1st Junior European Calcium Society Virtual Meeting

October 20-21, 2020

Programme
&
Abstract Book

Tuesday, October 20th

Technical chairs: Emily Coode (OU, UK) / Malene Brohus (AAU, Denmark)

13:00-13:10 Welcome
Chairs: Malene Brohus (AAU, Denmark) and Michael Vaughan (UCC, Ireland)

13:10-14:40 Session 1. Calcium Signalling in Disease
Scientific chairs: Luca Hegedus (UDE, Germany) and Silke Chalmers (UQ, Australia)

13:10-13:40	**Martin Schepelmann** (Medical University Vienna, Austria): *Walking the winding ways of the Calcium-Sensing Receptor*
13:40-14:00	**Jessica Moore** (Yale University, USA): *Investigating the role of Ca^{2+} signaling in regulating epidermal stem cell behaviors via live imaging*
14:00-14:20	**Flore Sneyers** (KU Leuven, Belgium): *The Ca^{2+}-buffering agent BAPTA-AM kills malignant B-cell models: is it all about Ca^{2+} chelation?*
14:20-14:40	**Thomas Gebert** (Baylor College of Medicine, USA): *Rotavirus induces activation of the Volume-Regulated Anion Channel (VRAC) to mediate remote calcium dysregulation in uninfected cells*

14:40-15:00 Break

15:00 – 16:30 Session 2. Organellar Calcium Signaling
Scientific chairs: Tim Vervliet (KU Leuven, Belgium) and Helene Halkjær Jensen (AAU, Denmark)

15:00-15:30	**Ana Rossi** (University of Cambridge, UK): *Quantal Ca^{2+} release mediated by very few IP_3 receptors that rapidly inactivate allows graded responses to IP_3*
15:30-15:50	**Feng Gu** (University Medical Center Hamburg-Eppendorf, Germany): *NAADP forming enzymes in T cell activation*
15:50-16:10	**Maria Calvo-Rodriguez** (Harvard Medical School, USA): *Mitochondrial calcium dysregulation in vivo in a mouse model of Alzheimer's disease*
16:10-16:30	**Jillian Weissenrieder** (University of Pennsylvania, USA): *The Mitochondrial Calcium Uniporter Contributes to Pancreatic Cancer Development and Invasion*

16:30-17:30 jECS General Assembly
Chairs: Helene Halkjær Jensen (AAU, Denmark) and Malene Brohus (AAU, Denmark)

1. Introduction to the jECS Board
2. Summary of jECS work 2019-2020
3. Expressions of interest for 2021 board membership
4. Future directions and plans
5. Questions and incoming suggestions

Wednesday, October 21st

Technical chairs: Emily Coode (OU, UK) / Malene Brohus (AAU, Denmark)

9:00-9:10 Welcome
Chairs: Malene Brohus (AAU, Denmark) and Michael Vaughan (UCC, Ireland)

9:10-10:40 Session 3. Calcium Signaling in Non-mammalian Systems
Scientific chairs: Björn-P. Diercks (UKE, Germany) and Pablo J Sáez (UKE, Germany)

09:10-09:40	**Alba Delrio Lorenzo** (University of Valladolid, Spain): *Sarcoplasmic reticulum Ca^{2+} dynamics in aging Drosophila and correlation with sarcopenia*
09:40-10:00	**Annalisa Bellandi** (John Innes Centre, UK): *Wound-induced calcium waves in plants - Dynamics and cell-to-cell transmission*
10:00-10:20	**Nishit Srivastava** (Institute Curie, France): *Pressure sensing through Piezo channels controls whether cells migrate with blebs or pseudopods*
10:20-10:40	**Scarlett E. Delgado** (Interdisciplinary Center of Neuroscience of Valparaíso, Chile): *Following calcium under mec-4d neurodegeneration and regeneration in Caenorhabditis elegans*

10:40 - 11:00 Break

11:00-11:45 Equity and Diversity in Science Roundtable
Enikö Kállay, (Dept. Pathophysiology and Allergy Research, Medical Univ. Vienna)
Sarah Roberts-Thomson (School of Pharmacy, Univ. of Queensland)
Geert Bultynck (Laboratory of Molecular and Cellular Signaling, KU Leuven)
Chairs: Felicity Davis (Univ. of Queensland, Australia) and Luca Hegedus (Univ. Duisburg-Essen, Germany)
All attendees are welcome and encouraged to participate

11:45-12:15 Break

12:15-13:45 Session 4. Structure and Molecular Mechanism of Calcium Binding Proteins Scientific chairs: Roya Tadayon (UWO, Canada) and Malene Brohus (AAU, Denmark)	
12:15-12:35	**Lucía González Gutiérrez** (University of Extremadura, Spain): *Channel complexes involved in remodeling of store-operated channels in colon cancer cells and their reversal by DFMO*
12:35-12:55	**Jenny van der Wijst** (Radboud University Medical Centre, Netherlands): *Structural insight into TRPV5 channel function and modulation*
12:55-13:15	**Vikas Arige** (Univ. of Rochester, USA): *Functional characterization of the calcium coordinating residue in human type 1 inositol 1,4,5-trisphosphate receptor*
13:15-13:45	**Gary Shaw** (UWO, Canada): *Mechanisms of calcium regulation by the dysferlin C2A domain*

13:45-14:00 Final remarks and best talk awards
Chairs: Roya Tadayon (UWO, Canada) and Malene Brohus (AAU, Denmark)

Notes:

Invited talks are 20 min + 10 min questions
Short talks from abstracts are 10 min + 10 min questions
All times are according to CEST time zone (Berlin, Paris, Rome, and Copenhagen)

1. Martin Schepelmann

Department of Pathology and Allergy Research
Medical University Vienna, Austria

Walking the winding ways of the Calcium-Sensing Receptor

Abstract:

2. **J. Moore**[1], S. Ganesan[1], C. Martone[1], E. Lathrop[1], V. Greco[1]

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Investigating the role of Ca^{2+} in regulating epidermal stem cell behaviors via live imaging

Abstract

Each day our bodies make and lose billions of cells. This regenerative process relies on our tissues' ability to orchestrate behaviors of an actively cycling stem cell pool, resulting in a balanced production of new stem cells (by division) and loss of cells (by differentiation/apoptosis). Imbalances in this process are associated with disease states, highlighting the importance of cellular coordination. Because Ca^{2+} signaling plays a key role in differentiation within the skin epidermis, I have established an in vivo approach that allows me to expand our understanding to epidermal stem cell behaviors by tracking them in the context of calcium dynamics in a live mouse. By further implementing a high-throughput image analysis pipeline, we have shown that in addition to single cell flashes, synchronized clusters of signaling occur among neighborhoods of 2-10 cells. Intriguingly, our previous work has shown that this size of cellular neighbourhood regulates local coordination of stem cells in various cell cycle stages, resulting in balanced self-renewal and differentiation behaviors. Daily revisits of the same epidermal cells show that regions with high calcium activity change along the temporal framework of stem cell behaviors. To understand how calcium regulates cell cycle progression, we combined the calcium reporter with a cell cycle reporter (Fucci2) and used drug treatments to enrich for cells in specific cell cycle stages. Through these approaches, we demonstrate that basal cells participate in heterogeneous calcium signaling throughout their cell cycle, up to but excluding division, and that synchronization in G1 but not S/G2 suppresses overall calcium signaling activity. This may signify the importance of calcium signaling during S/G2 phase in maintaining a heterogeneous population of cell cycle states in the basal layer. Finally, we are modulating calcium signaling patterns through optogenetic tools and Cx43 conditional knockout mice to define the role of intercellular communication in cell cycle coordination. Preliminary results indicate that loss of Cx43 leads to calcium signaling with abnormal spatiotemporal dynamics, disrupting the coordination of stem cell behaviors. Overall, we shed light on how cell-to-cell signaling can control and organize cellular processes within the context of a homeostatic tissue, establishing a foundation to understand pathologies.

3. **F. Sneyers[1]**, T. Vervloessem[1], J.B. Parys[1], M.D. Bootman[2], G. Bultynck[1]

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The Ca^{2+}-buffering agent BAPTA-AM kills malignant B-cell models: is it all about Ca^{2+} chelation?

Abstract

One of the hallmarks of lymphoid malignancies such as diffuse large B-cell lymphoma (DLBCL) is anti-apoptotic Bcl-2 overexpression. Venetoclax, a BH3-mimetic, selectively antagonizes Bcl-2 by binding to its hydrophobic cleft. Previous research revealed that venetoclax neither influenced intracellular Ca^{2+} dynamics nor affected the interaction between Bcl-2 and the IP_3R. Yet, combining venetoclax with BAPTA-AM, a well-known intracellular Ca^{2+} chelator, significantly increased apoptosis. These results suggest an interplay between venetoclax and Ca^{2+} signaling. However, caution is needed for the interpretation of these findings since several studies have shown that BAPTA-AM has intracellular targets that are unrelated to its Ca^{2+} chelating properties. Therefore, we set out to elucidate the mechanisms underlying this synergism by examining the link between BAPTA-AM, Bcl-2-family members and apoptosis.

We show that BAPTA-AM treatment induced apoptotic cell death in several DLBCL cell lines, although with different sensitivity. We assessed the effect of BAPTA-AM on the expression of different pro- and anti-apoptotic Bcl-2-family members. BAPTA-AM treatment resulted in a complete and rapid loss of anti-apoptotic Mcl-1, preceding the cell death occurrence in these cells. Moreover, overexpression of a non-degradable Mcl-1 variant could rescue BAPTA-AM-induced apoptosis. This suggests that the heterogeneity of BAPTA-AM-induced cell death among DLBCL cells may relate to their addiction towards Mcl-1. Surprisingly, different BAPTA-AM variants with varying affinity for Ca^{2+} provoked a similar decrease in cell survival as well as in Mcl-1-protein expression, indicating a "Ca^{2+}-independent" effect of BAPTA-AM. Moreover, EGTA-AM, a structurally different Ca^{2+} chelator, but with similar affinity for Ca^{2+} as BAPTA-AM, was much less effective in lowering Mcl-1-protein levels and provoking cell death. We show that BAPTA-AM and its variants neither impacted Mcl-1 mRNA levels nor influenced proteasomal degradation of Mcl-1, rather we found that they cause a decrease in general translational activity. It is well established that mammalian target of rapamycin (mTOR), a major sensor of cells' energy status, signaling drives Mcl-1 expression through its translation. Excitingly, BAPTA-AM treatment resulted in a rapid loss of p70S6 kinase phosphorylation, a substrate of mTOR.

In any case, our results reveal an unconventional, "Ca^{2+}-independent" mechanism by which BAPTA-AM limits malignant B-cell survival, exploiting their dependence towards Mcl-1 by provoking Mcl-1 downregulation.

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4. **J. T. Gebert**[1,2,3], J. Kellen[1], J. L. Perry[1,4], F. Scribano[1,4], J. Hyser[1]

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Rotavirus induces activation of the Volume-Regulated Anion Channel (VRAC) to mediate remote calcium dysregulation in uninfected cells

Abstract

Worldwide, infectious diarrhea is the second leading cause of mortality among children under the age of five. Despite the introduction of multiple vaccines, rotavirus (RV) remains the most common etiology. While the pathogenesis of RV is incompletely understood, dysregulation of intracellular calcium has long been a hallmark of RV infection. Until recently however, the nature of this dysregulation has been oversimplified. Far from the monophasic increase in intracellular calcium initially described, RV calcium dysregulation manifests as hundreds of discrete signals that occur throughout infection. Furthermore, our lab recently discovered that this dysregulation extends beyond the infected cell. Through the release of ADP, RV-infected cells remotely dysregulate calcium signaling pathways in uninfected neighboring cells *via* the P2Y1 receptor. We have yet to determine how ADP is released from the cell and the mechanism through which RV induces this release, but inhibiting these signals by blocking the P2Y1 receptor reduces symptom severity in mice and intestinal organoids. This suggests that remote dysregulation of calcium signaling pathways in uninfected cells plays an integral role in RV pathogenesis. By using long-term time-lapse calcium imaging in infected monolayers, we are able to quantitate calcium dysregulation at the single-cell level throughout the course of RV infection. Here we have employed this system to identify the mode of ADP release. We show that incubation with DCPIB, an inhibitor of the volume-regulated anion channel (VRAC) or knock out of the *LRRC8-A* gene encoding the obligatory subunit of VRAC both inhibit intercellular calcium waves in RV-infected monolayers. These findings implicate the Volume Regulated Anion Channel (VRAC) as the primary conduit for ADP release. This work reveals a new facet of RV pathogenesis and a new target for anti-RV therapeutics. Given that there are currently no targeted therapies approved for viral diarrhea, this is an important advancement in the effort to reduce the global burden of diarrheal disease.

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5. **A. Rossi**[1]**,** A. M. Riley[2], G. Dupont[3], B. Wacquier[3], T. Rahman[1], B. V. L. Potter[2], C. W. Taylor[1]

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Quantal Ca^{2+} release mediated by very few IP_3 receptors that rapidly inactivate allows graded responses to IP_3

Abstract

Inositol 1,4,5-trisphosphate receptors (IP3Rs) are intracellular Ca^{2+} channels linking extracellular stimuli to Ca2+ signals. Ca2+ release from intracellular stores is 'quantal': low IP3 concentrations rapidly release a fraction of the stores; Ca^{2+} release then slows orterminates without compromising responses to further IP3 additions. The mechanisms areunresolved. We synthesised a high-affinity partial agonist of IP3Rs to demonstrate thatquantal responses do not require heterogenous stores. IP3Rs respond incrementally to IP3and close after the initial response to low IP3 concentrations. Comparing functionalresponses with IP3 binding shows that a tiny fraction of a cell's IP3Rs mediate incremental Ca^{2+} release; inactivation does not therefore affect most IP3Rs. We conclude, and test bysimulations, that Ca^{2+} signals evoked by IP3 pulses arise from rapid activation and theninactivation of very few IP3Rs. This allows IP3Rs, despite their ability to evokeregenerative reponses, to behave as increment detectors, mediating graded Ca^{2+} release.

6. **F. Gu[1],** A. Krüger[1], H. G. Roggenkamp[1], B.-P. Diercks[1], R. Alpers[1], D. Lodygin[2], V. Jaquet[3], F. Möckl[1], L. C. Hernandez[1], A. Bauche[1], A. Rosche[1], H. Grasberger[4], J. Y. Kao[4], D. Schetelig[5], R. Werner[5], K. Schröder[6], M. Carty[7], A. G. Bowie[7], C. Meier[8], H.-W. Mittrücker[9], J. Heeren[1], K.-H. Krause[3], A. Flügel[2], A. H. Guse[1]

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NAADP forming enzymes in T cell activation

Abstract

Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most potent endogenous Ca^{2+} mobilizing second messenger known to date. It is effective in the low nanomolar range in many cells. NAADP functions as a Ca^{2+} trigger in T cells by evoking an initial local Ca^{2+} release, termed Ca^{2+} microdomain, from intracellular stores, which is then amplified through second messengers e.g. IP_3 and cADPR, and Ca^{2+} induced Ca^{2+} release (CICR). In T cells, NAADP is rapidly formed within 10 to 20 seconds after T cell activation. Then, there is a 2nd phase, in which NAADP is elevated between 5 to 20 minutes. *In vitro,* CD38 can catalyze the formation of NAADP from NADP through base-exchange reaction. However, this reaction only proceeds at acidic pH and in the presence of excess nicotinic acid. Since both subcellular and global Ca^{2+} signals were identical in wt and *CD38*$^{-/-}$ murine primary T cells, CD38 does not seem to be the NAADP forming enzyme in T cells.
We discovered a family of NAADP forming enzymes able to generate NAADP *in vitro* under physiological conditions. However, only 2 out of the multiple isozymes of the family appear to be involved in NAADP-dependent local Ca^{2+} microdomains of T cells. The strongest phenotype was observed in a functional double knock-out of the two isoenzymes mentioned above, while single knock-outs of these isozymes showed only weak phenotypes.
Taken together we discovered a family of NAADP forming enzymes that it is capable of synthesizing NAADP *in vitro* under physiological conditions.

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7. **M. Calvo-Rodriguez[1],** S. Hou[1], A. Snyder[1], E. K. Kharitonova[1], A. N. Russ[1], S. Das[1], Z. Fan[1], A. Muzikansky[2], M. Garcia-Alloza[3], A. Serrano-Pozo[1], E. Hudry[1], B. J. Bacskai[1].

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Mitochondrial calcium dysregulation in vivo in a mouse model of Alzheimer's disease

Abstract

Mitochondrial dysfunction is implicated in the pathogenesis of almost all neurological diseases, including Alzheimer's disease (AD). AD is characterized by the deposition of extracellular amyloid beta (Aβ) plaques, intraneuronal fibrillary tangles and cell death. Aβ oligomers (Aβo) surrounding plaques, rather than amyloid plaques themselves, are thought to be the neurotoxic species of Aβ and upstream of tau aggregation. Calcium (Ca^{2+}) dyshomeostasis resulting in Ca^{2+} overload in the cytosol of neurons and neurites, and mitochondrial dysfunction resulting in oxidative stress have been proposed as early pathogenic events in AD according to the amyloid cascade hypothesis. However, whether there are alterations in mitochondrial Ca^{2+} contributing to a more general cytosolic Ca^{2+} dyshomeostasis in AD was unknown.
Using a ratiometric Ca^{2+} indicator targeted to mitochondria in neurons (hsyn.2mtYC3.6) and intravital multiphoton imaging, we measured Ca^{2+} levels in neuronal mitochondria in the AD transgenic mouse model APP/PS1, which develops amyloid plaques similar to human AD. We observed an increase in the mitochondrial Ca^{2+} concentration in APP/PS1 mice, compared to wild-type mice, and after plaque deposition. Moreover, naturally secreted soluble Aβ application onto the healthy brain increased Ca^{2+} concentration in individual mitochondria, which was prevented by blocking the mitochondrial Ca^{2+} uniporter. Interestingly, high levels of mitochondrial Ca^{2+} in the neuronal soma preceded cell death. Taken together, these data support the idea that there is a mitochondrial Ca^{2+} dyshomeostasis linked to neuronal cell death in AD, and that it is, at least partially, due to soluble Aβo.

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8. **J. S. Weissenrieder[1]**, J. R. Pitarresi[2], B. Z. Stanger[3,4], A. K. Rustgi[5], J. K. Foskett[1]

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The Mitochondrial Calcium Uniporter Contributes to Pancreatic Cancer Development and Invasion

Abstract

Cancer is one of the top three causes of death in the United States. While many cancers respond well to newer targeted therapies, pancreatic ductal adenocarcinoma (PDAC) still has a very poor prognosis, with about 50,000 new cases and 45,000 deaths each year. Poor prognosis in PDAC is partially attributable to metastasis, which often occurs before diagnosis and contributes to mortality. More research is needed to enhance our understanding of PDAC disease mechanisms and potential new therapeutic strategies. We previously showed that cancer cell lines may be "addicted" to constitutive uptake of Ca^{2+} by mitochondria through the mitochondrial calcium uniporter (MCU) at endoplasmic reticulum-mitochondria contact sites. We hypothesized that mitochondrial Ca^{2+} influx through MCU contributes to cancer cell development, proliferation, and metastasis in PDAC. We here report that high MCU expression is associated with KRAS mutations, which commonly drive PDAC, as well as poor survival outcomes. MCU gene expression also correlates with genes associated with metastasis and poor patient outcomes, such as ADAM proteins, KRAS, and RAC1. To study the role of MCU in PDAC in vitro and in vivo, we employed the $Pdx1^{cre}$; KrasLSL-G12D/+; $p53^{fl/+}$; Rosa26LSL-YFP/LSL-YFP; $Mcu^{fl/fl}$ (KPCY) murine model of PDAC, generating KPCY-McuKO animals and cell lines from their YFP-positive tissues for further analysis. Cell lines developed from KPCY-McuKO pancreatic tissues lacked mitochondrial Ca^{2+} uptake, which was rescued by stable re-expression of MCU. MCU re-expression increased cell motility, self-renewal, proliferative, and invasive behaviors. It also associated with a change to a fibroblastic morphology indicative of epithelial- to-mesenchymal transition (EMT), including decreased surface expression of e-cadherin. MCUKO cells did not undergo morphological changes when exposed to TGFβ, while MCU-expressing cells became more fibroblastic and motile. Such findings suggest that MCU may contribute to the development of a partial EMT phenotype. In an immunocompetent orthotopic model of PDAC, MCU knockout ablated tumor growth and metastasis in a manner reversed by stable re-expression of MCU. Our findings suggest MCU may contribute to growth and metastasis, potentially through partial EMT. In conclusion, MCU-mediated mitochondrial Ca^{2+} uptake contributes to PDAC development and metastasis and may present a therapeutic target for cancer treatment.

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9. **A. D. Lorenzo**

University of Valladolid, Spain

Sarcoplasmic reticulum Ca 2+ dynamics in aging Drosophila and correlation with sarcopenia

Abstract

10. **A. Bellandi[1]**, J. Joyce[1], M. Johnston[1], J. De Keijzer[1], A. Breakspear[1], R. Morris[1], C. Faulkner[1]

[1]John Innes Centre, Norwich, UK

Dynamics and cell-to-cell transmission of wound-induced calcium waves in plants

Abstract

Plants can perceive a variety of external cues and respond to these by generating local and systemic signals that allow the coordination of appropriate responses across the whole plant body. It is known that when wounded, plant cells respond with an increase of cytoplasmic calcium concentrations and that this response propagates in a wave-like manner through the neighbouring non-wounded tissue. However, the molecular machinery and dynamics responsible for the generation and propagation of such responses are poorly understood.
Plant cells are enclosed and separated from each other by a cell wall. Plasma membrane-lined channels called plasmodesmata span across the cell wall and allow for regulation of cytoplasmic connectivity among neighbouring plant cells. Previous models and studies have hypothesized that the wound-induced calcium wave propagates cell-to-cell via plasmodesmata but have not shed light on the mechanisms by which this occurs.
Here, we developed novel experimental and computational methods that allow the study of dynamics and cell-to-cell transmission of wound-induced local calcium waves with high spatio-temporal resolution, in live and intact soil-grown plants. By using this method, we were able to show that the local calcium response to wounding in aerial tissues has at least three temporally distinct phases (a local burst, a propagating wave and a late diffuse calcium increase) differing in their dynamics and underlying mechanisms. Moreover, we show that the cell-to-cell propagation of the calcium wave does not rely on cytoplasmic connectivity and is dependent on glutamate-like receptor channels.

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11. **N. Srivastava**[1,3,4], D. Traynor[2,5], M. Piel[3,4], A. J. Kabla[1], R. R. Kay[2]

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Pressure sensing through Piezo channels controls whether cells migrate with blebs or pseudopods

Abstract

Blebs and pseudopods can both power cell migration, with blebs often favoured in tissues, where cells encounter increased mechanical resistance. To investigate how migrating cells detect and respond to mechanical forces we used a 'cell squasher' to apply uniaxial pressure to *Dictyostelium* cells chemotaxing under soft agarose. As little as 100 Pa causes a rapid (<10 sec), sustained shift to movement with blebs rather than pseudopods. Cells are flattened under load and lose volume; the actin cytoskeleton is reorganized, with myosin-II recruited to the cortex, which may pressurize the cytoplasm for blebbing. The transition to bleb-driven motility requires extra-cellular calcium and is accompanied by increased cytosolic calcium. It is largely abrogated in cells lacking the Piezo stretch-operated channel; under load, these cells persist in using pseudopods and chemotax poorly. We propose that migrating cells sense pressure through Piezo, which mediates calcium influx, directing movement with blebs instead of pseudopods.

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12. **S. E. Delgado**[1], C. Q. Chiu[1,2], A. Calixto[1]

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[2] Max-Planck Tandem Group

Following calcium under mec-4d neurodegeneration and regeneration in Caenorhabditis elegans

Abstract

MEC-4 is the pore-forming unit of the mechanosensory channel expressed in the Touch Receptor Neurons (TRNs) in *Caenorhabditis elegans*, that transduces changes on the membrane through sodium currents. An amino acid switch (A713T) in the second transmembrane domain changes MEC-4 selectivity and gating properties (Driscoll and Chalfie, 1991; Kellenberger and Schild, 2002; Brown *et al*., 2007). This mutant channel is known as MEC-4d and may permeate calcium in addition to sodium (Goodman *et al*., 2002; Bianchi *et al*., 2004; O'Hagan *et al*., 2005), causing calcium increases inside the neuron. As a result, MEC-4d expression disrupts normal homeostasis, causes protease activation, and necrotic death (Syntichaky *et al*., 2002; Calixto *et al*., 2012). In *mec-4d* animals, the chronic increase of ions inside the cell produces the breakage of axons, swelling of the soma and later, the death of the neuron in a stereotypical way. Interventions that delay or stop neurodegeneration include specific bacterial metabolites and diapause entry (Urrutia *et al*., 2020; Caneo *et al*., 2019). Under diapause, damaged TRNs exhibit an extraordinary capacity for functional regeneration, even from a soma alone (Caneo *et al*., 2019). This regeneration is mediated by DLK-1 and requires calcium, but the threshold could be lower. Using multiphoton microscopy, we aim to reveal the changes in calcium homeostasis in the TRNs that occur under diapause. To this end, we used GCaMP6m and tagRFP to follow spontaneous activity from the TRN in diapausing animals, also known as *dauers*, and the L2 stage as a control that shares parallel metabolic and developmental characteristics. We found that the *mec-4d* mutation generates increased calcium signals. Our findings suggest that non-physiological calcium concentrations are maintained while TRN regenerates. Then, *dauer* regeneration occurs despite the damage signal, dealing with the signal associated.

13. L. G. Gutiérrez[2]**,** L. Núñez[1], J. A. Rosado[2], C. Villalobos[3]

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Channel complexes involved in remodeling of store-operated channels in colon cancer cells and their reversal by DFMO

Abstract

Store-operated Ca^{2+} entry (SOCE) is the most important Ca^{2+} entry pathway in non-excitable cells. Colorectal cancer (CRC) show decreased Ca^{2+} store content and enhanced SOCE that are associated to remodeling of store-operated channels (SOCs), in addition its correlate with cancer hallmarks. Ca^{2+} remodeling in CRC may consist of changes in expression of Ca^{2+} channels and pumps and are associated to interaction among different molecular players: non-selective currents driven by Orai1, Orai3 and TRPC1 channels and Stim1 and Stim2 sensors. Difluoromethylornithine (DFMO), a suicide inhibitor of ornithine decarboxylase (ODC), is a strongly preventor of CRC. We asked whether polyamine depletion could be reverse Ca^{2+} remodelling interactions in CRC. We found that the principal players in normal mucosa cells in SOCE are Orai1 and Stim2, TRPC1 and Stim1 also are presented in low levels. It seems that all this molecules make week interactions between them. However, store-operated channels in CRC display enhanced dual interactions between TRPC1, Stim1 and Orai1 compared to normal cells. Moreover, DFMO treatment decreases specifically the interaction between TRPC1 and Stim1. These data are consistent with previous results that looked like DFMO treatment in CRC cells, specifically affecting the suppression of TRPC1 and Stim1. These results suggest that polyamines contribute to Ca2+ channel remodelling interactions in CRC and DFMO may prevent CRC by reversing channel remodeling.

14. S. Roig[1,2], N. Thijssen[1], M. van Erp[3], J. Fransen[3], Y. Cheng[4,5], J. Hoenderop[1], **J. van der Wijst**[1]

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Structural insight into TRPV5 channel function and modulation

Abstract

Introduction: TRPV5 (transient receptor potential vanilloid) is a uniquely calcium-selective TRP channel that plays a crucial role in the maintenance of calcium homeostasis. Unlike other TRPV channels, TRPV5 and its homologue TRPV6 do not exhibit thermosensitivity or ligand-dependent activation, but are constitutively open at physiological membrane potentials. Channel activity is regulated by a negative feedback via dynamic interaction with calmodulin (CaM). CaM is a calcium-binding protein that consists of two lobes (N- and C-lobe), each containing two calcium binding sites. Together with recent structural insight into the TRPV5-CaM complex, we aim to decipher the functional link towards channel regulation.
Methods: Full length and truncated TRPV5 proteins, expressed in human embryonic kidney (HEK293) cells, were affinity-purified and reconstituted into lipid nanodiscs (disc-shaped membrane mimics) or detergent micelles. Following size-exclusion chromatography, pure TRPV5 protein was used for single particle cryo-electron microscopy (cryo-EM). The interaction of TRPV5 with CaM was further studied with fluorescence life time imaging microscopy (FLIM)-based FRET and single molecule photobleaching TIRF (total internal reflection fluorescence) microscopy, using calcium-binding mutants of CaM. The link with TRPV5 channel activity was examined by Fura-2-AM calcium imaging.
Results: We report high resolution cryo-EM structures of TRPV5 and a complex structure of TRPV5 with CaM. This demonstrates interaction of CaM with specific carboxy-terminal regions of TRPV5. FLIM-FRET analyses with lobe-specific calcium-binding CaM mutants display intermediate states of TRPV5-CaM interaction. We also show significant differences in FRET efficiency of TRPV5 binding mutants with wildtype CaM, and thereby unravel the binding interface of TRPV5-CaM, and the calcium-dependency of interaction. Fura-2-AM imaging suggests a link between different CaM binding states and TRPV5 channel function. In addition, preliminary TIRF data corroborates the 3D classification of our TRPV5-CaM structure suggesting a preferred 1:2 TRPV5:CaM stoichiometry.
Conclusion: Our structural work has opened new avenues towards better understanding of TRP channel function. This follow-up study now provides insight into the dynamics behind TRPV5-CaM binding with the aim to shape a model for CaM-dependent channel inactivation. Future work should identify whether this working model is unique to the calcium-selective TRPV5 and TRPV6 or can be extrapolated to other TRPV channels.

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Functional characterization of the calcium coordinating residue in human type 1 inositol 1,4,5-trisphosphate receptor

Abstract

Numerous calcium (Ca^{2+}) channels, pumps, transporters, and binding proteins function to regulate intracellular Ca^{2+} levels which in turn regulate a large variety of physiological processes. Amongst these Ca^{2+} channels, the tetrameric inositol 1,4,5-trisphosphate receptor (IP_3R) mediates Ca^{2+} flow from the endoplasmic reticulum (ER) into the cytosol following activation of G protein-coupled receptor/phospholipase C-β, or tyrosine kinase receptor/phospholipase C-γ. Binding of both the intracellular messengers IP_3 and Ca^{2+} are a prerequisite for IP_3R channel opening. Although it is well established that IP_3 binds to the IP_3-binding domain at the N-terminus, the residues in IP_3Rs which constitute the Ca^{2+} binding site are not fully understood. A recent report detailing the structure of full-length human type 3 IP_3R indicates a critical role of glutamate residue (E1946, corresponding to E2002 of human type 1 IP_3R) for Ca^{2+}-binding. In order to investigate this, we generated stable cell lines harboring substitutions at the E2002 residue including E2002D and E2002Q in HEK-293 cells in which all three endogenous IP_3Rs were disrupted by CRISPR/Cas9 genome editing technology. Ca^{2+} imaging using Fura-2 acetoxymethyl ester (AM) and IP_3-generating agonist carbachol (CCh) revealed a significant attenuation in CCh-induced Ca^{2+} release across various concentrations in E2002D when compared to wild-type stable cell line. Interestingly, the E2002Q cell line did not respond to CCh-stimulation suggesting that negative charge on E2002 side chain residue may be required for Ca^{2+} coordination. A similar trend was observed using trypsin as an agonist to generate IP_3. Furthermore, we used time-resolved-total internal reflection microscopy (TIRF) to record Ca^{2+} puffs from these cell lines. CCh-evoked puff sites and the number of puffs were markedly decreased in E2002D and E2002Q cell lines when compared to stable wild-type cells. The distribution of peak puff amplitudes, however, did not differ between these cell lines. Taken together, our data suggest that the E2002 residue of IP_3R1 is crucial for the regulation of channel activation. Future studies are required to delineate the roles of this and other residues in Ca^{2+}_mediated gating of IP_3Rs.

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Mechanisms of calcium regulation by the dysferlin C2A domain

Abstract