suppressing LC3-II formation. These findings indicate that BAPTAic directly inhibits an essential proximal autophagy effector downstream of mTORC1 and upstream of LC3-II, independently of its Ca^{2+}-chelating function. To identify the autophagy effector that BAPTAic inhibits, we monitored ULK1 and ATG13, early autophagic markers that form the autophagy initiation complex. Upon mTORC1 inhibition, ULK1 is activated, resulting in ULK1 and ATG13-punctae formation. Strikingly, BAPTAic and TF-BAPTAic, but not EGTAic, suppressed ULK1 and ATG13 punctae formation. These results hint towards a Ca^{2+}-independent effect on the formation of the autophagy initiation complex by BAPTAic. Although low energy levels in the cell are expected to induce autophagy, a certain threshold of ATP levels and metabolites is essential for autophagy initiation (Mandic et al., 2024). Therefore, we measured the impact of the BAPTA-AM variants on glycolysis. We observed that BAPTAic and TF-BAPTAic, but not EGTAic, suppressed glycolytic activity in HEK293. Furthermore, inhibition of glycolysis with a high dose of 2-deoxyglucose prevented WIPI2 punctae formation, another early autophagy marker. These findings indicate that initiation of autophagy requires glycolysis, a process hampered by BAPTAic. Conclusion: BAPTAic independently of its Ca^{2+}-chelating activity, blocks the early steps of autophagy by drastically decreasing glycolytic activity.

Abstract 14

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MASTER-NAADP – Membrane permeAble, STabilized, biorEversibly pRotected precursor of the Ca^{2+} mobilizing second messenger NAADP

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Abstract:
Nicotinic acid adenine dinucleotide phosphate (NAADP) is considered the most potent endogenous Ca^{2+} mobilizing second messenger in T cells. When the T cell receptor is stimulated, NAADP is quickly produced by NADPH oxidases, reaching low nanomolar levels that can induce Ca^{2+} microdomains. These microdomains often merge into larger Ca^{2+} signals amplified by IP3 and later by cADPR. The exact activation mechanism and the specific channels targeted by NAADP are still widely debated. Both ryanodine receptor type 1 (RyR1) on the endoplasmic reticulum (ER) membrane and endo-lysosomal two-pore channels (TPCs) have been suggested as targets. Recent research from our group describes how NADPH oxidases produce NAADP, which then binds to the NAADP-binding protein HN1L/JPT2, leading to Ca^{2+} release via RyR1 in T cells. Here, the newly developed compound MASTER-NAADP, a Membrane permeAble, STabilized, biorEversibly pRotected precursor of NAADP, is characterized by high-resolution live-cell imaging. Addition of 100nM MASTER-NAADP and consequent release of its active NAADP mimetic reliably evoked Ca^{2+} microdomains in Jurkat T cells, primary human and primary murine T cells, eventually merging into global Ca^{2+} signals. Moreover, using a control compound or knock-out cells of either NAADP-binding protein (HN1L/JPT2) or knock-down of RyR, decreased Ca^{2+} microdomains. Furthermore, using the deprotected, active NAADP mimetic produced by PLE digestion in lipid-planar bilayer and permeabilized Jurkat cell assays, confirmed the specificity of MASTER-NAADP. Our results identify MASTER-NAADP as a novel active NAADP mimetic, providing a useful novel addition to the NAADP signaling toolbox.

Abstract 15

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Ryanodine receptors dynamically control lysosomal trafficking in neurons

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Abstract:
Ca^{2+} signaling at membrane contact sites critically control cell function, while its dysregulation drives pathogenesis. Here, we identify ATP6v0a1 as a novel partner of ryanodine receptors (RyRs), contributing to ER-lysosomal tethering and controlling intracellular availability of lysosomes to participate in autophagic flux. Recent work revealed that ER-resident RyRs suppress autophagosome turnover by the lysosomes. In familial Alzheimer's disease, hallmarked by excessive RyR activity and lysosomal dysfunction, inhibition of RyR activity restored autophagic flux by normalizing lysosomal vacuolar H+-ATPase (vATPase) levels. Yet, the mechanisms by which RyRs control lysosomal function and how it involves the vATPase remain unknown. We found that RyR2, the neuronal RyR isoform, directly interacts with the ATP6v0a1 subunit of the vATPase, contributing to ER-lysosomal contact site formation. Moreover, the RyR2::ATP6v0a1 complex was impaired in cortical samples derived from 3xTG Alzheimer Disease mouse models. ATP6v0a1 itself suppressed RyR2-mediated Ca^{2+} release, which impacts the handling of lysosomal cargo. Pharmacological inhibition of RyR activity was sufficient to mimic these effects on lysosomal cargo handling. In iPSC-derived cortical neurons, endogenous RyRs promoted lysosomal exocytosis, while inhibiting this spontaneous RyR activity limits lysosomal exocytosis, thus retaining lysosomes inside cells. The latter increases ER-lysosomal contact site formation, rendering lysosomes more available for autophagic flux. This places RyRs as dynamic control centers directing lysosomes towards secretion versus autophagy.

Abstract 16

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Calmodulin G133V: Disrupted Calcium Signaling and Potential Drug Interactions in Arrhythmia

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Abstract:
Calmodulin is a ubiquitous calcium sensor encoded by three genes (CALM1, CALM2, CALM3). Rare variants in these genes can cause fatal cardiac arrhythmia, particularly when calcium-binding EF-hands are affected. We identified the variant CALM1-G133V in a 5-year-old boy who suffered cardiac arrest during moon-car driving in the kindergarten. He was diagnosed with long QT syndrome and treated with metoprolol and an implantable cardioverter-defibrillator. Notably, ten weeks prior to the arrest, he had initiated methylphenidate therapy for attention deficit-hyperactivity disorder (ADHD). These observations prompted us to investigate how this variant alters calmodulin's calcium-sensing function and whether methylphenidate may contribute to arrhythmia risk. We purified and expressed the G133V calmodulin protein and found a ~10-fold reduction in calcium affinity, severely impairing sensor function. We further examined its regulation of two key calcium channels---voltage-gated calcium channel 1.2 and ryanodine receptor 2---both central to calmodulinopathy mechanisms. The G133V variant markedly reduced calcium-dependent binding to channel domains and impaired channel closure. To explore potential interactions with methylphenidate, we generated patient-derived induced pluripotent stem cells that were differentiated to cardiomyocytes. Further, we have also introduced the variant into Caenorhabditis elegans. These models will allow us to test whether methylphenidate exacerbates arrhythmia phenotypes in the context of calmodulin dysfunction.

Abstract 17

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Neutrophil Calcium Dynamics and Recruitment to Acetaminophen-Injured Hepatocytes

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Abstract:
Background: Neutrophils are the most abundant circulating immune cells and play a central role as first responders to tissue injury. In the liver, their recruitment is particularly prominent during drug-induced injury, where they contribute both to damage amplification and to the initiation of repair processes. Calcium signalling is a fundamental regulator of neutrophil functions, controlling chemotaxis, adhesion, degranulation, and the release of reactive oxygen species. However, while the importance of calcium signals in neutrophil activation is well established, the dynamics and patterns of calcium signalling in neutrophils upon contact with injured liver tissue remain poorly understood. Objective: Our goal is to investigate calcium signalling in neutrophils when interacting with injured hepatocytes. Methodology: We isolated human neutrophils from the blood of healthy volunteers (Ethical Committee: 70650723700005149), using a density gradient separation method with HISTOPAQUE-1077. Neutrophils were incubated with the calcium-sensitive fluorophore Fluo-4/AM and placed in contact with liver spheroids of HepG2 cells treated with 10 mM acetaminophen (APAP) for 24 h. Images were acquired using a Nikon Eclipse Ti Confocal Microscope, and analysis was performed using ImageJ in selected regions of interest. We evaluated neutrophil movement using Trackpy Software Version 0.7. Results: We evaluated calcium signalling in neutrophils located at three different distances from the spheroids, denoted as proximal, intermediate, and distal zones. We found that the amplitude and profile of calcium signalling in neutrophils were similar upon contact with control or injured spheroids, and across zones. However, the number of responsive neutrophils was higher in the proximal region compared to the distal region (83.54±10.32 vs. 66.24±2.42, p=0.0437). The distance traveled by neutrophils increased when in contact with APAP-treated spheroids (75.85±32.73 $_3$m for control spheroids vs. 138.0±96.80 μm for APAP-treated spheroids, p<0.0001). After initiation of the calcium response, neutrophil motility increased, followed by greater accumulation in the proximal zone of APAP-treated spheroids (p=0.0342). Conclusion: Our findings indicate that neutrophil calcium signalling is not altered in amplitude or profile by contact with APAP-injured liver spheroids. Instead, the main differences arise in the proportion of responsive neutrophils and their migratory behavior, with enhanced motility and accumulation near the injured site. Together, these results suggest that the number of calcium-responsive neutrophils and spatial context modulate neutrophil recruitment dynamics more than the calcium signalling intensity itself.

Abstract 18

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Long-QT syndrome associated calmodulin mutations disrupt key phospho-regulatory proteins involved in calcium cycling

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Abstract:
Long QT syndrome (LQTS) is an inherited cardiac disorder characterised by delayed ventricular repolarisation, leading to prolonged QT intervals on the electrocardiogram. Affecting approximately 1 in 2,500 individuals, LQTS can result in syncope, seizures and potentially fatal arrhythmias. While most cases are caused by mutations in cardiac ion channels, recent evidence implicates mutations in calmodulin (CaM), a ubiquitous Ca^{2+}-sensing protein, as contributors to the disease. CaM plays a central role in cardiac excitation-contraction coupling by binding and regulating numerous target proteins, with its activity tightly linked to intracellular Ca^{2+} fluctuations. However, the mechanisms by which CaM dysfunction leads to LQTS remain poorly understood. This study investigates how disease-associated CaM variants alter cardiac calcium homeostasis, focusing on key phospho-regulatory pathways involving CaM-dependent kinase II (CaMKII) and Calcineurin (CaN), as well as calcium (re)cycling via the Na^{+}-Ca^{2+} exchanger (NCX) and Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA). Five recently identified CaM disease-linked variants were examined using isothermal titration calorimetry (ITC) to assess their binding to CaMKII, CaN, and NCX. LQTS-associated CaM proteins exhibited substantially reduced binding affinity for all three targets (up to 9-fold, 15-fold and 21-fold respectively). Equilibrium Ca^{2+}-binding titration experiments, measured via intrinsic tyrosine fluorescence, revealed that disease-associated variants have up to 400-fold lower affinity for Ca^{2+} (K_a = 105 ± 23 μM) compared to wild-type CaM (0.24 ± 0.07 μM). To measure CaN activity, we have used a spectrophotometric assay, using pNPP as a substrate and have found CaM mutants have an up to 2-fold decrease in CaM-dependent CaN activity. Furthermore, we have used a real-time kinetic fluorometric assay to study CaMKII activity and demonstrated significantly decreased kinase activity in vitro for disease mutations. Ongoing work includes live-cell Ca^{2+} imaging and whole-cell recordings to determine how these variants affect NCX and SERCA function. Previous studies have largely focused on how CaM mutations influence ion channel behaviour, whereas their impact on calcium regulatory and recycling mechanisms has been largely overlooked. Our findings suggest that disruptions in calcium transport and signalling contribute to arrhythmogenesis in LQTS. This work provides new insights into the molecular basis of CaM-associated LQTS and may inform the development of targeted therapeutic strategies. Funding: This work was supported by British Heart Foundation Intermediate Basic Science Research Fellowships (FS/17/56/32925; FS/EXT/22/35014), British Heart Foundation Project Grant (PG/24/11830), British Heart Foundation Non-Clinical PhD Studentships (FS/PhD/20/29025; FS/PhD/22/29339), Biochemical Society General Travel Grant (GTG-0000001900), DMM Conference Travel Grant (CTG-DMM25082256).

Abstract 19

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Novel ryanodine receptor isoform (RyR1alt) expressed in the liver and its role in Ca^{2+} signaling

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Abstract:
Inositol-1,4,5-trisphosphate (IP_3) dependent Ca^{2+} oscillations evoked by hormones and other agonists play a key role in the regulation of liver physiology. While these Ca^{2+} oscillations are primarily driven by IP_3 receptor (IP_3R) Ca^{2+} channels, we have also identified a role for ryanodine receptor (RyR) Ca^{2+} channels in hepatocytes. RyRs in SR/ER are well known for controlling skeletal and cardiac muscle contraction, and also play important roles in other excitable cells. Despite being widely expressed, the role of RyR in nonexcitable cells is less understood. Our previous study demonstrated the presence in the liver of a novel alternative isoform of RYR type 1, referred to here as RyR1alt. In hepatocytes, the RyR1alt isoform participates in the generation of Ca^{2+} oscillations and waves that are generated through positive feedback on the IP_3R. To further investigate this novel RyR isoform, the two ends of the RyR1alt were identified by Rapid Amplification of cDNA Ends (RACE) and the entire sequence of the RyR1alt clone was obtained. The RyR1alt mRNA is 6445 bp long compared to the predicted 15220 of the full-length rat RyR1. RyR1alt displays complete homology with the 3' half of RyR1 except for a 50 bp sequence spliced out in the 5'-end of exon 61, resulting in a N-terminal truncated protein. Mass spectrometry analysis confirmed the N-terminal open reading frame sequence, and that RyR1alt comprises 1943 amino acids yielding a protein of 218 kDa, about 40% the size of full-length RyR1. In 3D structural predictions, both homology and AlphaFold models show that RyR1alt adopts a tetramer channel-like fold, similar to the full length RYR1. In HEK cells, overexpressed RyR1alt co-localizes with calnexin, confirming that this novel RyR isoform is localized in the ER. RyR1alt is functional and increases intracellular Ca^{2+} mobilization, exhibiting a more robust Ca^{2+} release in response to carbachol in HEK cells. Additionally, the presence of RyR1alt changes the kinetics of Ca^{2+} release in response to thapsigargin, showing a rapid burst phase compared to the control cells. In permeabilized primary hepatocytes and in intact HEK-RyR1alt cells, cADPR elicits intracellular Ca^{2+} increase, suggesting cADPR as modulator, that enhances RyR1alt-mediated Ca^{2+} release.

Abstract 20

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Calcium oscillations and early embryogenesis -novel paradigms for phospholipase C zeta

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Abstract:
Cases of male infertility are increasingly associated with the efficacy of oocyte activation. Mammalian oocyte activation occurs in response to specific profiles of calcium (Ca^{2+}) oscillations driven by sperm-specific phospholipase C zeta (PLCζ), abrogation of which in sperm is extensively linked with male infertility where oocyte activation is deficient (OAD). However, less is known whether sperm PLCζ underlies cases of defective embryogenesis and failed pregnancy following fertility treatment. We propose that a significant causative factor underlying poor global ART success are abnormalities/insufficiencies in Ca^{2+} oscillation-driven early embryogenic events, mediated by abnormalities/insufficiencies in sperm PLCζ. Indeed, Ca^{2+} oscillation profiles are necessary for early embryonic competency, mediated via the efficacy of PLCζ-driven oocyte activation. Ultimately, we aim to establish the link(s) between such PLCζ-mediated parameters with fertility treatment outcome. We have been evaluating PLCζ profiles in sperm from human couples undergoing fertility treatment in correlation with time-lapse morphokinetic analysis of resultant embryos, correlating such profiles to pregnancy status. Concurrently, we have also generated two strains of mutant PLCζ mice using CRISPR/Cas, using mutant sperm to generate embryos with wild-type (WT) oocytes via in vitro fertilisation (IVF). We found that minimal levels of PLCζ within a specific range were required for optimal early embryogenesis, correlating with increased pregnancy. Levels of sperm PLCζ below specific thresholds did not correlate to effective embryogenesis and pregnancy, despite eliciting successful fertilisation, indicating that minimal PLCζ levels are required to initiate fertilisation and support effective embryogenesis. Transgenic sperm exhibited severely reduced PLCζ levels, with transgenic mice yielding a drastically reduced litter size and reduced numbers of embryos reaching developmental milestones. Furthermore, embryos generated with transgenic sperm and WT oocytes exhibited high rates of polyspermy, alongside delayed embryonic progression compared to embryos generated using WT sperm and oocytes. Our data suggests that utilisation of PLCζ may benefit the larger population of couples seeking fertility treatment and could be used to enhance the success rates of fertility treatments.

Abstract 21

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Glucose-Dependent Modulation of Cholinergic Ca^{2+} Signaling in Pancreatic β -Cells

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Abstract:
Calcium (Ca^{2+}) signaling is a central regulator of insulin secretion in pancreatic β-cells, acting as the principal messenger that links glucose metabolism to insulin exocytosis. However, β-cell secretory function does not rely solely on changes in extracellular glucose concentration; it is also shaped by neuronal and paracrine inputs that modulate β-cell excitability. Among these, cholinergic stimulation mediated by acetylcholine (ACh) plays a key role in enhancing insulin release in synchrony with autonomic nervous system activity. Despite its importance, the interplay between cholinergic signaling and glucose metabolism in regulating subcellular Ca^{2+} dynamics remains not fully understood. We have studied the role of glucose in Ach-induced intracellular Ca^{2+} responses of cytosolic ($[Ca^{2+}]c$) and endoplasmic reticulum ($[Ca^{2+}]ER$) in pancreatic β-cells. To this end, Fura-2 was loaded into islets from an in-house-generated transgenic mouse model (Ins-erGAP3) that expresses the ER-targeted Ca^{2+} sensor erGAP3 exclusively in pancreatic β-cells. This approach enables simultaneous monitoring of $[Ca^{2+}]c$ and $[Ca^{2+}]ER$ dynamics without disrupting the islet's native architecture. Both resting $[Ca^{2+}]c$ and $[Ca^{2+}]ER$ were elevated in response to increasing extracellular glucose concentration. Cholinergic Ca^{2+} responses in β-cells were modulated by cytosolic Ca^{2+} levels in a biphasic manner, such that ACh elicited maximal Ca^{2+} release at intermediate glucose concentrations. Ach-induced Ca^{2+} responses were dampened at low and high glucose levels because the former had a low $[Ca^{2+}]ER$, while the latter involved a rapid Ca^{2+}-induced inactivation of IP_3R. These findings show that glucose finely tunes cholinergic Ca^{2+} signaling in pancreatic β-cells. Moreover, the Ins-erGAP3 mouse model represents a novel tool to study the integration of metabolic and neuronal Ca^{2+}-mediated signals that govern β-cell secretory function. This project was funded by the Spanish Ministry of Science and Innovation (PID2023-146434NB-I00), and the IBGM Strategic Programme, Escalera de Excelencia, Junta de Castilla y Leon (CLU2025-02-01).

Abstract 22

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Mechanistic Role of BDNF Polymorphism in Oxaliplatin-Induced Neuropathy via KCNN4 Calcium-Activated Potassium Channel Upregulation

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Abstract:
Oxaliplatin-induced peripheral neuropathy (OIPN) is a major dose-limiting toxicity in colorectal cancer treatment, with genetic factors significantly influencing individual susceptibility. The BDNF Val66Met polymorphism (rs6265, c196 G>A) affects neurotrophin signaling, yet its mechanistic contribution to chemotherapy-induced neuropathy through glial-neuronal interactions remains poorly understood. This study investigates how BDNF genetic variants modulate calcium homeostasis and ion channel expression in OIPN pathogenesis. We employed BV2 microglial cells engineered to express BDNF Val66Met genetic variants alongside dorsal root ganglion (DRG) neurons from BDNF knock-in mice to model glial-neuronal communication during oxaliplatin exposure. Rationetric calcium imaging (Fura-2) demonstrated that neurons co-cultured with BDNF Val/Val (G>G) BV2 cells exhibited dramatically elevated calcium influx upon depolarization (50 mM KCl) following oxaliplatin treatment compared to those with Met-variant (G>A) BV2 cells. Store-operated calcium entry (SOCE) assays using thapsigargin revealed that BDNF Val/Val conditions produced severe calcium dysregulation, with impaired endoplasmic reticulum calcium handling. Mechanistic analysis identified KCNN4 (KCa3.1), an intermediate-conductance calcium-activated potassium channel, as a critical mediator of genotype-dependent responses. Immunofluorescence quantification showed threefold upregulation of neuronal KCNN4 protein in BDNF Val/Val conditions compared to vehicle controls, while Met/Met variant showed minimal induction. Gene expression profiling revealed differential regulation of potassium channel genes (Kcng1, Kcnq2, Kenk5, Kcnn4) between genotypes, indicating compensatory transcriptional responses to calcium overload in Val-carrier neurons. The p75NTR modulator LM11a-31 demonstrated remarkable therapeutic efficacy by normalizing KCNN4 expression and restoring calcium homeostasis across both BDNF variants. Furthermore, LM11a-31 significantly attenuated microglial activation (GFAP upregulation) in oxaliplatin-treated cultures across all genotypes including wildtype controls, suggesting its neuroprotective mechanism involves modulation of glial inflammatory responses. These findings establish that BDNF polymorphism in microglia critically determines neuronal calcium handling and KCNN4 channel upregulation during oxaliplatin exposure. The calcium-potassium channel axis represents a genotype-dependent vulnerability mechanism in OIPN, with therapeutic targeting of p75NTR signaling offering protection regardless of genetic background. This work provides mechanistic rationale for genotype-stratified neuroprotective interventions and highlights the importance of glial-neuronal crosstalk in chemotherapy-induced neurotoxicity.

Abstract 23

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Mechanisms Involved in Aging-Enhanced Protein E–Induced Calcium Release from Intracellular Stores in Hippocampal Neurons

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Abstract:
Infection with SARS-CoV-2, the etiological agent of COVID-19, is associated not only with respiratory and vascular pathology but also with significant neurological alterations. The viral envelope (E) protein is a small structural component with ion channel–forming properties that plays an essential role during the viral life cycle. Nevertheless, its direct impact on neuronal cells has remained largely unexplored. We previously described that E protein rapidly penetrates hippocampal neurons and predominantly localizes to endoplasmic reticulum (ER) membranes in both short-term (6-8 days in vitro, DIV) and long-term (20-22 DIV) cultures of rat hippocampal neurons, considered as models of young and aged neurons, respectively. Notably, exposure to the E protein triggered apoptotic cell death selectively in aged neurons, whereas younger neurons remained largely resistant. Ca^{2+} release from intracellular stores triggered by the E protein was minimal in young neuronal cultures but substantially enhanced in aged neurons. In this study, we have investigated mechanisms involved in Ca^{2+} release induced by E protein in young and aged hippocampal neurons, Ca^{2+} signaling alterations and mitochondrial dysfunction in aged rat hippocampal neurons, as well as potential neuroprotective mechanisms. Calcium imaging experiments performed in Ca^{2+}-free conditions to study the possible implication of inositol 1,4,5-trisphosphate receptors (IP_3Rs), using the pharmacological inhibitor 2-APB. Interestingly, this compound failed to reduce, and instead enhanced, E protein–evoked Ca^{2+} release. Given the close functional coupling between the ER and mitochondria, we next examined the role of mitochondria in buffering E protein-induced Ca^{2+} release. Mitochondrial depolarization using the uncoupler FCCP significantly increased cytosolic Ca^{2+} elevations elicited by the E protein, indicating that mitochondria actively take up part of the Ca^{2+} released from intracellular stores. Despite this mitochondrial Ca^{2+} uptake, acute exposure to the E protein did not induce the opening of the mitochondrial permeability transition pore (mPTP), suggesting that early mitochondrial integrity is preserved. Finally, we found that a novel antiviral inhibits both Ca^{2+} release and neuron cell death evoked by E protein suggesting a critical role of Ca^{2+} mobilization in COVID19 neurotoxicity and a novel strategy for neuroprotection. Funding: This work was supported by the grants PID2024-159238OB-I00 and PID2021-125909OB-I00, the Junta de Castilla y León Excellence Program CLU-2025-2-01 and the CSIC DeeP-MaX excellence program. SLV was AM were supported by predoctoral fellowships from JCyL and the Spanish Association against Cancer (AECC), respectively.

Abstract 24

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The genetics of defective spermiogenesis in men of consanguineous origin

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Abstract:
Asthenoteratozoospermia is a condition resulting from aberrant spermiogenesis and it is characterized by abnormal sperm morphology and motility, resulting in male infertility. Despite its clinical importance, the genetic basis of this condition is not well understood. This study aims to identify genetic mutations in familial cases of asthenoteratozoospermia and understand their effects on sperm morphology and function, with a specific focus on the gene SPEM2, a testis enriched gene, necessary for mouse spermiogenesis. METHODS. Whole-genome sequencing (WGS) and Sanger sequencing were performed on affected individuals from one consanguineous family diagnosed with asthenoteratozoospermia and their fertile relatives. Detailed genealogic analysis and assessments of sperm morphology were conducted using immunostaining and confocal microscopy to identify and quantify abnormalities. RESULTS. Our analysis identified homozygous loss-of-function mutations in the gene SPEM2 in the affected individuals. These frameshift mutations followed an autosomal recessive inheritance pattern. Immunostaining and confocal microscopy revealed significant abnormalities in sperm structure, such as vacuolated or elongated nuclei, absent acrosomes, and coiled or broken flagella. Quantitative analysis demonstrated that this homozygous genetic mutation results in severe defects in sperm morphology and motility. CONCLUSION. The findings indicate that the identified loss-of-function mutation in SPEM2 is a major contributor to the asthenoteratozoospermia phenotype. These results enhance our understanding of the genetic basis of asthenoteratozoospermia and may inform future diagnostic and therapeutic strategies for male infertility. KEYWORDS. Infertility; human genetics; sperm; precision medicine.
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Abstract 25

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Association between histone deacetylase compartmentalization and survival of primary hippocampal neurons

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Abstract:
Class IIa histone deacetylases (HDAC4 and HDAC5) are critical transcriptional regulators whose function depends on intracellular localization. Whether class IIa HDACs are neuroprotective or pro-apoptotic is controversial. Class IIa HDACs when localized to the nucleus bind MEF2 on chromatin and mediate gene repression. In this study, it was tested if nuclear or cytoplasmic localization of HDAC4/5 and inhibition of MEF2A activity will promote neuronal survival and/or axon outgrowth in primary neurons. Neuronal survival and axon growth were studied using primary cultures of rat E18 hippocampal neurons. Cells were transfected with plasmids encoding wild-type (WT) forms and mutants of HDAC4 and HDAC5 with constitutive nuclear (3SA, S/A) or cytoplasmic (L175A, ANLS) localization. Surviving neurons spontaneously exhibit neurite outgrowth, and measurement of the longest neurite represents an assay for axon growth as a model.

Abstract 26

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Therapeutic Peptides Target Calcium Signaling Dysregulation in Hepatic Steatosis and Fibrosis.

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Abstract:
Disrupted intracellular calcium (Ca^{2+}) signaling is increasingly recognized as a hallmark of chronic liver diseases, including steatosis and fibrosis. These conditions represent progressive stages of non-alcoholic fatty liver disease (NAFLD), which affects nearly 25% of the global population and significantly contributes to liver-related mortality. Early pathological changes in Ca^{2+} dynamics offer a mechanistic window for therapeutic intervention prior to irreversible tissue remodeling. Objective: To evaluate whether peptides from the protective arm of the Renin-Angiotensin System (RAS) can restore ATP-induced Ca^{2+} signaling in 3D human liver spheroid models of steatosis and fibrosis. Methodology: Spheroids were generated using HepG2 hepatocytes and hepatic stellate cells. Steatosis was induced by exposure to 2 mM oleic acid for 48 hours, and fibrosis was induced by treatment with 10 ng/mL TGF-β for 4 days. Peptides (100 nM) were applied for 24 to 72 hours, and Ca^{2+} responses were assessed in Fluo-4/AM-loaded spheroids upon ATP stimulation (50 µM). Immunofluorescence images were acquired using a SPINNING DISK YOKOGAWA-3i Confocal Microscope, and analysis was performed using ImageJ in selected regions of interest to evaluate pathological markers. Results: Both steatotic and fibrotic spheroids exhibited significant attenuation of Ca^{2+} signaling amplitude (Steatosis: 159.9±32.9 vs. 231.2±110.6 in controls; Fibrosis: 153±32 vs. 301±20; $p<0.0001$, $n>70$ cells per condition). Additionally, pathological spheroids showed altered temporal dynamics of Ca^{2+} signaling, including delayed onset and a shift from compound to wave-like or oscillatory profiles. Notably, treatment with Angiotensin-(1-7), Alamandine-(1-5), and Angiotensin-(2-7) restored Ca^{2+} amplitude and temporal signaling patterns to levels comparable to controls. This restoration was accompanied by a significant decrease in lipid droplet accumulation ($p<0.0002$) in steatotic spheroids and reduced collagen type I deposition in the fibrotic model. Conclusion: These findings identify intracellular Ca^{2+} dysregulation as a sensitive functional indicator of liver pathology in human 3D spheroid models. Furthermore, peptides from the alternative RAS-axis effectively restore Ca^{2+} signaling homeostasis, highlighting their potential to reverse steatotic and fibrotic liver phenotypes. The use of this advanced 3D liver model, combined with detailed evaluation of intracellular Ca^{2+} dynamics, provides a robust platform to assess the anti-steatotic and anti-fibrotic potential of alternative RAS peptides.

Abstract 27

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Mechanism of intracellular Ca^{2+} waves upon mechanical stimulation of retinal epithelium cells

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Abstract:
Intercellular calcium (Ca^{2+}) waves (ICWs) represent the propagation of increases in intracellular Ca^{2+} through a population of cells and appear to be an important mechanism for coordinating multicellular responses. The ICW has been described in many cell types, such as anterior pituitary cells, astrocytes, cochlear cells, vascular endothelium, and epithelial cells of different organs. Although significant variations can be found in specific cell types, a general mechanism has proposed a combination of a paracrine signalling, through a signal molecule released to the extracellular space and a cytosolic molecule travelling through intercellular gap junctions. ICW has been studied mostly by monitoring cytosolic Ca^{2+}. However, the underlying mechanisms could have independent requirements on the two sources of Ca^{2+}, the extracellular and the ER Ca^{2+} stores, and this cannot be easily distinguished by exclusively recording $[Ca^{2+}]c$. Therefore, we addressed the propagation of the ICW by simultaneously monitoring the cytosolic- and ER-Ca^{2+} dynamics using fura-2 and the ER-targeted low-affinity Ca^{2+} indicator erGAP3, respectively, in human retinal pigment epithelium cell line ARPE-19 cell. Calibration of the Ca^{2+} signals allowed quantitative characterization of the Ca^{2+} dynamics in both intracellular compartments. ICW is triggered by mechanical stimulation of a single cell in the middle of the culture and provoked a wave that can be propagated up to 7 layers of a confluent monolayer. Mechanical stimulation in control conditions provoked two antiparallel Ca^{2+} waves, one of increased Ca^{2+} in the cytosol and one reverse wave of released Ca^{2+} in the ER lumen. Removal of extracellular Ca^{2+} or application of nickel, a general Ca^{2+}-channel antagonist, did not significantly alter the cytosolic Ca^{2+} wave, although it hampered the refilling of the ER, so the level of ER remained lower after the ICW. In contrast, depletion of ER Ca^{2+} stores with a SERCA blocker blocked both the cytosolic and the ER Ca^{2+} waves, demonstrating the reticular origin of the cytosolic Ca^{2+} wave. Interestingly, several manoeuvres to interfere with purinergic signalling uncoupled the cytosolic and the ER Ca^{2+} waves, uncovering previously overlooked Ca^{2+} players implicated in the ICW propagation. Funding: Financed by Ministerio de Ciencia e Innovación de España (PID2023-146434NB-I00) and Programa Estratégico del IBGM, Escalera de Excelencia, Junta de Castilla y León (Ref. CLU2025-02-01).

Abstract 28

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The Information in IP3-induced Ca² Spike Sequences

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Abstract:
The inositol 1,4,5-trisphosphate/calcium (IP_3/Ca^{2+}) signaling pathway transmits information on the concentration of extracellular agonists of plasma membrane receptors to intracellular targets by dynamic changes of the cytosolic Ca^{2+} concentration. We investigate spike sequences with regard to the information they provide on spike generation and feedbacks shaping spikes and interspike intervals (ISIs), and with regard to the information they transmit. The timing of spikes within a cell is random because each ISI has a large stochastic component. Cell-to-cell variability of the average ISI of individual cells in response to identical stimulation is very large. That is difficult to reconcile with the signal transmission function of the pathway. We report on cell specific spike sequence properties and general properties, which are not subject to cell variability. The moment relation between standard deviation and average of the ISI distribution is highly conserved. The concentration response relation of stimulation has cell specific and general parameters. It indicates possibilities for modes of information transmission by melody encoding, which reconciles earlier knowledge on frequency encoding with cell variability. Information on feedbacks acting during spike sequences can be found in the second moments of ISI and amplitude distributions. Not the average ISI but the moment relation indicates the time scale of recovery from the negative feedback which terminates spikes. ISI-amplitude anti-correlation indicates additional negative feedback acting during this recovery. Both together suggest that spike initiation and amplitude are determined by different release channel populations. All general properties are related to noise and fluctuations. We suggest stochastic modelling concepts taking them into account.

Abstract 29

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Characterization of Ca^{2+} oscillations in glioma cells using computational modelling

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Abstract:
Gliomas are aggressive brain tumors originating from glial cells. During tumor progression, interconnected glioma cells communicate through microtubes and display repetitive Ca^{2+} pulses that contribute to the activation of MAPK and NF-κB pathways, thereby promoting tumor growth. These oscillations depend on classical inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} release and require Ca^{2+}-activated K^+ channels, which are strongly upregulated in glioblastoma [1]. We first analyzed Ca^{2+} traces from human glioma cell lines BG5 and S24 to classify cells on the basis of their activity pattern. The pipeline discriminates the cells based on the width and the shape of the Ca^{2+} spikes, as well as depending on the frequency of oscillations. We then developed a detailed single-cell computational model that incorporates IP_3 synthesis and degradation, Ca^{2+} fluxes through IP_3 receptors, Ca^{2+} ATPases, the Na^+–Ca^{2+} exchanger, ORAI channels, and membrane-voltage dynamics including KCa3.1(KCNN4), K(V), Ca(V), and Na(V) channels [2]. Activation of KCa3.1 by cytosolic Ca^{2+} hyperpolarizes the membrane and enhances Ca^{2+} influx through ORAI1 channels, allowing tight control of IP_3-dependent Ca^{2+} oscillations. Model predictions on the impact of selectively inhibiting Ca^{2+} pathways agree with experimental data. Using transcriptomic analyses, we plan to further validate the model by testing if the different oscillatory behaviors that we have identified can be reproduced when changing the simulated activity of the channels according to the expression data. Extending the framework to a coupled two-cell system demonstrated that electrical and Ca^{2+} coupling can induce oscillations in a non-oscillatory cell when paired with an oscillatory partner. This result captures a potential mechanism for signal propagation within glioma networks. Future work will investigate how Ca^{2+} waves propagate across multicellular networks and hopefully predict how to decrease global oscillatory activity, and thus tumor progression. [1] Hausmann D., Hoffmann, D., Venkataramani, V., … and Winkler, F. (2022) Autonomous rhythmic activity in glioma networks drives brain tumour growth. Nature 613, 179-186. [2] Dupont, G., Falcke, M., Kirk, V. and Sneyd, J. (2016) Models of calcium signalling. Springer.

Abstract 30

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SPCA1-Dependent Golgi Regulation Controls Coronavirus Spike Processing and Fusion Activity

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Abstract:
Secretory pathway calcium-ATPase 1 (SPCA1) actively transports cytosolic calcium (Ca^{2+}) and manganese (Mn^{2+}) ions into the Golgi lumen, playing a crucial role in maintaining Golgi ion homeostasis and supporting Golgi-dependent functions. Several Golgi-resident enzymes, including glycosyltransferases and proprotein convertases, have activity that is sensitive to Ca^{2+} and/or Mn^{2+} ions. Ca^{2+}/Mn^{2+} homeostasis in the Golgi is therefore essential for correct protein folding, post-translational modification and efficient trafficking through the secretory pathway. This may be especially important during viral infection. Indeed, SPCA1 is required for proper processing of viral glycoproteins by supporting the maturation of a variety of RNA viruses, including members of the Paramyxoviridae, Flaviviridae, and Togaviridae families. The SARS-CoV-2 spike protein undergoes extensive post-translational modification in the Golgi, including proteolytic cleavage and glycosylation. We hypothesized that SPCA1-mediated Golgi Ca^{2+}/Mn^{2+} homeostasis is required for the proper processing of SARS-CoV-2 spike protein. Using CRISPR/Cas9-mediated SPCA1 depletion and rescue experiments, together with cell-cell fusion assays, mass spectrometry-based glycoproteomics, and proteolytic cleavage analyses, we found that SPCA1 is a regulator of SARS-CoV-2 spike protein maturation and function. Loss of SPCA1 reduced spike-mediated cell fusion, altered glycosylation patterns and impaired spike cleavage. A Ca^{2+}/Mn^{2+} transport-deficient SPCA1 mutant failed to rescue spike cleavage, suggesting that SPCA1's ion transport activity is essential for proper SARS-CoV-2 spike processing. These findings support the role of SPCA1 as a viral host factor, offering a novel therapeutic approach to limit coronavirus infection.

Abstract 31

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Cellular Context Determines the Outcome of ER Stress in Colorectal Cancer: Role of p53 and Differentiation.

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Abstract:
Introduction: Colorectal cancer (CRC), the second leading cause of cancer-related deaths worldwide, is characterized by chronic inflammation and endoplasmic reticulum (ER) stress. The ER is the calcium (Ca^{2+}) store and is where protein synthesis and folding occur. ER Ca^{2+} depletion disrupts the synthesis and turnover of many essential structural and functional proteins in proliferating and differentiated cells. Organic cation transporter 2 (OCTN2) and organic anion transporter 2 (OAT2), which are involved in L-carnitine and drug uptake and are associated with a good prognosis in CRC patients treated with FLOPOX, are increased by 60% and 30%, respectively, in colon tumor cells. The mechanism underlying their differential expression in CRC cells is unclear. Emerging evidence indicates that the tumor suppressor p53 regulates Ca^{2+} homeostasis, the disturbance of which is linked to ER stress. Whether the differential expression of OCTN2 and OAT2 depends on how various cell types with distinct p53 functions respond to chronic ER stress or Ca^{2+} disturbance remains unclear. Aim: This study examines how different CRC cells with distinct p53 functions respond to ER stress and, consequently, their roles in protein synthesis. To achieve this aim, Thapsigargin (TG), an inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump, is used to induce ER stress and to evaluate the transcription and translation of OCTN2 and OAT2, as well as Ca^{2+} mediators involved in Ca^{2+} homeostasis, in proliferating and differentiated CRC cells. Methods: MTT and Trypan blue assays were used to assess the inhibitory effect exerted by TG-induced ER stress on the metabolic activity and proliferation of two CRC cells that differ in their p53 function, HCT-116 (wild type) and Caco-2 (dysfunctional). RT-PCR and western blot analysis were performed to assess ER stress-induced differential modulation on the mRNA and protein expression levels of OCTN2, OAT2, mediators of Ca^{2+} homeostasis (PMCA1/4, TRPV6, NCX1, Oral-1, Stim 2, Serca2/3, ITRP2/3), and junctional proteins E-cadherin (Ecad), connexin 43 (Cx-43) in HCT-116 and Caco-2 cells. Differentiated Caco-2 cells were used to assess how proliferating and differentiated cells respond to ER stress. Results: TG-induced ER stress differentially inhibits the metabolic activity and proliferation of HCT-116 and Caco-2 cells in a dose and time-dependent manner, with HCT-116 cells (p53 wild-type) being more sensitive than Caco-2 cells (dysfunctional wild-type p53 pathway). Bip and CHOP, ER stress markers, were significantly increased by TG-induced ER stress in both the proliferating (HCT-116 and Caco-2) and differentiated (Caco-2) cells. Interestingly, TG-induced ER stress decreased the mRNA levels of OCTN2 and OAT2 transporters in proliferating cells (HCT-116 and Caco-2) but increased their levels in the differentiated cells. Notably, the observed decrease in mRNA levels does not correlate with the lack of change at the protein level. Moreover, differential modulation of junctional proteins (E-cad and Cx-43) and Ca^{2+} mediators (PMCA-1, SERCA-2, and TRPV6) was observed across the different cells. Conclusion: The data indicate that the impact of ER stress on the studied Ca^{2+} mediators, transporters, and structural proteins is shaped by p53 functionality and by the proliferative vs. differentiated state of CRC cells.

Abstract 32

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Two-pore channel-2 controls calmodulin-dependent STIM1 inactivation

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Abstract:
Lysosomes and the endoplasmic reticulum (ER) are Ca^{2+} stores that interact to generate Ca^{2+} signals regulating fundamental cellular processes. NAADP-sensitive TPC2 channels on lysosomes generate local Ca^{2+} elevations that sensitize ER Ca^{2+} release channels, triggering global Ca^{2+} signals. The ensuing ER Ca^{2+} depletion activates store-operated Ca^{2+} entry (SOCE) operated by STIM1-gated ORAI1 channels to sustain long-lasting Ca^{2+} signals. How TPC2 channels interact with STIM1 to integrate distinct intra and extracellular cues is unclear. Here, we show that TPC2 activation inhibits SOCE by enforcing rapid and persistent Ca^{2+}-CaM-dependent inactivation of the STIM1 channel activating domain (CAD). The TPC2 agonists NAADP and TPC2-A1-N abrogated SOCE in multiple cell lines and enhanced the slow Ca^{2+} dependent inactivation (SCDI) of STIM1-gated ORAI1 channels. TPC2 engagement triggered lysosomal Ca^{2+} release and mobilized ER Ca^{2+} stores independently of inositol trisphosphate receptors but prevented RFP-STIM1 recruitment to the TIRF plane by thapsigargin and disassembled RFP-STIM1 clusters forming after store depletion, preventing and acutely reversing SOCE. These effects persisted in STIM1 mutants truncated after the CAD and were prevented by genetic or pharmacological invalidation of TPC2, Calmodulin (CaM) inhibition, and cytosolic Ca^{2+} chelation. We conclude that Ca^{2+} ions released by TPC2 channels on lysosomes regulate CaM-dependent SOCE deactivation.

Abstract 33

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TRPM2 regulates pigmentation via activation of type 1-IFN-ISG15-MITF axis

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Abstract:
Transient Receptor Potential Mclastatin 2 (TRPM2), a Ca^{2+}-permeable cation channel, plays a critical role in innate and adaptive immunity, positioning it as a potential therapeutic target in disease contexts. TRPM2 functions as immunomodulator in immunocytes contributing to generate immune response and regulating immune cell function. However, its role in modulating innate immune response within non-immune cells remains elusive. Melanocytes, traditionally recognized for melanogenesis, are active participants in generating an immune response. However, an ion channel mediated regulation of immune cascade within melanocytes is completely unexplored in the field of pigmentation biology. Here, we demonstrate that TRPM2-mediated activation of type-1 interferon (IFN) response in melanocytes acts as a critical regulator of melanogenesis. We show that TRPM2 is a negative regulator of melanogenesis both via in vivo and in vitro studies. TRPM2$^{-/-}$ mice exhibit an increased pigmentation phenotype in the tail epidermis, demonstrating that TRPM2 negatively regulates melanogenesis. Further, TRPM2 zebrafish morphants also show increased melanogenesis. Subsequent in vitro assays involving pharmacological inhibition, gene silencing, and over-expression studies recapitulate our in vivo findings. Further, TRPM2 silencing in lightly pigmented (LP) primary melanocytes also showed an increased pigmentation phenotype. With unbiased transcriptomics analysis, we further identify that downstream of TRPM2, IFN stimulated gene 15 (ISG15) acts as a novel positive regulator of melanogenesis. We demonstrate that TRPM2 silencing activates type-1IFN response which leads to ISG15 induction. Further, activated ISG15 counteracts the global ubiquitination status in the melanocytes. Furthermore, we reveal ISG15 as a novel positive regulator of microphthalmia-associated transcription factor (MITF), a master regulator of melanogenesis. Taken together, our study unveils a novel pathway wherein TRPM2-mediated autonomous activation of the type-1IFN-ISG15-MITF axis regulates the process of melanogenesis.

Abstract 34

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IP_3Rs critically contribute to ferroptosis-induced cell death

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Abstract:
Ferroptosis is a recently characterized, iron-dependent form of regulated cell death, defined by the accumulation of lipid peroxides within cellular membranes. This process results from iron-driven oxidation of phospholipids, ultimately leading to membrane rupture and finally cell death. Emerging evidence implicates the endoplasmic reticulum-mitochondria contact sites (ER-mitochondria contact sites, EMCSs) as crucial early hotspots for phospholipid peroxidation and as signaling hubs controlling the initiation of ferroptosis. These inter-organelle regions also host essential players of calcium homeostasis and apoptosis regulation, including IP_3Rs and members of the BCL-2 family. However, the crosstalk between ferroptosis, calcium signaling, and BCL-2/IP_3R modulation remains poorly understood. This project aims to elucidate how BCL-2 and IP_3Rs influences ferroptosis-induced cell death. Specifically, it investigates whether BCL-2, known for its anti-apoptotic functions and capacity to inhibit IP_3R-mediated calcium fluxes, can suppress ferroptosis by altering IP_3R activity, calcium transfer, and ER-mitochondria redox signaling. Preliminary data show that HEK IP_3R triple knockout (TKO) cells display markedly higher resistance to erastin-induced ferroptosis compared to wild-type counterparts, suggesting a key role for IP_3Rs in ferroptotic signaling (Dr. Maria Livia Sassano, KU Leuven, Belgium). By recomplementing each IP_3R isoform in HEK TKO cells, IP_3R1 and IP_3R2 appear to mediate susceptibility to ferroptosis, while IP_3R3 does not. Supporting this, diffuse large B-cell lymphoma (DLBCL) cells with high IP_3R2 expression are particularly sensitive to both RSL3 and erastin-induced ferroptosis. Calcium chelation experiments using BAPTA-AM or EGTA-AM further confirm that calcium contribute to ferroptotic cell death, as chelators significantly delay RSL3 and erastin-triggered lethality. This was also confirm in two small lung cancer cell lines (H441 and A549) in Dr. Silvia von Karstedt laboratory (University of Cologne, Germany). To dissect the molecular interplay between BCL-2 and IP_3Rs in ferroptosis regulation, a series of targeted BCL-2 mutants were employed. The BCL-2 K17D mutant disrupts IP_3R binding while retaining Bax-binding capacity, whereas the BCL-2 GR/AA mutant loses Bax interaction but continues to inhibit IP_3Rs. However, our data show that overexpression of either wild-type BCL-2 or these mutants does not significantly alter ferroptosis-induced cell death, indicating that modulation of IP_3R binding by BCL-2 is not sufficient to suppress ferroptotic signaling. This suggests that BCL-2's anti-apoptotic mechanisms may not directly extend to ferroptosis, or that additional regulatory pathways override its inhibitory effects under ferroptotic conditions. In parallel, I will use CySLess-IP_3R1 constructs, in which all cysteine residues are replaced by alanine, preventing receptor oxidation and allowing assessment of redox-sensitive IP_3R modulation to determine whether IP_3R oxidation is a key event linking ferroptosis inducers to calcium dysregulation and ER-mitochondria oxidative stress. Future experiments will quantify the impact of ferroptosis inducers on ER-mitochondria calcium fluxes, redox changes, and contact site dynamics using split-FAST fluorescence reporters. In DLBCL cell lines, genetic modulation of IP_3R2 expression, via knockdown in SUDHL4 or overexpression in OCILY1 cells, will clarify the relationship between IP_3R2 abundance and ferroptotic vulnerability. Comparing responses to erastin and RSL3 will also reveal whether ferroptosis suppression through calcium modulation is universal or pathway-specific, and would open new therapeutic targets in B-cell malignancies.

Abstract 35

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ZDHHC20-mediated S-acylation of Oral3 controls calcium signaling and cell death in Triple-Negative Breast Cancer

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# contributed equally to this work

Abstract:
S-acylation, also known as palmitoylation, is a highly dynamic lipid post-translational modification that regulates protein localisation and function. It is mediated by a family of membrane protein acyltransferases (PATs) containing a catalytic ZDHHC domain and is reversed by acyl-protein thioesterases (APTs). Here, we investigated the role of ZDHHC20-mediated S-acylation of the calcium-signaling protein Oral3 in triple-negative breast cancer (TNBC). Bioinformatic analyses revealed that high expression of ZDHHC20 and Oral3 correlates with poor survival in breast cancer patients. Using acyl-resin-assisted capture (AcylRAC) assays and mutagenesis, we found that Oral3 is S-acylated at cysteine 143, located in the intracellular loop between transmembrane domains 2 and 3. This modification was required for functional store-operated calcium entry (SOCE) in HEK-293 cells. AcylRAC analyses further showed that Oral3 exhibits higher S-acylation levels in MDA-MB-231 TNBC cells compared with ER-positive MCF7 and non-tumorigenic MCF10A cells. Consistent with its role as a negative regulator of SOCE in certain cellular contexts, silencing Oral3 reduced SOCE in MCF7 and MCF10A cells but enhanced SOCE in MDA-MB-231 TNBC cells. Silencing zDHHC20 abolished Oral3 S-acylation, and downregulation of zDHHC20 reduced SOCE in non-tumorigenic MCF10A and ER-positive MCF7 cells while enhancing SOCE in TNBC MDA-MB-231 cells. Furthermore, silencing Oral3 or zDHHC20 impaired proliferation of MDA-MB-231 cells, but not MCF10A cells, in a calcium-dependent manner. Cell-cycle analysis revealed no significant changes in cell-cycle distribution; however, silencing Oral3 or zDHHC20 in TNBC cells significantly increased the apoptotic cell population, as assessed by Annexin V staining. Together, these findings identify zDHHC20 as a key regulator of Oral3-dependent calcium signalling and highlight its potential as a therapeutic target in aggressive TNBC.

Abstract 36

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S-Acylation by zDHHC20 targets STIM1 to cholesterol-rich ER-PM contact sites to sustain Ca^{2+} signalling at the immune synapse

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Abstract:
Store-operated calcium entry (SOCE) is a fundamental signalling pathway that underlies the ability of immune cells to mount long-lasting effector responses. Mutations in STIM1 and ORA11 genes that mediate SOCE cause severe immunodeficiencies. Depletion of endoplasmic reticulum (ER) Ca^{2+} stores activates the ER-resident Ca^{2+} sensor STIM1 by triggering its oligomerisation and translocation to ER-plasma membrane (PM) contact sites, where it traps and gates ORAI1 Ca^{2+}-permeable channels. The sequence of molecular steps underlying STIM1 activation is well characterized, but how S-Acylation impact the targeting of STIM1 to ER-PM junctions and its interaction with ORAI1 remains poorly understood. S-Acylation, a reversible post-translational lipid modification, can target proteins to lipid domains enriched in cholesterol. We previously showed that S-Acylation regulates the activity ORAI1 channels in T-cells. Here, we identify a critical role for S-Acylation in promoting the efficient translocation of STIM1 to ER-PM contact sites. Using biochemical assays, mutagenesis, Ca^{2+} imaging, and TIRF microscopy, we show that STIM1 is S-acylated on cysteine 437, and that zDHHC20 (PAT20) catalyses this lipidation. Substitution of Cys-437 with alanine (C437A) markedly impaired SOCE in HEK-293 and Jurkat T cells and prevented the recruitment of STIM1 to the PM upon store depletion. To identify the conformational activation step controlled by S-Acylation, we used a $STIM1^{1-448}$ truncation mutant that binds to PM phosphoinositides (PI) via a stretch of positively charged residues (4K) within its STIM-ORA1 activating region (SOAR), independently of distal cytosolic STIM1 domains. The C437A mutation strongly reduced the recruitment of $STIM1^{1-448}$ to ER-PM junctions, indicating that SOAR-PI interactions are not sufficient for efficient PM trapping. We then used a shorter $STIM1^{1-431}$ mutant lacking the critical cysteine that does not translocate to ER-PM contact sites upon store depletion. Remarkably, re-introducing a cysteine at position 432 restored PM translocation, demonstrating that S-Acylation is sufficient to drive STIM1 membrane targeting, likely by promoting its integration into specific lipid microdomains. To assess the implication of membrane lipids, we acutely depleted cholesterol from cells with methyl-β-cyclodextrin (MβCD). MβCD treatment dramatically inhibited the translocation of wild-type STIM1 to ER-PM contact sites upon store depletion, phenocopying the behaviour of the non-acylated C437A mutant, which itself was not impacted by MβCD. These findings indicate that S-Acylation targets STIM1 to ER-PM contact sites enriched in cholesterol. In T-cells, these molecular defects impaired immunological synapse formation and diminished downstream signalling. Wild-type STIM1 accumulated robustly at synapses formed on anti-CD3-coated coverslips or with antigen-presenting Raji cells, whereas the C437A mutant displayed significantly reduced enrichment. Consistently, NFAT-driven transcriptional activity was strongly attenuated in cells expressing the S-Acylation-deficient mutant. Our results identify S-Acylation as a regulatory mechanism controlling STIM1 recruitment to PM domains enriched in cholesterol. This reversible change in lipid affinity enables STIM1 to sequentially interact with PM domains enriched in phosphoinositides via its polybasic and SOAR domains and with cholesterol via its S-Acylated C437, facilitating interactions with ORAI1 channels at immune synapses. These insights shed new light on how the membrane environment shapes Ca^{2+} signalling and may inform strategies aiming to modulate immune responses by targeting lipid modifications.

Abstract 37

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The architecture of the cortical endoplasmic reticulum supports Ca^{2+} tunneling

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Abstract:
Ca^{2+} signaling intervenes in virtually all cellular processes and relies on two main sources of Ca^{2+}: the extracellular space and the endoplasmic reticulum (ER). After store depletion, both compartments are coupled through Store-Operated Ca^{2+} Entry (SOCE) to refill the ER stores using the extracellular Ca^{2+} pool. Ca^{2+} entering the cell through SOCE also activates intracellular Ca^{2+} sensitive targets and because of the compactness of the SOCE microdomain those targets need to be localized very near to the entry point of Ca^{2+}. To activate distal effectors using SOCE cells rely on a mechanism termed Ca^{2+} tunneling (Courjaret and Machaca, 2025). Ca^{2+} tunneling develops when both IP_3-receptors and SOCE are active. Extracellular Ca^{2+} enters the cell through SOCE, is transported by SERCA pumps into the ER and channeled towards distant cortical targets using the ER lumen as a tunnel, isolating the signal from the cytosol. Cortical delivery of Ca^{2+} to PM effectors such as ion channels is favored by tunneling while deeper targets receive less Ca^{2+} (Courjaret and Machaca, 2025). We recently outlined the 3D localization of the elements of Ca^{2+} tunneling relative to each other: SOCE cluster, SERCA pump, PMCA, and IP_3 receptors (Courjaret and Machaca, 2025). Here, using 3D electron microscopy we identify a "basket" structure in the cortical ER joining membrane contact sites where SOCE occurs. Mathematical modeling shows that the ER Basket greatly enhances the efficiency of tunneling. To further evaluate the importance of the cortical ER, we manipulated the actin cytoskeleton and ER structure and measured the functional consequences on SOCE and tunneling. Disrupting actin polymerization had little effect on SOCE and tunneling, while stabilizing the cytoskeleton favored tunneling. Brefeldin treatment increased the amount of cortical ER and enhanced Ca^{2+} tunneling. Together those results support a critical role for a stable cortical ER architecture in Ca^{2+} tunneling. Further investigation will be required to identify differences in this architecture depending on the cell type and/or physiological conditions, particularly in secretory cells. Courjaret et al., J. Cell. Biol. 2025 Courjaret and Machaca, Cold Spring Harb. Perspect. Biol. 2025

Abstract 38

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Mechanistic Evidence for Dual-Channel Modulation by Eact via TRPV4 in Cystic Fibrosis

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Abstract:
Cystic fibrosis (CF) is a life-limiting genetic disorder characterized by impaired airway hydration, defective mucociliary clearance, and progressive lung disease resulting from dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR). While CFTR modulators have improved outcomes for selected patients, their mutation-specific efficacy and limited accessibility highlight the need for complementary, CFTR-independent strategies to restore airway surface liquid homeostasis. Alternative epithelial ion channels that regulate calcium signaling, chloride secretion, and ciliary activity represent promising therapeutic targets. Fact is a small-molecule activator of the Ca^{2+}-activated chloride channel TMEM16A, and emerging evidence suggests that it also modulates transient receptor potential vanilloid 4 (TRPV4), a Ca^{2+}-permeable cation channel involved in ciliary beat regulation and epithelial ion transport. However, the molecular basis and functional consequences of this interaction have not been fully elucidated. Here, we utilized an integrated computational and experimental approach to investigate whether Eact directly binds and activates human TRPV4. Structure-based molecular docking identified Eact binding within a known allosteric pocket of TRPV4 associated with channel gating. Molecular dynamics simulations in an explicit lipid bilayer demonstrated stable ligand engagement and increased conformational flexibility in regions linked to channel activation. Binding free-energy analysis supported a favorable interaction profile consistent with agonist-like behavior. Functional validation using whole-cell patch-clamp electrophysiology in TRPV4-expressing HEK293 cells revealed that Eact significantly increased TRPV4-mediated currents compared with baseline, confirming direct channel activation. Together, these findings provide structural and functional evidence that Eact acts as a direct TRPV4 ligand while retaining its activity on TMEM16A. This dual-channel mechanism highlights a CFTR-independent strategy to enhance epithelial ion transport and mucociliary clearance. By integrating molecular modeling with electrophysiological validation, this work supports dual-channel modulation as a translational approach for improving airway function in cystic fibrosis and related respiratory diseases.

Abstract 39

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Computational modelling of Ca^{2+} signalling in Endoplasmic Reticulum-Plasma Membrane junctions

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Abstract:
Ca^{2+} is a ubiquitous signalling ion that regulates virtually all cellular processes. Beyond well-studied whole-cell responses, highly local and transient Ca^{2+} signals emerge within Endoplasmic Reticulum-Plasma Membrane (ER-PM) junctions, known as Ca^{2+} nanodomains, typically ~200 nm wide and ~15 nm deep that host the machinery for Store-Operated Ca^{2+} Entry. These signals contribute not only to global Ca^{2+} responses but also to intrinsically local physiological readouts; however, their millisecond timescale and nanoscale confinement make mechanistic understanding difficult with imaging alone. We developed a three-dimensional ER-PM junction model that couples deterministic reaction-diffusion and can incorporate stochastic channel gating in realistic geometries. The framework explicitly represents the spatial organisation and kinetics of Oral channels, IP_3 receptors (IP_3Rs) and Plasma Membrane Ca^{2+}-ATPases (PMCAs), and permits controlled variation of junctional architecture, channel/pump distributions and Ca^{2+} buffering. Applied in two independent studies, the model has explained (i) Adhesion-Dependent Ca^{2+} Microdomains (ADCM) in T cells by showing that local IP_3R-mediated release synergises with Oral influx, while PMCA critically shapes amplitude and spatial extent (published work); and (ii) predicts how exogenous buffers (e.g., BAPTA, EGTA, TF-BAPTA) modulate junctional Ca^{2+} signals---altering peak size and detectability within the junction, with consequences for physiological readouts (ongoing work). These results establish a versatile computational framework for nanoscale Ca^{2+} signalling in ER-PM junctions, complementing experiments to uncover molecular mechanisms controlling Ca^{2+} nanodomain formation and to quantify how channel distribution and junctional architecture govern local signalling in cells.

Abstract 40

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Decoding the role of the cyclic GMP -- PKG and downstream organelles to control the last step of the intraerythrocytic cycle of the malarial parasite Plasmodium falciparum

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Abstract:
Malaria is a deadly disease caused by the parasite of the Plasmodium genus. The parasite must complete its sexual cycle in the mosquito vector of the Anopheles genus and undergo a sexual cycle in the vertebrate host. The cycle is tightly regulated to guarantee that the parasite successfully transitions between complex and different stages. In the vertebrate host, the asexual replication of the parasites in red blood cells (RBCs) is the primary cause of the main symptoms of malaria. Especially the process of egress of daughter parasites from the RBCs, which occurs synchronously in the bloodstream around midnight, causing the intermittent fevers characteristic of malaria. In Plasmodium falciparum, the deadliest species of the parasite, the fever cycles repeat every 48 hours. The regulation of the egress process of parasites is dependent on the activation of the cyclic GMP--PKG pathway, which leads to an increase in cytosolic Ca^{2+} concentration, activation of Calcium--Dependent Protein Kinase--5 (CDPK5), causing the breakdown of the membranes of the vacuole that contains the parasite and of the red blood cells, releasing the parasites into the bloodstream. In this work, we focused on understanding the organelles downstream of PKG in the egress process. We used a strain of the parasite that expresses the genetically encoded Ca^{2+} indicator PfGCaMP3 in the cytosol to study the dynamics of this ion in the cytosol prior to egress, identifying an increase in Ca^{2+} with oscillatory characteristics 30 to 25 minutes before the release of parasites into the bloodstream. Additionally, we employed a combination of pharmacological tools to modulate the cyclic GMP-PKG pathway and elucidate the downstream organelles activated by PKG, identifying that this kinase causes an increase in cytosolic Ca^{2+} through the endoplasmic reticulum, the lysosomal-like compartment called the food vacuole, and also extracellular Ca^{2+}, to orchestrate an oscillatory pattern resulting from the interplay between these three Ca^{2+} sources. Understanding how PKG controls this phenomenon is important for the targeted search for antimalarials, as there is a spread of antimalarial resistance strains of Plasmodium worldwide.

Abstract 41

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Role of STIM1 Phosphorylation in Dissociation of ER from the Mitotic Spindle

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Abstract:
The endoplasmic reticulum (ER) is a highly dynamic organelle that undergoes several elongation, fusion, and junction sliding events supported by microtubules. During cell division, the ER undergoes extensive remodeling, with its membranes coalescing around the spindle poles and the cell cortex, while being largely excluded from the mitotic spindle. The mechanisms underlying this remodeling are poorly understood, however, one mechanism involves the ER Ca${}^{2 +}$ sensor stromal interaction molecule 1 (STIM1). STIM1 couples ER membranes to microtubules through its interaction with the microtubule +TIP protein end binding 1 (EB1). Phosphorylation of STIM1 during mitosis has been argued to cause its dissociation from EBI, and hence prevention of mis-localization of ER to the mitotic spindle. However, work employing endogenous expression systems reported that phosphorylation of STIM1 is dispensable for normal mitotic ER organization. This project aims to reconcile these discrepancies and further dissect the contribution of STIM1 phosphorylation towards ER exclusion from the mitotic spindle, using two strategies: first, transient overexpression of wild-type, non-phosphorylatable (IOA: ten CDK phosphorylation consensus sites mutated to alanine), and phosphomimetic (IOE: ten CDK phosphorylation consensus sites mutated to glutamate) forms of STIM1 in STIM1-knockout HEK293 cells, to examine STIM1 association with the microtubules in mitotic cells; and second, using mouse embryonic fibroblasts (MEFs) derived from STIM1--IOA knock-in mouse line that expresses the non-phosphorylatable STIM1 mutant at endogenous physiological levels. We observe that indeed overexpression of non-phosphorylatable STIM1--IOA drives its accumulation within the mitotic spindle and causes apparent ER-microtubule association, whereas overexpressed wild-type and phosphomimetic IOE constructs do not mis-localize. Strikingly, this phenotype is absent in the MEFs expressing STIM1--IOA mutant at endogenous levels, where ER exclusion from the spindle remains intact. However, the STIM1--IOA MEFs exhibit alternative phenotypes, including reduced proliferation and an increased frequency of multipolar spindle formation, suggesting subtler mitotic disturbances without overt ER mislocalization. Together, these findings support a model in which mitotic ER exclusion is preserved under physiological non-phosphorylatable STIM1 expression, arguing that the ER mis-localization to the spindle likely reflects dosage-dependent saturation of ER-microtubule tethering mechanisms rather than a primary role of phosphorylation alone. Ongoing work will focus on expression titration, correlation of phenotypes with STIM1 protein abundance, and validation using other ER-microtubule linker proteins. Ultimately, this study refines our understanding of mitotic ER organization and highlights mechanisms whose failure could contribute to diseases linked to defective cell division.

Abstract 42

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Oral3 orchestrates gemcitabine resistance in pancreatic cancer via NFATc1-SLIT3 axis

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Abstract:
Pancreatic Cancer (PC) is one of the most aggressive cancers and is associated with poor prognosis. While first-line PC chemotherapy, i.e., gemcitabine, offers survival benefits, the acquired gemcitabine-resistance leads to recurrence, metastasis, and the long-term prognosis remains poor. Although there is substantial clinical evidence of gemcitabine resistance, the cellular and molecular mechanisms that drive this resistance remain largely unappreciated. Here, we reveal that Oral3, a $Ca^{2}{}_{3}$-selective channel, is a crucial inducer of gemcitabine resistance. We demonstrate that Oral3 is overexpressed and hyper-functional in gemcitabine-resistant PC cells. Oral3 silencing in gemcitabine-resistant cells enhances chemo-sensitivity, inhibits migration, stalls invasion, and moderates stemness characteristics. Notably, studies in the zebrafish model corroborate Oral3's significance in gemcitabine-resistance in vivo. Mechanistically, our unbiased RNA-seq analysis, coupled with robust functional studies, reveals that SLIT3 acts downstream of Oral3 to induce gemcitabine resistance. Finally, we report that the NFATc1 transcription factor bridges Oral3 to SLIT3 transcription. Taken together, this study reveals Oral3 as a promising target to combat gemcitabine-resistance and uncovers a unique Oral3-NFATc1-SLIT3 signaling module that drives chemoresistance.

Abstract 43

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S-Acylation of STIM1 splice variants differentially regulate Store Operated Ca^{2+} Entry

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Abstract:
Skeletal muscle contraction depends on plasma membrane (PM) depolarization and activation of voltage-gated Ca^{2+} channels (VGCCs), which trigger Ca^{2+} release from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyRs). Sustained contraction and resistance to fatigue involves replenishment of SR Ca^{2+} stores through STIM--ORAI--mediated store-operated Ca^{2+} entry (SOCE). Stromal interaction molecule proteins (STIMs) act as SR Ca^{2+} sensors that, upon store depletion, oligomerize and translocate to SR--PM junctions to activate Oral channels. S-acylation, the reversible attachment of long-chain fatty acids, most commonly palmitate, to cysteine thiols, is mediated by membrane protein acyltransferases (PATs), of which 23 isoforms are encoded in the human genome. This lipid modification represents a novel mechanism for modulating SOCE through lipidation of Oral1 at cysteine C143 and STIM1 at cysteine C437, promoted by PAT20. Here, we aimed to determine whether STIM1 and its muscle-specific splice variant STIM1 Long (STIM1L), which contains an additional 106 amino acids encoding an actin-binding domain, are S-acylated and to understand the physiological relevance of this modification in skeletal muscle function. Using the Acyl-Resin Assisted Capture (AcylRAC) assay, we found that both STIM1 and STIM1L are S-acylated at cysteine C437 located in the SOAR (STM-Orai Activating Region) domain. In HEK-293 cells, S-acylation of STIM1 enhanced SOCE, as measured by intracellular Ca^{2+} imaging, whereas S-acylation of STIM1L had the opposite effect. To assess basal channel activity mediated by STIM1 (and its C437A mutant) or STIM1L (and its C437A mutant), we performed basal manganese (Mn^{2+}) quench experiments, which revealed that Mn^{2+} entry mediated by STIM1L C437A was significantly reduced. Using total internal reflection fluorescence (TIRF) microscopy, we observed that STIM1 C437A translocated less efficiently to the PM upon activation and exhibited reduced co-localization with Orai1 channels. In contrast, STIM1L C437A translocated and co-localized with Orai1 as efficiently as the wild-type protein. However, the fraction of cells displaying preclustered STIM1L, a well-established characteristic of this splice variant, was significantly reduced, consistent with the diminished basal activity observed in Mn^{2+} quench experiments. AcylRAC and SOCE assays further revealed that STIM1, but not STIM1L, functions downstream of PAT20. In human myoblasts, STIM1, STIM1L and Orai1 were all S-acylated during myogenic differentiation. PAT20 overexpression increased SOCE in myotubes that was followed by increased expression of myogenic markers MEF2c and myosin heavy chain. In conclusion, S-acylation of cysteine C437 in STIM1 is critical for sustaining SOCE after store depletion and for efficient interaction with Orai1, whereas in STIM1L, this residue is essential for basal Orai1 activity.

Abstract 44

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Investigating the interplay between lysosomal and ER Ca^{2+} channels

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Abstract:
Calcium (Ca^{2+}) is a ubiquitous signaling messenger that regulates diverse physiological functions including contraction, gene expression, metabolism, and cell proliferation. Moreover, Ca^{2+} is involved in the activation of diametrically opposite cellular responses such as cell proliferation and cell death exemplifying the need for exquisite specificity of Ca^{2+} signaling events. Many of these Ca^{2+} signaling events occur in specific nanodomains where intracellular organelles interact with each other or with the plasma membrane. The endoplasmic reticulum (ER) is the largest Ca^{2+} store in the cell. Inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) are the major Ca^{2+} release channels located in the ER membrane and exhibit subtype specific regulation and properties. In addition, acidic organelles such as lysosomes and endosomes also participate in intracellular Ca^{2+} signaling. Two pore channels (TPCs) are a class of ubiquitously expressed ion channels that reside in this compartment. These channels switch their ion permeability when stimulated with nicotinic acid adenine dinucleotide phosphate (NAADP) or upon direct activation with phosphatidylinositol-3,5-bisphosphate ($PI(3,5)P_2$). The discovery of cell-permeable, specific, TPC2 agonists that mimic NAADP and $PI(3,5)P_2$ has made it possible to study signaling through TPC2 in an endogenous setting. TPC2-A1-N mimics NAADP, promoting Ca^{2+} currents, while TPC2-A1-P is a $PI(3,5)P_2$ mimetic, activating robust Na^+ currents. We hypothesize that local Ca^{2+} signals originating from TPC2 sensitize ER Ca^{2+} release channels resulting in global Ca^{2+} responses. Preliminary data from our lab suggests that TPC2-evoked Ca^{2+} signals sensitize IP_3Rs and RyRs; however, the spatiotemporal constraints and characteristics of this crosstalk remain unresolved. Data will be presented using molecular tools combined with state-of-the-art live-cell Ca^{2+} imaging techniques investigating the functional and structural interactions between endolysoosomal TPC2 and the individual isoforms of ER-resident IP_3Rs and RyRs.

Abstract 45

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Distinct expression of the three calmodulin--encoding genes, CALM1, CALM2, and CALM3 in the brain

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Abstract:
Calmodulin is a highly conserved calcium sensor protein which is essential for neuronal signaling and synaptic plasticity. Several recent studies have identified calmodulin variants in patients with neurodevelopmental disorders, yet the role of calmodulin in neurological disease is largely unexplored. In humans, three independent genes, CALM1 (chr14q31), CALM2 (chr2p21), and CALM3 (chr19q13), encode the exact same 149 amino acid sequence for calmodulin. The evolutionary rationale for maintaining three conserved calmodulin genes remains unclear. In patients, the genetic variants are unevenly distributed between the three genes, suggesting different usage and/or role of the genes. These observations raise the question of whether their transcription is regulated differently across the human brain. Understanding how CALM1-3 are expressed under normal conditions may provide insight into how CALM variants contribute to neurodevelopmental disorders. We therefore sought to establish an overview of CALM expression patterns under normal conditions and determine whether the three genes exhibit distinct expression profiles in the brain. To explore the CALM gene expression, we analyzed publicly available bulk RNA-sequencing datasets from human induced pluripotent stem cell (iPSC)-derived neurons and post mortem brain tissue. All three CALM genes were consistently expressed across datasets, but with distinct relative abundances. In iPSC-derived neurons, CALM3 showed the highest expression, whereas in human brain tissue, CALM1 and CALM3 showed higher expression than CALM2. These findings suggest that the three CALM genes are subject to differential transcriptional regulation, despite encoding an identical protein. Such differences in expression are likely to be important when considering the functional impact of CALM variants in neurodevelopmental disorders.

Abstract 46

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Microglial CX3CR1 receptor undergoes endocytosis and vesicular trafficking upon extracellular Tau exposure

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Abstract:
Misfolded Tau protein clearance from extracellular space is highly impaired in the Alzheimer's disease brain. Microglia, the brain resident macrophage cells, are majorly involved in the active clearance of Tau oligomers and aggregated fibrils during the initial stages of disease progression. In recent research, GPCRs have gained more attention in the field of Alzheimer's disease therapy. Extracellular Tau protein is involved in direct binding to several GPCRs that promote receptor activation, downstream signalling, Tau internalization, and receptor endocytosis. Upon Tau binding, receptors undergo vesicular trafficking by several cellular mechanisms, leading to extracellular Tau internalization and degradation. CX3CR1 is a microglia chemokine receptor that maintains microglia at rest upon fractalkine binding in neurons. Extracellular Tau also binds the CX3CR1 receptor for microglial activation and Tau phagocytosis. In this study, we are interested in understanding CX3CR1 interaction with Tau oligomers and aggregates promoting clathrin-mediated receptor endocytosis. Further, we studied the association of the Tau-CX3CR1 complex with early, late, recycling endosomal and lysosomal markers to visualize the role of CX3CR1 in extracellular Tau clearance. Here, we have used confocal fluorescence microscopy to visualize the immunostained samples at different time intervals. Our study clearly demonstrated the association of the microglial chemokine CX3CR1 receptor in sensing and promoting extracellular Tau internalization, accumulation, and degradation, which would shed some light in the field of misfolded Tau protein clearance by microglial GPCRs in Alzheimer's disease. Key findings: - Microglia phagocytose full-length Tau (hTau40wt) oligomers via actin remodelling. - Tau oligomers interact with CX3CR1 that mediates microglial migration, lamellipodia-filopodia formation and MTOC polarization. - Microglia degrade extracellular Tau oligomers' deposition by CX3CR1-mediated chemotaxis via forming actin microstructures. - Microglia internalize Tau oligomers by CX3CR1-mediated endocytosis and accumulated in Rab7+ vesicles, but monomer traffics towards lysosomal degradation.

Abstract 47

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Atrial Shear-mechanotransduction and local Ca^{2+} Adaptation

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Abstract:
Pathological shear stress induces Ca^{2+} waves in quiescent atrial myocytes via gap junction hemichannel-mediated ATP release. We examined whether and how shear stress alters atrial local Ca^{2+} signaling and its underlying mechanisms. Using two-dimensional confocal Ca^{2+} imaging in combination with a microfluidic jet system, we assessed the effects of shear stress on peripheral junctional and central non-junctional Ca^{2+} transients in field-stimulated rat and mouse atrial myocytes and in the HL-1 mouse atrial cell line. Pharmacological and genetic interventions as well as local mitochondrial Ca^{2+} measurements were adopted. In intact murine atrial cells, shear stress (~16 dyn/cm^2) elicited transient enhancements in local Ca^{2+} transients and diastolic Ca^{2+} increases, with larger effects in the center than in the periphery. This stimulatory effect was rapidly reversed to inhibitory effects on Ca^{2+} transients by prolonged shear stress. In cardiac-specific connexin 43 conditional knockout mouse atrial myocytes, this shear effect was maintained. Shear-induced stimulatory effects on Ca^{2+} transients were significantly suppressed by pretreatment with the pannexin blocker probenecid (800 μM), P2 purinoceptor antagonist suramin (30 $_3$M) or the inositol 1,4,5-trisphosphate receptor (IP_3R) inhibitor 2-APB (3–10 μM). To test the role of mitochondrial Ca^{2+} mobilization in the shear-Ca^{2+} response, cytosolic and mitochondrial Ca^{2+} were simultaneously imaged (60–120 Hz) using rhod-2 and CEPIA2mt in monolayered HL-1 cells, respectively. Similar shear-induced local Ca^{2+} changes were observed in highly confluent (>90%) HL-1 cells. Mitochondrial Ca^{2+} increased following cytosolic Ca^{2+} rise, with larger and faster changes in the periphery than in the center. Inhibition of pannexin, P2 purinoceptors (by suramin), or the sarcoplasmic reticulum (SR) Ca^{2+} pump (by thapsigargin) almost completely suppressed the shear-induced Ca^{2+} increases in both the cytosol and mitochondria. Pretreatment with 2-APB suppressed both cytosolic and mitochondrial Ca^{2+} increases under shear stress in a concentration-dependent manner (3–10 $_3$M), with more potent effects in the mitochondria than in the cytosol. Blockade of the mitochondrial Ca^{2+} uniporter (MCU) with Ru360 (10 μM) selectively inhibited mitochondrial Ca^{2+} increase under shear stress. Our data suggest that shear stress enhances Ca^{2+} transients in beating atrial myocytes via pannexin–P2 receptor signaling-mediated SR Ca^{2+} release, resulting in rapid Ca^{2+} loading in peripheral mitochondria via the IP_3R and MCU. This shear-induced local Ca^{2+} signaling pathway may allow atrial myocytes to measure atrial blood volume or regurgitant jets, thereby immediately adapting by enhancing both contractility and Ca^{2+}-dependent mitochondrial function.

Abstract 48

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Calcium-Dependent Control of a Perinuclear cAMP Compartment Promotes Neurite Extension

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Abstract:
Ca^{2+} acts as the initiating signal that couples neuronal activity to perinuclear cAMP signaling and axon growth. We previously identified a perinuclear cAMP compartment organized by the scaffold protein muscle A-kinase anchoring protein α (mAKAPα/AKAP6α) as necessary and sufficient for axon elongation in cultured rat hippocampal neurons. Here, we demonstrate that activity-dependent Ca^{2+} influx through voltage-gated L-type Ca^{2+} channels selectively elevate Ca^{2+} levels at the nuclear envelope, where it locally regulates cAMP signaling within mAKAPα signalosomes. Membrane depolarization induced a Ca^{2+}-dependent activation of perinuclear protein kinase A, while Ca^{2+} elevations elsewhere in the cell failed to elicit comparable PKA responses, indicating that Ca^{2+} acts in a spatially restricted manner. Both perinuclear PKA activation and downstream axon elongation were abolished by buffering Ca^{2+} specifically at the nuclear envelope, demonstrating that local Ca^{2+} signaling is required upstream of cAMP/PKA activation. These findings establish Ca^{2+} as the proximal regulator of perinuclear cAMP signaling, functioning through mAKAPα-organized microdomains to translate electrical activity into localized cAMP/PKA activation that drives neurite extension.

Abstract 49

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Bcl–2 upregulates calcium efflux through PMCA and NCX1 to preserve intracellular calcium homeostasis and confer resistance to apoptosis

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Abstract:
Bcl-2 is a key regulator of intracellular calcium (Ca^{2+}) homeostasis, impacting cellular metabolism, survival, and apoptosis. While its modulation of Ca^{2+} dynamics within the endoplasmic reticulum and mitochondria has been extensively studied, the role of Bcl-2 in controlling plasma membrane Ca^{2+} flux remains unclear. Here, we show that Bcl-2 enhances store-operated calcium entry (SOCE)-mediated Ca^{2+} influx, which sustains spontaneous Ca^{2+} oscillations. In parallel, Bcl-2 upregulates plasma membrane calcium ATPase (PMCA) and sodium–calcium exchanger 1 (NCX1), facilitating Ca^{2+} extrusion. Pharmacological inhibition of PMCA with resveratrol (RES) or NCX1 with ORM-10103 suppressed spontaneous Ca^{2+} oscillations, with PMCA exerting a more prominent effect. Both inhibitors also aggravated thapsigargin-induced Ca^{2+} cytotoxicity. Together, these findings identify a dual role for Bcl-2 in maintaining Ca^{2+} homeostasis: promoting Ca^{2+} influx to support oscillatory signaling and facilitating Ca^{2+} efflux to prevent toxic accumulation. This dual regulation provides new insights into Bcl-2-mediated calcium signaling and its contribution to apoptosis suppression, independent of interactions with other Bcl-2 family proteins.

Abstract 50

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Molecular partnerships of CRISPs in calcium expulsion pathways

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Abstract:
CRISP/s (Cysteine Rich Secretary Proteins) show an expression bias in the male reproductive tract where they are sequentially acquired on sperm during epididymal transit. Additionally, CRISPs are reported in the venom secreting ducts of insects and reptiles. CRISPs harbor an ion channel regulatory motif in the C-terminus; however, identity of ion channels regulated by mammalian CRISPs is limited. To address this, an interactome analysis of CRISP4 from rat caudal spermatozoa was carried out. Rat CRISP4 (rCRISP4) was immunoprecipitated from caudal spermatozoa and subjected to LC-MS analysis. Plasma membrane localizing proteins were shortlisted and the interaction of PMCA4b (Plasma membrane calcium ATPase, isoform b), a calcium extrusion pump, was validated by co-immunoprecipitation and co-localization. The effect of CRISPs on PMCA4b mediated intracellular calcium [Ca^{2+}] efflux was determined using ratiometric fluorescent dye Fura-2-AM. Impact of presence or absence of binding partners of CRISPs on calcium expulsion was also compared. Membrane interactome of rCRISP4 from caudal spermatozoa revealed PMCA4b as a novel binding partner and their interaction was validated. Multiple CRISPs interacted with hPMCA4b where association of hPMCA4b with human CRISP1 and rCRISP4 delayed hPMCA4b mediated calcium extrusion but not human CRISP3. Effect of different CRISPs on PMCA4b mediated calcium expulsion was correlated with their secretion propensity and cholesterol binding. Our study unveils a previously unknown mechanism for the role of CRISPs in modulating intracellular calcium levels via PMCA4b.

Abstract 51

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Spontaneous mutations in Inositol Trisphosphate Type 3 receptor cause idiopathic multicentric Castleman disease

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Abstract:
Idiopathic multicentric Castleman disease (iMCD) is an atypical lymphoproliferative disorder characterized by enlarged lymph node tissue with associated histopathological abnormalities. However, the cause of iMCD and the underlying molecular mechanisms remain unclear. To detect somatic mutations, whole exome sequencing using the Tempus xE platform was performed in a cohort of iMCD (n=62), healthy donors (n=6), and diffuse large B cell lymphoma patients (n=6). Notably, several novel variants were identified in the type 3 inositol trisphosphate receptor (ITPR3), ryanodine type 1 and type 3 receptors. The identified variants were predicted to be pathogenic by at least 5 of the 7 pathogenic prediction softwares (SIFT, Polyphen, LRT, FATHMM, M-CAP, ClinPred, AlphaMissense). These observations indicate potential dysregulation of intracellular calcium (Ca^{2+}) signaling in iMCD. Three missense mutations in ITPR3 - Asp203Asn, Val210Gly, and Val432Met were identified in the N-terminal beta-trefoil 1 (β-TF1, suppressor domain) and β-TF2 (proximal IP_3 binding core) domains. The Asp203 and Val210 residues are located in the β-TF1 domain and are conserved in IP_3R sub-types. Asp203Asn mutation results in loss of negative charge on the Asp side chain while the Val210Gly mutation causes a change in hydrophobicity index (V-hydrophobic, G-polar). Similarly, the Val432 in the β-TF2 domain is conserved in IP_3R sub-types. We hypothesize that these mutations alter IP_3 and/or Ca^{2+} binding affinity of IP_3R3 resulting in altered channel activity. To test this, we generated point mutations in IP_3R3 expression plasmid to determine the consequences of these mutations on IP_3R3 expression, sub-cellular localization, and channel function. Transient transfection of these mutations in a CRISPR modified HEK-293 cell line lacking all the three native IP_3Rs previously generated in our laboratory followed by western blotting revealed that the three mutants migrate at the appropriate size indicating that these mutations do not interfere with IP_3R3 protein expression. Furthermore, single cell Fura-2 AM Ca^{2+} imaging indicates that mutant homo-tetrameric channels retain function in response to IP_3 generating agonists. Our current ongoing investigations are aimed at deciphering whether these mutations are - (a) gain-of-function or loss-of-function mutations, (b) mutant channels localize properly to the endoplasmic reticulum, (c) form tetramers, and (d) to compare the kinetics of mutant channels to the wild-type IP_3R3. Overall, our investigations suggest somatic mutations in IP_3R3 leading to altered intracellular Ca^{2+} signaling patterns as a potential mechanism causing iMCD.

Abstract 52

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Intracellular Ca^{2+} depletion induces FOXM1 SUMOylation and accumulation on the inner nuclear membrane resulting in accelerated G2/M cell cycle transition

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Abstract:
Intracellular Ca^{2+} have important functions in regulating the activity of numerous transcription factors and cancer development. FOXM1 is a key transcription factor and an important oncogenic protein involved in tumorigenesis, but whether Ca^{2+} regulates the function of FOXM1 remains unclear. BAPTA-AM was used to cause intracellular Ca^{2+} depletion, and immunofluorescence staining was used to observe the distribution of FOXM1 in cells. Here, we found that Ca^{2+} promotes the entry of FOXM1 into the nucleus in many cancer cell lines, but Ca^{2+} depletion causes FOXM1 to accumulate at the inner nuclear membrane (INM). Further experiments revealed that sequestered FOXM1 colocalized with lamin B in the INM and was influenced by the activity of nuclear exportin 1 (XPO1). To investigate the mechanism by which intracellular Ca^{2+} affects FOXM1 sequestration, we found that among posttranscriptional modifications, only FOXM1 SUMOylation showed a significant increase under conditions of Ca^{2+} depletion, while inhibition of FOXM1 SUMOylation using siRNAs or inhibitors rescued FOXM1 sequestration. Furthermore, Ca^{2+}-dependent SUMOylation of FOXM1 appears to enhance the G2/M transition of the cell cycle and reduce apoptosis. In conclusion, our results provide a molecular basis for the regulatory relationship between Ca^{2+} signaling and FOXM1, and we look forward to elucidating the biological functions associated with Ca^{2+}-dependent FOXM1 SUMOylation in the future.

Abstract 53

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Functional Studies of ATP Binding Sites in Inositol (1,4,5)-Trisphosphate receptors

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Abstract:
Inositol (1,4,5)-trisphosphate receptors (IP_3Rs) are essential elements of the calcium signaling "toolkit" and play key roles in numerous physiological processes by mediating Ca^{2+} release from the endoplasmic reticulum. ATP is known to potentiate IP_3-induced Ca^{2+} release without hydrolysis, but the structural basis for this regulation has not been elucidated. Recent Cryo-EM analyses have identified conserved ATP-binding residues, E2149/K2221, K2152/K2224, and K2560/K2633 in human IP_3R3 and rat IP_3R1 respectively, while the AlphaFold-predicted structure of mouse IP_3R2 suggests a comparable ATP-binding pocket involving R2174, K2177, and K2584. These residues are largely conserved across all IP_3R subtypes, derived from multiple species. To study the functional roles, the conserved lysine residues were substituted with leucine in IP_3R1 and IP_3R3. In these mutants, ATP no longer enhanced Ca^{2+} release, confirming the importance of these residues for ATP coordination. Moreover, the electrophysiological recordings showed that the IP_3R2 double mutant (K to L at both sites) exhibited an open probability in the presence of ATP similar to that of the wild-type receptor under no ATP conditions, indicating that IP_3R2 likely has a similar ATP-binding pocket as $IP_3R1/3$, consistent with predictions from its AlphaFold structure. Subtype-specific regulation was further examined by exchanging the negatively charged glutamic acid (E2149) in IP_3R3 with arginine (E2149R) and substituting the corresponding arginine (R2174) in IP_3R2 with glutamic acid (R2174E). The IP_3R3 E2149R mutant exhibited increased ATP sensitivity, whereas the IP_3R2 R2174E mutant displayed decreased ATP sensitivity compared with their respective wild types. These findings support the idea that the negatively charged glutamic acid in IP_3R3 contributes to its lowest ATP sensitivity (Kd~500 nM) relative to IP_3R2 (highest, Kd~40 nM) and IP_3R1(intermediate, Kd~200 nM), where the corresponding residues are arginine and lysine, respectively. Collectively, these findings demonstrate that the conserved lysine residues are functionally responsible for ATP's regulatory effect on IP_3Rs, while charge variation at the glutamic acid /arginine/lysine site accounts for subtype-specific ATP sensitivity. Ongoing electrophysiological studies on different IP_3R isoforms and mutants will help clarify biophysically how ATP allosterically regulates IP_3-induced Ca^{2+} release.

Abstract 54

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Mathematical modeling of spatial Ca^{2} dynamics in acinar cells during salivary secretion

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Abstract:
Saliva is secreted by acinar cells located at the ends of the salivary glands, and the salivation process is controlled by intracellular Ca^{2+} signals. Water secretion is ultimately driven by Ca^{2+} activated Cl^- channels located on the apical cell membrane. Elevated Ca^{2+} increases channel permeability, promoting Cl^- efflux from the cell. This ion movement is followed by the transport of other ions, ultimately driving osmotic water flow from the interstitium into the lumen. To date, many mathematical models have been developed to study the secretion process and Ca^{2+} dynamics within acinar cells. In acinar cells, the region between the tight junction and the apical cell membrane is referred to as the apical region, and the Ca^{2+} concentration in this region has previously been assumed to be an indicator of water flow. However, an extremely small region located near the cell membrane has not been well studied, and this gap becomes increasingly significant as experimental techniques improve. Our study is motivated by recent experimental results obtained from acinar cells in two groups of mice: healthy control mice and DMXAA-treated mice exhibiting Sjögren's syndrome (SS)-like symptoms. These studies revealed new insights into a microdomain between the cortical ER membrane and the apical plasma membrane. The Ca^{2+}-activated Cl^- channel is localized within this microdomain, suggesting that Ca^{2+} dynamics within the microdomain play a key role in regulating water secretion. These new studies introduce a vital problem for mathematical modelling. Most existing models treat Ca^{2+} dynamics in the apical region as the controller of water flow, and it remains unclear whether changing this assumption will alter previous modelling results. At the same time, the microdomain is extremely small, making direct experimental measurement of Ca^{2+} dynamics within it very difficult. Nevertheless, these dynamics can be predicted using mathematical models, which may also help elucidate the possible composition of the microdomain. In this study, computations were performed for multiple possible microdomain compositions. Computational results reveal that Ca^{2+} dynamics in the microdomain may be more sensitive to changes in stimulation level than those in the apical region, with oscillations arising at lower agonist levels. Moreover, microdomain activity can influence global Ca^{2+} signaling through changes in cell volume. Simulations were initially performed using a two-dimensional mesh. More recently, simulations based on experimentally observed three-dimensional cortical ER geometries of acinar cells have revealed similar Ca^{2+} dynamics, further supporting the predictions from the two-dimensional simulated mesh.

Abstract 55

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Elucidating the functional role of calcium release-activated calcium (CRAC) channels in fibroblast activation during renal tubulointerstitial fibrosis.

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Abstract:
Regardless of the cause, interstitial fibrosis is an inevitable process in chronic kidney disease (CKD). The progression of interstitial fibrosis is positively correlated with the degree of decline in kidney function. Due to the lack of detailed understanding of the mechanisms, current treatment options for CKD remain limited. This study found that the activation and expression of calcium release-activated calcium channels (CRACs) are involved in TGF-β1-induced fibroblast activation. In a UUO-induced renal fibrosis model, both the expression and activation of CRACs were increased, while blocking CRACs inhibited renal fibrosis.. Although the protein expression of STIM1 and Orai1, the two main components of the CRAC channel, was significantly increased in fibrotic kidneys, their mRNA levels did not show an equivalent increase. This suggests the possible existence of a post-translational regulatory mechanism. In cell studies, we found that increasing matrix stiffness can enhance the protein levels of STIM1 and Orai1 without affecting their mRNA expression by culturing cells on matrices of varying stiffness. Furthermore, increased matrix stiffness enhances the activation of CRAC channels. On the one hand, inhibiting collagen crosslinking and tissue sclerosis can suppress the upregulation of STIM1 and Orai1 induced by TGF-β1 in collagen gel cultured cells, as well as the upregulation of STIM1 and Orai1 induced by UUO during renal fibrosis. Our findings suggest that enhanced mechanical signal transmission is key to coordinating inflammatory responses and fibrotic events, and that the expression and activation of CRAC channels play a crucial role.

Abstract 56

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Hyperexcitability in TRPC4 knockout hiPSC-derived neurons is reversed by endocannabinoid 2 receptor agonists for potential therapeutics of autism

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Abstract:
Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition characterized by repetitive behaviors and deficits in social communication. ASD risk genes converge on shared molecular and cellular pathways, including calcium signaling. Patient-specific human induced pluripotent stem cell (hiPSC)-derived neurons provide a powerful platform to model convergent disease mechanisms. TRPC4 is a potential ASD risk gene identified from a Qatar ASD cohort. TRPC4 knockout (KO) in hiPSC-derived neurons also resulted in neuronal and network hyperexcitability and reduced SOCE. Using multi-electrode array (MEA) recordings, we found that hiPSC-derived cortical neurons carrying ASD-associated genetic variants exhibit robust neuronal hyperexcitability, suggesting hyperexcitability phenotype represents a common functional outcome of ASD risk variation. We examined β-caryophyllene (BCP), a selective cannabinoid type-2 receptor (CB2R) agonist and a major constituent of traditional bakhoor used in Arabic aromatherapy. Chronic BCP and JWH-133 (a selective CB2R agonist) treatment significantly attenuated hyperexcitability. Together, these findings identify BCP and JWH-133 as a culturally grounded and therapeutic candidate for ASD, supporting potential translational value of bakhoor for therapeutic strategies targeting hyperexcitability.

Abstract 57

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Mutation-Induced Rewiring of the Conformational Dynamics of the N-Terminal of Human Inositol 14,5–Trisphosphate Receptor Type 1 I

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Abstract:
Inositol 14,5–trisphosphate receptors (IP_3Rs) represent a major family of intracellular Ca^{2+} channels, residing mainly within the membrane of the endoplasmic reticulum (ER). These ion channels play critical role in the initiation as well as subsequent propagation of intracellular Ca^{2+} signals when cell surface receptors are stimulated by various agonists that activate phospholipase C enzymes. A functional IP_3R is a tetramer of large subunits, each of which has modular architecture comprising of the N terminus, the intermediary regulatory domain and the distal C terminus harbouring the channel pore. Their N-terminal region plays a pivotal role in ligand recognition, inter-domain communication, and allosteric regulation of channel gating. A growing number of disease-associated mutations, together with variants predicted to be functionally disruptive, have been identified within this region. However, the molecular mechanisms by which these sequence perturbations alter protein dynamics and lead to dysfunctional Ca^{2+} signalling remain incompletely understood. To address this challenge, an integrative ensemble-based computational strategy was employed to characterise the conformational landscapes of the wild-type IP_3R1 N-terminus and a curated set of pathogenic and predicted mutations. Amongst the three mammalian IP_3R isoforms, IP_3R1 was chosen, given this being the most heavily mutated isoform so far with clear pathophysiological relevance. Conformational ensembles were first generated using the generative modelling framework BioEmu, enabling efficient sampling of equilibrium-like structural heterogeneity beyond static structural representations. Joint projection of all ensembles into a common low-dimensional embedding space revealed mutation-specific redistribution of conformational populations, as well as altered dynamical pathways connecting functionally relevant states when compared with the wild type. Representative conformations from distinct regions of the embedding space were subsequently subjected to extensive atomistic molecular dynamics simulations. By combining equilibrium and non-equilibrium sampling approaches, Markov state models were constructed to quantify both the thermodynamic stability and kinetic connectivity of key conformational states. This multi-scale analysis reveals that mutations exert their effects primarily by reshaping the underlying conformational energy landscape and modulating collective motions within the N-terminal domains, rather than by inducing isolated local structural changes. These dynamical perturbations provide a mechanistic basis for altered allosteric communication and impaired IP_3-dependent regulation. Overall, this work highlights the importance of an ensemble-centric perspective for understanding mutation-driven dysfunction in IP_3R1 and offers a dynamic framework for linking sequence variation in the N-terminal region to altered regulatory behaviour and aberrant Ca^{2+} signalling.

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