9th Workshop of the European Calcium Society

Mitochondrial Ca^{2+} Signaling in Health and Disease

Programme & Abstracts

Padua, Italy
7-9 September 2023

https://ECSW2023.azuleon.org

Scientific Committee

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Programme

Palazzo Bo, Nievo Room

12:30 **Registration**

13:30 **Intoduction**
Welcome by ECS Secretary-General Geert Bultynck *(KU Leuven, Belgium)*

14:00-14:50 **"Tullio Pozzan Memorial Lecture"** ***(supported by Cell Calcium)***
Introduction by Paola Pizzo (University of Padua, Italy)
Anant Parekh *(University of Oxford, United Kingdom)*
Mitochondrial regulation of store-operated calcium channels

Chair: Marisa Brini (University of Padua, Italy)

14:50-15:25 **Guy Rutter** *(University of Montreal, Canada)*
Mitochondrial dynamics in pancreatic beta cell function and heterogeneity

15:25-16:00 **Fabiana Perocchi** *(Technical University Munich, Germany)*
Unbiased mapping of the MCU interactome reveals MCNR1 as a potential molecular target in cancer

16:00-16:35 **Amado Carreras Sureda** *(University of Geneva, Switzerland)*
CaMPARI applied to ion channel genomic interrogation during ER stress

17:00-19:00 ***Tour Palazzo Bo (2 turns, 25 people each)***

19:30 ***Welcome spritz @ Pedrocchi***

Palazzo Bo, Archivio Antico Room

Chair: Riccardo Filadi (Neuroscience Institute, Italian National Research Council, Italy)

9:00-9:35 **Maria Patron** *(Max Planck Institute for Biology of Ageing, Cologne, Germany)*
Proteolytic control of mitochondrial Ca^{2+} signalling

9:35-10:10 **Israel Sekler** *(Ben-Gurion University, Israel)*
Physiological roles and mode of regulation of the mitochondrial Na^{+}/Ca^{2+} exchanger NCLX

10:10-10:45 **Cláudia Pereira** *(University of Coimbra, Portugal)*
Perturbation of ER-mitochondria contacts in mental illness

10:45-11:15 ***Coffee break***

Short communications

Chair: Nicolas Demaurex (University Geneva, Switzerland)

11:15-11:30 **Alejandro Marmolejo Garza** *(University of Groningen, The Netherlands)*
Calcium signaling is impaired in iPSC-derived Alzheimer's Disease models

11:30-11:45 **Alex Whitworth** *(University of Cambridge, United Kingdom)*
Partial loss of the mitochondrial calcium uniporter (MCU) mitigates pathology *in vivo* across a diverse range of neurodegenerative disease models

11:45-12:00 **Tânia Fernandes** *(University of Coimbra, Portugal)*
Impaired ER-mitochondria contacts affect lipid droplets degradation in an *in vitro* Alzheimer's disease model

12:00-12:15 **Anna Rita Cantelmo** *(INSERM, Institut Pasteur de Lille, France)*
Single-cell analysis reveals mitochondrial calcium signaling as a novel modulator of endothelial cell plasticity

12:15-12:45 Eurobioimaging *(sponsor)*

Introduction by Riccardo Filadi *(Neuroscience Institute, Italian National Research Council, Italy)*

Micaela Zonta and Michele Gintoli *(Neuroscience Institute, Italian National Research Council, Italy)*

13:00-15:30 ***Lunch and Posters***

Chair: Jan Parys (KU Leuven, Belgium)

15:30-16:05 **Patrizia Agostinis** *(VIB-KU Leuven, Belgium)*
The autophagy-mitochondria metabolism link in lymphangiogenesis

16:05-16:40 **Amalia Dolga** *(University of Groningen, The Netherlands)*
Alterations in mitochondrial metabolism and calcium pathways in familial Alzheimer's disease iPSC-derived neuronal cells and brain organoids

16:40-17:15 **Mayte Montero** *(University of Valladolid, Spain)*
Calcium signaling as a new target for ageing and neurodegenerative diseases

Short communications

17:15-17:30 **Michael Feng** *(Helmholtz Munich, Germany)*
Systematic identification of signaling pathways regulating mitochondrial calcium uptake

17:30-17:45 **Diego de Stefani** *(University of Padua, Italy)*
Uncovering the functional role of TMEM65 protein in mitochondrial homeostasis

18:00 ***Tour Palazzo Bo (3rd turn)***

20:00 ***Gala dinner***
Ristorante Antico Brolo *(Corso Milano, 22 - 35139 Padova https://anticobrolo.it)*

Palazzo Bo, Archivio Antico Room

Chair: Javier Alvarez (University of Valladolid, Spain)

9:00-9:35 **Araceli Del Arco** *(Universidad Autónoma de Madrid, Spain)*
Ca^{2+} regulation of neuronal metabolism; role of MCU and Aralar/MAS pathways

9:35-10:10 **Geert Bultynck** *(KU Leuven, Belgium)*
Anti-apoptotic Bcl-2 and its network controlling Ca^{2+} signaling

10:10-10:45 **Mounia Chami** *(CNRS UMR-7275, Valbonne, France)*
Mitophagy failure in Alzheimer's disease: a hope for a diagnostic application and therapeutic targeting

10:45-12:00 ***Coffee and Posters***

Short communications

Chair: Tito Calì (University of Padua, Italy)

12:00-12:15 **Jan Parys** *(KU Leuven, Belgium)*
The role of pyruvate kinase M2 in mitochondrial Ca^{2+} handling and cell death

12:15-12:30 **Lenka Radonova** *(National Institute of Environmental Health Sciences, NC, USA)*
Unmasking the connections: mitochondria-endoplasmic reticulum domains in Ca^{2+} signaling at fertilization

12:30-12:45 **Seemanti Aditya** *(Indian Institute of Technology Madras, India)*
Deciphering the role of PIGBOS1 in cellular calcium homeostasis

12:45-13:00 **Giulia Dematteis** *(University Piemonte Orientale, Italy)*
Investigation of the interplay between endoplasmic reticulum-mitochondria distances and mitochondrial Ca^{2+} uptake

13:00-14:30 ***Lunch and Posters***

14:30-15:20 **Closing Lecture**

Chair: Paola Pizzo (University of Padua, Italy)

Rosario Rizzuto *(University of Padua, Italy)*
MCU and mitoKATP in the inflammatory response

15:30-16:00 ***Group Photo***

16:00-16:30 **Final remarks and prizes**

Invited Speakers

PATRIZIA AGOSTINIS

VIB-KU Leuven, Leuven, Belgium

Patrizia Agostinis (PA) received a Master in Biology from the University of Padua (Italy) and a PhD in BioMedical Science from the University of Leuven KU Leuven. After a short postdoctoral period, PA became an independent Group Leader of the Research Fundation (FWO) Flanders-Belgium. She is currently full Professor and head of the Cell Death Research & Therapy lab at the Department of Cellular and Molecular Medicine and a Group Leader of the Center for Cancer Biology of the VIB at KU Leuven. Her group made important contributions to the cancer biology and immunotherapy fields by unravelling molecular underpinnings and the robust in vivo anticancer vaccination properties of immunogenic cancer cell death and how cancer- or endothelial cell-intrinsic autophagy pathways impact tumor angiogenesis, metastasis and therapy responses. PA was elected President of the European Cell Death (ECDO) Society, honorary member of the European Academy of Tumor Immunology and received the 2019 Outstanding Achievement Award of the International Cell Death Society. The overarching goal of PA lab is to unveil how key cell-fate decision networks (i.e. cell death pathways, metabolic/quality control mechanisms) in cancer cells and stromal cells impact local and peripheral tumor microenvironment and responses to anticancer therapies. PA is also a strong advocate for Women in Science and mentor of several PhD and Postdoctoral researchers, who continued their independent carrier as scientists or specialists.

GEERT BULTYNCK

KU Leuven, Leuven, Belgium

Geert Bultynck (PhD, KU Leuven, 2001) heads the Laboratory of Molecular & Cellular Signaling, KU Leuven, Belgium. Since 2008 as a PI, he has been studying the role Ca^{2+} signaling in health, disease & therapy with a focus on the role of the Bcl-2-protein family. He aims to decipher the non-canonical functions of the anti-apoptotic Bcl-2 family underlying direct modulation of intracellular Ca^{2+}-transport systems, particularly IP3 receptors and ryanodine receptors and thereby contributing to cell death resistance. The team wants to unravel the molecular determinants underlying IP3R/Bcl-2 complexes and by doing so develop novel therapeutic cell death strategies by targeting & disrupting these complexes in cancer cells. The team also exploits the properties of Bcl-2 proteins in suppressing Ca^{2+} signaling in diseases associated with excessive Ca^{2+}-release events such as neurodegeneration and rare genetic diseases.

Geert Bultynck currently serves as Executive Editor of BBA – Molecular Cell Research journal and is the Secretary-General of the European Calcium Society (ECS), a community of more than 200 researchers worldwide.

AMADO CARRERAS SUREDA

University of Geneva, Geneva, Switzerland

Amado obtained his Biology and Master degree at the Univeristy Pompeu Fabra (UPF) in Barcelona. In 2010, he joined the Ph.D. program in the department of Department of Experimental and Health Sciences also in the UPF in Barcelona. He did his Ph.D. at the Molecular Physiology and Channelopathies group leaded by Miguel Angel Valverde and worked on T cell activation and intracellular calcium homeostasis. After, he did a postdoc in the lab of prof. Claudio Hetz, granted by the FONDECYT program where he studied protein folding and ER stress coupled to mitochondrial biology, focusing on ER and mitochondria contact sites. During these periods he was able to work in the NYU, or KU Leuven, profiting from career awards like EMBO short term fellowship or the Boehringer Ingelheim travel grant. He came back to Europe on 2019, where he currently works as a Junior lecturer in the University of Geneva in the laboratory of Prof. Nicolas Demuarex. He intends to dedicate his scientific career to the study of ER proteostasis, interorganellar ionic and metabolic communication and how this impacts cellular adaptation to stress environments.

MOUNIA CHAMI

CNRS UMR-7275, Valbonne, France

Dr. Chami is a Senior Researcher at the University Côte d'Azur, at the Institute of Molecular and Cellular Pharmacology. She received her Doctoral degree on Molecular and Cellular Biology from the University of Paris XI. She worked at the University of Ferrara, Italy, as a post-doctoral fellow, served as a Junior researcher in Paris V University, France, and as a team leader at the Italian Institute of Italy. Dr. Chami Mounia is expert in the field of subcellular calcium signaling and intracellular organelles dysfunctions namely the endoplasmic reticulum (ER) and mitochondria (http://cvscience.aviesan.fr/cv/185/mounia-chami). In the field of Alzheimer's disease (AD), she demonstrated the impact of ER calcium homeostasis in AD pathogenesis through the control of Amyloid precursor protein processing, the development of cognitive deficits and synaptic plasticity dysfunction, and also neuroinflammation. These studies reveal the potential therapeutic benefit in targeting ER Calcium release to alleviate or treat AD. Dr. Chami also reported the localization and the metabolism of APP in Mitochondria Associated Membranes thus interfering with lipid metabolism and mitochondria dyfunctions in AD. Recent studies by Dr. Chami highlight novel mechanisms linking mitochondrial dysfunction and mitophagy failure to AD development.

ARACELI DEL ARCO

Universidad Autónoma de Madrid, Madrid, Spain

Araceli del Arco obtained a Bachelor of Biology (1988) and a Ph.D. in Sciences (1993) from the Autonomous University of Madrid (UAM), Spain, in the Marta Izquierdo' group one of the promoters of Molecular Biology in Spain. In 1994 she joined the group of Jorgina Satrústegui, a pioneering scientist in the study of calcium regulation of mitochondrial metabolism. In 1997 she incorporated as Assistant Professor to the Universidad de Castilla-La Mancha (Toledo, Spain) where obtained a permanent position in 2003. However, her scientific career has continued to develop in close collaboration with J. Satrustegui in the UAM. In the late 1990s, their research focused on Ca^{2+} signaling systems in mitochondria led to the discovery of the Ca^{2+} regulated mitochondrial carriers, CaMCs, metabolite transporters of the inner mitochondrial membrane with Ca^{2+}-binding domains facing the intermembrane space. In contrast with the well-known system of Ca2+-signaling in mitochondria, which depends on Ca^{2+} entry to the matrix along the mitochondrial calcium uniporter (MCU), CaMCs activation by Ca^{2+} is independent of matrix Ca^{2+}. This group first identified the mitochondrial transporters of aspartate/glutamate (AGCs) showing that they were the Ca^{2+}-regulated component of the malate-aspartate NADH shuttle. Later, they reported the existence of the short CaMCs, or SCaMCs, which were shown to correspond to the mitochondrial transporters of ATP-Mg^{2+}/Pi or ADP/Pi. Since then, her efforts have focused on understanding how cytosolic calcium signals, through CaMCs activation, modulate mitochondrial function.

AMALIA DOLGA

University of Groningen, Groningen, The Netherlands

Amalia Dolga is Associate Professor on Regenerative Neuropharmacology at the University of Groningen, the Netherlands. Her laboratory aims at identifying the molecular mechanisms responsible for cell death (particularly ferroptosis) and at elucidating their involvement in human brain diseases. The focus of her research is on mitochondrial dysfunction, calcium and inflammatory pathways. To unravel the complex web of factors that contribute to these pathways, her group is using human induced pluripotent stem cell-differentiated brain cells, including neurons/microglia and brain organoids. Furthermore, her team is employing organ-on-a-chip technology to study how brain cells communicate with each other. Through these innovative approaches, her group aims to gain valuable insights into the underlying mechanisms of brain diseases and propose improved treatments.

MAYTE MONTERO

University of Valladolid, Valladolid, Spain

I have worked on calcium signaling since my Ph.D., in which I studied the control of the Ca^{2+} permeability of the plasma membrane by the level of filling of intracellular Ca^{2+} stores (1991), Valladolid, Spain. I then did a postdoc in Padova (Italy) (Dpto Scienze Biomediche, Universitá di Padova, with Profs. Pozzan and Rizzuto) on the development of a new method to measure the [Ca^{2+}] in the endoplasmic reticulum using the recombinant photoprotein aequorin targeted to the ER (1993-95). I then returned to Valladolid, Spain and together with Professor Javier Alvarez we continued working on subcellular calcium homeostasis in the IBGM (Instituto de Biomedicina y Genética Molecular, Valladolid, Spain). I took a faculty position in Biochemistry and Molecular Biology, University of Valladolid, becoming full Professor in 2018. In 2012 I spent a sabbatical stay at the Laboratory of Molecular Biology/ Medical Research Council, Cambridge, UK, with Prof. W.R.Schafer, learning the issues surrounding calcium imaging in live C. elegans pharynx, both cytosolic and mitochondrial. Then I returned to my lab where our efforts are now focused in the relationship between ageing and calcium homeostasis in *C. elegans*, as well as in the diseases associated to ageing, in particular neurodegenerative diseases such as Alzheimer, Parkinson and Huntington, using models of these diseases in *C. elegans*.

ANANT PAREKH

University of Oxford, Oxford, United Kingdom

Anant Parekh was a medical student at Oxford University. He stayed on for his graduate studies and received a D.Phil. from the Department of Pharmacology on E-C coupling in smooth msucle. He completed a postdoctoral fellowship at the Max Planck Institute for Biophysical Chemistry in Goettingen, Germany with Nobel Laureate Erwin Neher, Ph.D., and Reinhold Penner, M.D., Ph.D. He then joined the Department of Physiology, Anatomy and Genetics at the University of Oxford and became Professor of Physiology and Director of the Centre of Integrative Physiology and Fellow of Merton College. He is now Chair of the Department of Signal Transduction at the NIH, USA. Parekh has received several awards for his research, including the Wellcome Prize in Physiology and the GL Brown Prize in Physiology. He was elected a member of Academia Europaea in 2002, the U.K. Academy of Medical Sciences in 2012 and became a Fellow of the Royal Society in 2019. He was awarded the Annual Prize from the UK Physiological Society in 2023. His group studies intracellular calcium signalling, particularly the function of store-operated calcium channels. A major focus is on how aberrant calcium signals contribute to disease in humans, particularly allergies and asthma.

MARIA PATRON

Max Planck Institute for Biology of Ageing, Cologne, Germany

Maria focused all her scientific career on mitochondrial calcium signaling. She did her PhD in Padova with Rosario Rizzuto where she characterized how MICUs family regulates mitochondrial calcium uniporter activity. She moved then for her postdoc to Cologne in the lab of Thomas Langer where she discovered TMBIM5, a new channel that extrudes Ca^{2+} from mitochondria.

CLÁUDIA PEREIRA

University of Coimbra, Coimbra, Portugal

C Pereira is Biochemist (1991), Master in Cell Biology (1994) and received, in 2000, a PhD in Cell Biology, University of Coimbra (UC). Presently, she is Investigator with tenure at the Fac. Medicine, UC, and leads the research line "Endoplasmic reticulum (ER) stress response and ER-mitochondria axis" at CNC. She published > 80 peer-reviewed articles, mainly in Q1 journals. The scientific impact of her research is reflected in the high number of citations (>4000) and the H index (39).

The mission of C Pereira research is to investigate the molecular basis of neuropsychiatric disorders associated with metabolic and inflammatory alterations, namely age-related neurodegenerative disorders, such as Alzheimer's disease, and mood and psychotic disorders, such as bipolar disorder and schizophrenia, respectively, in order to identify potential applications in the diagnosis, prognosis and therapy. Using several disease models like cell lines and primary cell cultures and also sub-cellular fractions, control- versus patient-derived cells and transgenic mice, together with multiple experimental approaches including biochemistry, molecular and cellular biology, and animal behavior analysis, the group focus on the disease-associated deregulation of ER and mitochondria stress responses, and is also interested in understanding how disturbances of the ER-mitochondria axis under pathological conditions affect proteostasis, lipid and energetic metabolism, calcium homeostasis and inflammation. C Pereira's studies can contribute to the development of novel therapies for the above diseases promoting human health and well-being.

FABIANA PEROCCHI

Technical University Munich, Munich, Germany

Professor Perocchi (b. 1977) conducts research at the interface of biology, medicine and computational biology. Her group integrates systems-level multi-omics strategies with traditional bottom-up biochemical, genetic and physiological approaches to discover and functionally characterize hitherto unknown molecular components of mitochondrial machineries and pathways. A central aspect of her long-term research goals is to understand how mitochondria can sense and integrate calcium-dependent signalling in a cell and tissue-specific manner in order to decipher mitochondrial-dependent pathomechanisms of neuro-metabolic diseases.
Professor Perocchi studied Biology in Rome, and then moved to Heidelberg where she completed a PhD in Functional Genomics at the European Molecular Biology Laboratory. In 2007, she joined the Department of Systems Biology at Harvard Medical School and Massachusetts General Hospital (USA) as a postdoctoral researcher, where she discovered the molecular components involved in mitochondrial calcium transport. After a short postdoc (2011-2012) at the Center for Genomic Regulation in Barcelona (Spain), she joined the Gene Center of the Ludwig-Maximilian University (LMU) as a Junior Group Leader of the Bavarian Research Network for Molecular Biosystems. In 2013 she was also appointed as Young Investigator Group Leader at the Helmholtz Munich, funded by the Emmy Noether Research Program. In 2018 she was appointed head of the Research Unit in Functional Genomics of Mitochondria at the Institute for Diabetes and Obesity of the Helmholtz Munich. Since 2021, she is an Associate Professor for Systems Biology of Metabolism at TUM School of Medicine.

ROSARIO RIZZUTO

University of Padua, Padua, Italy

Prof. Rizzuto is Professor of General Pathology in the Medical School of the University of Padua.
After acting as Director of the Department of Biomedical Sciences and Member of the Academic Senate, from 2015 to 2021 Prof. Rizzuto was the Rector of the University of Padua. In 2022 he coordinated the presentation of the proposal for the establishment of the National Center for Gene Therapy and Pharmacological Drugs (Mission 4, Component 2 of the PNRR MUR). After approval by the Ministry and funding with €320,036,606.03, Prof. Rizzuto was nominated President of the governing body ("Fondazione") of the National Center.
Prof. Rizzuto received multiple awards, including the Chiara D'Onofrio award, the Biotec Award, the Theodor Bucher Medal and in 2014 the Antonio Feltrinelli Award from the Accademia dei Lincei. He served as a Chairman for three international congresses (12th International Symposium on Calcium Binding Proteins and Calcium Function in Health and Disease in 2002, EMBO Workshop on "Calcium signaling and diseases" in 2004 and the Gordon Research Conference on Calcium Signaling in 2011) and >300 invited talks at International conferences and research institutions. He is a member of the Academia Europaea and of the European Molecular Biology Organization (EMBO). He acts as a reviewer for numerous funding agencies (BBSRC, Wellcome Trust, UMDF, etc.) and top international journals (Nature, Science, Cell, etc.).
Prof. Rizzuto research is focused on cellular signals and in particular the mechanisms and pathological alterations of mitochondrial homeostasis of calcium ions. His activity was funded through the years by National and International agencies, such as European Research Council (Ideas Advanced "Mitocalcium" project), National Institute of Health (NIH), Italian Association for Cancer Research (AIRC), Telethon, Ministries of Education and Health and the Cariparo and Cariplo Foundations. He is the author of more than 300 publications in international journals listed by Pubmed (>47,000 citations; h-index 106, from Scopus).

GUY RUTTER

Imperial College London, London, United Kingdom

Guy Rutter is a Professor of Medicine at the University of Montreal and Principal Fellow at the CRCHUM in Montreal. He is also Professor of Cell Biology at Imperial College London and Visiting Professor at Nanyang Technological College, Singapore. His research focusses on diabetes mellitus, a condition which currently affects almost 10 % of the population worldwide, and specifically on the signalling pathways through which glucose, incretins and other hormones control insulin secretion from the pancreatic β cell. His research career began as a graduate student with Dick Denton FRS in the Department of Biochemistry at Bristol University U.K. fist established by Sir Philip Randle, examining the regulation of the then recently-discovered intramitochondrial Ca^{2+} sensitive dehydrogenases. In 1991 he won an MRC Travelling Fellowship to study under Claes Wollheim in Geneva, Switzerland, and with Rosario Rizzuto and Tullio Pozzan in Padua, where he demonstrated the importance of these enzymes in insulin secretion. He established his own laboratory at Bristol University in 1995, and moved to Imperial College London in 2006 to lead the Section of Cell Biology and Functional Genomics and later the Imperial Network of Excellence in Diabetes. He moved to Montreal in 2021 where his current interests include beta cell heterogeneity and mitochondrial dysfunction. He is a Wellcome Trust Investigator also funded by UKRI-MRC, CIHR, NIH, JDRF and Innovation Canada.

ISRAEL SEKLER

Ben-Gurion University, Be'er Sheva, Israel

My research is focused on calcium homeostasis. The mitochondrial Na^+/Ca^{2+} exchanger is a key player in mitochondrial and cellular calcium homeostasis but although its existence was documented 40 years ago its molecular identity remained unknown until now. By combining molecular silencing, ectopic expression, and dominant negative analysis with imaging of mitochondrial and cellular calcium levels, **we have identified NCLX as the long-sought exchanger**. This finding opened the door to molecular analysis of the mitochondrial Ca^{2+} transport machinery and interrogation of diverse physiological studies ranging from cardiac activity to insulin secretion neuronal activity and hepatic endocrine control which we are actively pursuing. We also interrogate the structure function and regulation of NCLX. We identified the regulatory domain of NCLX that controls mitochondrial Ca^{2+} signaling by phosphorylation and is sensing the mitochondrial membrane potential.

Abstracts

Deciphering the role of PIGBOS1 in cellular calcium homeostasis

Seemanti Aditya, A.K. Bera
Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences, Indian Institute of Technology Madras, Chennai, Tamil Nadu, India

Calcium ions (Ca^{2+}) play a crucial role in various physiological processes, including memory formation, cell-cell communication, and apoptosis. Maintaining an optimal concentration of Ca^{2+} within the cell is essential for proper cellular functioning. Disruptions in Ca^{2+} homeostasis can result in protein aggregation, leading to neurodegenerative disorders such as Alzheimer's and Parkinson's disease (PD). Extensive evidence suggests that changes in cellular Ca^{2+} homeostasis along with oxidative stress, and endoplasmic reticulum (ER) stress are closely linked to the progression of PD. Recently, a mitochondrial microprotein called PIGBOS1 has emerged as a key regulator of the ER stress response through its interaction with an ER-resident chloride channel called CLIC-like1 (CLCC1). Suppression of either PIGBOS1 or CLCC1 individually has been shown to intensify ER stress and increase cell death. In this study, we aimed to investigate the role of PIGBOS1 in maintaining cellular Ca^{2+} homeostasis by monitoring Ca^{2+} dynamics in the ER, cytoplasm, and mitochondria using genetically encoded Ca^{2+} indicators. Our findings revealed that HEK-293T cells overexpressing PIGBOS1 exhibited a higher basal level of Ca^{2+} in the ER. Furthermore, these cells showed an increased rise in cytosolic and mitochondrial Ca^{2+} levels following ionomycin/histamine treatment. Collectively, these results suggest that PIGBOS1 is an integral component of the cellular Ca^{2+} signaling network and may have implications in the development of diseases like PD.

Single-cell analysis reveals mitochondrial calcium signaling as a novel modulator of endothelial cell plasticity

M. Lebas[1], G. Chinigò[2,3], C. Dejos[2], J. Goveia[4], A. Beatovic[4], A. Fiorio Pla[2,3], D. Gkika[5], Anna Rita Cantelmo[1]

Endothelial cells (ECs) are highly plastic cells able to give raise to other cell types and undergo fate change. The transdifferentiation towards a mesenchymal-like phenotype, a process referred as to endothelial-to-mesenchymal transition (EndMT), is critical for embryonic development, and in adults it is one of the major contributors to the onset of diseases, including cancer, organ fibrosis and a number of cardiovascular disorders. Therefore, EndMT targeting holds therapeutic promise for treating some of the most prevalent diseases. However, the field lacks a precise molecular and functional definition of EndMT, and more importantly, targeting the EndMT master-regulatory transcription factors remains challenging.

Here, we performed single-cell RNA-sequencing on three in vitro models of induced EndMT differentiation to identify novel conserved EndMT regulators. Our analysis revealed that mitochondrial calcium signalling controls EndMT. We functionally validate that mitochondrial calcium uptake influences endothelial transdifferentiation,whereas pharmacological blockade of the mitochondrial calcium uniporter (MCU) prevents EndMT. Conditional deletion of *mcu* in ECs confirms loss of mesenchymal activation in a hind limb ischemia mouse model. Consistent with these findings, human tissues from patients with critical limb ischemia showing features of EndMT express significant higher levels of endothelial MCU.

Together, these data identify mitochondrial calcium signaling as a novel regulatory mechanism of endothelial transdifferentation, thus potentially allowing for the development of new therapeutic interventions for EndMT-based diseases.

Uncovering the functional role of TMEM65 protein in mitochondrial homeostasis

M. Vetralla , V. Cadenelli , R. Rizzuto, Diego De Stefani
Department of Biomedical Science, University of Padua, Italy

Mitochondria are organelles that play a pivotal role in calcium (Ca^{2+}) handling and signalling. They can take up and release Ca^{2+} ions through the concerted action of influx and efflux pathways to shape the spatio-temporal dynamics of cytosolic Ca^{2+}
transient. Mitochondrial Ca^{2+} homeostasis has been extensively studied and functionally linked to the regulation of diverse physiological processes such as energy metabolism, tissue growth and development, neurotransmitter release, muscle
contraction, autophagy and cell death. Examples of diseases associated with impairment of mitochondrial Ca^{2+} homeostasis include ischaemia–reperfusion injury, neurodegenerative diseases, stroke, metabolic syndrome, muscle atrophy and arrhythmia.
Despite recent advancements, the molecular identity of molecules involved in mitochondrial calcium dynamics is still not completely understood. In this work we explored the possibility that the orphan protein TMEM65 could be involved in
mitochondrial calcium handling. Our data clearly indicate that TMEM65 is a protein of the inner mitochondrial membrane. Most importantly, it is an essential component of the molecular machinery that mediates Na+-dependent mitochondrial Ca^{2+}
efflux. The identification of the functional role of TMEM65 protein represents an opportunity to develop new pharmacological modulators of mitochondrial Ca^{2+} signaling with relevant therapeutic potential.

Investigation of the interplay between endoplasmic reticulum-mitochondria distances and mitochondrial Ca^{2+} uptake

Giulia Dematteis[1], L. Tapella[1], R. MIggiano[1], M. Brini[2], T. Calì[3], A.A. Genazzani[1], D. Lim[1]
[1]Department of Pharmaceutical Sciences, Università del Piemonte Orientale, Novara, Italy
[2]Neurodegeneration Study Center (CESNE), University of Padova, Padova, Italy, Department of Biology, University of Padua, Padova, Italy
[3]Department of Biomedical Sciences, University of Padua, Padova, Italy, Padova Neuroscience Center (PNC), University of Padova, Padova, Italy, Neurodegeneration Study Center (CESNE), University of Padova, Padova, Italy

A low affinity mitochondrial Ca^{2+} uptake occurs mostly at the Endoplasmic Reticulum-Mitochondria (ER-Mit) contact sites (MERCS), via IP3R-VDAC complex. MERCS dysfunctions have been correlated with altered mitochondrial Ca^{2+} handling, and identified in pathological conditions such as neurodegeneration, tumours and metabolic disorders. Although it is hypothesized that the ER-Mit distance for Ca2+ transfer lays in a range of 10-25 nm, the optimal distance and its regulation remain unknown.
To address this issue, we used available and newly designed ER-Mit artificial linkers that span the range between 5 and 30nm. Following ATP-induced Ca^{2+} dynamics in Mit matrix of HeLa and other cells, 20nm was identified as the optimal distance to promote ER-Mit Ca^{2+} transfer. Using Proximity Ligation Assay (PLA) we show that, in presence of 20nm ER-Mit linker (20nm-EML), there is a drastic increase of IP3R-VDAC interaction, justifying the increased Ca2+ transfer. Unexpectedly, 20nm-EML resulted in increase of basal [Ca2+] in the cristae and inter-membrane space, but not in the matrix. A recently identified MCU activator amorolfine discharged the Ca2+ from cristae into the matrix inducing Mit Ca2+ overload, opening of mPTP and cell death.
To investigate physiological occurrence and modulation of 20nm contact sites, we generated a split-GFP contact sites sensor for 20nm distance (20nm-SPLICS), that allowed monitoring the dynamic increase of ER-Mit interactions at 20nm. In Airway Smooth Muscle Cell, upon stimulation Absintin→hTAS2R46→EPAC signalling pathway, increased interaction at 20nm mediated augmented Histamine-induced MIt Ca^{2+} uptake.
In conclusion: 1) 20nm is the optimal distance for ER-Mit Ca2+ transfer which allows spontaneous assembly of IP3R-VDAC1 complexes; 2) Modulation of the ER-Mit distance is a valuable strategy to finely tune Mit calcium uptake, thereby regulating cell fate; 3) Dynamic modulation of 20nm ER-Mit contact sites physiologically regulates Mit Ca2+ signals.

Systematic identification of signaling pathways regulating mitochondrial calcium uptake

Michael S. Feng[1,3,5], F. Perocchi[1,2,4]
[1]Institute for Diabetes and Obesity, Helmholtz Munich, Germany
[2]Munich Cluster for Systems Neurology (SyNergy), Germany
[3]International Max Planck Research School for Molecular Life Sciences (IMPRS-LS), Munich, Germany
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Mitochondria serve as both encoder and decoder of intracellular Ca^{2+} signaling through a highly selective mitochondrial Ca^{2+} uniporter channel (MCUC). Over the last decade, the identification of several MCUC components has supported the hypothesis of existing mt-Ca^{2+} signaling checkpoints, whereby increased Ca^{2+} levels regulate mitochondrial metabolism, while an overload activates cell death, thus affecting the pathophysiology of many human diseases. However, molecular mechanisms linking intracellular Ca^{2+} signaling and mitochondrial functions remain unknown. Several lines of evidence, including the identification of mitochondria-localized kinases and phosphatases and of mitochondrial enzymes and MCUC subunits regulated by Ca^{2+}-dependent changes in their phosphorylation state, suggest that the integration of mitochondria into Ca^{2+} signaling cascades is regulated through reversible phosphorylation. Therefore, we applied a systematic and unbiased approach to identify mitochondria-associated kinases and phosphatases regulating organelle Ca^{2+} dynamics. We show that the mitochondrial phosphatome of several mouse tissues changes upon mt-Ca^{2+} uptake, and employ a quantitative, MS-based chemical proteomic strategy to purify mitochondrial kinases. Moreover, we integrate publically available datasets to predict a candidate list of 133 kinases and 63 phosphatases associated with mitochondria. We then perform three complementary siRNA-based screens to quantify the effect of gene knockdown on mitochondrial and cytosolic Ca^{2+} dynamics through ER-dependent or extracellular Ca^{2+} sources. We identify several mitochondrial proteins and pathways that regulate mt-Ca^{2+} through protein interactions with MCUC and ER-mitochondrial tethering, supporting the involvement of phosphorylation in the regulation of inter-organelle-mediated mt-Ca^{2+} signaling.

Impaired ER-mitochondria contacts affect lipid droplets degradation in an *in vitro* Alzheimer's disease model

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by deposition of extracellular neuritic plaques, and intracellular neurofibrillary tangles[1]. Mitochondria- associated membranes (MAMs)[2] are endoplasmic reticulum (ER) subdomains that contact with mitochondria[2], and modulate several cellular events, namely Ca2+ transport [3], lipids synthesis and transfer[4], and lipid droplets (LDs) formation[5], ER stress response[6], and autophagy[7]. It has been recently proposed that MAMs dysfunction is involved in early AD, upstream of neuropathological and cognitive changes [8]. This study was aimed to investigate whether AD-associated MAMs alterations affect lipid metabolism, focusing on LDs, by using an in vitro model of early AD. MAMs were analyzed by microscopy in wild-type cells (N2A- WT) and N2A cells overexpressing mutant APP (APPswe cells). Ca2+ homeostasis, LDs and reactive oxygen species (ROS) content were analyzed with fluorescent probes, and free fatty acids (FFA) levels and lipid peroxidation extent were determined using colorimetric assays. The role of macroautophagy (MA), chaperone-mediated autophagy (CMA), and ubiquitin- proteosome system (UPS) in LDs degradation was also studied. Decreased ER-mitochondria contacts, levels of the tethering protein MFN2, FACL-4 (lipid biosynthetic MAM-resident enzyme) and LDs and inter-organelle Ca2+ transfer, together with an increase in FFA concentration, ROS accumulation and lipid peroxidation were observed in AD cells. We also observed enhanced LDs-lysosome colocalization in AD cellular model and LDs accumulation after chloroquine (CQ) treatment in both cell lines while disturbances in LDs were found to be associated with CMA and UPS impairment in AD cells. In conclusion, altered MAMs in AD impair LDs degradation increasing lipid toxicity and promoting oxidative distress. These observations help to clarify the physiopathological mechanisms involved in AD.

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Calcium signaling is impaired in iPSC-derived Alzheimer's Disease models

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Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by the presence of amyloid beta plaque deposition, tau hyperphosphorylation and decline in cognition. Mechanistically, these proteostatic changes are able to induce mitochondrial calcium overload that could lead to cell death. Conventional preclinical 2D *in vitro* AD models do not accurately recapitulate these hallmarks. As an effort to improve AD modeling, we have established iPSC-derived brain organoids (BO) and microglia-like cells (iMGLs) from AD patients harboring the PSEN1 delta E9 mutation and their CRISPR-Cas9-mutation-corrected isogenic controls. We have established a protocol to measure live calcium imaging with Fluo-4 in matrigel-embedded single organoids. We observed that during differentiation, microglia upregulate important mitochondrial calcium regulators such as MICU1 and EMRE. We observed a strong phagocytic defect in diseased microglia that is able to be recovered by inhibitors of mitochondrial calcium uptake. Additionally, we have co-cultured both BOs and iMGLs to obtain a more complex organoid model in which microglia is engrafted in a 3D brain-like structure. We characterized the structural, transcriptomic and functional traits of such systems. Ongoing work of an unbiased transcriptomic study of co-cultured organoids may shed light into microglial gene signatures in health and disease. Dissecting these differences may allow for the development of more effective therapeutic strategies for AD.

The role of pyruvate kinase M2 in mitochondrial Ca^{2+} handling and cell death

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PKM1 and PKM2 are the two isoforms of pyruvate kinase M. PKM2 controls the final, rate-limiting step of glycolysis, contributing to the Warburg effect, and supports anabolic processes during tumorigenesis. PKM2 also shows non-metabolic roles, such as promoting gene expression, cell cycle progression and cell survival. Recently, our group showed that in addition PKM2 modulates intracellular Ca^{2+} signaling by suppressing inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) activity [1].
Using newly developed HeLa and HEK293 PKM2 knock-out (KO) cells, we demonstrate that the inhibition of IP_3-dependent Ca^{2+} release occurs without interfering with either IP_3R or SERCA expression levels, ER Ca^{2+} store content, or store-operated Ca^{2+} entry. While PKM2 KO leads to increased IP_3-induced Ca^{2+} release in the cytosol, Ca^{2+} uptake in the mitochondria is decreased. Interestingly, PKM2 KO impacts mitochondria at multiple levels: loss of PKM2 resulted in fewer ER-mitochondria contact sites, smaller and less branched mitochondria, a more negative mitochondrial membrane potential and increased respiration.
Disruption of the PKM2:IP_3R interaction can be achieved using the IP_3R-derived TAT-D5SD peptide [1]. Consequently, TAT-D5SD treatment (but not with a control peptide) led to a transient Ca^{2+} release from the ER, both into the cytosol and into the mitochondria as well as to apoptotic cell death. Importantly, the TAT-D5SD peptide evoked similar phenomena in wild type and PKM2 KO cells, strongly suggesting the involvement of an additional protein. The possibility for a role for PKM1 and/or Bcl-2 herein is presently being investigated.
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Unmasking the connections: mitochondria-endoplasmic reticulum domains in Ca^{2+} signaling at fertilization

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Embryo development starts at fertilization when sperm trigger oscillations in egg cytoplasmic Ca^{2+} levels. Ca^{2+}oscillations depend on Ca^{2+} release from endoplasmic reticulum (ER) stores, followed by a return to basal Ca^{2+} levels due to Ca^{2+} reuptake into the ER by SERCA pumps and Ca^{2+} efflux by PMCAs. Each Ca^{2+} transient stimulates mitochondrial Ca^{2+} uptake, leading to the generation of ATP that powers these pumps and other cellular activities. Meiotic maturation from germinal vesicle stage oocytes to metaphase II-arrested eggs is accompanied by reorganization of the ER to form cortical clusters that may serve to facilitate Ca^{2+} release following sperm-egg fusion. In somatic cells, the ER and mitochondria closely associate at mitochondria-ER-associated membrane domains (MAMs), which are essential for cell processes like lipid synthesis, metabolism, and Ca^{2+} homeostasis. It is unknown if MAMs are present or have important functions in eggs. We performed focused ion beam-scanning electron microscopy to determine the ultrastructure of ER cortical clusters and mitochondria in mouse eggs. Our preliminary findings indicated that ER cortical clusters were composed of compact groups of ER tubules. The mitochondria were spherical and often in small groups in the egg cortex, many of which were closely associated with ER tubules. We also observed MAMs in live mouse oocytes and eggs using a splitFAST-based probe designed to detect ER-mitochondrial interactions at less than 10-15 nm. There were differences in the MAM distribution in oocytes and eggs: In oocytes, the MAMs localized strongly around the nucleus, with some at the cell periphery. In eggs, MAMs surrounded the meiotic spindle and exhibited a more homogeneous localization through the cytoplasm. Our preliminary results provide the basis for future experiments involving the disruption of MAMs in eggs to study their impact on Ca^{2+} signaling during fertilization.

Partial loss of the mitochondrial calcium uniporter (MCU) mitigates pathology *in vivo* across a diverse range of neurodegenerative disease models

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Mitochondria perform many essential cellular functions in bioenergetics, metabolic regulation, calcium (Ca^{2+}) homeostasis, cell death processes and more. Consequently, highly metabolic tissues such as neurons are particularly vulnerable to disruption of diverse mitochondrial processes, and mitochondrial dysfunction is a common feature in most, if not all, neurodegenerative diseases (NDs). Ca^{2+} dysregulation is also a common feature of numerous NDs including Parkinson's (PD), Alzheimer's (AD), Huntington's (HD) and amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD); however, a specific role for mitochondrial Ca^{2+} is less well elaborated. Mitochondria take up Ca^{2+} from the cytosol via the Mitochondrial Calcium Uniporter (MCU) complex. MCU has a relatively low affinity for Ca^{2+} so uptake is achieved via microdomains of high [Ca^{2+}] released from the endoplasmic reticulum (ER) at sites where the ER closely apposes mitochondria, so-called mitochondria-ER contact sites (MERCs). Conversely, mitochondrial Ca^{2+} efflux is principally mediated by NCLX. While mitochondrial Ca^{2+} uptake facilitates energy production, excessive Ca^{2+} uptake leads to mitochondrial permeability transition, loss of membrane potential and energy production, and ultimately cell death. Therefore, limiting mitochondrial Ca^{2+} overload is a potential therapeutic strategy for NDs. Using Drosophila models for various NDs we found that excess mitochondrial Ca^{2+} was a common feature in these models along with excess MERCs. Importantly, we found that reducing MCU or overexpressing NCLX mitigates much of the pathology in these models, implicating this as an attractive therapeutic avenue.

P.1

Partial knockdown of sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) improves lifespan and healthspan in a rotenone model of Parkinson disease in *C. elegans*

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The progressive ageing of advanced societies has led to an increase in the prevalence of neurodegenerative diseases in our population, and the lack of effective treatments encourages the search for new therapeutic targets for these pathologies. We have recently described that submaximal inhibition of the Sarco-Endoplasmic Reticulum Ca^{2+} ATPase (SERCA), the main responsible for ER calcium storage, is able to increase lifespan in *C. elegans* worms by mechanisms involving mitochondrial metabolism and the nutrient-sensitive pathways AMP-dependent kinase and TOR. We have studied here the effects of submaximal SERCA inhibition in a chemical model of Parkinson's disease induced in *C. elegans* worms by treatment with the mitochondrial complex I inhibitor rotenone. Specific SERCA inhibition was achieved by feeding the worms with 10% RNAi against *sca-1*, the sole orthologue of SERCA in *C. elegans*. Our results show that rotenone produces alterations in worms that include decreased lifespan, smaller size, reduced fertility, decreased motility, much smaller rates of defecation and pharynx pumping, reduced mitochondrial membrane potential and oxygen consumption rate, increased mitochondrial ROS production, altered mitochondrial structure, and altered ethanol preference in behavioral studies. Most of these alterations were either fully or partially reversed in worms treated with 10% *sca-1* RNAi, suggesting that SERCA inhibition could be a novel pharmacological target in the prevention or treatment of neurodegeneration. The mechanism of this effect is probably a consequence of the decrease in ER Ca^{2+} levels following partial SERCA knockdown. This would decrease the amount of Ca^{2+} released during cell stimulation, as well as ER-mitochondria Ca^{2+} transfer, thus reducing mitochondrial metabolism and ROS production.

P.2

Untangling inter organelle contact dynamics *in vivo*

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To tune their activities to the cellular environment, organelles need to communicate among each other inside the cell. These communications can occur via the establishment of contact sites, which are emerging as critical signalling hubs involved in a growing variety of physiological and pathophysiological processes, hence the current need to deeply characterize them in both health and disease. To this end, we exploited a palette of recently designed fluorescent sensors, the SPLICS reporters, to visualize and characterize different inter-organelle interactions in physiological conditions, both *in vitro* and *in vivo*. Specifically, we have screened the reporters for ER-mitochondria, lysosomes-mitochondria, peroxisomes-mitochondria, and ER- plasma membrane contacts *in vitro* using SHSY5Y cells. I*n vivo*, we tested the same reporters either in the Rohon Beard sensory neurons of *Danio rerio* by transiently expressing them or in the wing neurons of newly generated *Drosophila melanogaster* transgenic lines constitutively expressing the fluorescent probes. We have found a different cellular distribution of the different contacts in neurons, in accordance with the organelle distribution itself, together with a different dynamics of the contact movements along neuronal axons in the analysed models. Our data further validate the applicability of the SPLICS system both in cells and in animals and represent a starting point for the *in vivo* investigation of inter-organelle communications not only in physiological conditions, but also in disease-relevant backgrounds.

P.3

Mild excitotoxic glutamate effect on mitochondrial membrane potential, Ca^{2+} homeostasis and energy production on hippocampal neurons of an Alzheimer's disease mouse model

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Neurons maintain a tight control over their cellular homeostasis in order to precisely regulate ion fluxes for the generation of action potential and neurotransmission. In particular, the energetic balance has to be finely adjusted since its alteration may hamper cellular functionality with consequent cell suffering and death. Mitochondria play a central role in the homeostatic regulation within the cells due to the many vital tasks they perform in terms of energy production, modulation of intracellular signaling and specifically in participating in the shaping of Ca^{2+} signaling. We observed that in a mouse model of Familial Alzheimer's disease (AD), hippocampal neurons are sensitive to a modest excitotoxic signal that produces a transient cytosolic and mitochondrial matrix Ca^{2+} increase with consequent partial loss of mitochondrial membrane potential and delayed recovery of mitochondrial matrix $[Ca^{2+}]$, which might contribute in the activation of the mitochondria permeability transition pore (PTP) with possible impairment of mitochondrial and cellular functions. A brief treatment with inhibitors of the PTP is able to reduce these effects and might have protective role against exogenous insults and help mitigate neurodegeneration.

P.4

α-synuclein regulates lysosomes-mitochondria contact sites

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Mitochondrial and lysosomal activities are crucial to maintain cellular homeostasis: they must be harmonized and fine-tuned to avoid cellular dysfunctions. Defects in mitochondria and lysosome represent a common hallmark of many neurodegenerative diseases, including Parkinson's disease. Optimal coordination between organelles activities is achieved at organelles membrane contact sites where distinct tethering/untethering protein machineries regulate organelle network dynamics, metabolite transfer and exchange of ions and second messengers. We had previously demonstrated that α-synuclein positively affects Ca^{2+} transfer from the endoplasmic reticulum (ER) to mitochondria, augmenting the mitochondrial Ca^{2+} transients elicited by agonists that induce ER Ca^{2+} release. This effect was dependent on an increase in the number of ER-mitochondria interactions. Exaggerated accumulation of α-synuclein into the cells, which caused its redistribution to localized foci, reduced ER-mitochondria apposition, and the ability of mitochondria to uptake Ca^{2+} and, in turn, compromised cellular bioenergetics.
Here we describe that the overexpression of wt α-synuclein or the Parkinson's disease-related A30P and A53T mutants strongly reduces the apposition of mitochondria/lysosomes membranes resulting in reduced mitochondrial Ca^{2+} buffering upon Ca^{2+} release from lysosomes, calcineurin activation and enhanced TFEB nuclear translocation.
In summary, we propose that the modulation by α-synuclein of lysosome-mitochondria contact sites interferes with TFEB signalling, suggesting that their targeting could open avenues to counteract dysregulation of different biological processes linked to defective TFEB dependent pathways, including those leading to neurodegenerative diseases.

P.5

Bcl-2-based strategies to target dysregulated Ca^{2+} signaling as an early event in Alzheimer's disease

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OBJECTIVES. Ca^{2+} homeostasis is crucial for maintaining different neuronal functions and aberrant Ca^{2+} signaling plays an important role in the early pathogenesis of Alzheimer's disease (AD). Familial AD is mainly caused by mutations in presenilin 1 (PSEN1). These PSEN1 mutations provoke Ca^{2+} dysregulation and were shown to sensitize the inositol 1,4,5-trisphosphate receptor (IP_3R) and ryanodine receptor (RyR), thereby eliciting exaggerated Ca^{2+}-signaling patterns. An important regulator of IP_3Rs and RyRs is the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2). Bcl-2 can inhibit both receptors and thus could be exploited to counteract dysregulated Ca^{2+} release caused by PSEN1 mutations. Our ambition is to provide new perspectives on the early pathophysiology of AD and to exploit Bcl-2 to normalize aberrant Ca^{2+} release.
RESULTS. Using Fura2-loaded HeLa PSEN dKO cells expressing either wild-type (WT) PSEN1 or PSEN1 mutants, we conducted single-cell Ca^{2+} measurements. Unexpectedly, our analysis revealed an inhibitory effect of PSEN1 mutants on IP_3R -mediated Ca^{2+} release. Additionally, we measured mitochondrial Ca^{2+} influx and cytosolic Ca^{2+} release in HEK293 cells overexpressing RyR2, the major RyR isoform in neurons, with overexpression of PSEN1 WT/mutants. Our preliminary data suggest that PSEN1 mutants affect mitochondrial Ca^{2+} influx. Furthermore, we evaluated the impact of Bcl-2 and a Bcl-2 mutant (Bcl-2K17D), which fails to inhibit IP_3Rs, on amyloid and synapse protection by overexpressing Bcl-2 proteins in brains of 5xFAD mice. These *in vivo* results support the pivotal role of RyRs in AD pathology and indicate the neuroprotective potential of Bcl-2 through the inhibition of RyRs.
CONCLUSIONS. Mutations in PSEN1 impact RyR2-mediated mitochondrial Ca^{2+} flux, and this may contribute to other defects observed in early-AD pathology. Moreover, Bcl-2-based strategies show promise in restoring normal Ca^{2+} signaling and counteracting the development of early AD features.

P.6

Unravelling the role of mitochondrial calcium homeostasis in Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative pathology characterised by the loss of dopaminergic neurons and motor impairments. Despite the complex pathogenesis, accumulating evidence is supporting a role of calcium dyshomeostasis in PD, and other neurodegenerative diseases (NDs), to the point of suggesting a "calcium hypothesis of neurodegeneration". Although calcium can modulate different physiological functions, the ion levels require tight regulation, as mitochondrial calcium overload can have detrimental effects. A key player in calcium homeostasis is the Mitochondrial Calcium Uniporter (MCU), which has a crucial role in regulating the organelle metabolism. Interestingly, data from our lab indicate that the partial loss of MCU rescues different fly models of NDs, including a PD-model characterised by the loss of the mitochondrial kinase PINK1 (*Pink1*$^{-}$). In particular, reducing MCU levels rescues lifespan, dopaminergic neuron loss, locomotion, and mitochondrial calcium levels. To investigate the metabolic role of calcium in *Pink1*$^{-}$ model, we performed an untargeted metabolomics analysis, which showed that *Pink1*$^{-}$ flies present a general metabolic up-regulation. Remarkably, the partial loss of MCU rebalances more than the 50% of *Pink1*$^{-}$ metabolome, including some lipidic classes, carbohydrates, and redox markers. Of note, metabolic dysregulation is acquiring growing awareness in NDs, as an important and still missing piece of the complex neurodegenerative puzzle. Thus, to identify the mechanisms underlying the beneficial effects of MCU in PD, we are currently integrating the metabolomics dataset with a transcriptomics approach, which will contribute to better define the pathways affected in the disease and potentially open new therapeutic avenues.

P.7

IP_3 receptor modulation by anti-apoptotic Bcl-2: impact of disease-related mutations and role of novel interaction partners

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The inositol 1,4,5-trisphosphate receptors (IP_3R), located in the endoplasmic reticulum (ER), play a central role in intracellular Ca^{2+} signaling. Through the control of the Ca^{2+} flow from the ER lumen into the cytosol and mitochondria, these Ca^{2+}-release channels influence many intracellular processes among which cell proliferation, mitochondrial bioenergetics and apoptosis. Moreover, IP_3Rs function as a hub for plenty regulatory proteins, including at the level of the ER-mitochondria contact sites. The anti-apoptotic Bcl-2 protein is one of those proteins that binds and inhibits the IP_3R via its BH4 domain.
In the context of cancer, several recurrent mutations in BCL-2 have been identified. We have investigated the functional implications for selected recurrent mutations located in the hydrophobic cleft of Bcl-2, namely G101V, D103Y, A113G, H129L and V156D. These mutations were also identified in chronic lymphocytic leukemia patients with acquired resistance against venetoclax, a BH3 mimetic Bcl-2 antagonist. HDX-MS analysis of the different mutants reveal clear structural rigidity shifts in comparison to $Bcl\text{-}2^{wt}$ in both the hydrophobic cleft and the structurally isolated BH4 domain. While all mutants retain the ability to inhibit staurosporine-induced cell death, which is largely Ca^{2+}-mediated, H129L- and V156D-mutated Bcl-2 displayed an increased anti-apoptotic effect.
We are also further elucidating the effects of two other IP_3R-accessory proteins, CISD2 and PKM2 that may impact the regulation of IP_3Rs by Bcl-2. Wolfram syndrome related protein, CISD2 directly binds both IP_3R1 and Bcl-2, with CISD2 supporting IP_3R-mediated ER-mitochondrial Ca^{2+} flux, ER-mitochondria contact formation, as well as Bcl-2 functioning. PKM2 also interacts with Bcl-2, while evidence indicate that PKM2 modulates IP_3R function in a direct and/or indirect way.
Our data highlight IP_3R-mediated ER-mitochondrial Ca^{2+} flux and Bcl-2 as an axis in cell death control and disease.

P.8

Systematic and unbiased mapping of the *in vitro* and *in vivo* MCU interactome

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Mitochondria play a key role in Ca^{2+} homeostasis and the regulation of metabolism and cell death. The MCU is an ion channel that mediates the highly selective uptake of Ca^{2+} into the mitochondrial matrix. To date, a deep characterization of the molecular links that couple Ca^{2+} entry with mitochondrial function is lacking.
We perform 40 immunoprecipitations from HEK cells and mice tissues expressing a near-endogenous level of the three membrane components of the MCUC as baits and built a protein network of significant interactors. Furthermore, as the expression and assembly of uniporter components were found to be greatly interdependent, we characterized how the MCU interactome remodels upon genetic perturbation in one or the other uniporter subunits.
Our interactome identifies 95 protein interactors of the MCU Complex and provides novel molecular links for mt-Ca^{2+}-mediated metabolic coupling, oxidative stress and cell death activation, and suggests previously overlooked cross-talks between mt-Ca^{2+} uptake and mitochondrial transcription, translation and protein import. Notably, two-thirds of the MCUC interactors identified are annotated as disease genes in cancers, metabolic, and neurological dysfunctions, highlighting the interactome's potential for uncovering novel genetic underpinnings and pharmacological targets for disease.
We spotlighted a role for MCUB as a decoy preventing the assembly of MCU within higher molecular weight complexes in human cells and mouse tissues.
Moreover, we identified EFHD1 as a specific negative regulator of MCU-dependent Ca^{2+}-uptake. We show that EFHD1 loss-of-function results in increased mt-Ca^{2+} level in HeLa cells upon histamine stimulation, SOCE activation, or digitonin permeabilization and that its EF-hands are essential to regulate mt-Ca^{2+} uptake, pointing to a direct regulatory effect on MCUC. Finally, we showed that EFHD1 knockdown leads to decreased cell viability an increased susceptibility to pro-apoptotic stimuli.

P.9

Dynamic chemogenetic reporters to investigate Ca^{2+}-mediated regulation of organelle contacts

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The maintenance of Ca^{2+} homeostasis is critical for the correct modulation of several cell pathways including cell death, autophagy and ER stress. Importantly, the regions of close apposition between different organelles, such as those between the endoplasmic reticulum (ER) and mitochondria (known as Mitochondrial Associated Membranes, MAM), are strategic platforms whereby organelles can communicate through Ca^{2+}. Remarkably, MAM dysregulation has been associated with the genesis and/or progression of numerous pathologies, including neurodegenerative diseases and cancer.
Here, we developed and characterized a set of reversible fluorescent probes, based on splitFAST, to detect the juxtaposition between endoplasmic reticulum (ER) and either mitochondria or plasma membrane (PM). We characterized these probes by confirming the previously reported effects of ER stressors and familial Alzheimer's disease (FAD)-linked presenilin-2 (PS2) mutations on ER-mitochondria contact sites, as well as by measuring the kinetics of cortical ER-PM association upon Store Operated Ca^{2+} Entry activation.
Moreover, the expression of these reporters in different cell types allowed us to identify ER Ca^{2+} content as a regulator of ER-mitochondria contact formation. Specifically, ER Ca^{2+} depletion leads to an increase in the percentage of mitochondrial surface engaged in close contacts with the ER. Interestingly, by taking advantage of the reversibility of the probe, we observed that this process can be reversed upon ER Ca^{2+} refilling.
Our results suggest a hitherto unknown effect of luminal ER-Ca^{2+} in the modulation of ER-mitochondria contacts. Currently, our efforts are focused on the definition of the molecules mediating this process.

P.10

Potassium-dependent regulation of mitochondrial Ca^{2+} fluxes and cellular networks

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We recently discovered the molecular identity of the long sought mitochondrial ATP-sensitive potassium channel ($mitoK_{ATP}$), i.e. a macromolecular complex composed by a K^+-permeant subunit named MITOK, and a regulatory subunit named MITOSUR. Although the contribution of $mitoK_{ATP}$ to multiple physiopathological conditions is well documented, the fundamental mechanisms underlying potassium-dependent control of organelle physiology is still poorly understood. Within mitochondria, ion homeostasis (especially for Ca^{2+} and K^+) is tightly interconnected with metabolism. However, the systematic analysis of the impact of each ion at organellar and cellular level is still missing, especially in terms of regulation of metabolic and transcriptional networks. Therefore, we aim to test if changes in organelle K^+ fluxes are able to affect mitochondrial Ca^{2+} influx and/or efflux and the mechanisms underlying this integration in normal conditions and in the presence of organelle stress. To address this problem, we developed cellular models where we can reversibly tune the expression of MITOK, which will be also combined with classical pharmacological strategies to either open or inhibit $mitoK_{ATP}$ channels. Thanks to these novel tools, we will define the Ca^{2+} /K^+ flux-dependent metabolic remodelling occurring in different physiopathological conditions.

P.11

Impaired mitochondrial ATP production downregulates Wnt signaling via ER stress induction

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Wnt signaling affects fundamental development pathways by regulating cell differentiation and proliferation and, when aberrantly activated, it promotes the development of several cancers. Wnt signaling is modulated by different factors, but whether mitochondrial energetic state affects Wnt signaling is unknown. Here we show that sub-lethal concentrations of different compounds that decrease mitochondrial ATP production downregulate Wnt/β-catenin signaling in HEK293 cells, in two Wnt-dependent colon cancer cell lines and *in vivo* in zebrafish reporter lines. In addition, impaired respiratory chain complex III function in human fibroblasts from a GRACILE syndrome patient and in a respective, newly generated zebrafish model led to reduced Wnt signaling. Other signaling pathways were unaffected even *in vivo*, indicating specificity of the mitochondria-Wnt signaling axis. We identified a mechanism whereby a decrease in mitochondrial ATP reduces calcium uptake into the endoplasmic reticulum (ER), leading to ER stress and to impaired Wnt signaling. In accordance, reduction of ATP export from mitochondria as well as ER stress inducers tunicamycin and thapsigargin led to inhibition of Wnt signaling. In turn, recovery of ATP level using creatine and phosphoenolpyruvate or an ER stress inhibitor restored Wnt activity, even in GRACILE fibroblasts. These findings reveal an unexpected mechanism that links mitochondrial energetic metabolism to the control of Wnt pathway, indicating that agents affecting mitochondrial ATP production may be beneficial against several pathologies ranging from cancer to neurological disorders that are related to altered Wnt signaling.

P.12

The role of mitochondrial cations in the mtDNA release from mitochondria upon inflammation

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Mitochondria, central platforms for a plethora of functions, are responsible for the majority of downstream signaling cascades involved in health and diseases. It has been extensively studied how mitochondria play a pivotal role in innate and adaptive immunity, from induction to maintenance. Notably, maintaining Ca^{2+} and K^{+} homeostasis is one of the crucial tasks carried out by these organelles. Our preliminary results, in agreement with previous works, demonstrated that altered mitochondrial Ca^{2+} or K^{+} fluxes reduce NLRP3 inflammasome induction; however, the precise molecular mechanisms are still under investigation. Here, we examine whether and how mitochondrial Ca^{2+} and K+ regulate mtDNA release during NLRP3 inflammasome induction. To this purpose, we developed a fluorescent probe based on GFP-SPLICS technology to follow mtDNA release into the cytoplasm upon inflammasome induction. Finally, to test this probe, we are setting up a cellular system based on HEK293T cells expressing all the components of NLRP3 inflammasome together with the silencing of MCU, the mitochondrial Ca^{2+} uniporter, responsible for Ca^{2+} entry into mitochondria, or MITOK, the mitochondrial ATP-sensitive K+ channel, which plays a central role in controlling mitochondrial matrix volume, respiration, and membrane potential. Our results indicate that MCU or MITOK silencing decreased NLRP3 inflammasome induction, suggesting MCU and MITOK as strong candidates to prevent the negative consequences of inflammation.

P.13

Regulation of endoplasmic reticulum–mitochondria tethering and Ca^{2+} fluxes by TDP-43 via GSK3β

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TDP-43 is an essential nuclear DNA/RNA-binding protein involved in several steps of RNA processing, including transcription, translation, alternative splicing, mRNA transport and stability and microRNA and long non-coding RNA modification.
Both the discovery of missense mutations in the TDP-43 gene in familial amyotrophic lateral sclerosis (ALS) cases and the identification of TDP-43 as the major component of cytosolic inclusions in almost all sporadic and familial ALS patients pointed to the association of TDP-43 with ALS pathogenesis. However, the details of how TDP-43 dysfunction causes progressive degeneration of motor neurons in ALS are still elusive.
Several studies have revealed an intimate relationship between ALS and perturbed intracellular Ca^{2+} homeostasis. For example, many pathogenic changes in ALS -affected neurons, including protein misfolding and aggregation, proteasome and autophagy impairment, endoplasmic reticulum (ER) stress, mitochondria-ER contacts (MERCS) dysfunctions, mitochondrial abnormalities, hyper-excitability and glutamate excitotoxicity, are closely related to Ca^{2+} signalling.
Growing evidence demonstrated that cytosolic mislocalization and aggregation of TDP-43 trigger neurodegeneration not only by the acquisition of a potentially toxic activity in the cytoplasm ("gain-of-function" hypothesis) but also by the loss of its physiological functions ("loss-of-function" hypothesis).
In light of the above notions, to clarify whether TDP-43 loss of function disrupts ER-mitochondria cross-talk and Ca^{2+} homeostasis, as a possible pathogenic mechanism in ALS, we analysed both MERCs and ER-mitochondria Ca^{2+} fluxes by different genetic/biochemical approaches in HeLa cells transfected, or not, with siRNAs for TDP-43 knock-down.
We showed that TDP-43 depletion causes alterations of both MERCS formation/maintenance and mitochondrial Ca^{2+} fluxes, possibly through the deregulation of RNA metabolism and GSK3β expression/activity.

P.14

The Parkinson's disease-related protein DJ-1 shapes mitochondrial contact sites with other organelles

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DJ-1, encoded by the PARK7 gene, is a protein with different functions, including antioxidant and chaperone activities, that plays a significant role in regulating mitochondrial homeostasis. Mutations in the PARK7 gene not only result in reduced DJ-1 protein levels leading to mitochondrial dysfunction and increased susceptibility to oxidative stress, but they are also causative of familial forms of Parkinson's disease (PD). Thus, understanding the role of DJ-1 and its role in regulating mitochondrial homeostasis could be resourceful for the development of novel therapeutic strategies. We previously demonstrated that the overexpression of DJ-1 leads to an increase in mitochondrial calcium uptake and ATP production, suggesting a role of DJ-1 in the formation of mitochondrial contact sites with the endoplasmic reticulum (ER). To investigate whether DJ-1 could be involved in the regulation of other mitochondrial contact sites, we used a set of genetically encoded split-green fluorescence protein (GFP)-based probes, called SPLICSs. Employing this technology, we can easily detect organelles juxtaposition targeting the first ten ß-strands and the eleventh GFP ß-strands of the GFP to two different organelles. Our results suggest that DJ-1 overexpression affects the mitochondrial interaction network with other organelles differently, increasing the number of contact sites with the ER, and changing those with the nucleus and lysosomes compared to control conditions. We further investigated this aspect by studying the effect of DJ-1 mutants, including the artificial variants C106T (a not oxidable form of DJ-1) and E18Q (a variant localized to mitochondria), and the pathological mutations, M26I and L166P. To characterize how the rearrangement of contact sites affects mitochondrial metabolism and fitness, we also investigated how the overexpression of DJ-1 both wild-type and mutants, affect mitochondrial calcium uptake stimulating calcium release both from the ER ad lysosomes.

P.15

Dysfunction of mitochondrial Ca^{2+} in a mouse model of Alzheimer's disease

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The pathogenic processes of Alzheimer's disease (AD) are still elusive and there is no cure to halt the disease. So far, therapeutic strategies targeting amyloid beta (Aβ) and tau have failed. The impairment in neuronal intracellular calcium (Ca^{2+}) handling, by altering synaptic transmission and brain cell communication, as well as metabolic dysfunctions, by causing neuronal degeneration and death, may contribute to AD development and progression. Interestingly, mitochondrial Ca2+ (mCa^{2+}) is a key regulator of these processes. Although various mitochondrial dysfunctions - including mCa^{2+} alterations - have been described in several AD models and samples, their role in AD pathogenesis is still largely unclear. To date, neither mCa^{2+} during AD progression nor the impact of an altered mCa^{2+} buffering capacity in shaping neuronal cytosolic Ca^{2+} (cCa^{2+}) dynamics have been investigated. The aims of our study are to follow mCa^{2+} dynamics during AD progression, investigate its impact on cCa^{2+} responses and test whether mCa^{2+} can be an interesting therapeutic target. Exploiting different AD mouse models, we observed a reduced mCa^{2+} uptake in the early stages of AD (before the onset of cognitive deficits and the appearance of Ab plaques), while mCa^{2+}overload appears in the late stages of the disease. We are currently investigating the mechanism behind these mCa^{2+} phenotypes, their impact on cCa^{2+} dynamics and on cognitive dysfunctions. Our preliminary data indicate that mCa^{2+} mishandling appears early in AD development and a differential mCa^{2+} phenotypes are associated with different AD stages.

P.16

Novel imaging tools to investigate mitochondrial calcium dynamics

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Cellular physiology relies on Ca^{2+} signalling. Since the first Ca^{2+} indicators were developed, tremendous advances have been made in understanding the pathways controlling cell life. Despite this success, some areas of Ca^{2+} imaging, in particular those aimed at investigating organelle Ca^{2+} handling, still require efforts to expand the variety of imaging tools available.
Ca^{2+} handling by mitochondria is crucial for cell life and the direct measure of mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_m$) is of pivotal interest. Traditionally, mitochondrial Ca^{2+} imaging relied on genetically-encoded Ca^{2+} indicators (GECIs), that can be easily and specifically targeted. Notably, GECIs can be employed for the generation of transgenic animals, with important implications for the study of Ca^{2+} dynamics in living tissues. Surprisingly, few examples are available of transgenic animals expressing mitochondria-localized GECIs. In an effort to overcome this limitation, we recently developed a transgenic mouse line expressing a mitochondria-targeted, FRET-based Cameleon Ca^{2+} probe. Going along this path, we recently developed a transgenic fly line for the expression of a mitochondria-targeted Cameleon Ca^{2+} sensor and successfully employed it in *in vivo* Ca^{2+} imaging experiments.
Despite their obvious advantages, GECIs need invasive procedures to be delivered, which are not applicable to all cell types and hardly suitable for high-throughput screenings. Synthetic Ca^{2+} indicators represent an easily deliverable alternative. A few years ago, we developed a fura-2-based fluorescent Ca^{2+} sensor, named mt-fura-2, to monitor real-time $[Ca^{2+}]_m$. Despite the probe is functional, it presents a major flaw *i.e.* low quantum yield. To overcome this limitation, we rationally optimized its molecular structure and obtained a brighter version, named mt-fura-2.3, that shows proper mitochondrial localization and accurately measures $[Ca^{2+}]_m$, even in cell types where the delivery of GECIs is troublesome.

P.17

Impact of ETC complex I dysfunction on mitochondrial Ca^{2+} homeostasis

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Complex I (CI), also known as NADH ubiquinone oxidoreductase, is a ubiquitous enzyme involved in the generation of the proton-motive force exploited for ATP synthesis in the mitochondrial respiratory chain. CI is a central player of cellular bioenergetics, it owns a pivotal contribution in controlling cell metabolism, adaptation to hypoxia, and sensitivity to apoptosis. Indeed, defects in CI are not only the primary cause of inherited mitochondrial diseases, but are also present in a broad spectrum of pathological conditions, such as diabetes and cancer. Our group is investigating whether and how ablation of CI can impinge on mitochondrial Ca^{2+} homeostasis, i.e. a key signal regulating organelle physiology.
We thus analyzed mitochondrial Ca^{2+} handling in WT and KO for NDUFS3 cell lines, wherein NDUFS3 being a non-catalytic core subunit essential for CI assembly. The goal is to understand whether a specific dysregulation of mitochondrial Ca^{2+} homeostasis is present in models of complex I dysfunction. Our results show that genetic ablation of NDUFS3 can induce significant changes in mitochondrial Ca^{2+} fluxes in some models, although sustained by a global rearrangement of Ca^{2+} signaling at whole cell level. Accordingly, no major differences can be appreciated at the level of expression of MCU complex components. Overall, our data suggest that dysregulation of mitochondrial Ca^{2+} homeostasis could play a role on complex I dysfunction, depending on the cellular context.

P.18

ERALL: a comprehensive protein compendium to characterize endoplasmic reticulum-mitochondrial crosstalk

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The endoplasmic reticulum (ER) and mitochondria are endowed with several functionalities, among them a direct line of communication between these two organelles through the formation of direct membrane contact sites. The ER-mitochondria contacts are conserved from yeast to humans and mediate several fundamental biological processes. However, we lack a comprehensive understanding of the molecular basis of both the physical and functional connections between the ER and mitochondria and how they are remodelled in response to cell challenges.
As a first step to address these open questions, we have performed a systematic in silico analysis to predict a comprehensive and accurate compendium of ~1300 ER proteins, which we name ERALL.
To this goal, we developed a machine learning-based approach integrating 33 large-scale datasets that provide complementary clues on ER localization. ERALL includes over 300 predicted proteins that have not been previously linked to the organelle, several of which contain transmembrane, ATP-binding or EF-hand domains and may represent novel ER transporters, channels, and protein complexes. Importantly, one-third of the ERALL proteins have already been associated with human diseases in the OMIM database.
As proof of concept that this resource can be utilized to analyse ER-mitochondrial communication, we performed a series of genetic loss-of-function screens in HeLa cells to identify ER proteins that affect ER-dependent mitochondrial Ca^{2+} ($mtCa^{2+}$) uptake, mitochondrial membrane potential which drives $mtCa^{2+}$ uptake, and formation of ER-mitochondrial contacts. The inter-cross of hits from all screens identified several candidates with either an enhancing or an inhibitory regulatory role on $mtCa^{2+}$ signalling and ER-mitochondria tethering, including a neuronal protein that we propose as a novel tether with pathophysiological relevance in Hereditary Spastic Paraplegia.

P.19

Lack of Tmbim5 affects response to oxidative stress

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Mitochondria are important cell organelles and their dysfunction is involved in many human diseases including cancer and neurodegenerative disorders. Mitochondrial Ca^{2+} levels regulate ATP synthesis and affect cellular signaling, gene expression and apoptosis. Nevertheless, our knowledge about Ca^{2+} transport in mitochondria is still insufficient. Recently, TMBIM5 was identified as being involved in Ca^{2+} and H^+ exchange in mitochondria (published in Patron et al., 2022 and Zhang et al., 2022).
Here, we investigated the effects of *tmbim5* knock-out in zebrafish. We found that *tmbim5*$^{-/-}$ fish are viable, but they display signs of neurodegeneration as evidenced by an increased number of dead cells in the brains of mutant larvae in comparison to wild-type (WT). Reactive oxygen species (ROS) level, measured *in vivo* in larvae brain did not differ between WT and mutants. However, we observed significant ROS elevation in WT fish after oxidative stress induction with paraquat (PQ), while no change in ROS level was detected in *tmbim5*$^{-/-}$ larvae. Moreover, behavior of WT larvae was affected by PQ and it treatment had no effect on mutants.
Altogether, these data suggest that Tmbim5 is important in regulation of response to the oxidative stress.
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