16th FASEB Science Research Conference on Calcium and Cell Function: Calcium channels and signaling in health and disease

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The 16th FASEB Science Research Conference on Ca^{2+} and Cell Function took place in Lisbon, Portugal, on June 12–17, 2016. Chaired by Stefan Feske (New York University, organizer) and Murali Prakriya (Northwestern University, co-organizer), it brought together over 130 scientists from North America, Europe, and Asia. The 48 invited speakers discussed a wide range of topics relating to the molecular and cellular mechanisms of Ca^{2+} signaling and homeostasis and the physiological and pathological effects of Ca^{2+} in health and disease. Several research areas were particularly well represented, including store-operated Ca^{2+} entry (SOCE). SOCE is mediated by the Ca^{2+} release–activated Ca^{2+} (CRAC) channel that is formed by ORAI and STIM proteins in the plasma and ER membranes, respectively. Many talks explored the molecular regulation of the CRAC channel, including its protein structure, subunit stoichiometry, gating mechanism, and principles of the ORAI–STIM interaction. In addition, several talks explored its physiology and pathophysiology in processes as varied as neuronal cell function, immune responses, and cancer based on insights from CRAC channel–deficient patients and gene-targeted animals. Given the important role of CRAC channels in immunity, the role of Ca^{2+} signals in the regulation of immune responses was a particular focus of the meeting, as was the pathogenesis of immune-related disorders, especially infectious, inflammatory, and allergic diseases. Another focus was mitochondrial Ca^{2+} homeostasis (Fig. 1), a research area that has attracted renewed attention since the discovery of the mitochondrial Ca^{2+} uniporter (MCU). The molecular regulation of mitochondrial Ca^{2+} homeostasis by the MCU and molecules associated with it were discussed in several presentations, including the endnote lecture. In addition to CRAC channels and the MCU, other Ca^{2+} signaling proteins were represented at the meeting, including transient receptor potential (TRP) channels, intracellular calcium release channels, and voltage-gated Ca^{2+} channels. A decade after the discovery of STIM and ORAI proteins and half a decade after the identification of the MCU, this meeting revealed how research exploring the molecular mechanisms and physiological roles of CRAC channels and mitochondrial Ca^{2+} homeostasis have been reinvigorated and will likely dominate the Ca^{2+} signaling field for years to come.

The meeting started with a keynote address by **David Clapham** (Harvard University, Howard Hughes Medical Institute) who provided a comprehensive discussion and perspectives on the current understanding of Ca^{2+} signaling in primary cilia. Primary cilia are solitary, nonmotile extensions of the centriole found on nearly all nucleated eukaryotic cells. Mutations in primary ciliary proteins are associated with severe developmental defects. Dr. Clapham showed that primary cilia are a unique Ca^{2+} compartment and that changes in the ciliary Ca^{2+} concentration occur without substantially altering global Ca^{2+} levels in the cytoplasm. A heteromeric TRP channel composed of PKD1L1-PKD2L1 acts as a ciliary Ca^{2+} channel from which his laboratory was able to directly record currents in primary cilia. Ca^{2+} influx via PKD1L1-PKD2L1 had measurable but modest effects on Hedgehog signaling. In contrast to reports in the literature that primary cilia sense mechanical force through Ca^{2+}-permeable ion channels, Dr. Clapham reported the complete lack of mechanically induced Ca^{2+} increases in primary cilia of various cell types, including kidney epithelial cells, crown cells of the embryonic node, and kinocilia of inner ear hair cells isolated from Arl13b-mCherry-GECO1.2 reporter mice, cultured in vitro and exposed to physiological or supraphysiological levels of fluid flow, suggesting that mechanosensation by primary cilia does not occur via Ca^{2+} signaling.

Session 1

Session 1 focused on imaging Ca^{2+} signals in living cells. **Alexander Flugel** (University of Göttingen) described the imaging of real-time Ca^{2+} signals during the activation of T cells as they interacted with their cognate receptors. His talk illuminated the mechanisms by which autoreactive T cells transmigrate through the blood–

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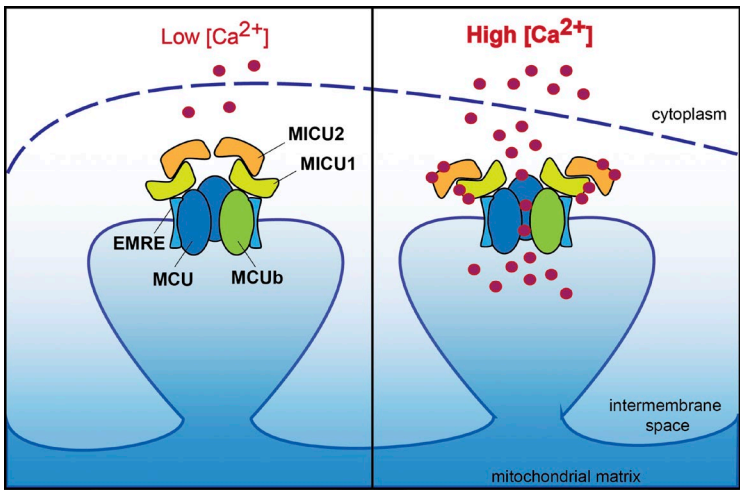

Figure 1. **Schematic representation of the MCU complex composition.** Mitochondrial Ca^{2+} uptake is controlled by a multiprotein complex composed of the pore-forming subunits MCU and MCUb together with MICU1, MICU2, and EMRE. In resting conditions (left), MICU1/MICU2 heterodimers act as the MCU gatekeeper, because of the inhibitory effect of MICU2. Once Ca^{2+} signaling is activated (right), the increase in cytosolic [Ca^{2+}] induces a conformational change in the dimer that releases MICU2-dependent inhibition. At the same time, MICU1 acts as a cooperative activator of the channel and thus stimulates the channel activity. Red dots indicate Ca^{2+}.

brain barrier (BBB) and recognize cognate antigens in situ. He used two-photon imaging to determine that resident macrophages within the leptomeninges represent the first antigen-presenting cells that the incoming T cells interact with. He also found that activated T cells undergo a complex reprogramming of their gene expression profile even before entering the brain, which enables their migration through the BBB. **Takeharu Nagai** (Osaka University) discussed his recent inventions with bioluminescent probes that are capable of video rate functional imaging of Ca^{2+} signals. He discussed the development of an enhanced Nano-lantern probe made by fusing mutants of fluorescent proteins with a previously developed bioluminescent probe called NanoLuc. This fluorescence resonance energy transfer (FRET)–based tool could be expressed at very low levels to image long-term Ca^{2+} signals in cardiomyocytes without physiological perturbations. **Anna Toth** (Northwestern University) presented a short talk on astrocyte gliotransmission and the contribution of SOCE signaling in gliotransmitter release. Her work indicated that CRAC channels are a major mechanism of Ca^{2+} entry in astrocytes and play a critical role in initiating gliotransmitter release, which may have important implications for neuron–glia cross-talk and modulation of neuronal network activity. **Oliver Griesbeck** (Max Planck Institute for Neurobiology) spoke on the development of FRET-based Ca^{2+} biosensors and specifically introduced a new "Twitch" Ca^{2+} biosensor with a minimized Ca^{2+}-binding domain based on the C-terminal lobe of troponin C and thus reduced number of Ca^{2+}-binding sites per sensor. His laboratory optimized the FRET response through a large-scale functional screen. This new sensor has excellent biocompatibility, a high dynamic range, and greatly improved brightness compared with current genetically encoded Ca^{2+} indicators. The biosensor was tested using high-resolution imaging of Ca^{2+} fluctuations during activation of T cells upon encountering their antigen. **Andreas H. Guse** (University of Hamburg) discussed the role of NAADP-mediated Ca^{2+} signaling in T cells and described the central role of ryanodine receptors in this process. Ca^{2+} microdomains were observed in close proximity to the plasma membrane (PM), indicating a role for Ca^{2+} entry early during the genesis of Ca^{2+} signals. His results pointed to a model wherein microdomains of Ca^{2+} entering through ORAI1/STIM1 channels evoke Ca^{2+}-induced Ca^{2+} release through the opening of ryanodine receptors and in which NAADP mediates a critical role in setting the setpoint for RyR activation by Ca^{2+}. **Keiko Nonomura** (Scripps Institute) described the mechanism and functions of the newly discovered Piezo channels, which transduce mechanical forces into biochemical cellular signals. Piezo1 and Piezo2 are large mechanosensitive proteins that generate cation-selective channels. She described several different roles of Piezo2 obtained by analysis of genetic knockout of Piezo2. Piezo2 is important for movement coordination, as mice with Piezo2 deficiency in proprioceptors display severe coordination as well as respiratory function deficits. Her results indicated that Piezo2 is the key mechanotransducer for light touch sensation. **Joseph Dynes** (University of California, Irvine) discussed single molecule approaches to probing ORAI calcium channel organization and function. Results from his transmission and freeze-fracture electron microscopy work indicated that STIM1 and ORAI1 aggregate at ER–PM junctions after store depletion. STIM1, visualized on the cytoplasmic surface of the ER, bridged a 12-nm ER–PM gap to the PM and showed clear arrangement into small clusters after store depletion of STIM1- and ORAI1-transfected HEK cells. Visualization of ORAI1 revealed aggregation into clusters where channels were nonrandomly spaced ~15 nm apart. He also showed his work on probing single channel activity of ORAI1 using a Ca^{2+} indicator (GECO1.2) fused to ORAI1. Results from the analysis of the Ca^{2+} signals showed that there exist at least two functional modes of

channel gating, at least one of which could reflect the kinetics of STIM1 binding/unbinding.

Session 2

Session 2 examined Ca^{2+} signaling in cellular organelles with a particular focus on mitochondria and the ER. **Gyorgy Hajnoczky** (Thomas Jefferson University) discussed the signaling between ER and mitochondria, including the structural specializations of the contacts between these organelles and the organization of the proteins mediating this signaling. A highlight of his talk was the generation of reactive oxygen species (ROS) produced in the intermembrane space between the inner and outer mitochondrial membranes and how this is regulated by the delivery of Ca^{2+} from the ER to the mitochondria. His talk also provided evidence that MICU1 that senses cytoplasmic Ca^{2+} for the MCU is required for local ER–mitochondrial Ca^{2+} transfer and is needed for survival in a new mouse model. **Madesh Muniswamy** (Temple University) discussed his recent findings on the molecular regulation of the MCU. He showed work indicating that MICU1 is an essential gatekeeper for MCU-mediated mitochondrial Ca^{2+} uptake that regulates cell survival. MICU1 resides in the mitochondrial matrix and modulates MCU activity by modulating Ca^{2+} permeation. His work also revealed that the newly discovered regulator, MCUR1, interacts with MCU to modulate its activity without affecting the mitochondrial membrane potential. MCUR1 directly binds MCU and EMRE to form the active MCU complex. **Thomas Simmen** (University of Alberta) gave a short talk on the regulation of ER–mitochondrial contacts. His data revealed that TMX1 knockout results in increased ER Ca^{2+} content, cytosolic Ca^{2+} clearance, and reduced mitochondrial metabolism, supporting a model wherein this protein is critical for the proper coupling of ER–mitochondrial contacts. The loss of these contacts also promotes tumor growth, establishing a physiological relevance for ER–mitochondrial contacts. **Aldebaran Hofer** (Harvard University) discussed her recent findings on cross-talk of Ca^{2+}/cAMP signaling in organelles. She first described her laboratory's development of a FRET-based PKA activity reporter, which could be used to assess the cAMP signals in various cellular compartments including primary cilia, which she showed is a privileged cAMP signaling region within the cell. She also described the strange phenomenon of red to green photo conversion of mCherry that was discovered serendipitously in her laboratory's work on attempts to label the primary cilium. **Susan Hamilton** (Baylor College of Medicine) described work on RyR function and links to myopathies and addressed the question of why mutations in RyR1, $Ca_V1.1$ channels, and the ER protein calsequestrin all cause similar myopathies. Through analysis of various RyR1 mutations and their effects on the distribution of calsequestrin and associated proteins including triadin, she proposed that the RyR1 p.I4895T mutation causes a persistent increase in both SR and ER mitochondrial Ca^{2+} uptake and ROS production, decreased protein synthesis, and ultimately cell death. **Kimberli Kamer** (Harvard University) finished the session with a short talk on the molecular assembly and regulation of the MCU and her laboratory's findings indicating how MCU activity is regulated by the associated proteins MICU1, MICU2, and EMRE.

Session 3

Session 3 discussed SOCE and mechanisms of CRAC channel function. The session began with a presentation from **Christoph Romanin** (University of Linz) who described several gain-of-function mutations in ORAI1 and STIM1 whose analysis revealed interesting insights into the molecular mechanisms of ORAI1 and STIM1. He described the domains and mutations that his laboratory previously identified as critical hotspots for the operation of STIM1 and ORAI1 and newer work on gain-of-function mutations that result in spontaneous activation of STIM1 and ORAI1. The STIM1 p.R304W mutation causes a human disease called Stormorken syndrome and occurs via the release of an autoinhibitory clamp within STIM1, thereby activating STIM1 and SOCE. **Patrick Hogan** (La Jolla Institute of Allergy and Immunology) described the conformational dynamics of ORAI1 channel gating, which his laboratory probed using advanced in vitro approaches with purified proteins and state of the art FRET-based techniques. His work showed that the hydrophobic region in the outer part of the ORAI1 pore is critical for channel gating and that STIM1 evokes a conformational change in the region around E106 that constitutes the selectivity filter of the channel. His work suggested that the nonpolar, hydrophobic stretch of the channel presents a barrier to ion flux, thereby implicating this region of the channel in gating. **Peter Stathopulos** (University of Western Ontario) discussed his recent work on the posttranslational modification the ER Ca^{2+} sensor, STIM1, and the implications for STIM1 structure and function. His work showed that N-glycosylation of the STIM protein in the luminal EF-hand region of the sensor decreases folding and stability of STIM1, thereby enhancing its sensitivity for activation, whereas S-nitrosylation evokes the opposite effect, enhancing the stability of the protein and therefore diminishing its sensitivity for activation by store depletion. **Donald Gill** (Penn State University) presented work showing that a remote region at the C terminus of ORAI1 regulates channel gating. His laboratory generated a constitutively open ORAI1 channel by mutating a stretch of residues (LVS HK) in the hinge region between M4 and the M4 extension helix to ANSGA. He suggested that interaction between the cytoplasmic sides of third and fourth

transmembrane domains are essential for relaying a signal from the ORAI1 C terminus to open the channel pore. Consistent with this model, mutations in this region that are predicted to destabilize the M3–M4 interaction eliminated the ability of STIM1 to gate the channel. He concluded that binding of STIM1 to gate the channel is restricted to the peripheral C-terminal extension helix, which remotely controls gating of the ORAI1 pore through a simple conformational switch mediated by the C-terminal nexus and transduced through the pore helices to open the channel. **Richard Lewis** (Stanford University) discussed the subunit stoichiometry of the ORAI1 channel and the energetic requirements for gating the channel. Using concatenated ORAI1 subunits, his laboratory showed that ORAI1 hexamers fully recapitulate the functional and biophysical hallmarks of the CRAC channel, questioning the validity of previous conclusions that the ORAI1 channel is a tetramer and supporting the hexameric *Drosophila* Orai crystal structure. Through mutagenesis of ORAI1 concatemers, they then investigated whether STIM1 activates the channel by binding to one or two ORAI1 C termini simultaneously. Their results indicated that STIM1 activates the ORAI1 channel preferentially through binding to pairs of ORAI1 subunits and that each binding event contributes an equal amount of energy toward channel opening. **Jonathan Soboloff** (Temple University) discussed inhibition of Ca^{2+} clearance by STIM1. His laboratory's results indicated that, during T cell activation, inhibition of PM Ca^{2+} ATPase (PMCA) activity is initiated when STIM1 expression is up-regulated in an EGR1- and EGR4-dependent manner. They further establish that this inhibition contributes significantly to NFAT-dependent gene transcription and, ultimately, T cell activation. **Gaiti Hasan** (National Centre for Biological Sciences, Bangalore), who presented in a later session, shed light on the physiological role of SOCE in the model organism *Drosophila melanogaster*. She showed that SOCE in fruit fly neurons is required for the maturation of the neuronal flight circuit by regulating the expression of specific genes at defined stages of neural circuit development. In particular, her work indicated that SOCE is essential for the development of dopaminergic neurons and for the up-regulation of tyrosine hydroxylase levels that occur during pupal development. This study adds to the growing evidence for the role of SOCE in regulating critical functions in the nervous system.

Session 4

Session 4 focused on Ca^{2+} in immunity and infection. **Roberto Docampo** (University of Georgia) spoke about the MCU and acidocalcisomes of *Trypanosoma brucei*, the agent of African sleeping sickness. Acidocalcisomes are acidic Ca^{2+} stores found in a diverse range of organisms from bacteria to humans and in trypanosomes are involved in phosphate metabolism, cation uptake, and calcium signaling. In his talk, Dr. Docampo showed that the MCU of *T. brucei* (TbMCU) is required for cell growth and infectivity and for the regulation of trypanosome mitochondrial bioenergetics and is closely linked to acidocalcisome function. Switching from pathogens to the immune response against them, **Nicolas Demaurex** (University of Geneva) provided evidence that STIM1-mediated Ca^{2+} signaling is involved in phagocytosis by neutrophils. STIM1 and the adapter protein junctate regulate phagocytosis by recruiting ER Ca^{2+} stores to phagosomes to generate pro-phagocytic local Ca^{2+} elevations via the opening of phagosomal Ca^{2+} channels. In addition, STIM1 is needed for the migration of another immune cell type, dendritic cells (DCs), to draining lymph nodes, the regulation of phagolysosome fusion, and cross-presentation of antigens by DCs to T cells, which may have important implications for antiviral and antitumor immunity. **Anant Parekh** (Oxford University) presented data showing the activation of CRAC channels in mast cells by house dust mite antigens, which are potent allergens causing allergic asthma. Given the central role of mast cells in asthma pathology and CRAC channels for mast cell activation, these findings indicate that some allergens can directly activate CRAC channels and promote allergic immune responses, reinforcing the view that targeting CRAC channels should be an effective therapeutic strategy to managing asthma and other airway diseases. **Masatsugu Oh-hora** (Kyushu University) reported on how STIM1 and STIM2 mediate SOCE in T cells and thereby provide immune homeostasis by controlling the development of regulatory T cells (T reg cells). The physiological role of T reg cells, suppression of autoreactive T cells and prevention of autoimmunity, is abolished in the absence of SOCE. In addition, SOCE may prevent autoimmunity by another mechanism, through the suppression of IL-4 production in conventional T cells and their ability to promote immunoglobulin class switching to IgE and IgG1. The immunopathological consequences of abnormal Ca^{2+} signaling were further highlighted by **Joseph Hyser** (Baylor College of Medicine) who showed that SOCE is a major Ca^{2+} signaling mechanism associated with rotavirus infection, the leading cause of acute viral diarrhea in children. Rotavirus disrupts Ca^{2+} homeostasis through its nonstructural protein 4 (NSP4), which causes Ca^{2+} release from the ER and activates SOCE via ORAI1 channels, thereby promoting intestinal fluid secretion. In addition to ORAI1, voltage-gated $Ca_V1.3$ channels also appear to mediate Ca^{2+} signaling in human intestinal enteroids. The session provided strong evidence that CRAC channels and SOCE control innate and adaptive immunity by a variety of immune cell types and thereby fine-tune immune function in response to infections and in autoimmunity.

Session 5

Session 5 explored the physiological and pathophysiological role of Ca^{2+} signals. **David Yule** (University of Rochester) presented new insights into IP_3 receptor (IP_3R) structure and function. IP_3Rs are ligand-gated, tetrameric Ca^{2+} channels that mediate Ca^{2+} release from intracellular stores in response to IP_3, Ca^{2+}, and ATP binding. Stable expression of concatenated IP_3R tetramers in IP_3R-null cell lines revealed that IP_3-induced Ca^{2+} release only occurs when each IP_3R monomer within the tetramer is occupied by IP_3. IP_3Rs come in three isoforms (IP_3R1, IP_3R2, and IP_3R3) that have distinct regulatory properties and may assemble in homo- or heterotetrameric complexes, but the stoichiometry and properties of heteromeric IP_3Rs are not well defined. Analyzing concatenated IP_3R tetramers of different isoform composition showed that IP_3R2 monomers within a heterotetramer determine channel regulation by ATP especially under suboptimal conditions when ATP concentrations vary. Heteromeric IP_3R tetramers likely extend the versatility of IP_3-induced Ca^{2+} signaling in cells expressing multiple IP_3R isoforms. **Kevin Foskett** (University of Pennsylvania) shed new light on the pathophysiological role of constitutive low-level ER to mitochondrial Ca^{2+} transfer, which is required for ATP production in many cell types, including cancer cells. Whereas insufficient Ca^{2+} transfer and mitochondrial ATP production can be rescued by AMPK-dependent autophagy in nontumor cells, cancer cells die by necrosis associated with mitotic catastrophe during proliferation. These data suggest that ER to mitochondria Ca^{2+} transfer is of particular importance for the bioenergetics and survival of cancer cells. **Hyun Jin Kim** (Sungkyunkwan University) presented new data regarding the role of another organellar Ca^{2+} channel, TRPML3, and showed that it is mainly functional in ATG5-positive phagophores where it is directly activated by PI_3P, resulting in increased autophagy.

The remaining talks in this session focused on the role of PM Ca^{2+} channels and Ca^{2+} influx. **Amy Lee** (University of Iowa) discussed the contributions of voltage-gated $Ca_V1.4$ channels in photoreceptor synapse development and maintenance. $Ca_V1.4$ subunits have a dual role as protein scaffolds that promote assembly of the presynaptic active zone and as conduits for Ca^{2+} to support transmission that may strengthen postsynaptic development at synapses between rods and rod bipolar cells. **Mohamed Trebak** (Penn State University) showed that endothelial barrier function, which is either disrupted or stabilized by distinct GPCR agonists, is independent of GPCR-induced Ca^{2+} release from internal stores and SOCE via ORAI1 channels. However, disruption of endothelial barrier function by the GPCR agonists thrombin and histamine required the Ca^{2+} sensor STIM1, presumably through SOCE- and Ca^{2+}-independent mechanisms. **Rodrigo Lacruz** (New York University) provided evidence that SOCE is required for amelogenesis, i.e., the formation of dental enamel, which contains a high percentage of crystalline $Ca(H_2PO_4)_2$, making it the hardest substance in the human body. Deletion of *Orai1* or *Stim1* and *Stim2* genes in mice impaired SOCE in ameloblasts, the enamel-forming cells, and resulted in severely hypomineralized enamel associated with increased attrition of teeth, a phenotype that is similar to that in human patients with mutations in *ORAI1* and *STIM1* genes. An additional role for SOCE was discussed in a short talk by **Axel Concepcion** (New York University), who showed that ORAI1- and STIM1-deficient patients are anhidrotic, i.e., lack the ability to sweat, similar to mice with targeted deletion of *Orai1* or *Stim1* and *Stim2* genes. This defect was caused by impaired activation of Ca^{2+}-activated chloride channels and Cl^- secretion by eccrine sweat gland cells. The session provided a remarkable survey of the versatile roles of Ca^{2+} signals originating from organellar Ca^{2+} stores or Ca^{2+} influx in the function of a wide range of cell types and the pathological consequences when these mechanisms are perturbed.

Session 6

Session 6 continued to explore the role of Ca^{2+} in disease. **Katsuhiko Mikoshiba** (RIKEN Institute for Brain Science) discussed a variety of diseases resulting from the dysregulation of distinct IP_3R isoforms and molecules interacting with them. IP_3R1 is highly enriched in neurons, and its dysfunction results in cerebellar ataxia. It interacts with many molecules, including the chaperone GRP78 that protects the brain from damage caused by ER stress, transglutaminase 2 that allosterically blocks IP_3R1 and is associated with Huntington's disease, the ER redox sensor ERp44, and disrupted-in-schizophrenia 1 (DISC1) that regulates synaptic plasticity. In contrast, IP_3R2 is required for the function of a variety of cells and tissues, including the secretory function of salivary, lacrimal, and eccrine sweat glands, osteoclasts, and bone formation, as well as astrocytes, where they control the release of thrombospondin for the rewiring of neural networks that causes severe neuropathic pain and regulate the metaplasticity of cortical astrocytes. **Ivana Kuo** (Yale University) presented her findings about Polycystin 2 (PC2), mutations of which are associated with autosomal-dominant polycystic kidney disease (ADPKD). PC2 resides in mitochondria-associated ER membranes and regulates mitochondrial Ca^{2+} entry, presumably by inhibiting IP_3R-mediated ER Ca^{2+} release. PC2 antagonized expression of the mitochondrial–ER tethering protein mitofusin 2 and promoted mitophagy by activating the mitophagic PINK1–Parkin pathway, which may contribute to cyst development and mitochondrial pathologies in tissues containing PC2. Continuing the theme of mitochondrial Ca^{2+} homeostasis, **Cristina Mammucari** (University of Padua) discussed how the

MCU regulates progression of triple-negative breast cancer. MCU expression correlated with tumor size and lymph node infiltration, suggesting that mitochondrial Ca^{2+} uptake may regulate cancer progression. Deletion of MCU in xenografted MDA-MB-231 breast cancer cells greatly reduced tumor growth and metastasis. In the absence of MCU, formation of ROS and expression of hypoxia-inducible factor HIF-1α were reduced, suggesting that mitochondrial Ca^{2+} uptake via the MCU is involved in tumor progression. Taking the Ca^{2+} and cancer theme from intracellular organelles to Ca^{2+} influx, **Natalia Prevarskaya** (Université Lille) presented data about different Ca^{2+} channels and how they mediate the initiation and progression of prostate cancer. Prostate cancer cells appear to use TRP, ORAI, and STIM protein redistribution to form novel channel complexes that are activated in a store-independent manner and promote more aggressive cancer growth and invasiveness. **Diana Bautista** (University of California, Berkeley) explored the molecular mechanisms of chronic itch, especially in the inflammatory skin disease atopic dermatitis. She showed that the serotonin receptor HTR7 is a key mediator of itch in atopic dermatitis and promotes the opening of TRPA1 channels, which trigger itch behavior. Itch-producing compounds (pruritogens) are released by a variety of cells in the skin, including keratinocytes and immune cells, and activate somatosensory neurons as well as the Ca^{2+}-dependent release of inflammatory mediators. One of these mediators is IL-8, which was increased in mice with atopic dermatitis and caused the recruitment of neutrophils to the skin, leukotriene B4 production, and activation of itch-mediating sensory neurons. The consequences of constitutive Ca^{2+} influx through CRAC channels were the topic of the presentation by **Thilini Gamage** (University of Oslo). Heterozygous gain-of-function mutations in STIM1 result in constitutive Ca^{2+} influx via CRAC channels and are associated with two distinct but overlapping Mendelian diseases: (1) Stormorken syndrome is caused by a singular STIM1 p.R304W mutation that presents with tubular aggregate myopathy (TAM), miosis, hematological defects, asplenia, and ichthyosis; (2) isolated TAM is caused by nine distinct gain-of-function mutations in the EF-hand domain of STIM1 (and three mutations in ORAI1). Although knock-in mice with heterozygous expression of the STIM1 p.R304W appear to only partially mimic the phenotype of patients with Stormorken syndrome, they may be a useful tool to study the effects of constitutive CRAC channel activation. Session 6 provided important new insights into the consequences of dysregulated Ca^{2+} homeostasis and the resulting disease states.

Session 7

Session 7 focused on the molecular and structural basis of Ca^{2+} channel function. It began with a talk by **Stephen Long** (Memorial Sloan Kettering Cancer Center) who presented his laboratory's crystal structure of the *Drosophila* Orai channel. He discussed several intriguing aspects of the channel structure, including its hexameric stoichiometry, the structural basis of Ca^{2+} selectivity and permeation, and potential models of the channel gating mechanism. **Youxing Jiang** (University of Texas Southwestern Medical Center) presented his work on the structure of the plant vacuolar two-pore channel from *Arabidopsis*, AtTPC1, and its functional analysis by patch-clamp recording. TPC channels are thought to mediate NAADP-dependent Ca^{2+} release from endolysosomes and have emerged as molecular players regulating NAADP signaling and lysosome function. Their analysis revealed interesting insights into how Ca^{2+} activates TPC and the role of the membrane potential in regulating this process. He also discussed the implications of the resting state voltage sensor revealed in this study. **Jianping Wu** (Tsinghua University) presented the structural characterization of the voltage-gated $Ca_V1.1$ channel, which mediates excitation-contraction coupling in skeletal muscle. The elucidation of the rabbit $Ca_V1.1$ channel structure by single-particle cryo–electron microscopy represents a major breakthrough, which revealed not only the overall structure of the pore-forming complex, but also the associated α2, β, and γ subunits and the way these subunits interact with the pore. The structure represents a key step in understanding the functional mechanisms and the potential effects of disease-causing mutations in the skeletal muscle Ca^{2+} channel. The talk by **Henry Colecraft** (Columbia University) presented interesting ways to engineer the $Ca_V1.2$ channel using genetically encoded methods to control channel pharmacology. Two approaches his laboratory tested included introducing a split-intein motif to generate $Ca_V1.2$ moieties with altered function or pharmacology and their effort to block channels through inactivation by membrane tethering the C terminus of the channel with the Ras family of GTPases. **Jen Liou** (University of Texas Southwestern) discussed the homeostatic regulation of Ca^{2+} signaling at ER–PM junctions. During receptor-induced Ca^{2+} signaling, both PIP_2 at the PM and Ca^{2+} in the ER are consumed to generate cytosolic Ca^{2+} signals. To support Ca^{2+} signaling, ER Ca^{2+} is replenished by SOCE mediated by STIM1-ORAI1 at ER–PM junctions, but little is known about the replenishment of PM PIP_2. Using fluorescent tools to track the dynamic changes of PIP_2 levels at the PM, her work indicated that replenishment of PIP_2 is facilitated by the ER membrane protein E-Syt1, which is targeted to ER–PM junctions after increases in cytosolic Ca^{2+} levels, together with the phosphatidylinositol transfer protein Nir2, which is targeted to ER–PM junctions by sensing the production of phosphatidic acid, a metabolite of PIP_2. The session ended with a short talk by **Barbara Niemeyer** (Saarland University) who presented her work on the regulation of ORAI function and signaling by H_2O_2. H_2O_2 evokes mul-

tiple effects on ORAI channels, including inhibition of ORAI1 (but not ORAI3) channel activation, slowing diffusion of ORAI1, and, while enhancing STIM1-ORAI1 binding, reducing ORAI1–ORAI1 interaction. However, the major effect inhibiting channel activation is a specific interaction of the key reactive cysteine in ORAI1, C195, in TM3, which, when oxidized or mutated into an oxidomimetic aspartic acid, interacts with S239 of TM4 of the same subunit, thereby locking channels in the closed state.

In two short talks selected from the poster abstracts, **Jianbo Yue** (City University of Hong Kong) discussed his recent work on the identification of GAPDH as a novel cyclic adenosine diphosphoribose (cADPR)–binding protein using an approach based on photoaffinity protein-ligand labeling, and **Enrico Zampese** (Northwestern University) discussed the link between Ca^{2+} entry into SNc dopaminergic neurons through PM Ca_V1 L-type channels and mitochondrial Ca^{2+} loading, speculating that this loading is part of a feed-forward metabolic control mechanism that results in sustained elevations in mitochondrial oxidant stress.

The meeting ended with an endnote lecture by **Rosario Rizzuto** (University of Padua) who provided a comprehensive overview of the regulation and role of the MCU in cell function with a particular focus on the molecular composition of the MCU complex (Fig. 1). Located in the inner mitochondrial membrane, the MCU plays critical roles in shaping global Ca^{2+} signals and controlling aerobic metabolism, as well as apoptotic cell death. The MCU complex is a macromolecular structure that includes the MCU itself, MCUb, EMRE, MICU1, and its isoforms MICU2 and MICU3. Recent evidence suggests that the composition of the MCU complex varies between cell types consistent with large variations in mitochondrial Ca^{2+} uptake between different cells and tissues. The ongoing elucidation of the identity of the MCU complex components not only provides a rapid increase in our molecular understanding of MCU function and mitochondrial Ca^{2+} uptake, but also the role of the MCU and mitochondrial Ca^{2+} signaling in health and disease.

Four poster presenters were selected for awards: **Rainer Schindl** (University of Linz) received the *Science Signaling*–sponsored award for his work on "Ca^{2+} signaling and gene regulation by carcinogenic ORAI mutants," **Daniele Guido** (University of Geneva) received the *Cell Calcium*–sponsored award for his work on "Junctate boosts phagocytosis by recruiting ER Ca^{2+} stores," **Gaia Gherardi** (University of Padua) won the PLOS-sponsored award for her work on "The mitochondrial Ca^{2+} uniporter controls skeletal muscle trophism in vivo," and **Megumi Yamashita** (Northwestern University) received *The Journal of General Physiology*–sponsored award for her work on "STIM1 activates CRAC channels through rotation of the pore helix to open a hydrophobic gate."

In summary, the talks at this conference covered a broad range of topics in Ca^{2+} signaling capturing recent progress and current excitement in the field. The topics covered included the role of Ca^{2+} channels and Ca^{2+} signals in CNS inflammation, immunity to infection, autoimmunity and allergy, myopathies, chronic itch, cardiomyopathy, and cancer. In addition to illuminating the current level of understanding of the field, the wealth of research presented at this conference also highlighted significant controversies or uncertainties that remain to be resolved, including gaps in mechanistic operation of Ca^{2+} signaling proteins, their links to diseases, and the structural basis of signaling proteins. The meeting also highlighted intriguing possibilities for pharmacological interventions to aid the development of novel therapeutics, targeting a variety of proteins and pathways, including CRAC channels, mitochondrial proteins, and transcription factors. Based on the current pace of work, we expect that new insights regarding the therapeutic potential of manipulating these and other pathways will be conspicuously featured at the next FASEB Science Research Conference in two years.

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S. Feske is a co-founder of CalciMedica. M. Prakriya declares no competing financial interests.

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