ABSTRACTS BOOK

XIV INTERNATIONAL MEETING OF THE EUROPEAN CALCIUM SOCIETY

Calcium Signaling in Renaissance

September 25-29, 2016
Valladolid, Spain

ABSTRACTS BOOK

XIV
INTERNATIONAL MEETING OF THE EUROPEAN CALCIUM SOCIETY

Calcium Signaling in Renaissance

September 25-29, 2016
Valladolid, Spain

Welcome letter from the chair

It is my great pleasure to welcome you all to the 14th International Meeting of the European Calcium Society (ECS). As you know, this meeting belongs to the biennial international meetings of the ECS, now well established in the scientific community and well known for their scientific excellence, their highly convivial atmosphere, and their large number of participants. At this meeting we again have over 225 participants, coming from 29 different countries, representing not only Europe, but also North-, Central- and South America, Asia from the Middle-East till the Far East and Oceania, both Australia and New Zealand. Moreover, we are especially proud that again we not only have excellent, established scientists attending but also a very large body of junior scientists who we hope will interact and discuss together for their mutual profit.

For the very first time in the history of the ECS, our International Meeting occurs in Spain, and more in particular in Valladolid, a city with a great and proud heritage of history, culture and science, and a city reputed for its sense of hospitality. The Local Organizing Committee, headed by Carlos Villalobos, has worked very hard to organize every aspect of this meeting which promises to be a huge success at all levels! The ECS Board and the ECS Scientific Committee therefore wish already to express their thanks for the excellent organization and the perfect collaboration. Personally, I also want to thank all the members of the Scientific Committee for their much appreciated input.

As you can see from the program, we have chosen for this meeting a number of new and exciting topics, including various aspects of Ca^{2+} signalling in disease and in therapy, new information on the structural aspects of the Ca^{2+} channels as well as novel functions of the Ca^{2+} ion and new technical approaches. Moreover, this year we have 3 Plenary lecturers, Prof. Javier García-Sancho, Nobel Prize winner Erwin Neher and last but not least Berridge lecturer James W. Putney. Another novelty is the inclusion of a late-breaking research session, which will feature novel, excellent work on topics otherwise not represented.

We also are particularly happy that you submitted so many abstracts on so many topics. The session chairs had therefore a particularly hard time to make the selection of the 18 abstracts that will be presented as oral communication. In addition, 15 of the total of 131 posters were selected for a quick 3-minute presentation in a special Flash session "The Ca^{2+} signalling network: form, function, failure", which is another novelty in the program.

Taken together, the ECS, the Local Organizers and the Scientific Committee think we will have a very exciting mix of high-level science, in which all the aspects of Ca^{2+} will be highlighted and discussed. Thanks to the efforts of the Local Organizers, this will be combined with extra-scientific activities that will let you see and taste the best of Valladolid and its surroundings!

Therefore, it only remains me to wish you all a very interesting and a very enjoyable week here in Valladolid, at our 14th International Meeting!

Jan B. Parys,
Chair of the Scientific Committee

Scientific Committee

Jan B. Parys (Chair)

Annette Draeger (Vice-Chair)
Javier García-Sancho (Vice-Chair)

Michael J. Berridge
Catherine Leclerc
Walter Chazin
Marek Michalak
Geneviève Dupont
Marc Moreau
Julia Gerasimenko
Stephen E. Moss
Volker Gerke
Roland Pochet
Andreas Guse
Natalia Prevarskaya
Jacques Haiech
Juan A. Rosado
Claus Heizmann
Luigia Santella
Enikö Kallay
Varda Shoshan-Barmatz
Joachim Krebs
Carlos Villalobos
Jacek Kuznicki

Local Organizing Committee

Carlos Villalobos (Head, Local Organizing Committee)

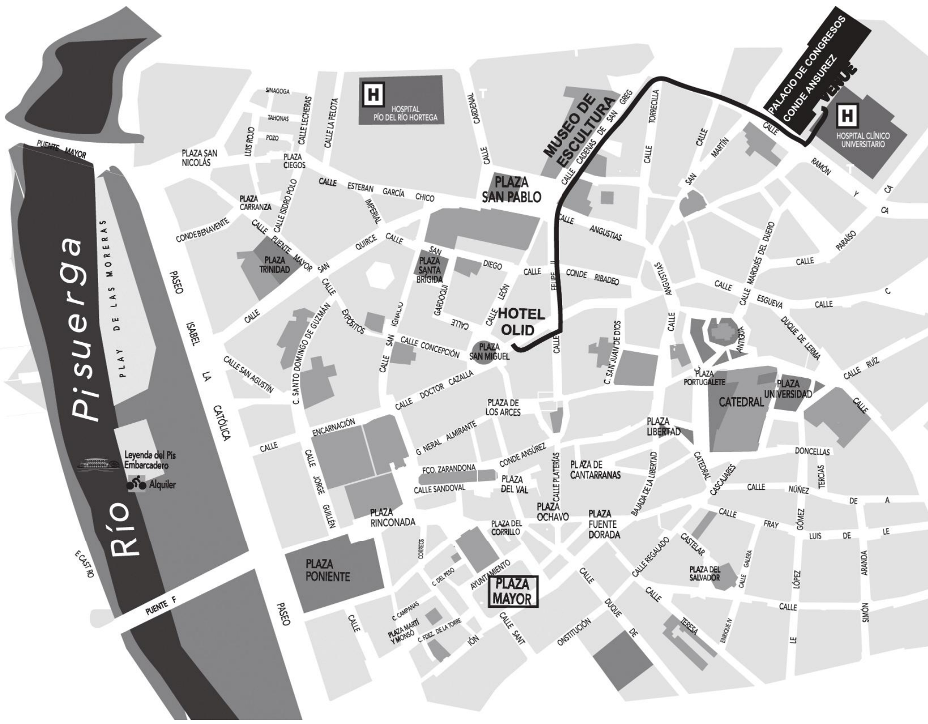
Lucía Nuñez (Treasurer)
Ana Sánchez
Javier García-Sancho
María Teresa Alonso
Javier Alvarez
Rosalba Fonteriz

General Information

Venue

The Conference Venue is the 'Palacio de Congresos Conde Ansúrez', the University of Valladolid Conference Centre. It is located in the heart of the city. It is an old building dating from the 17th century. It was originally the Archive for the Royal Chancery, next to the Palace where the wedding of the Catholic King and Queen, Isabel and Ferdinand, took place. It is also next to the former prison of the Royal Chancery, which today houses the University of Valladolid Library. The Conference Centre is between five and ten minutes' walk from the Main Square, some of the principal hotels, and the area for tapas and restaurants.

Palacio de Congresos Conde Ansurez. c/ Real de Burgos s/n 47011 Valladolid, Spain.



Walking Map from Headquarters Hotel Olid to Palacio de Congresos Conde Ansurez.

Registration and Desk

Registration will open at the main entrance hall of the venue on Sunday, September 25th, 2016 at 13:00 h and the desk will remain open until the walking tour starting at about 18:30 h. It also will be open during the entire congress. Name badges will be provided upon arrival and are requested to be visible and used at all times.

Presentations

All speakers (except the Flash presenters, which have already provided their presentations to their chair) are requested to provide their presentations to the speaker's office at the entrance of the Conference room in the second floor the day before presentations take place. To save time, we ask you not to use your own laptop for your presentation but to upload your power point files. It is very important to strictly keep to the allotted time for presentations:

Plenary conferences: 40 min and no questions.
Symposia: 20 min + 10 min of questions.
Late-breaking research: 15 min + 5 min of questions.
Oral Communications: 10 min + 5 min of questions.
Flash Presentations: 3 min and no questions.

Poster presentations: the posters are pooled by topic, similar to the 9 symposia. Poster boards are numbered according to the number in present Abstract booklet. Please put up your poster as soon as possible and keep it on display during the whole meeting. Presenting authors are requested to be available at the poster board for questions as follows: odd-numbered posters on Monday and even-numbered posters on Tuesday, each time from 14:00 h to 15:30 h. Please also note that photography of the posters is only permitted with the agreement of the presenting author.

Social Activities

SUNDAY, SEPTEMBER 25TH.
Guided, walking tour of historial Valladolid form the venue to the Palacio de Santa Cruz, first renaissance building in Spain. 5 groups will be formed, 3 of them in English, one in French and the remaining one in Spanish. The visit will end in Palacio de Santa Cruz for a welcome cocktail.

MONDAY, SEPTEMBER 26TH.
Speakers dinner at local restaurant "El Poniente". The other attendants will receive free tickets for tapas and drinks to be used at selected local tapas restaurants.

TUESDAY, SEPTEMBER 27TH.
Visit to the Museum of Sculpture. 8 groups with guides in English (3) and French (1) will visit the Museum. 4 groups starting at 17:15 h and the other 4 groups starting at 18:15 h.

WEDNESDAY, SEPTEMBER 28TH.
Visit to Ribera del Duero Vineyards and Winery Cellars. There will be 4 buses, 2 of them visiting the Peñafiel Castle and Matarromera Cellar and the other 2 cellar Protos and the Peñafiel Castle. Small wine tastes (Catas) will be provided. All groups will go to the banquet dinner at the Hotel Castilla Termal Monasterio de Valbuena, a 1.000 years old monastry for a Cava cocktail and lechazo dinner with Jazz music.

THURSDAY, SEPTEMBER 29TH.
Before leaving, a farewell cocktail will be served at the venue's gardens.

A shopping morning, guided tour is scheduled for accompanying persons.

SUPPORTED BY THE FOLLOWING INSTITUTIONS & COMPANIES

ECS2016 FULL PROGRAM

Sunday, September 25th

13:00 h Registration opens @ Venue "Palacio de Congresos Conde Ansúrez"

16:30 h Welcome addresses by Chair Jan B. Parys (Belgium), ECS president Marc Moreau (France) and Local Organizer Carlos Villalobos (Spain).

17:00 h Opening Conference
Prof. Javier García-Sancho (Spain).
Introduced by Carlos Villalobos (Spain)
Measuring Ca^{2+} inside intracellular organelles

18:30 h Walking Tour of Historic Valladolid (60-90 min)

20:00 h Welcome reception @ "Palacio de Santa Cruz".

Monday, September 26th

08:30 h Symposium #1.
Ca^{2+} and neurological disease: in search for novel targets.
Chair, Carlos Gutierrez-Merino (Spain).

Antonio G. García (Spain).
Altered Ca^{2+} and exocytotic fusion pore in neurodegenerative diseases.
Michael Duchen (UK).
The dialogue between mitochondria and Ca^{2+} in cell life and death.
Ana M. Mata (Spain) .
Functional modulation of plasma membrane Ca^{2+} pumps by molecular markers of Alzheimer's disease.
Elizabeth Yates (UK) Oral Communication.
Lysosomes, Ca^{2+} and LRRK2: a toxic relationship in Parkinson disease?.
Matej Hotka (Austria) Oral Communication.
Investigating the link between mitochondria, Ca^{2+} and epileptiform activity.

10:30 h Coffee break.

11:00 h Late-breaking research symposium.
Chair, Jan B. Parys (Belgium).

Andreas Guse (Germany).
2'-Deoxy-ADPR: a novel second messenger activating TRPM2.
Carlos Enrich (Spain).
Annexin A6 interacts with TBC1D15 to regulate Rab7 activity and cholesterol homeostasis.
Israel Sekler (Israel).
The optometabolic strategy; light-dependent control of mitochondria metabolism and Ca^{2+} signaling.

12:00 h Flash Symposium.
The Ca^{2+} signalling network: Form, function, failure.
Chair, Sandip Patel (UK). 3 min quick presentations of selected posters.

Form of the Ca^{2+} signalling network.
Yuequan Shen (China).
LETM1 forms a Ca^{2+}/H^{+} antiporter.
Christopher J. Penny (UK).
Isolated pores from TPC2 reveal functional redundancy in the evolution of asymmetric ion channels.

Tong Chun-Kit (Hong Kong, China).
Nuclear Membrane Patch for Type 2 Ryanodine Receptor Channel.
Gines Salido (Spain).
Role of Filamin A as a modulator of store-operated Ca^{2+} entry.
Arijita Ghosh (India).
LRRC8B: New associate of calcium signaling network.

Function of the Ca^{2+} signalling network
Bradley J. Stith (USA)
Lipid phosphatidic acid and an outwardly spiraling disk of calcium release leads to the fertilization calcium wave.
Marek Michalak (Canada).
Endoplasmic reticulum calcium dictates intracellular distribution of membrane cholesterol.
Amalia M. Dolga (Germany).
The role of SK channels at the ER-mitochondria interface.
Philippe Gailly (Belgium).
Role of TRPV4 in pressure-induced inhibition of renin secretion by juxtaglomerular cells.
Graham Ellis-Davies (USA).
BIST-2EGTA - a new caged calcium probe that is highly sensitive to 1P and 2P excitation.

Failure of the Ca^{2+} signalling network.
Geneviève Dupont (Belgium).
Modeling the interplay between Ca^{2+} and β-amyloids dysregulations in the onset of Alzheimer's disease.
Magali Savignac (France).
Cav1.2 and Cav1.3 calcium channels are critical during allergic asthma.
Gihan Gunaratne (USA) .
Targeting two-pore channels to inhibit MERS-Coronavirus cell entry.
Gustavo Benaim (Venezuela).
The intracellular Ca^{2+} regulation as a rational therapeutic target for new drugs against trypanosomatid agents of Chagas Disease and Leishmaniasis.
Silvia N.J. Moreno (USA).
A plastid Two Pore Channel and its role in organelle integrity and calcium signaling.

13:00 h Lunch

14:00 h Poster Session #1

15:30 h Symposium #2.
Good and bad sides of Ca^{2+} signaling in human disease.
Chair Natalia Prevarskaya (France).

Victoria Bolotina (USA).
Ca^{2+} signaling path to autophagic dysfunction and Parkinson's disease.
Barbara Ehrlich (USA).
Cancer treatment alters Ca^{2+} signaling and causes neuropathic pain, but the changes can be prevented.
Joost Hoenderop (Netherlands).
Renal Ca^{2+} handling and the anti-aging hormone klotho.
Katja Rietdorf (UK) Oral Communication.
How does fat cause heart diseases? Effects of epicardial adipocytes on cardiomyocyte signaling and contractility.
Miguel Burgos (Spain) Oral Communication.
E152K STIM1 mutation deregulates Ca^{2+} signaling contributing to chronic pancreatitis.

17:30 h Coffee break

18:00 h Symposium #3.
Targeting calmodulin receptor interaction in cancer chemotherapy.
Chair, David Sacks (USA).

G. Prem Veer Reddy (USA).
The functional interaction of calmodulin with androgen receptors in prostate cancer.
James Ames (USA).
Structural basis for Ca^{2+}-induced activation and dimerization of ERα by calmodulin.
Antonio Villalobo (Spain).
Regulation of the EGFR by calmodulin: mechanism and therapeutic implications.
Marie Potier-Cartereau (France) Oral Communication.
Lipid raft KCa/Ca^{2+} channel complexes: novel targets to reduce tumor development by lipids.
Alexia Vautrin (France) Oral Communication.
Type 3 Inositol (1,4,5)-trisphosphate receptor: a migratory modulator with a unique Ca^{2+} signature in breast cancer cells.

21:00 h Speakers dinner at Local Restaurant "Brasería Poniente" or free tapas tour for rest of attendants.

Tuesday, September 27th

08:30 h Symposium #4.
Ca^{2+} remodeling in cancer.
Chair, Gary S. Bird (USA).

Juan A. Rosado (Spain).
Ca^{2+} entry abnormalities in leukemia and breast cancer cells.
Geert Bultynck (Belgium).
Understanding and targeting the ER functions of Bcl-2 in cancer.
Carlos Villalobos (Spain).
Ca^{2+} remodeling in colon cancer.
John Bassett (Australia) Oral Communication.
Assessment of cytosolic free Ca^{2+} levels in GCaMP6m expressing MDA-MB-231 breast cancer cells undergoing cell death using time-lapse high-content imaging.
Maggie L. Kalev-Zylinska (New Zealand) Oral Communication.
Glutamate-mediated Ca^{2+} entry in megakaryocytic cells: evidence for a hijacking effect to support leukaemia growth.

10:30 h Coffee break.

11:00 h Symposium #5.
ER-mitochondrial Ca^{2+} signaling in disease & therapy
Chair, Geert Bultynck (Belgium)

Paolo Pinton (Italy).
Endoplasmic reticulum-mitochondria Ca^{2+} crosstalk in the control of tumor cell fate.
Darren Boehning (USA).
Novel Ca^{2+} signaling pathways in T cells: implications for autoimmunity and cancer.
Jennifer Rieusset (France).
Role of endoplasmic reticulum-mitochondria coupling in hepatic metabolic diseases.
Benjamin Delprat (France) Oral Communication.
ER-mitochondria crosstalk is regulated by WFS1/WIP1 interaction and is impaired in Wolfram syndrome.
Tomás Gutiérrez (Canada) Oral Communication.
Regulation of ER-mitochondria contacts and SERCA activity by the ER chaperones CNX and TMX1.

13:00 h Lunch

14:00 h Poster Session #2

15:30 h Tribute to Prof. Michael Sanderson and Prof. Roger Tsien.
Chair Martin D. Bootman (UK).

Juan Llopis (Spain), Katja Rietdorf (UK), and Luc Leybaert (Belgium). Video message from Tullio Pozzan (Italy).

16:00 h Plenary Conference.
Prof. Erwin Neher (Germany).
Introduced by Roland Pochet (Belgium).
Dynamics of [Ca^{2+}] changes during bursts of activity at the 'Calyx of Held' synapse

17:30 h Visit to National Museum of Sculpture @ Colegio de San Gregorio.

Wednesday, September 28th

08:30 h Symposium #6.
Structures of Ca^{2+} ion channels.
Chairs, Mitsu Ikura (Canada) and Walter Chazin (USA).

Peter B. Stathopulos (Canada).
Structural insights into mitochondrial Ca^{2+} regulation.
Irina Serysheva (USA).
Structure and function of the IP_3R_1 Ca^{2+} channel: insights from cryo-EM studies.
Qun Liu (USA).
Structural mechanism for a transmembrane Ca^{2+} leak.
Filip Van Petegem (Canada) Oral Communication.
Crystallographic investigation of the Ca^{2+} sensing apparatus of voltage-gated Na^{+} and Ca^{2+} channels: effect of arrhythmia-causing mutations.
Katrien Willegems (Belgium) Oral Communication.
Ryanodine receptor in the open state with and without ryanodine.

10:30 h Coffee break.

11:00 h Symposium #7.
New tools for Ca^{2+} imaging and other technologies.
Chair, Juan Llopis (Spain).

Matsumitsu Iino (Japan).
Use of CEPIA indicators for the study of intraorganellar Ca^{2+} dynamics.
Oliver Griesbeck (Germany).
Optimized ratiometric calcium Biosensors .
María T. Alonso (Spain).
In vivo imaging of Ca^{2+} in the endoplasmic reticulum with GAP sensors.
Ágota Apáti (Hungary) Oral Communication.
Genetically engineered Ca^{2+} indicators for visualization of Ca^{2+} signals: from single cells to tissues.
Fabiana Perocchi (Germany) Oral Communication.
From functional genomics to drug re-purposing: the mitochondrial uniporter case.
David Castrillo (Spain) Hamamatsu ´s brief presentation.
New Imaging tools from Hamamatsu Photonics.

13:00 h Lunch.

14:00 h Poster Session #3.

15:00 h ECS General Assembly.

16:00 h Berridge Lecture.
Prof. James W. Putney (USA).
introduced by Martin D. Bootman (UK).
Signaling functions of store-operated Ca^{2+} channels.

17:30 h Visit to a selected Ribera del Duero Vineyard and Winery Cellar.

21:00 h Conference Banquet at Hotel Castilla Termal Monasterio de Valbuena in Valbuena de Duero (Valladolid) at the heart of the Duero River Valley

Thursday, September 29th

08:30 h Symposium #8.
MicroRNA and calcium signaling.
Chair, Catherine Leclerc (France).

Na Li (USA).
MicroRNA and Ca^{2+} signaling in atrial fibrillation.
Sebastian Albinsson (Sweden).
Regulation of smooth muscle contractility and mechanosensing by microRNAs.
Enikö Kallay (Austria).
MicroRNAs and the Ca^{2+}-sensing receptor.
Agne Tilunaite (UK) Oral Communication.
Cellular Ca^{2+} signalling in the presence of single cell variability and dynamic stimuli.
Mª Cristina López-Méndez (Mexico) Oral Communication.
Ryanodine receptors of acidic Ca^{2+} stores generate a non-productive Ca^{2+} release in smooth muscle cells.

11:00 h Coffee break.

11:00 h Symposium #9.
Calcium, stem cells and regeneration.
Chair, Andrew Miller (Hong-Kong, China).

Jonathan Marchant (USA).
Ca^{2+} signaling and CNS regeneration in planaria.
Catherine Leclerc (France).
Ca^{2+} signaling in glioblastoma stem cells .
Jianbo Yue (Hong-Kong, China).
Role of CD38/cADPR/Ca^{2+} signaling in differentiation of mouse ES cells.
Carles Rentero (Spain) Oral Communication.
Annexin A6-mediated alanine uptake is necessary for liver regeneration in mice.
Sachie Kanatani (Sweden) Oral Communication.
Voltage-dependent Ca^{2+} channel signaling mediates GABAA receptor-induced migratory activation of dendritic cells infected by Toxoplasma gondii.

13:30 h Awards ceremony and concluding remarks.

14:00 h Farewell's cocktail at Venue's garden.

ABSTRACTS OF PLENARY LECTURES, SYMPOSIA AND ORAL COMMUNICATIONS

Opening Conference
C-1

Measuring Ca^{2+} inside intracellular organelles.

Javier García-Sancho.

Instituto de Biología y Genética Molecular (IBGM). Universidad de Valladolid y Consejo Superior de Investigaciones Científicas (CSIC). Valladolid, Spain.

jgsancho@ibgm.uva.es

Interest on Ca^{2+} signaling is moving from cytosol to organelles, as proper organellar functioning requires proper control of their luminal Ca^{2+} homeostasis. Subcellular Ca^{2+} signals in organelles are best monitored using targeted genetically encoded calcium indicators (GECI). Aequorins (AEQ) were the first GECI used and novelties including low affinity AEQ will be reviewed. Fluorescent GECI include several families, mostly derived from calmodulin and troponin, and they have been widely engineered for changes in affinity, brightness or gain. We shall focus on a new GECI family, GAP, based on the fusion of GFP and AEQ. GAP exhibits a unique combination of features: dual-excitation ratiometric imaging capability, high dynamic range, good signal-to-noise ratio, insensitivity to pH and Mg^{2+}, uncomplicated calibration, and targetability to at least five different organelles[1]. Low affinity GAP variants were engineered for measuring in high [Ca^{2+}] compartments, such as ER or Golgi[2]. As neither AEQ nor GFP are present in mammalian cells, perturbations by interactions with endogenous ligands are prevented and transgenic animals expressing ER-targeted GAP did not show phenotypic alterations and GAP was expressed in a variety of organs and tissues including nervous system. We shall provide proof of concept for the suitability of the new biosensors to monitor Ca^{2+} dynamics inside intracellular organelles, including ex vivo and in vivo measurements in transgenic animals. The new sensors fill a gap in the actual repertoire of Ca^{2+} indicators and open an avenue to explore complex Ca^{2+} signaling in animal models of health and disease.

1. RODRIGUEZ-GARCÍA et al. (2014) Proc. Nat. Acad. Sci. USA, 111: 2584-2589
2. NAVAS-NAVARRO et al. (2016) Cell Chem Biol. 23: 1-8.

Supported by MINECO (BFU2014-53469P) and ISCiii (RD12/0019/0036)

Symposium #1: Ca^{2+} and neurological disease: in search for novel targets.
S-1.1

Altered kinetics of single-vesicle exocytosis and calcium balance in neurodegenerative diseases.

Antonio G. García, Carmen Nanclares, Iago Méndez, Juan Fernando Padín, Antonio M.G. de Diego and Luis Gandía.

Instituto Teófilo Hernando, Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid, 28029 Madrid, Spain.

We have explored the kinetics of the exocytotic single-vesicle quantal release of catecholamine in the adrenal chromaffin cells of transgenic mouse models of neurodegenerative diseases (MCCs) with a carbon-fibre microelectrode and amperometry. In the aged APP/PS1 mouse model of AD (20 months), MCCs challenged with high K^+ fired bursts of secretory spikes with single-spike kinetics having the following characteristics with respect to age-matched wild-type (WT) mice: an averaged faster rise time and decay time, narrower $t_{1/2}$, lower spike amplitude (I_{max}), and reduced quantal size (Q); this suggests that single-vesicle exocytosis is faster with smaller transmitter release indicating rapid opening and closing of the fusion pore (de Diego et al. 2012). Whether this altered kinetics of exocytosis developed with the time course of disease progression was explored in 3xTg mice, a triple transgenic mouse model of AD carrying mutations in APP, PS1, and tau protein. We explored the exocytotic kinetics at 2, 6 and 12 months aged mice and found that the secretory averaged spike became narrower and faster with reduced Q at 6 and 12 months, with respect young pre-disease 2 months mice (Nanclares et al. 2016). We found the opposite in $SOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis (ALS) at advanced disease stages (postnatal days P90-P120): slower rise time and decay time, smaller I_{max}, but higher Q, indicating a slower opening and closing of the fusion pore with higher transmitter release, with respect age-matched WT mice (Calvo-Gallardo et al. 2015). Of interest was the observation that $SOD1^{G93A}$ and 3xTg MCCs at advanced disease stages had greater peak whole-cell Ca^{2+} current (I_{Ca}). Thus, the altered kinetics of single-vesicle exocytosis may be secondary to a primary cell Ca^{2+} imbalance, known to be present in neurodegenerative diseases (Berridge 2014). This may be linked to neuronal hyperactivity recently reported to occur at early stages of AD (Busche & Konnerth 2016).

Berridge, M. J. (2014) Calcium regulation of neural rhythms, memory and Alzheimer's disease. J Physiol, 592, 281-293.

Busche, M. A. and Konnerth, A. (2016) Impairments of neural circuit function in Alzheimer's disease.

Philos Trans R Soc Lond B Biol Sci, 371.

Calvo-Gallardo, E., de Pascual, R., Fernández-Morales, J. C. et al. (2015) Depressed excitability and ion currents linked to slow exocytotic fusion pore in chromaffin cells of the $SOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis. Am J Physiol Cell Physiol, 308, C1-19.

de Diego, A. M., Lorrio, S., Calvo-Gallardo, E. and García, A. G. (2012) Smaller quantal size and faster kinetics of single exocytotic events in chromaffin cells from the APP PS1 mouse model of Alzheimer's disease. Biochem Biophys Res Commun, 428, 482-486.

Nanclares, C, Colmena, I., García, A.G. and Gandía, L. Age-related changes of the exocytotic fusion pore in chromaffin cells of the 3xTg mouse model of Alzheimer's disease (unpublished), 2016.

Symposium #1: Ca^{2+} and neurological disease: in search for novel targets. S-1.2

The dialogue between mitochondria and calcium in cell life and death.

Gauri Bhosale, Jenny Sharpe, Gyorgy Szabadkai and Michael R Duchen.

Department of Cell and Developmental Biology and Consortium for Mitochondrial Research, University College London, Gower St., London WC1E 6BT.

m.duchen@ucl.ac.uk

Calcium signaling is fundamental to cell physiology, initiating core physiological processes that include fertilization, contraction, migration and secretion. A rise in intracellular Ca^{2+} concentration also activates multiple pathways that match energy supply to demand, driving increased rates of ATP synthesis or increased bioenergetic capacity. Increased cytosolic Ca^{2+} increases metabolite transfer across the inner mitochondrial membrane through activation of Ca^{2+}-regulated mitochondrial carriers. Increased matrix Ca^{2+} concentration, mediated by the calcium uniporter (MCU), stimulates the citric acid cycle and the ATP synthase. Calcium signals increase mitochondrial biogenesis and also control mitochondrial trafficking, positioning mitochondria at points of high energy demand. While the specific physiological importance of the MCU complex in work-induced stimulation of respiration remains unclear, abnormal mitochondrial calcium signaling causes pathology. Loss of function mutations in MICU1, which defines the threshold $[Ca^{2+}]c$ at which the MCU opens, are associated with learning difficulties, a progressive extrapyramidal motor disorder and muscle weakness in childhood. In MICU1 deficient fibroblasts derived from the patients, resting mitochondrial matrix $[Ca^{2+}]$ is increased and mitochondria fragmented. We propose that MICU1 acts as a signal-noise discriminator, and that mutations of this protein lead to futile calcium cycling. Thus, significant calcium uptake at rest is balanced by sodium calcium exchange which is in turn balanced sodium-proton exchange, undermining the proton flux that drives ATP generation. These mutations thus reveal the importance of differentiating signal from noise, and the energetic cost of futile calcium cycling, and demonstrate the importance of fine tuning of mitochondrial Ca^{2+} signals in shaping energy homeostasis.

We are grateful to the Muscular Dystrophy UK and to GSK for support.

Symposium #1: Ca^{2+} and neurological disease: in search for novel targets. S-1.3

Functional modulation of Ca^{2+} pumps by molecular markers of Alzheimer´s disease.

Ana M. Mata.

Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, Badajoz, Spain.

anam@unex.es

Dysregulation of Ca^{2+} homeostasis is widely involved in aging and aging-related diseases such as Alzheimer´s disease (AD). The presence of toxic aggregates of amyloid β-peptide ($A\beta$) and neurofibrillary tangles of tau protein constitutes two characteristic hallmarks of AD. It has been widely documented the association of intracellular calcium dysregulation with $A\beta$, but not with tau. Considering that Ca^{2+}-ATPases are high-affinity Ca^{2+} transporters responsible for the maintenance of optimal intracellular Ca^{2+} levels we have analyzed the putative involvement of these proteins in calcium dysregulation associated to AD. The Ca^{2+}-dependent activity of plasma membrane Ca^{2+}-ATPase (PMCA) is less sensitive to Ca^{2+} concentrations changes in AD than in control membrane preparations, being the $A\beta$ peptide actively involved in this effect. However, the intracellular SERCA and SPCA Ca^{2+} pumps do not show differences related to the disease. Both, $A\beta$ and tau are able to inhibit PMCA activity, but the molecular mechanisms underlying this adverse effect seem to be different. In fact, the ionic nature of the lipid environment is crucial to determine the inhibitory effect of tau, but not of $A\beta$ on PMCA. Functional analysis with specific native and truncated isoforms show the presence of different PMCA binding sites for $A\beta$ and tau. Besides, these inhibitions are antagonized by calmodulin. In summary, these results provide evidence to support the involvement of PMCA in the molecular mechanisms associated to intracellular Ca^{2+} impairment and neurodegeneration.

Supported by Spanish grants from Ministerio de Economía y Competitividad BFU2011-23313 and BFU2014-53641-P), Junta de Extremadura (GR10108 and GR15139), and FEDER.

Symposium #1: Ca^{2+} and neurological disease: in search for novel targets. ORAL COMMUNICATION O-1.1

Lysosomes, Ca^{2+} and LRRK2: A toxic relationship in Parkinson disease?

Yates E[1], Kilpatrick BS[1], Chau D[2], Schapira AHV[2] and Patel S[1]

[1]Department of Cell and Developmental Biology
[2]Department of Clinical Neuroscience, University College London, London, UK.

elizabeth.yates.13@ucl.ac.uk

The common G2019S mutation in leucine-rich repeat kinase 2 (LRRK2) is linked to familial and sporadic Parkinson disease (PD). Our previous work identified enlarged and clustered lysosomes in fibroblasts from patients with LRRK2 G2019S PD that were corrected by buffering local Ca^{2+} increases or inhibiting the endolysosomal Ca^{2+} release channel, TPC2. However, the consequences for stimulus-evoked Ca^{2+} signaling in PD remain unclear. Here we asked whether lysosome morphology is disturbed in a neuronal model of the disease and whether Ca^{2+} signaling is affected. In dopaminergic SH-SY5Y cells stably expressing LRRK2 G2019S, lysosome morphology was disrupted compared to wild type controls. Furthermore, these cells exhibited potentiated Ca^{2+} signals when stimulated with the cholinergic agonist, carbachol. Interestingly, there was no such difference in response to the peptide mediator, bradykinin. To assess the contribution of lysosomal Ca^{2+} to these signals, cells were pre-treated with the lysosomotropic agent, GPN or the novel TPC blocker, tetrandrine. Carbachol signals were inhibited but bradykinin signals were not. Our data associate defective lysosome morphology and agonist–evoked Ca^{2+} signals in LRRK2 G2019S neuronal cells with the release of Ca^{2+} from lysosomes. These findings may be an important consideration for the development of PD therapeutics.

Symposium #1: Ca^{2+} and neurological disease: in search for novel targets. ORAL COMMUNICATION O-1.2

Investigating the link between mitochondria, Ca^{2+} and epileptiform activity.

Matej Hotka[1], Michal Cagalinec[2] and Helmut Kubista[1]

[1] Department of Neurophysiology and Neuropharmacology, Center of Physiology and Pharmacology, Medical Univ. Vienna, Austria
[2] Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia.

matej.hotka@meduniwien.ac.at

In recent years a link between mitochondria and epilepsy has been proposed, which may at least partially be due to an increase of ROS production. Epileptogenesis is characterized by an abnormal electrical activity, termed the paroxysmal depolarization shift (PDS). PDS differ from normal action potentials by a prolonged depolarized shoulder lasting for several hundreds of milliseconds, mediated by an abnormal activity of the L-type calcium channels (LTCCs). LTCCs are sensitive to ROS, hence the possibility exists that ROS induced LTCC enhancement may play a role in PDS formation. Since PDS were suggested to be involved in the remodeling of neuronal networks, this may represent a crucial pathogenic mechanism in epileptogenesis. Here we investigated the link between mitochondria and epileptiform activity by studying the effect of paroxysmal depolarization shifts (induced by bicuculline + the LTCC agonist Bay K8644) on mitochondrial distribution, motility and morphology in neuronal networks formed from dissociated hippocampal cells. Likewise, the effect of experimentally induced changes in mitochondrial distribution (MGARP transfection), motility and morphology on epileptiform activity were studied. We found that PDS reversibly affected the mitochondria in the soma and the proximal neurites, by changing the mitochondrial morphology and inhibition of the movement up to 90%. We also found that neurons with disturbed mitochondrial distribution and morphology were more sensitive to PDS formation. These findings suggest the possible positive feedback mechanism in the epileptogenetic period which might be responsible for further neuronal pathology.

Funding: Austrian Science Fund (FWF, Project P-28179)

Late-Breaking Research Symposium LBR-1

2′-Deoxy-ADPR: a novel second messenger activating TRPM2.

Ralf Fliegert[1], Andreas Bauche[1], Adriana-Michelle Wolf Pérez[1], Joanna M. Swarbrick[2], Monika D. Rozewitz[1], Riekje Winzer[1], Mareike Janus[1], Angelika Harneit[1], Marianne Flato[1], Christelle Moreau[2], Tanja Kirchberger[1], Valerie Wolters[1], Barry V.L. Potter[2,3] and Andreas H. Guse[1]

[1]The Calcium Signalling Group, Department of Biochemistry and Molecular Cell Biology, University Medical Centre Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany
[2]Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK . [3]Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT, UK.

guse@uke.de

TRPM2 is a Ca^{2+}-permeable non-selective cation channel activated by adenosine 5′-diphosphoribose (ADPR). So far ADPR has been assumed to be the main agonist for TRPM2. Here, we show that 2′-deoxy-ADPR is a significantly better TRPM2 agonist inducing 10.4-fold higher whole cell currents at saturation. This effect is caused by a decreased rate of inactivation and a higher average open probability, as shown by single channel recordings of TRPM2. Using HPLC and mass spectrometry, endogenous 2′-deoxy-ADPR was detected in Jurkat T-lymphocytes. A metabolic pathway for generation of 2′-deoxy-ADPR may consist of cytosolic nicotinamide mononucleotide adenylyltransferase 2 (NMNAT-2) and NAD-glycohydrolase CD38. Taken together, 2′-deoxy-ADPR is a novel second messenger produced by CD38 and is the primary agonist of TRPM2.

Funding: Deutsche Forschungsgemeinschaft (to AHG), Wellcome Trust (to BVLP & AHG)

Late-Breaking Research Symposium LBR-2

Annexin A6 interacts with TBC1D15 to regulate Rab7 activity and cholesterol homeostasis.

Carlos Enrich[1], Elsa Meneses-Salas[1], Ana García-Melero[1], Thomas Grewal[2] and Carles Rentero[1].

[1]Departament de Biologia Cel·lular, Immunologia i Neurociències. Centre de Recerca Biomèdica CELLEX. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Facultat de Medicina. Universitat de Barcelona. 08036-Barcelona. Spain. [2]Faculty of Pharmacy, University of Sydney. Sydney NSW 2006. Australia.

Pathological accumulation of cholesterol in late endosomes (LE) is often observed in lysosomal storage diseases such as Niemann-Pick type C1. We have previously demonstrated that overexpression of Annexin A6 (AnxA6) induces LE-cholesterol accumulation, however the underlying molecular mechanism involved have remained unclear. A yeast two-hybrid screening revealed that AnxA6 interacts with TBC1D15 (Rab7-GAP). Rab7 overexpression rescues LE-cholesterol accumulation in NPC1 mutant cells, yet co-transfection of Rab7 with AnxA6 inhibits LE-cholesterol egress. This suggested that AnxA6, via TBC1D15 and Rab7-dependent pathways, is part of the machinery that regulates cholesterol export from LE. Importantly, silencing of AnxA6 not only significantly reduced the cholesterol accumulation in LE of NPC1 mutant cells, but also promoted a diversion of LDL-cholesterol into LD, in an ACAT-mediated manner, providing a potential alternative pathway to overcome cellular dysfunction due to LDL cholesterol accumulation in LE and restore intracellular cholesterol distribution. Furthermore, since the biogenesis of LD takes place in the endoplasmic reticulum (ER), we have first evidence that the transfer of free cholesterol from LE into LD occurs through membrane-contact sites (MCS) between LE and ER. ORP1L is a cholesterol transporter and sensor associated with LE protein, which is involved, through MCS, in transport of sterols from LE to the ER, in a Rab7-dependent manner. Therefore, the ability of AnxA6 to promote Rab7 inactivation via TBC1D15 (Rab7-GAP) and its possible involvement in cholesterol transfer to LD via the ER involving ORP1L and MCS represents a major advance in our understanding of AnxA6 function in cholesterol homeostasis.

This work is supported by grant BFU2015-66785-P from Ministerio de Economía y Competitividad, Spain.

Late-Breaking Research Symposium LBR-3

Optogenetic Control of Mitochondrial Ca^{2+} Signaling and Metabolism.

Israel Sekler

Dept. of Physiology and Cell Biology, Faculty of Health Sciences Ben-Gurion University Israel.

Israel.sekler@gmail.com

Key mitochondrial functions such as ATP production, Ca^{2+} shuttling, depend on the steep membrane potential across the mitochondrial membrane inner membrane. While uncouplers and protonophores can modulate mitochondrial membrane potential, they are often irreversible and cannot provide specific control of metabolic activity of cells population within a tissue or to interrogate distinct segments of the mitochondrial network within the cells. The optogenetic strategy, based on the use light-gated ion channels, revolutionized our ability to interrogate brain activity by providing light dependent control on neuronal firing in a time and cell specific manner. I will describe how targeting of light-gated ion channel to the inner mitochondrial membrane of cells provide light dependent , reversible control of mitochondrial membrane potential thereby of ATP synthesis, Ca^{2+} signaling and respiration. I will further discuss how this optometabolic approach allows fast, reversible and cell-specific interrogation of key physiological processes including light dependent spontaneous beats in cardiac myocytes and secretory signals in pancreatic _-cells. In the final part of my lecture I will focus on the use of the optometabolic tool in interrogating Ca^{2+} and mitochondrial membrane potential in a mitochondrial network within a single cell.

Symposium #2: Good and bad sides of Ca^{2+} signaling in human disease S-2.1

Ca^{2+} signaling path to autophagic dysfunction and Parkinson's disease.

Victoria Bolotina

Boston University, Boston, MS. USA

Symposium #2: Good and bad sides of Ca^{2+} signaling in human disease S-2.2

Cancer treatment alters Ca^{2+} signaling and causes neuropathic pain, but the changes can be prevented.

Barbara E. Ehrlich

Yale University, New Haven, CT, USA.

barbara.ehrlich@yale.edu

Major advances in chemotherapy regimens have increased the number of cancer survivors, but chemotherapy-induced peripheral neuropathy (CIPN) remains a debilitating, dose-limiting side effect for many classes of chemotherapeutics. CIPN manifests as numbness, tingling or a burning/stabbing pain in the hands and feet which can also affect the central nervous system. Most of the patients receiving paclitaxel, a taxane that is one of the most effective drug for treating breast and ovarian cancer, develop CIPN and in nearly half of these cases the neuropathy is irreversible. Although the chemotherapeutic action of the taxanes is by stabilizing microtubules and preventing cell division, the side effects appear to be microtubule independent because several other commonly used cancer chemotherapeutics also induce CIPN. We hypothesize that taxane-induced CIPN is initiated by its binding to neuronal calcium sensor 1 (NCS1) which leads to increased intracellular calcium. This increased calcium turns on calpain which then cleaves and inactivates a number of proteins, including NCS1. Mice lacking NCS1 do not experience taxane-induced CIPN. NCS1 binds to several proteins that regulate intracellular calcium. By disrupting these interactions, it is possible to prevent taxane-induced CIPN. These findings will make it possible to devise effective treatment regimens that avoid CIPN and other unwanted side effects of chemotherapy and will improve quality of life for cancer survivors.

Symposium #2: Good and bad sides of Ca^{2+} signaling in human disease
S-2.3

Renal calcium handling and the anti-aging hormone klotho.

Joost Hoenderop

Department of Physiology
Radboud University Medical Center, Nijmegen, Netherlands.

The kidney plays a crucial role in the maintenance of the body calcium balance. Ca^{2+} is an essential ion in all organisms and participates in a large variety of structural and functional processes. Of all Ca^{2+} filtered by the glomerulus, the majority (99%) is reabsorbed. Reabsorption of Ca^{2+} largely takes place through paracellular transport in the proximal part of the nephron (~70%) and the thick ascending limb (~25%). The final amount of Ca^{2+} excretion via the urine is determined in the distal part of the nephron, through a tightly regulated process of active Ca^{2+} reabsorption. Transcellular Ca^{2+} transport is specifically located in the second part of the distal convoluted tubule and connecting tubule, also known as the distal convolution. This active transcellular transport is hallmarked by the apical transient receptor potential vanilloid 5 (TRPV5) epithelial Ca^{2+} channel, regulated by an array of events, and mediated by hormones, including 1,25-dihydroxyvitamin D_3, parathyroid hormone, and estrogen. Once in the renal cell, Ca^{2+} is buffered by the Ca^{2+}-binding protein calbindin-D_{28K} and the complex diffuses intracellularly towards the basolateral side. Finally, Ca^{2+} is extruded towards the blood compartment by the ATP-dependent Ca^{2+}-ATPase and the Na^{+}-Ca^{2+} exchanger type 1. Novel molecular mechanisms have been identified, such as the direct regulatory effects of klotho and tissue kallikrein on the abundance of TRPV5 at the apical membrane. The newly discovered mechanisms could provide potential pharmacological targets in the therapy of renal Ca^{2+} wasting. In the presentation a guided molecular tour along the nephron will be made detailing the contribution of the individual segments to the overall renal Ca^{2+} handling.

Symposium #2: Good and bad sides of Ca^{2+} signaling in human disease
ORAL COMMUNICATION O-2.1

How does fat cause heart diseases? Effects of epicardial adipocytes on cardiomyocyte signalling and contractility.

Said Masoud[1], Martin D. Bootman[1], Fraser McDonald[2], Hilary MacQueen[1] and Katja Rietdorf[1]

[1]The Open University, Department of Life, Health and Chemical Sciences, Walton Hall, Milton Keynes, MK7 6AA. [2]Kings College London, Department of Orthodontics, Craniofacial Development and Stem Cell Biology, 310 Tower Wing, Guy's, London, SE1 9RT.

katja.rietdorf@open.ac.uk

The heart is surrounded by a layer of fat known as epicardial adipose tissue (EAT). EAT thickness is a predictor for several cardiac diseases. Adipocytes within the EAT cause harmful effects via secretion of adipokines and cytokines, which can act on neighbouring cardiomyocytes in a paracrine manner. However, EAT also has beneficial effects, and is critical to the heart's function by providing energy and offering physical protection. We found that adipocytes caused negative chronotropic (0.51 $\pm$ 0.01 vs. 0.32 $\pm$ 0.04 Hz contraction frequency) and negative inotropic responses (6.9 $\pm$ 0.3 vs. 3.8 $\pm$ 0.5 µm contraction amplitude) after 3 days of co-culture with neonatal rat ventricular cardiomyocytes. Using signalling arrays we found a significant up-regulation of cardiomyocyte pro-apoptic signalling pathways by the adipocytokines TNF-α and Activin A. In contrast, adiponectin reduced ERK and mTOR activity, in line with its proposed cardioprotective effects. These data illustrate that adipose-derived adipocytokines have complex effects on both contractility and signaling pathways in cardiomyocytes. Because mature adipocytes do not attach to cell culture surfaces it is impossible to study the paracrine interaction between adipocytes and cardiomyocytes for prolonged periods using conventional 2-dimensional co-cultures. Addition of adipocyte-conditioned medium is often used, but cannot replicate the paracrine interactions occurring between cells being in physical contact. To study the effects of adipocytes on adjacent cardiomyocytes, and vice versa, over prolonged periods of time we are establishing a 3-dimensional co-culture. This approach allows cellular phenotypes, calcium signalling and contraction to be measured within the cultures.

Funding: The Open University

Symposium #2: Good and bad sides of Ca^{2+} signaling in human disease
ORAL COMMUNICATION O-2.2

E152K STIM1 mutation deregulates Ca^{2+} signaling contributing to chronic pancreatitis.

Miguel Burgos[1,2], Fabrice Antigny[3], Pauline Dubar[1], Reginald Philippe[1], Peter Stathopoulos[4,5], Mitsuhiko Ikura[5], Wesley Brooks[6], Juan Llopis[2], Beatriz Domingo[2], Jian-Min Chen[1], Thierry Capiod[7], Claude Ferec[1] and Olivier Mignen[1]

[1]INSERM U1078 Génétique, Génomique fonctionnelle et biotechnologies, Faculté de Médecine et des Sciences de la Santé, Université de Bretagne Occidentale, Brest, France; [2]Centro Regional de Investigaciones Biomédicas (CRIB) and Facultad de Medicina de Albacete, Universidad Castilla-La Mancha, Albacete, Spain; [3]Dept of Basic Neurosciences, 1, Rue Michel Servet, 1211 Geneva 4, Switzerland; [4]Princess Margaret Cancer Centre and Dept of Medical Biophysics, University of Toronto, Canada; [5] Dept of Physiology and Pharmacology, University of Western Ontario, Canada; [6] Dept of Chemistry, University of South Florida, Tampa, FL USA; [7] INSERM U1151 – Institut Necker Enfants Malades (INEM) Faculté de Médecine Paris Descartes, Paris, France

Miguel.burgos@uclm.es

Pancreatic acinar cell damage observed in pancreatitis can result from an abnormal regulation of $[Ca^{2+}]i$ leading to enhanced intracellular trypsin activation. We identified different mutations in the Stromal Interaction Molecule 1 (STIM1) gene in 3 cohorts of patients suffering from chronic pancreatitis. A mutation in the STIM1 EF-SAM domain (E152K) induced an increase in endoplasmic reticulum (ER) Ca^{2+} release in fibroblasts obtained from patients and STIM1-E152K transfected HEK293 cells. This increase resulted from higher calcium levels in the ER due to enhanced SERCA pump activity. Differences in STIM1-SERCA interactions between wild type and mutated STIM1 may explain this change in SERCA activity caused by modifications of STIM1 biophysical characteristics. In AR42J cells expressing STIM1-E152K, perturbations in Ca^{2+} signaling is correlated with defects in trypsin activation and secretion and also an increased cytotoxicity after cell stimulation with cholecystokinin. Altogether, STIM1-E152K induces deregulation of Ca^{2+} homeostasis that could lead to cell damage in acinar cells and contribute to the development of chronic pancreatitis in patients bearing the mutation.

We acknowledge MINECO/FEDER grants, reference BFU2012-39548-C02-01 and BFU2015-69874-R, and group grant GI20163512 from Vicerrectorado de Investigación y Política Científica, UCLM. We also acknowledge the Cystic Fibrosis association "Gaetan Saleun", Brest, France.

Symposium #3:Targeting calmodulin receptor interaction in cancer chemotherapy
S-3.1

The functional interaction of calmodulin with androgen receptors in prostate cancer.

G. Prem Veer Reddy, Ph.D.

Vattikuti Urology Institute, Henry Ford Health System and Department of Pathology, Wayne State University School of Medicine, Detroit, MI, USA

Androgen receptors (AR) play a critical role at all stages of prostate cancer (PCa). Therefore, AR-antagonist therapies are a mainstay for the treatment of metastatic PCa. However, currently available AR-antagonists, which target primarily the AR ligand binding domain (AR-LBD), do not provide a lasting remission and the disease invariably recurs to become castration-resistant (CRPC), for which there is no cure. Therefore, there is an urgent need for AR-antagonists that can inhibit AR without relying on the AR-LBD for the treatment of CRPC. We discovered calmodulin (CaM) co-localization and co-immunoprecipitation with AR in PCa cells. CaM binds to the AR functional amino-terminal domain (AR-NTD) and regulates AR function and AR protein stability; CaM-antagonists, including W-7, trifluoperazine and CaM-siRNA, inhibit AR activity, compromise AR protein stability, and suppress proliferation of PCa cells. CaM protects AR from calpain-mediated proteolysis. CaM is overexpressed in prostate tumor tissues and its increase in the nucleus is associated with the elevated nuclear AR protein levels. A novel curcumin derivative, hydrazinobenzoylcurcumin (HBC) that selectively binds and inhibits CaM, disrupts CaM-AR interaction, suppresses the phosphorylation of AR serine 81, and blocks AR from binding to androgen response elements, which are critical for AR transcriptional activity. Interestingly, HBC represses the expression of oncogenes and stimulates expression of tumor-suppressor genes in PCa. Importantly, HBC suppresses xenograft CRPC tumor growth in nude mice. Together, these studies reveal an important role of CaM in promoting AR-dependent growth of PCa and offer a novel strategy targeting the AR-NTD for an effective treatment of CRPC.

Symposium #3:Targeting calmodulin receptor interaction in cancer chemotherapy S-3.2

Structural basis for Ca^{2+}-induced activation and dimerization of ER-α by calmodulin.

James B. Ames and David Sacks

Department of Chemistry, University of California, Davis, CA 95616 USA

jbames@ucdavis.edu

The estrogen receptor alpha (ER-_) regulates expression of target genes implicated in development, metabolism, and breast cancer. Calcium-dependent regulation of ER-_ is critical for activating gene expression and is controlled by calmodulin (CaM). In my talk, I will present the NMR structure of Ca^{2+}-bound CaM bound to two molecules of ER-_ (residues 287-305). The two lobes of CaM each bind to the same site on two separate ER-_ molecules (residues 292, 296, 299, 302 and 303), which explains why CaM binds two molecules of ER-_ in a 1:2 complex and stabilizes ER-_ dimerization. Exposed glutamate residues in CaM (E11, E14, E84, E87) form salt bridges with key lysine residues in ER-_ (K299, K302 and K303), which is likely to prevent ubiquitination at these sites and inhibit degradation of ER-_. CaM facilitates dimerization of ER-_ in the absence of estrogen, and stimulation of ER-_ by either Ca^{2+} and/or estrogen may serve to regulate transcription in a combinatorial fashion.

Supported by a grant from the National Institutes of Health (EY012347 to JBA).

Symposium #3:Targeting calmodulin receptor interaction in cancer chemotherapy S-3.3

Regulation of the EGFR by calmodulin: mechanism and therapeutic implications.

Antonio Villalobo

Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Madrid, Spain.

antonio.villalobo@iib.uam.es

The epidermal growth factor receptor (EGFR/HER1/ErbB1) is a tyrosine kinase that belongs to the ErbB family, and is implicated in multiple cellular functions including: cell proliferation, cell survival, differentiation and cell migration, and is mutated or overexpressed in multiple solid tumors. We have demonstrated that the EGFR, and its cousin ErbB2/HER2, binds calmodulin (CaM) at the cytosolic juxtamembrane region in a Ca^{2+}-dependent manner, participating in the ligand-dependent activation of the receptor as an intracellular co-activator. Deletion or mutation of the CaM-binding domain (CaM-BD) of the EGFR inactivated or strongly decreased the tyrosine kinase activity of the receptor. Inhibition of CaM with specific cell-permeable chemical antagonists or downregulation of CaM in conditional-knockout cells stably expressing the human EGFR also inhibited the ligand-dependent activation of the receptor. Furthermore, CaM can be phosphorylated by the EGFR at both Tyr99 and Tyr138 in the absence of Ca^{2+}. We will present results showing that phospho-Tyr-CaM also acts as an activator of the EGFR in the presence but not in the absence of receptor ligand, increasing therefore the tyrosine kinase activity of the receptor toward exogenous substrates. A cell-permeable peptide corresponding to a modified sequence of the CaM-BD of the EGFR also inhibited the ligand-dependent activation of the receptor and the proliferative capacity of cells overexpressing this receptor. A model describing how Ca^{2+}/CaM and phospho-Tyr-CaM exert its activator action, and how targeting the CaM-BD of the EGFR, and ErbB2, could be a valid therapeutic strategy against tumors with overexpressed or dysregulated hyperactive receptors will be discussed.

This work was funded by the Secretaría de Estado de Investigación, Desarrollo e Innovación (SAF2014-52048-R), and the CSIC program i-COOP+2014 (COOPA20053).

Symposium #3:Targeting calmodulin receptor interaction in cancer chemotherapy
ORAL COMMUNICATION O-3.1

Lipid raft KCa/Ca^{2+} channel complexes: novel targets to reduce tumor development by lipids.

M. Guéguinou[1,9], T. Harnois[2,9], D. Crottes[3], A. Uguen[6,7], N. Deliot[2,9], A. Gambade[1],, A. Chantôme[1,9], JP. Haelters[4,9], PA. Jaffrès[4,9], ML. Jourdan[1,10], G. Weber[1], O. Soriani[8], P. Bougnoux[1,9, 10], O. Mignen[6,9], N. Bourmeyster[2,9], B. Constantin[2,9], T. Lecomte[5,9,10], C. Vandier[1,9*] and M. Potier-Cartereau[1,9*‡].

[1]INSERM UMR 1069, Université de Tours, France.
[2]Equipe ERL 7368 CNRS Université de Poitiers, France.
[3]Department of Physiology, University of California, San Francisco.
[4]CNRS-UMR 6521-Université de Brest, France.
[5]GICC– UMR 7292 Université de Tours, France.
[6]INSERM- UMR 1078 Université de Brest, France.
[7]CHRU Brest, Service d'anatomie et cytologie pathologiques Brest, France.
[8]CNRS UMR 7299, INSERM-UMR 1099, Université de Nice Sophia-Antipolis, France. [9]Ion channels Network and Cancer-Cancéropôle Grand Ouest, (IC-CGO).
[10]CHRU Tours, Tours, France.

marie.potier-cartereau@univ-tours.fr

Membrane lipid rafts are distinct plasma membrane nanodomains that are enriched with cholesterol, sphingolipids and gangliosides, with occasional presence of saturated fatty acids and phospholipids containing saturated acyl chains. It is well known that they organize receptors (such as Epithelial Growth Factor Receptor), ion channels and their downstream acting molecules to regulate intracellular signaling pathways. Among them are Ca^{2+} signaling pathways, which are modified in tumor cells and inhibited upon membrane raft disruption. In addition to protein components, lipids from rafts also contribute to the organization and function of Ca^{2+} signaling microdomains. Here we focus on the lipid raft KCa/Ca^{2+} channel complexes that regulate Ca^{2+} in cancer cells, and discusses the potential modification of these complexes by lipids as a novel therapeutic approach in tumor development.

Supported by Ligue Nationale contre le cancer, SNFGE, Région Centre Val de Loire, Cancéropole Grand Ouest, Ion channel network of Cancéropole Grand Ouest, INCa, ANR.

Symposium #3:Targeting calmodulin receptor interaction in cancer chemotherapy
ORAL COMMUNICATION O-3.2

Type 3 Inositol (1,4,5)-trisphosphate receptor: a migratory modulator with a unique Ca^{2+} signature in breast cancer cells.

Alexia Vautrin, Abdallah Mound, Arthur Foulon, Béatrice Botia, Frederic Hague, Halima Ouadid-Ahidouch, Lise Rodat-Despoix and Fabrice Matifat

Laboratory of Cellular and Molecular Physiology (EA-4667), "ion channels in breast cancer", University of Amiens, 33 Rue Saint-Leu, 80039 Amiens France.

alexia.vautrin@u-picardie.fr

As the most common lethal cancer in women worldwide, breast cancer remains a research priority. Though the involvement of ion channels in cancer cell signaling pathways is now well established, the role of inositol (1,4,5)-trisphosphate (IP_3) receptors (IP_3Rs) remains enigmatic. In this context, we investigated the involvement of the three IP_3Rs (IP_3R1, 2 & 3) in migration processes of human breast cancer cells. Migratory behavior of cancer cells was studied on three breast cancer cell lines: the low-migrating MCF-7 cell line and the highly migrating and invasive MDA-MB-231 and MDA-MB-435s cell lines. We demonstrated that a higher IP_3R3 expression at both mRNA and protein levels is correlated to a stronger migration capacity. Gene silencing of IP_3R3 leads to a significant decrease of their migration abilities without changing their proliferation rate. We then investigated the IP_3R3 calcium signature in these three cell lines. Calcium imaging assays reveal an increasing calcium resting ratio according to cellular migration capacities. IP_3R3 silencing caused a drastic modification of the temporal feature of ATP (5 µM)-induced Ca^{2+} signaling, displaying a pattern of sinusoidal Ca^{2+} oscillations instead of a plateau phase. We hypothesized that the migration capacity of breast cancer cells could be related to the temporal feature of the IP_3R3 dependent Ca^{2+} signal. Altogether, our results demonstrate that IP_3R3 is a key target in human breast cancer migration processes. It modulates the migration capacity of cells by affecting the calcium signaling profile.

Supported by the "Région Picardie" and "Fonds Européen de Développement Economique et Regional" (FEDER).

Symposium #4: Ca^{2+} remodelling in cancer S-4.1

Ca^{2+} entry abnormalities in leukemia and breast cancer cells.

Juan A. Rosado

Department of Physiology, Cell Physiology Research Group, University of Extremadura, Cáceres, Spain.

Emerging evidence has revealed that dysfunction of the mechanisms involved in intracellular Ca^{2+} homeostasis has a critical role in the development or maintenance of a several cancer hallmarks, including enhanced proliferation, invasion and apoptosis resistance. Ca^{2+} entry, especially receptor-operated Ca^{2+} influx, is a major mechanism for the regulation of Ca^{2+} homeostasis, where the proteins of the STIM, Orai and TRPC families play a central role. STIM and Orai proteins up- or downregulation in cancer cells has been reported to result in Ca^{2+} homeostasis remodeling associated to cell proliferation and evasion of apoptosis in a variety of cancer cell types. In HL60 human leukemia cells Orai2 is overexpressed both at the transcript and protein level. Orai2 silencing significantly attenuates TG-induced store-operated Ca^{2+} entry (SOCE), as well as cell migration and invasion, which it has not effect on cell proliferation. However, when we tested the expression pattern of the molecular components of Ca^{2+} entry in breast cancer cells we found overexpression of the channel TRPC6 in the luminal MCF-7 and basal MDA-MB-231 cell lines as compared to the non-tumoral MCF-10A cell line. Using fluorescence Ca^{2+} imaging, MTT proliferation, wound healing and transwell migration assays, we have found that in MCF-7 and MDA-MB-231 cells, TRPC6 plays a relevant role in SOCE, proliferation, cell migration and invasion. Summarizing, cancer cells undergo phenotypic changes leading to distinct Ca^{2+} entry abnormalities that contributes to cancer hallmarks.

Supported by grant BFU2013-45564-C2-1-P from Ministerio de Economía y Competitividad and Junta de Extremadura-FEDER (GR15029), Spain.

Symposium #4: Ca^{2+} remodelling in cancer S-4.2

Understanding and targeting the ER functions of Bcl-2 in cancer.

Geert Bultynck

KU Leuven, Leuven, Belgium.

geert.bultynck@kuleuven.be

Anti-apoptotic Bcl-2 proteins not only neutralize pro-apoptotic Bcl-2-family members at the mitochondria but also control ER Ca^{2+} fluxes. Bcl-2 directly targets different Ca^{2+} transport systems, including IP_3 receptors (IP_3R), ryanodine receptors and voltage-dependent anion channels, impacting their functional properties. These "Ca^{2+}-signaling" functions of Bcl-2 proteins contribute to their anti-apoptotic effects in cells. Moreover, different anti-apoptotic Bcl-2-family members, like the related Bcl-2 and Bcl-XL, appear to target the same Ca^{2+}-transport systems, though often via different molecular mechanisms, domains and affinities resulting in different functional outcomes. Bcl-2 inhibits excessive, pro-apoptotic IP_3R activity via its BH4 domain and C-terminal domain, but not its hydrophobic cleft. In contrast, Bcl-XL enhances spontaneous/low-level, pro-survival IP_3R activity via its hydrophobic cleft. Moreover, cancer cells exploit these "Ca^{2+}-signaling" functions of Bcl-2 proteins as a survival strategy. Tools, like BIRD-2 (Bcl-2 / IP_3R Disrupter-2) that target the BH4 domain of Bcl-2 elicit pro-apoptotic intracellular Ca^{2+} overload in B-cell cancers, including diffuse large B-cell lymphoma and chronic lymphocytic leukemia. Thus, some B-cell cancer cells are addicted to Bcl-2 at the ER to suppress Ca^{2+} signaling. This addiction to Bcl-2 is due to (i) high IP_3R2-expression levels and (ii) elevated basal IP_3 signaling downstream the B-cell receptor. BH3-mimetic drugs that target Bcl-2's hydrophobic cleft do neither impact Ca^{2+} signaling nor kill Bcl-2-dependent cancer cells in a Ca^{2+}-dependent manner, correlating with the dispensability of Bcl-2's hydrophobic cleft for IP_3R targeting and regulation. Hence, Bcl-2 proteins are critical regulators of Ca^{2+} dynamics, a property engaged by cancer cells as a pro-survival strategy.

Supported by grants obtained from the KU Leuven, Research Foundation – Flanders (FWO) and Stichting Tegen Kanker.

Symposium #4: Ca^{2+} remodelling in cancer S-4.3

Ca^{2+} remodeling in colon cancer.

Carlos Villalobos.

Instituto de Biología y Genética Molecular (IBGM). Consejo Superior de Investigaciones Científicas (CSIC) 47003 Valladolid, Spain.

carlosv@ibgm.uva.es

Ca^{2+} is involved in critical cancer processes including proliferation, invasion and survival. Recently, interest is emerging on Ca^{2+} channel remodeling in cancer. Nevertheless, the molecular and functional mechanisms of intracellular Ca^{2+} remodeling in colon cancer are unknown. We have investigated the molecular basis of intracellular Ca^{2+} handling in human colon adenocarcinoma cells and its contribution to cancer features. Fluorescence Ca^{2+} imaging, patch-clamp electrophysiology, conventional and quantitative PCR, western-blotting, gene silencing and cell proliferation and survival assays were employed for this purpose. We found that store-operated Ca^{2+} entry (SOCE), Ca^{2+} release and Ca^{2+} entry induced by agonists, and Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}) are larger in human adenocarcinoma colon cells (HT29) compared to normal human colonic mucosa cells (NCM460). Furthermore, tumor cells display a nonselective store-operated current (I_{SOC}) that is missing in normal cells. In addition, Ca^{2+} stores are partially depleted in tumor cells relative to normal cells. Enhanced SOCE and depleted Ca^{2+} stores correlate with increased proliferation and survival characteristic of tumor cells. Moreover, tumor cells display enhanced expression of TRPC1, TRPC4, ORAI1, ORAI2 and ORAI3, and loss of expression of TRPC7, TRPV6 y TRPM8. In addition, STIM2 protein expression is decreased in tumor cells compared to normal cells. Gene silencing experiments show that changes in SOCE and I_{CRAC} could be mediated by differences in ORAI1 expression whilst the change in I_{SOC} could be mediated by enhanced expression of TRPC1. In addition, Ca^{2+} store depletion and acquisition of cell death resistance is mediated, at least in part, by changes in the ratio STIM1 to STIM2. We conclude that colon cancer cells undergo a profound intracellular Ca^{2+} channel remodeling that contributes to cancer hallmarks.

Supported by grants BFU2012-37146, BFU2015-70131R and BIO/VA46/14 from Ministerio de Economía y Competitividad, Spain and Junta de Castilla y León, Spain.

Symposium #4: Ca^{2+} remodelling in cancer ORAL COMMUNICATION O-4.1

Assessment of cytosolic free calcium levels in GCaMP6m expressing MDA-MB-231 breast cancer cells undergoing cell death using time-lapse high-content imaging.

John Bassett, Amelia Peters, Sarah Roberts-Thomson and Gregory Monteith.

School of Pharmacy, The University of Queensland, Brisbane, Australia; Mater Research, The University of Queensland, Brisbane, Australia.

j.bassett@uq.edu.au

Processes important in cancer including cell death and migration occur over long periods of time making the measurement of Ca^{2+} signaling during such events suited to the use of genetically encoded calcium indicators. This study assessed alterations in cytoplasmic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) during cell death using an MDA-MB-231 triple negative breast cancer cell line stably expressing the genetically encoded calcium indicator GCaMP6m. Following treatment with ceramide (100 µM) or staurosporine (1 µM) relative changes in $[Ca^{2+}]_{CYT}$ were investigated. This was achieved by employing time-lapse high-content imaging over 6 hours in low-fluorescence FluoroBrite™ DMEM media using an ImageXpress Micro (Molecular Devices) system, in the presence of propidium iodide 1 µg/mL as an indicator of cell death. Ceramide treatment invoked a significant increase in $[Ca^{2+}]_{CYT}$. The time to reach peak $[Ca^{2+}]_{CYT}$ showed considerable variance between individual cells, from immediate to 5 hours. Peak relative $[Ca^{2+}]_{CYT}$ was greater in the subset of cells that underwent cell death (~10%). Moreover, increases in $[Ca^{2+}]_{CYT}$ always preceded increases in propidium iodide permeability (cell death). Current studies are assessing the consequences of silencing plasma membrane Ca^{2+} ATPase isoforms on ceramide-induced $[Ca^{2+}]_{CYT}$ increases and the relationship with propidium iodide permeability. Staurosoporine treatment induced an initial rapid increase in $[Ca^{2+}]_{CYT}$ and morphological changes followed by sustained oscillations in $[Ca^{2+}]_{CYT}$. Assessment of the mechanism of $[Ca^{2+}]_{CYT}$ oscillations and their association with cell death pathways may provide new insight into the relationship between calcium signaling and cell death in triple negative breast cancer cells.

Supported by National Health and Medical Research Council of Australia Project Grants (1079671, 1079672), Australian Postgraduate Award.

Symposium #4: Ca^{2+} remodelling in cancer
ORAL COMMUNICATION O-4.2

Glutamate-mediated calcium entry in megakaryocytic cells: evidence for a hijacking effect to support leukaemia growth.

Tania Kamal[1]*, James I. Hearn[1]*, Yohanes Nursalim[1], Taryn N. Green[1], Marie-Christine Morel-Kopp[2], Christopher M. Ward[2], Matthew J. During[3], Emma C. Josefsson[4] and Maggie L. Kalev-Zylinska[1,5]

[1]The University of Auckland, Auckland, New Zealand;
[2]The University of Sydney and Royal North Shore Hospital, Sydney, Australia;
[3]The Ohio State University, Columbus, OH, USA;
[4]The Walter and Eliza Hall Institute of Medical Research and University of Melbourne, Melbourne, VIC, Australia;
[5] LabPlus Haematology, Auckland Academic Health Alliance, Auckland, New Zealand.
**These authors contributed equally to this work.*

m.kalev@auckland.ac.nz

We have previously reported a novel mechanism for calcium (Ca^{2+}) entry into leukaemic megakaryoblasts that increases cell proliferation. This mechanism engages N-methyl-D-aspartate receptors (NMDARs) that are glutamate-gated calcium ion channels located in the plasma cell membrane. Intriguingly, we saw that NMDAR inhibition reduces proliferation of leukaemic cells, suggesting a novel way to interfere with megakaryocytic cancers. Here, we continued to interrogate NMDAR functionality and its contribution to the growth of normal and leukaemic megakaryocytes. Meg-01, Set-2 and K-562 cells were used as models of leukaemic megakaryoblasts. NMDAR effects in cultured cells were tested using well-established NMDAR agonists and antagonists. The role of NMDARs in normal megakaryocytes was examined using a conditional knock-out mouse model (Pf4-Grin1$^{-/-}$ mice) and in ex vivo cultures of primary mouse megakaryocytes. The NMDAR-mediated Ca^{2+} fluxes were detected in megakaryocytic cells loaded with Fluo-4-AM. When cultured in the presence of NMDAR inhibitors, leukaemic cells underwent differentiation and acquired cytoplasmic vacuoles. This pro-differentiation effect was associated with mild induction of apoptosis but more evidence for autophagy. In contrast, NMDAR antagonists reduced megakaryocytic differentiation of normal lineage-negative mouse progenitors and diminished proplatelet formation ex vivo. Further, Pf4-Grin1$^{-/-}$ mice had lower platelet counts. In conclusion, glutamate-mediated Ca^{2+} entry appears hijacked in leukaemic cells away from supporting differentiation towards supporting proliferation. We are now using a CRISPR/Cas9 system to confirm effects of pharmacological modulators in cultured cells and characterising mechanisms through which Pf4-Grin1$^{-/-}$ mice develop thrombocytopaenia. Elucidation of these effects may help design new strategies to modulate human megakaryopoiesis.

Supported by Child Cancer Foundation (project 12/17); Leukaemia and Blood Cancer New Zealand and donation from Anne, David and Victoria Norman.

Symposium #5: ER-mitochondrial Ca^{2+} signalling in disease & therapy
S-5.1

Endoplasmic reticulum-mitochondria Ca^{2+} crosstalk in the control of the tumor cell fate.

Paolo Pinton.

Dept. of Morphology, Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology, Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy.

paolo.pinton@unife.it

Mitochondria-associated membranes (MAMs) are juxtaposed between the endoplasmic reticulum and mitochondria and have been identified as a critical hub in the regulation of cell death and tumor growth. Importantly, a drastic dysregulation in Ca^{2+} signaling has been associated to different pathological states implying that Ca^{2+} communication between ER and mitochondria is vital for the physiological functioning of several intracellular processes. One key function of MAMs is to provide asylum to a number of proteins with tumor suppressor and oncogenic properties. I will discuss how manipulation of Ca^{2+} fluxes represents a key mechanism underlying the action of several oncogenes and tumor-suppressor genes.

Symposium #5: ER-mitochondrial Ca^{2+} signalling in disease & therapy
S-5.2

Novel Ca^{2+} signaling pathways in T cells: implications for autoimmunity and cancer.

Darren Boehning

University of Texas Health Science Center at Houston

Darren.F.Boehning@uth.tmc.edu

Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are critical regulators of cell death in response to multiple stimuli, including engagement of the Fas death receptor (FasR). We have shown that the FasR directly engages and activates components of the T cell receptor complex to elicit apoptotic calcium release from IP_3R channels. More recently, we demonstrated that very rapid Fas-dependent palmitoylation of the Src kinase Lck is essential for recruiting this protein into lipid rafts and initiating Fas-dependent calcium release and cell death. Surprisingly, Lck was depalmitoylated with equally rapid kinetics in the continued presence of Fas ligand. Here we show using our recently developed and highly sensitive pulse labeling technique using alkyne lipids combined with acyl-biotin exchange that there are large-scale rapid and reversible changes in the palmitoyl-proteome after Fas stimulation. We identified stimulus-dependent palmitoylation of many signaling proteins encompassing the calcium release machinery of T cells, including phospholipase C gamma-1, ZAP-70, and Orai1. Surprisingly, metabolic labeling approaches revealed that some proteins could be modified with a large variety of lipids in addition to palmitate including both saturated and unsaturated lipids of varying lengths. Our results indicate that the lipidation of signaling proteins controlling calcium release is a much more heterogeneous and widespread than previously thought with significant implications for autoimmunity and cancer.

Supported by GM081685 and GM087285 from the National Institutes of Health, USA

Symposium #5: ER-mitochondrial Ca^{2+} signalling in disease & therapy
S-5.3

Role of endoplasmic reticulum-mitochondria coupling in hepatic metabolic diseases.

Jennifer Rieusset

Institution/s: INSERM U1060, CarMeN Laboratory, Lyon, FRANCE.

Jennifer.rieusset@univ-lyon1.fr

The liver plays a central role in glucose homeostasis by consuming and producing glucose during periods of feeding and fasting, thus maintaining blood glucose concentration within a physiological range. Disruption of hepatic glucose sensing and hepatic insulin resistance predispose to hyperglycemia and to the development of liver metabolic diseases, including type 2 diabetes and non-alcoholic fatty liver diseases. Mitochondria and endoplasmic reticulum (ER) are important metabolic organelles and nutrient sensors, and their dysfunction has been extensively and independently implicated in hepatic metabolic diseases. An interesting and underestimated field of research is that both organelles interact at sites known as mitochondria-associated membranes (MAM), in order to exchange metabolites and calcium and regulate cellular homeostasis. Therefore, miscommunication between ER and mitochondria may participate to both organelle dysfunction and altered hepatic metabolism. In this context, I will present recent data showing that MAM could be an important hub for insulin and nutrient signaling in liver, thus adapting mitochondria physiology and cellular metabolism to nutrient availability. Lastly, I will highlight how chronic MAM disruption could participate to both hepatic insulin resistance and metabolic inflexibility associated with hepatic metabolic diseases. Collectively, these data suggest that targeting MAM might be a novel strategy for the treatment of hepatic metabolic diseases.

Symposium #5: ER-mitochondrial Ca^{2+} signalling in disease & therapy
ORAL COMMUNICATION O-5.1

ER-mitochondria crosstalk is regulated by WFS1/WIP1 interaction and is impaired in Wolfram syndrome.

Benjamin Delprat[1,2], Claire Angebault[1,2], Jérémy Fauconnier[3], Jennifer Rieusset[4], Jolanta Jagodzinska[1,2], Corentin A. Affortit[1,2], Camille Mégy[1,2], Chantal Cazevieille[1,2], Julia Korchagina[1,2], Delphine Bonnet- Wersinger[1,2], Dan Milea[5,6], Alain Lacampagne[3], Christian Hamel[1,2,7] and Cécile Delettre[1,2]

[1]NSERM U1051, Institute of Neurosciences of Montpellier, 34090 Montpellier, France; [2]University of Montpellier, 34090 Montpellier, France; [3]INSERM U1046, UMR CNRS 9214, University of Montpellier, 34295 Montpellier, France; [4]INSERM U1060, UMR INRA 1397, CarMeN Laboratory, Lyon 1 University, F-69003 Lyon, France; [5]Department of Ophthalmology, Angers University Hospital, 43933 Angers, France; [6]Singapore National Eye Centre, Singapore; Singapore Eye Research Institute, Singapore; Duke-NUS, Neuroscience and Behavioral Diseases, 169857 Singapore; [7]CHRU Montpellier, Centre of Reference for Genetic Sensory Diseases, CHU, Gui de Chauliac Hospital, 34090 Montpellier, France.

benjamin.delprat@inserm.fr

Communication between endoplasmic reticulum (ER) and mitochondria plays a pivotal role in calcium (Ca^{2+}) signalling, energy metabolism and cell survival. Dysfunctions of this crosstalk lead to metabolic and neurodegenerative diseases. Wolfram syndrome (WS) is a fatal neurodegenerative disease due to mutations of the ER resident protein WFS1. However, clinical phenotype of WS resemble mitochondrial disorders. Here, we show that WFS1 forms a complex with WIP1, inositol 1,4,5-triphosphate receptor (ITPR) and voltage-dependent anion channel 1 (VDAC1) to promote ER-mitochondrial Ca^{2+} transfer in response to stimuli that generate inositol-1,4,5-triphosphate. Moreover, we demonstrate that WFS1 associates with WIP1 to prevent its degradation by the proteasome. Finally, we show that WIP1 regulates VDAC expression and mitochondrial respiratory chain. Our results describe an unexpected key role of WFS1 and WIP1 in ER-mitochondria crosstalk and reconcile the ER expression of WFS1 with the mitochondrial phenotype, underlining a novel pathogenic mechanism for WS and opening new insights into the biogenesis of other neurodegenerative diseases.

Supported by grants from the Agence Nationale pour la Recherche (ANR-12-JSV1-0008-01), France

Symposium #5: ER-mitochondrial Ca^{2+} signalling in disease & therapy
ORAL COMMUNICATION O-5.2

Regulation of ER-mitochondria contacts and SERCA activity by the ER chaperones CNX and TMX1.

Tomás Gutiérrez, Arun Raturi, Carolina Ortiz-Sandoval, Gabriel Chan and Thomas Simmen.

Department of Cell Biology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada.

gutirrez@ualberta.ca

The endoplasmic reticulum (ER) and mitochondria lie in close apposition forming physical contacts in a specialized membrane domain, where a flux of Ca^{2+} between these two organelles takes place. This ion-based signaling regulates several cell functions, including mitochondrial metabolism and apoptosis. We have identified two transmembrane ER chaperones that regulate this signaling mechanism, calnexin (CNX) and TMX1. CNX KO cells have increased ER-mitochondria contacts as well as increased Ca^{2+} transfer to mitochondria, resulting in increased mitochondrial metabolism and ATP levels. This function is accompanied by reduced ER Ca^{2+} content and reduced Ca^{2+} clearance from the cytosol compared to WT cells, suggesting that CNX activates SERCA. Accordingly, CNX co-immunoprecipitates with SERCA, suggesting a direct regulation. In contrast, TMX1 has an opposite effect. TMX1 also co-immunoprecipitates with SERCA, but TMX1 KO cells show increased ER Ca^{2+} levels and their cytosolic Ca^{2+} clearance is faster compared to WT cells, suggesting that TMX1 inhibits SERCA. TMX1 KO cells also show reduced ER-mitochondria contacts and reduced Ca^{2+} transfer to the mitochondria, resulting in reduced ATP levels. Finally, TMX1 KO cells have increased CNX-SERCA interaction, indicating that CNX and TMX1 competitively interact with SERCA exerting opposite effects on its activity.

Supported by CCSRI grant 2010-700306 and CRS grant 18325. Tomás Gutiérrez was supported by an Alberta Innovates Health Solutions fellowship.

Plenary Conference
C-2

Dynamics of [Ca^{2+}] changes during bursts of activity at the 'Calyx of Held' synapse.

Erwin Neher

Nobel Price in Physiology in 1991
Max-Planck-Institute for biophysical Chemistry,
Membrane Biophysics, Göttingen, Germany

eneher@gwdg.de

Although neurotransmitter release is triggered by short-lived high-amplitude microdomains of elevated [Ca^{++}] during action potentials (APs), the cell-averaged global [Ca^{++}] controls many aspects of synaptic transmission - in particular the 'priming' of synaptic vesicles for release. We recently described a special form of priming, so-called 'superpriming', which confers high release probability to a fraction of release-ready vesicles (Taschenberger et al. 2016; PNAS, 113: E4548-57). Superpriming is a slow, but highly Ca^{++}-dependent process, providing extra strength for synaptic transmission at the onset of burts-like activity and mediating post-tetanic potentiation.
In order to be able to simulate such modulation during diverse patterns of neuronal activity, we measured [Ca^{++}]-changes using the low affinity Ca^{++}-indicator Fura6F in the Calyx of Held, a giant glutamatergic nerve terminal, which can be voltage-clamped and dialyzed through a patch pipette. This way the ionic composition of the cytosol can be controled. We studied [Ca^{++}]-changes during and after Ca^{++}-influx under various conditions of intracellular dialysis. First, we measured the endogenous Ca^{++}-binding capacity, κ_s, of immobile cytoplasmic Ca^{++}-buffers. Then we determined the Ca^{++}-clearance kinetics after step-depolarizations in the near absence of any mobile Ca^{++}-buffers. Next, we added 0.5 mM EGTA to the intracellular milieu in order to determine the kinetic parameters of this chelator. We found that Ca^{++}-affinity in the cytosolic environment seems to be lower and kinetics to be faster than in-vitro. Finally, we measured [Ca^{++}]-changes in near unperturbed terminals and asked what kind of additional cytosolic Ca^{++}-buffer we would have to postulate for optimal fitting. Again, high dissociation rates had to be postulated, compared to Parvalbumin, which had been postulated to be relevant at the Calyx.

Symposium #6: Structures of Ca^{2+} ion channels
S-6.1

Structural mechanisms of MCU regulation by divalent cations.

Samuel K. Lee[1], Santhanam Shanmughapriya[2], Muniswamy Madesh[2] and Peter B. Stathopulos[1]

[1]Department of Physiology and Pharmacology, University of Western Ontario, London, ON, Canada; [2]Lewis Katz School of Medicine, Temple University, Philadelphia, PA.

peter.stathopulos@schulich.uwo.ca

Mitochondrial matrix calcium (Ca^{2+}) regulates a plethora of physiological processes ranging from the energy required for sustaining life, to signaling processes which lead to cell death. Principal molecular machinery involved in regulating matrix Ca^{2+} include the pore forming mitochondrial Ca^{2+} uniporter (MCU) located on the inner mitochondrial membrane. While a host of proteins that regulate MCU have been identified which bind directly or indirectly to MCU, little is known about MCU autoregulation. We crystallized the large soluble N-terminal domain of MCU that resides in the matrix, revealing a contiguous negative electrostatic surface patch intimately involved in the autoregulation of MCU. We used in vitro biophysical assays coupled with cellular functional analyses to show that this MCU regulating acidic patch (MRAP) is responsive to changes in divalent cation concentrations, playing a role in modulating MCU activity. Disruptions in MRAP either by divalent cation binding or by introducing mutations destabilize and promote monomerization of the N-terminal domain, leading to inhibited MCU activity. We will present the structural and functional highlights of these findings at the ECS 2016.

Supported by grants from the NIH (to M.M.) and from CFI and NSERC (to P.B.S.).

Symposium #6: Structures of Ca^{2+} ion channels
S-6.2

Structure and Insights into the Mechanism of the IP_3-activated Calcium Release Channel.

Irina Serysheva.

Department of Biochemistry and Molecular Biology. The University of Texas Medical School. 6431 Fannin, MSB 6.210, Houston, TX 77030. USA.

Irina.I.Serysheva@uth.tmc.edu

The inositol 1,4,5-trisphosphate receptors (IP_3Rs) are Ca^{2+} release channels found in virtually all eukaryotic cells and are responsible for the release of Ca^{2+} from the endoplasmic reticulum. IP_3R channel gating is coupled to the binding of the primary ligands IP_3 and Ca^{2+}, and its activity can be tuned by binding of small molecules and regulatory proteins. Our recent structure of the IP_3R1 from rat cerebellum was solved to near-atomic resolution (4.7 Å) and resulted in the first structural model of the full-length tetrameric IP_3R channel. Over 85% of the protein backbone was resolved allowing us to identify structural elements involved in channel gating and modulation. Recently, using optimized data acquisition and analysis methods we have determined the structure of IP_3R1 in an apo-state at resolution beyond 4 Å. Using single-particle cryo-EM, we have also performed structural analysis of purified tetrameric IP_3R1 vitrified in the presence of adenophostin A, a structural mimetic of IP_3, that is a high-affinity, full agonist of IP_3Rs. These structures provide basis for understanding of the mechanistic principles by which ligand-binding triggers movements of the channel gate leading to the Ca^{2+} conductive activated state of IP_3R1 channel. All together these studies demonstrate the power of single-particle cryo-EM that is now a proven alternative to crystallography for 3D structural analysis of ion channels.

Supported by grants from NIH (R01GM072804, R21AR063255, R01GM080139, and R21GM100229), Department of Defense (R038318-1), the American Heart Association (14RNT1980029), the Muscular Dystrophy Association (295138) and National Science Foundation (DBI-1356306)

Symposium #6: Structures of Ca^{2+} ion channels
S-6.3

Structures and Mechanisms for TMBIM-mediated Ca^{2+} Leak.

Gerry Guo[1], Yanqi Chang[2], Vito Graziano[1], Sean McSweeney[1], Wayne A. Hendrickson[3], and Qun Liu[1]

[1]Brookhaven National Laboratory, [2]NGM Bio, [3]Columbia University

qunliu@bnl.gov

Ca^{2+} is a ubiquitous intracellular messenger that regulates cellular activities in plants, animals and humans. Cytosolic Ca^{2+} is kept at a low level, but subcellular organelles such as the endoplasmic reticulum (ER) maintain Ca^{2+} stores. Under resting conditions, Ca^{2+} homeostasis is dynamically regulated to equilibrate between active calcium uptake and passive calcium leak. Ca^{2+} homeostasis mediated by the Transmembrane BAX Inhibitor-1 Motif-containing (TMBIM) proteins is cytoprotective. An overloaded Ca^{2+} store promotes cell death. We determined crystal structure of a TMBIM homolog and characterized its biochemical function. The structure has a seven-transmembrane-helix fold consisting of a centralized C-terminal helix wrapped by two triple-helix sandwiches. Lateral displacement of transmembrane helix TM2 by change of pH leaves a transmembrane pore, allowing a leak of Ca^{2+} across membranes. The leak seems to be regulated by a di-aspartyl pH sensor consisting of two conserved aspartate residues. The Ca^{2+} leak is intrinsic to all kinds of cells and is cytoprotective for living organisms from stress.

Supported in part by NIH grant GM107462 and by Brookhaven National Laboratory LDRD 15-034.

Symposium #6: Structures of Ca^{2+} ion channels
ORAL COMMUNICATION O-6.1

Crystallographic investigation of the calcium sensing apparatus of voltage-gated sodium and calcium channels: effect of arrhythmia-causing mutations.

Kaiqian Wang, Jocelyn Liu, Bernd Gardill, Ching-Chieh Tung, and Filip Van Petegem

University of British Columbia, Dept. of Biochemistry and Molecular Biology, Canada

filip.vanpetegem@gmail.com

Voltage-gated sodium (NaV) and calcium (CaV) channels are membrane proteins that allow for the selective passage of Na^+ or Ca^{2+} ions across the plasma membrane. Interestingly, both have the ability to sense cytosolic Ca^{2+} levels. In several CaV channels, Ca^{2+} entering through the pore can result in accelerated inactivation, a process known as 'Calcium dependent Inactivation'. In some cases it can also increase ionic currents through the process of 'calcium dependent facilitation'. In NaV channels, cytosolic Ca^{2+} has been shown to affect channel inactivation, and to interfere with the so-called 'late sodium current'. These are essential electrophysiological properties of the NaV and CaV channels, shaping the action potentials in many excitable cells. Any interference can result in devastating conditions, such as cardiac arrhythmias and epilepsy. In both channels, the calcium-dependent feedback mechanisms have been shown to be mediated by Calmodulin. The cytosolic components that interact with Calmodulin, as well as Calmodulin itself, are targets for a multitude of disease-causing mutations. In the cardiac variants, these are known to result in Long-QT and Brugada syndromes, two types of arrhythmias. Here we present high-resolution crystal structures of these regions in both their WT and disease mutant forms, along with an electrophysiological analysis. The results show that different disease mutations are able to affect the calcium-dependent feedback mechanisms in different ways. Intriguingly, some mutations in calmodulin affect the function of the Ryanodine Receptor, whereas others selectively affect CaV channels. Comparing structures of these disease mutant forms helps explain these differential effects.

Supported by Canadian Institutes of Health Research (CIHR)

Symposium #6: Structures of Ca^{2+} ion channels
ORAL COMMUNICATION O-6.2

Ryanodine receptor in the open state with and without ryanodine.

Katrien Willegems and Rouslan Efremov

Structural Biology Research Center, Vrije Universiteit Brussel (VUB), Vlaams instituut biotechnologie (VIB), Pleinlaan 2, 1030 Brussels. Belgium

kwillege@vub.ac.be

Ryanodine receptors (RyRs) are calcium channels in the sarcoplasmic and endoplasmic reticulum crucial in the excitation-contaction mechanism in both skeletal and cardiac muscles. Recently, high-resolution structures of skeletal RyR1 in the closed state were obtained using single-particle cryo-electron microscopy (SP cryo-EM). Here we present the structures of skeletal RyR1 in the open state and in the open state bound to one of its many ligands, ryanodine.

Supported by a IWT sbo fellowship from agentschap innoveren en ondernemen, Flanders, Belgium.

Symposium #7: New tools for Ca^{2+} imaging
S-7.1

Use of CEPIA indicators for the study of intraorganellar Ca^{2+} dynamics.

Masamitsu Iino[1,2]

[1]Nihon University School of Medicine, Division of Cellular and Molecular Pharmacology, Tokyo, Japan, [2]The University of Tokyo, Graduate School of Medicine, Department of Pharmacology, Tokyo, Japan

Changes in the Ca^{2+} concentrations within the endoplasmic reticulum (ER) and mitochondria are important not only for the regulation of the cytosolic concentration of Ca^{2+} but also for the regulation of cell functions such as ER stress and cell death. We aimed at generating ER and mitochondrial Ca^{2+} indicators by changing the amino acid residues within the EF-hands of GECO, a family of GFP-based genetically encoded Ca^{2+} indicators. Considerable trial and error was required, because changes in the EF-hands often reduced not only the Ca^{2+} affinity but also the dynamic range. With appropriate optimization and addition of ER-targeting signals, we generated G-CEPIA1er, R-CEPIA1er, and GEM-CEPIA1er, which have, respectively, green, red, and blue/green fluorescence. We also generated CEPIA2mt, CEPIA3mt, and CEPIA4mt to cover the broad range of mitochondrial Ca^{2+} concentrations. The color palette of CEPIA allows us to measure ER Ca^{2+} concentrations simultaneously with other fluorescent reporters. Thus, we have succeeded in the simultaneous Ca^{2+} imaging in the ER, mitochondria and cytosol. Expressing G-CEPIA1er in central neurons, we were able to observe ER Ca^{2+} dynamics in response to synaptic input. We have generated transgenic mice in which G-CEPIA1er expression is controlled by a tetracycline-regulated system. Using these mice, we can now monitor ER Ca^{2+} dynamics in live animals. Thus CEPIA indicators are useful tools to study intraorganellar Ca^{2+} dynamics.

Supported by JSPS KAKENHI Grant Number JP21229004 and JP25221304 to MI.

Symposium #7: New tools for Ca^{2+} imaging S-7.2

Optimized Ratiometric Calcium Biosensors.

Oliver Griesbeck

Max-Planck-Institut für Neurobiologie, Martinsried, Germany

griesbeck@neuro.mpg.de

I describe a collection of FRET-based calcium biosensors with a minimized calcium binding domain and thus a reduced number of calcium binding sites per sensor. They are based on the C-terminal lobe of Troponin C and were characterized by NMR, SAXS and X-ray. Their FRET responses were optimized by a large scale functional screen in bacterial colonies, refined by a secondary screen in hippocampal neurons. Further improvements in brightness lead to sensors with excellent properties in vivo. When imaging neuronal activity in mouse cortex and olfactory bulb the performance of the most sensitive variants matched that of synthetic calcium dyes. Moreover, improved Twitch sensors allowed for high resolution imaging of calcium fluctuations during tissue migration and activation of T-lymphocytes upon encountering their antigen. The sensitivity, brightness, biocompatibility and linear response properties should make them widely useful for cellular imaging applications.

Symposium #7: New tools for Ca^{2+} imaging S-7.3

In vivo Ca^{2+} imaging in the endoplasmic reticulum with GAP sensors.

María Teresa Alonso

Instituto de Biología y Genética Molecular (IBGM), Universidad de Valladolid y Consejo Superior de Investigaciones Científicas (CSIC), Valladolid, Spain

Proper functioning of organelles such as the endoplasmic reticulum (ER) or the Golgi apparatus requires luminal accumulation of Ca^{2+} at high concentrations. Direct intra-organellar measurement of Ca^{2+} concentrations in intact preparations is essential to address complex physiological questions. Few genetically encoded Ca^{2+} indicators have been developed for high-Ca^{2+} organelles, and none of these have been used in vivo in the context of transgenic expression. We have recently developed a new family of fluorescent Ca^{2+} sensors based on the fusion of two Aequorea victoria proteins, GFP and apoaequorin, named GAP. Here we report the generation of a novel low affinity Ca^{2+} indicator, optimized for measurements in high Ca^{2+} concentration environments. Transgenic animals (mice and flies) expressing the ER-targeted sensor enabled monitoring fast physiological Ca^{2+} responses under minimally disturbing conditions, both ex vivo and in vivo.The applicability of the sensor was demonstrated under three experimental paradigms: i) ER Ca^{2+} oscillations in cultured astrocytes, ii) ex vivo functional mapping of cholinergic receptors triggering ER Ca^{2+} release in acute hippocampal slices from transgenic mice, and iii) in vivo sarcoplasmic reticulum Ca^{2+} dynamics in the muscle of transgenic flies. Our results provide proof of concept for the suitability of the new biosensors to monitor Ca^{2+} dynamics inside intracellular organelles under physiological conditions and open an avenue to explore complex Ca^{2+} signalling in animal models of health and disease.

Supported by grants from MINECO (BFU2014-53469P) and ISC III (TerCel; RD12/0019/0036 and RD16/0011/0003).

Symposium #7: New tools for Ca^{2+} imaging
ORAL COMMUNICATION O-7.1

Genetically engineered calcium indicators for visualization of calcium signals; from single cells to tissues.

Tünde Berecz[1], Kornélia Szebényi[1], András Füredi[1], László Homolya[1], Tamás Orbán[1], János Réthelyi[2], Balázs Sarkadi[1] and Ágota Apáti[1]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary.
[2]National Brain Research Project (NAP) Molecular Psychiatry Research Group, Hungarian Academy of Sciences, Budapest, Hungary

apati@biomembrane.hu

The cell permeable small molecular calcium sensitive dyes are commonly used in calcium signaling studies, while their applications have numerous drawbacks, especially in sensitive cell types such as human pluripotent stem cells (hPSC) and in their differentiated offsprings. The recently developed variants of genetically encoded Ca^{2+} indicators (GECIs) serve as alternatives for calcium imaging. To reveal the applicability of GECIs, we have generated hPSCs which stably express the GCaMP2 calcium indicator and tested the effects of various ligands in hPSCs and their differentiated derivatives (cardiomyocytes, neural and mesenchymal cell types) in parallel with similar cells loaded with Fluo4. We found no significant changes in ligand induced Ca^{2+}-signals in GCaMP2-expressing cells as compared to signaling in Fluo4-loaded hPSCs. To extend our knowledge towards neural tissues we have also generated GCaMP6fast-expressing neural cell cultures from hPSC-derived neural progenitor cells. We found that spontaneous activity and connectivity of neural tissues could be efficiently studied both in GCaMP6fast-expressing and Fluo4-loaded cells. We have also established a transgenic rat strain with one copy of the GCaMP2 transgene per allele with a defined insertion pattern, without major genetic or phenotypic alterations. Calcium signals in primary cell cultures established from the heart and kidney of transgenic rats and the proximal tubular cells in in vivo experiments were studied. From our data we concluded that this system provides a new model for studying in vitro or in vivo cellular calcium signalling and opens new possibilities for physiological and pharmacological investigations.

This work was supported by grants Hungarian Brain Research Program [KTIA VKSZ_12, NAP-A-1.10] and by the National Research, Development and Innovation Office [KTIA_AIK_12-1-2012-0025, KMR_12-1-2012-0112].

Symposium #7: New tools for Ca^{2+} imaging
ORAL COMMUNICATION O-7.2

From functional genomics to drug re-purposing: the mitochondrial uniporter case.

Fabiana Perocchi[1,2]

[1]Gene Center Munich and Department of Biochemistry, Ludwig-Maximilians-Universität, 81377 München, Germany. [2]Institute of Human Genetics, Helmholtz Zentrum München, 85764 Neuherberg, Germany.

Perocchi@genzentrum.lmu.de

Mitochondria are essential hubs of calcium-mediated signaling networks. The organelle can take up, buffer, and release calcium ions to effectively shape intracellular calcium transients, stimulate ATP production and regulate cell death. Although, the basic mechanisms of mitochondrial calcium homeostasis have been firmly established for decades, the molecular identity of the mitochondrial calcium signaling toolkit has evaded classical bottom-up approaches. Our previous studies [1,2,3] have provided a compelling example of the power of systems approaches applied to mitochondrial calcium signaling to discover hitherto unknown molecular components of the calcium uniporter. We developed computational and experimental frameworks for a systematic reconstruction of calcium-dependent signal transduction cascades in mitochondria. By combining evolutionary genomics and loss-of-function genetic and chemical screens, our systems approach holds the potential to shed light on yet unanswered questions in the field of mitochondrial calcium signaling.

1. Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliansky V, Mootha VK (2011). Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature. 476(7360):341-5.
2. Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, Mootha VK (2010). MICU1 encodes a mitochondrial EF hand protein required for calcium uptake. Nature. 467(7313):291-6.
3. Cheng Y, Perocchi F (2015). ProtPhylo: identification of protein-phenotype and protein-protein functional associations via phylogenetic profiling. NAR. 43(W1):W160-8.

Berridge Lecture
C-3

Signalling functions of store-operated calcium channels.

James W. Putney, Gary S. Bird, Felicity M. Davis, Pooja Desai, Diane D'Agostin, Shilan Wu and Natacha Steinckwich-Besançon.

Calcium Regulation Group, Signal Transduction Laboratory, National Institute of Environmental Health Sciences-NIH, Research Triangle Park, NC USA.

putney@niehs.nih.gov

Numerous physiological functions are initiated or regulated by calcium signaling. In general, Ca^{2+} signals arise from the release of Ca^{2+} from intracellular stores, from entry of Ca^{2+} across the plasma membrane, or more commonly a combination of the two. Store-operated Ca^{2+} entry is activated when the release of Ca^{2+} stores (for example by 1,4,5-IP_3) lowers endoplasmic reticulum Ca^{2+} concentration which in turn activates a Ca^{2+} sensor STIM1 (or STIM2). STIM1 aggregates at endoplasmic reticulum-plasma membrane junctions where it interacts with store-operated channels composed of Orai1 (or Orai2 or 3) subunits. To better understand the role of store-operated channels in various physiological functions, mouse models have been generated with deletions of STIM1 or 2, or Orai1, 2 or 3. Our laboratory has focused primarily on mice lacking the predominant store-operated channel subunit, Orai1. These mice are deficient in both innate and acquired immunity, in bone formation and resorption, in keratinocyte differentiation and wound healing, and in lacrimal secretion. Surprisingly, female Orai1 knockout female mice are fertile. However, pups born to Orai1 knockout females do not survive due to a failure of lactation in the dams. This appears due to a loss of Ca^{2+} oscillations in mammary gland myoepithelial cells. On the other hand, male Orai1 knockout mice are sterile. This results from a loss of sperm late in spermatogenesis. The use of mouse models with tissue-specific deletions or modifications of STIM and Orai genes should yield important new information on physiological and pathological roles of store-operated channels.

Symposium #8: MicroRNA and Ca^{2+} signalling
S-8.1

MicroRNA and calcium signaling in atrial fibrillation.

Na Li.

Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas, USA.

nal@bcm.edu

Atrial fibrillation (AF) is the most common sustained arrhythmia with an increasing prevalence worldwide. Calcium (Ca^{2+}) dysregulation in atrial myocytes is a hallmark of AF pathophysiology. Reduction of L-type Ca^{2+} current (ICa,L) and increased sarcoplasmic reticulum (SR) Ca^{2+} leak via ryanodine receptor type-2 (RyR2) contribute to the shortening of action potential duration (APD) and triggered activity respectively, predisposing to AF development. Changes in the protein level of LTCC and RyR2 have been observed in atrial samples of AF patients, and may be attributed to an altered microRNA-mediated posttranscriptional regulation. In this talk, I will review the known microRNA-mediated regulations of calcium handling proteins in the context of AF, and the possible therapeutic approaches targeting microRNAs.

Supported by the grant from American Heart Association (14SDG20080008)

Symposium #8: MicroRNA and Ca^{2+} signaling S-8.2

Regulation of smooth muscle contractility and mechanosensing by microRNAs.

Sebastian Albinsson, Karolina Turczynska, Diana Dahan, Anirban Bhattachariya, Per Hellstrand and Karl Swärd.

Department of Experimental Medical Science, Lund University, Lund, Sweden.

sebastian.albinsson@med.lu.se

Small noncoding RNAs called microRNAs play an important role for smooth muscle development and contractile function. Using smooth muscle specific Dicer KO mice we have in earlier studies demonstrated that the expression of microRNAs is essential for survival, both during embryonic development and in adult mice. Dysregulation of specific microRNAs in smooth muscle may contribute to vascular and urinary bladder disease. We therefore aimed to investigate the specific role of the highly smooth muscle enriched microRNAs, miR-143 and miR-145, in vascular and bladder smooth muscle. This was performed in a series of studies where we 1) investigated the importance of miR-143/145 for stretch-induced contractile differentiation, 2) compared the difference in sensitivity to miR-143/145 deletion in bladder and vascular smooth muscle, 3) evaluated the role of miR-143/145 for myogenic tone in small mesenteric arteries. Our results demonstrate a reduced stretch-induced contractile differentiation and an abolished myogenic constriction in miR-143/145 KO mice. This effect may partially be dependent on a decreased expression of L-type calcium channels which we have previously shown to be indirectly regulated by miR-145. Furthermore, contractile function was reduced in miR-143/145 KO vascular and airway smooth muscle but essentially maintained in in the bladder. A potential explanation for this effect is a low expression of the miR-145 target, angiotensin converting enzyme, in bladder smooth muscle. Thus, microRNAs may have varying impact on cellular function depending on the expression of their targets genes.

Symposium #8: MicroRNA and Ca^{2+} signaling S-8.3

MicroRNAs and the calcium-sensing receptor.

Enikö Kallay

Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology & Immunology, Medical University of Vienna, Austria.

enikoe.kallay@meduniwien.ac.at

The calcium sensing receptor (CaSR) is a G protein-coupled receptor involved in several signalling pathways beyond its main role in regulating calcium homeostasis. It is expressed ubiquitously. Altered CaSR signalling is associated with several pathophysiological states. In cancer, CaSR expression either increases or is reduced, even lost. As its function is tissue-specific, the regulation of its expression differs also in the different tissues. Epigenetic mechanisms, such as DNA hypermethylation, histone deacetylation, and unbalanced expression of microRNAs (miR) might be responsible for changes in CaSR expression. We and others have shown that DNA methylation reduced CaSR expression in colorectal tumours and in unfavourable neuroblastomas. However this mechanism is not responsible for the CaSR loss in parathyroid tumours.
MicroRNAs comprise a crucial group within epigenetic mechanisms that regulate gene expression post-transcriptionally. Current knowledge is limited regarding microRNAs and CaSR expression. Only few reports associated miRs with the CaSR expression. Increasing calcium concentrations through the CaSR regulated the expression of miR-9 and miR-374 in a reciprocal manner374 in the kidneys. A recent study has reported upregulation of miR-21, miR-135a/b, and downregulation of miR-145 in CaSR null colon cancer cells compared with cells that express the CaSR. A microarray study identified 22 differentially expressed miRs that potentially target the CaSR in colon cancer cells. In vitro gain- and loss-of-function studies revealed that miR-135b and miR-146b regulated CaSR protein levels while miR-9 and miR-27a had no effect. Identification of miRs that target the CaSR might prove extremely useful in finding ways to modulate CaSR levels.

Supported by the European Union Marie Curie Initial Training Network "Multifaceted CaSR", FP7-264663, and Horizon 2020 MSCA European Training Network "CaSR Biomedicine" 675228.

Symposium #8: MicroRNA and Ca^{2+} signaling
ORAL COMMUNICATION O-8.1

Cellular calcium signalling in the presence of single cell variability and dynamic stimuli.

[1]Agne Tilūnaite, [2]Wayne Croft, [3]Noah Russell, [2]Tomas C. Bellamy and [1]Rüdiger Thul

[1]School of Mathematical Sciences, University of Nottingham, Nottingham, UK;
[2]School of Life Sciences, University of Nottingham, Nottingham, UK;
[3]Department of Electrical and Electronic Engineering, University of Nottingham, Nottingham, UK.

pmxat6@nottingham.ac.uk

Under physiological conditions, cells often experience time dependent stimuli such as transient changes in neurotransmitter or hormone concentrations, but it remains an open question how cells transduce such dynamic stimuli. We exposed HEK293 cells and astrocytes to dynamically varying time courses of carbachol and ATP, respectively, and investigated the corresponding cellular calcium spike sequences. While single cells generally fail to follow the applied stimulation due to their intrinsic stochasticity and heterogeneity, faithful signal reconstruction is observed at the population level. We provide a simple transfer function that explains how dynamic stimulation is encoded into ensemble calcium spike rates. When dynamically stimulated, different cells often experience diverse stimuli time courses. Furthermore, cell populations may differ in the number of cells or exhibit various spatial distributions. In order to understand how these conditions affect population responses, we compute the single cell response to a given dynamic stimulus. Single cell variability and the small number of calcium spikes per cell pose a significant modelling challenge, but we demonstrate that Gaussian processes can successfully describe calcium spike rates in these circumstances and outperform standard tools such as peri-stimulus time histograms and kernel smoothing. Having the single cell response model will allow us to compare responses of various sets of cells to the observed population response and consequently obtain insight into tissue-wide calcium oscillations for heterogeneous cell populations.

Supported by Leverhulme Trust (RPG-2012-628).

Symposium #8: MicroRNA and Ca^{2+} signaling
ORAL COMMUNICATION O-8.2

Ryanodine receptors of acidic Ca^{2+} stores generate a non-productive Ca^{2+} release in smooth muscle cells.

Mª Cristina López-Méndez, Ubaldo García and Agustín Guerrero-Hernández

Centro de Investigación y de Estudios Avanzados del IPN (CINVESTAV-IPN Unidad Zacatenco), México.

crismas2005@hotmail.com

Ryanodine receptors (RyRs) in the sarcoplasmic reticulum produce $[Ca^{2+}]_i$ transients of various sizes, small as sparks or large as global Ca^{2+} waves. The former activate BKCa channels in smooth muscle cells and are associated with relaxation, while the latter are associated with contraction. We have shown that RyRs are expressed in three different intracellular compartments, perinuclear, cytoplasmic and superficial; however, SERCA pump is present only in the perinuclear and superficial compartments. To study the role of cytoplasmic RyRs that are devoid of SERCA pump, we have carried out simultaneous recordings of changes in the cytoplasmic (with Fura-red) and intraluminal (with Mag-fluo-4) $[Ca^{2+}]$ in freshly isolated smooth muscle cells from guinea pig urinary bladder with confocal microscopy. Transient applications of caffeine produced reproducible $[Ca^{2+}]_i$ responses both in perinuclear and cytoplasmic compartments, that were inhibited by ryanodine or thapsigargin (inhibitor of SERCA pump). However, the $[Ca^{2+}]_i$ response in cytoplasmic region was of smaller amplitude and delayed with respect to perinuclear one. The luminal $[Ca^{2+}]$ responses required external Ca^{2+} for recovery and were inhibited by thapsigargin but only in the perinuclear region, while those in the cytoplasmic region were inhibited by bafilomycin (inhibitor of V-type H^+ ATPase). These data suggest that RyRs in the perinuclear region produce the $[Ca^{2+}]_i$ response that travels to the cell surface, while RyRs in cytoplasmic region are in an acidic compartment that produces basically no $[Ca^{2+}]_i$ response to maximal activation by caffeine either because the Ca^{2+} response is short-lived or comes from a small capacity acidic Ca^{2+} store.

Supported by grant 377848 from Consejo Nacional de Ciencia y Tecnologia (MX).

Symposium #9: Ca^{2+}, stem cells and regeneration
S-9.1

Calcium signalling and CNS regeneration in planaria.

John D. Chan and Jonathan S. Marchant

Department of Pharmacology & Stem Cell Institute, University of Minnesota MN55455 USA.

march029@umn.edu

The robust regenerative capacity of planarian flatworms depends on the orchestration of signalling events from early wounding responses through the stem cell enacted differentiative outcomes that restore appropriate tissue types. Acute signaling events in excitable cells play an important role in determining regenerative polarity, rationalized by the discovery that subepidermal muscle cells express critical patterning genes known to control regenerative outcomes. These data imply a dual conductive (neuromuscular signaling) and instructive (anterior-posterior patterning) role for Ca^{2+} signaling in planarian regeneration. Here, to facilitate study of acute signaling events in the excitable cell niche, we report a de novo transcriptome assembly from the planarian Dugesia japonica allowing characterization of the diverse ionotropic portfolio of this model organism. We demonstrate the utility of this resource by proceeding to characterize the individual role of planarian voltage-operated Ca^{2+} channels during regeneration, and demonstrate that knockdown of a specific voltage operated Ca^{2+} channel (Ca_V1B) that impairs muscle function uniquely creates an environment permissive for anteriorization through dysregulation of bioaminergic signaling. The bioinformatic dataset should assist wider adoption of this model for investigations of the role of Ca^{2+} signaling in acute planarian physiology and regeneration

Supported by the NSF (MCB1615538, JSM) and a Stem Cell Biology Training Grant (T32 GM113846, JDC).

Symposium #9: Ca^{2+}, stem cells and regeneration
S-9.2

Calcium signalling in glioblastoma stem cells.

Francisco Aulestia[1], Isabelle Néant[1], Jihu Dong[2], Jacques Haiech[2], Marie-Claude Kilhoffer[2], Marc Moreau[1] and Catherine Leclerc[1]

[1]Centre de Biologie du Développement CNRS UMR5547, Université Toulouse 3, 118 route de Narbonne, F31062 Toulouse, Cedex 04, France. [2]Laboratoire d'Innovation Thérapeutique, Université de Strasbourg, UMR CNRS 7200, 74 route du Rhin, 67401 Illkirch, France

Cancer stem cells (CSCs) exist in a reversible cell-cycle arrest called quiescence. Quiescence represents one option for CSCs to evade killing following conventional therapies and quiescent CSCs are therefore the main cause of cancer recurrence. In glioblastoma, the most common and aggressive primary brain tumours, the quiescent glioblastoma stem-like cells (GSCs) are localized to hypoxic and acidic microenvironments. The functional characterization of these quiescent GSCs is a major challenge because of the potential cell cycle re-entering of these cells following microenvironmental changes. Here, we show that GSCs can be induced and maintained in a quiescent state by lowering the extracellular pH. Through RNA-seq analysis we characterized the RNA signatures of quiescent versus proliferating GSCs. In particular we identified the Ca^{2+} toolbox genes differentially expressed. Using the bioluminescent Ca^{2+} reporter EGFP-aequorin targeted to the mitochondria or the cytosol we explored how proliferating and quiescent GSCs maintain Ca^{2+} homeostasis. We showed that the switch to quiescence is characterized by both an increased capacity of quiescent GSCs' mitochondria to capture Ca^{2+} and the modification of the kinetic of Ca^{2+} influx through store-operated channels (SOC). This remodelling of Ca^{2+} homeostasis is associated with the reversible change of mitochondrial morphology from a tubular to a donut shape. Our data suggest that the remodelling of the Ca^{2+} homeostasis and the reshaping of mitochondria during the transition from proliferation to quiescence constitute a protective mechanism that favours cancer stem-like cells' survival and their aggressiveness in glioblastoma.

This work was supported in France by the Centre National de la Recherche Scientifique (CNRS), Université Toulouse 3, Université de Strasbourg and by a joint grant from the Agence Nationale de la Recherche (ANR) given between France and Hong Kong to CL, JH and MM (CalciumGlioStem ANR-13-ISV1-0004)

Symposium #9: Ca^{2+}, stem cells and regeneration
S-9.3

Role of the CD38/cADPR/Ca^{2+} signaling in differentiation of mouse ES cells.

Jianbo Yue

Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, China

jianbyue@cityu.edu.hk

Cyclic adenosine diphosphoribose (cADPR) is an endogenous Ca^{2+} mobilizing messenger that is formed by ADP-ribosyl cyclases from nicotinamide adenine dinucleotide (NAD). The main ADP-ribosyl cyclase in mammals is CD38, a multi-functional enzyme and a type II membrane protein. Embryonic stem (ES) cells are promising resources for both scientific research and clinical regenerative medicine. We found that the mouse ES cells are responsive to cADPR and possess the key components of the cADPR-signaling pathway. Therefore, we explored the role of CD38-cADPR-Ca^{2+} in the cardiomyocyte (CM) differentiation of mouse ES cells. We demonstrate that the CD38-cADPR-Ca^{2+} signaling pathway inhibits the CM differentiation of mouse ES cells. In addition, we found that the CD38-cADPR pathway negatively modulated the FGF4-Erks1/2 cascade during CM differentiation of ES cells, and transiently inhibition of Erk1/2 blocked the enhancive effects of CD38 knockdown on the differentiation of CM from ES cells. Likewise, we studied the role of CD38/cADPR in the neural differentiation of mouse ES cells. Perturbing the CD38/cADPR signaling by either CD38 knockdown or treatment of cADPR antagonists inhibited the neural commitment of mouse ES cells, whereas overexpression of CD38 promoted it. Moreover, CD38 knockdown dampened reactive oxygen species (ROS) production during neural differentiation of ES cells by inhibiting NADPH oxidase (NOX) activity, while CD38 overexpression enhanced it. Taken together, our data indicate that the CD38 signaling pathway is required for neural differentiation of mouse ES cells by modulating ROS production.

Symposium #9: Ca^{2+}, stem cells and regeneration
ORAL COMMUNICATION O-9.1

Annexin A6-mediated alanine uptake is necessary for liver regeneration in mice.

Anna Alvarez-Guaita[1], Elsa Meneses-Salas[1], Thomas Grewal[2], Carlos Enrich[1] and Carles Rentero[1]

[1]Departament de Biomedicina, Universitat de Barcelona, Barcelona, 08036, Spain; [2]Faculty of Pharmacy, University of Sydney, Sydney, NSW, 2006, Australia.

carles.rentero@ub.edu

Annexin A6 (AnxA6) belongs to a conserved family of Ca^{2+}- and phospholipid-binding proteins which interact with membranes in a calcium-dependent manner. Although AnxA6 is highly abundant in the liver (0.25% total protein), its function in the physiology of this organ remains unknown. Thus, we aimed to investigate the in vivo liver function of AnxA6 using the AnxA6 null-mutant (AnxA6$^{-/-}$) mouse model. AnxA6$^{-/-}$ mice show a highly reduced survival rate after two-thirds partial hepatectomy (PHx) associated to a prolonged hypoglycemia. A comprehensive analysis of glucose metabolism pointed to an impairment in liver gluconeogenesis in AnxA6$^{-/-}$ mice, revealing a new function for AnxA6 in liver glucose production during the regeneration process and fasting. AnxA6$^{-/-}$ hepatocytes are incapable of performing gluconeogenesis specifically from alanine, which is the main gluconeogenic substrate during liver regeneration and starvation. Interestingly, we found that AnxA6 deficiency cause an impairment of alanine uptake in hepatocytes. The results here presented suggest that AnxA6 regulates the intracellular trafficking of SNAT2, the major liver alanine transporter, to the hepatocyte sinusoidal plasma membrane. Notably, the exogenous administration of glucose rescued AnxA6$^{-/-}$ mice survival after surgery, supporting the lack of glucose production in this strain as the cause of the reduced survival after partial PHx. This study demonstrates that AnxA6 is a new regulator of hepatic gluconeogenesis and critical for mice survival after PHx. A novel role for AnxA6 in alanine uptake and as a liver gluconeogenesis regulator is described, being essential for maintaining blood glucose levels during both liver regeneration and fasting.

Supported by the Ministerio de Economía y Competitividad (MINECO) [grant numbers BFU2012-36272 and CSD2009-00016] and Fundació Marató TV3 [grant number PI042182] (Spain) to CE and The University of Sydney, Australia [grant numbers 2010-02681, U7007 and U7042] (Australia) to TG.

Symposium #9: Ca^{2+}, stem cells and regeneration
ORAL COMMUNICATION O-9.2

Voltage-dependent calcium channel signaling mediates GABAA receptor-induced migratory activation of dendritic cells infected by Toxoplasma gondii.

Sachie Kanatani[1,2], Jonas M. Fuks,[1,2] Linda Westermark,[1,2] Manuel Varas-Godoy[3], Per Uhlén[3] and Antonio Barragan, [1,2]

[1]Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, 106 09 Stockholm, Sweden, [2]Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, 141 86 Stockholm, Sweden, [3] Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden

Sachie.kanatani@su.se

The obligate intracellular parasite Toxoplasma gondii exploits cells of the immune system to disseminate. Upon T. gondii-infection, γ–aminobutyric acid (GABA)/GABAA receptor signaling triggers a hypermigratory phenotype in dendritic cells (DCs) by unknown signal transduction pathways. Here, we demonstrate that calcium (Ca^{2+}) signaling in DCs is indispensable for T. gondii-induced DC hypermotility and transmigration in vitro. We report that Toxoplasma infection modulates the expression of the GABAA receptor regulator NKCC1 with an impact on Ca^{2+} signaling and hypermotility. We found that GABA induces calcium entry in DCs. We report that murine bone marrow-derived DCs preferentially express the L-type voltage-dependent Ca^{2+} channel (VDCC) subtype CaV1.3. Silencing of CaV1.3 by short hairpin RNA or pharmacological antagonism of VDCCs abolished the hypermigratory phenotype. The present data establish that Ca^{2+} is a second messenger to GABAergic signaling in DCs and that T. gondii-induced migration of DCs requires signaling via VDCC subtype CaV1.3. The findings define a novel motility-related signaling axis in DCs. The findings also unveil that interneurons and immune cells share common GABAergic motogenic pathways and that T. gondii employs non-canonical pathways to induce host cell migration.

Supported by grants from the Swedish Research Council and the Hedlund Foundation.

ABSTRACTS OF POSTER COMMUNICATIONS

Alvarez Illera, Pilar Long-term monitoring of Ca^{2+} dynamics in C. elegans pharynx: an in vivo energy balance sensor. **P-106**

Apati, Agota Genetically engineered calcium indicators for visualization of calcium signals; from single cells to tissues. **P-98**

Arias del Val, Jessica Functional roles of MICU1 and MICU2 in mitochondrial Ca^{2+} uptake. **P-85**

Asghar , Muhammad Yasir STIM1 and Orai1 modulate the migration and proliferation of human thyroid cancer ML-1 cells. **P-56**

Aulestia, Francisco Remodeling of mitochondrial Ca^{2+} uptake capacity during the transition proliferation to quiescence of glioblastoma stem-like cells. **P-127**

Baraibar , Andres Mateo Long lasting steady-state secretory responses in ouabain- treated bovine chromaffin cells. **P-120**

Bassett , John Assessment of cytosolic free calcium levels in GCaMP6m expressing MDA-MB-231 breast cancer cells undergoing cell death using time-lapse high-content imaging. **P-54/O-4.1**

Benaim, Gustavo The intracellular Ca^{2+} regulation as a rational therapeutic target for new drugs against trypanosomatid agents of Chagas Disease and Leishmaniasis. **P-43**

Bogdanova, Anna Ca^{2+} reducing therapy for symptomatic treatment of sickle cell disease: first results of a phase IIa-b trial. **P-37**

Boglarka , Zambo Decreased PMCA4b levels in the red blood cells of healthy individuals is connected to a minor haplotype in the ATP2B4 gene. **P-36**

Bong , Alice H.L. Changes in calcium signaling as a consequence of doxorubicin treatment in MDA-MB-231 breast cancer cells expressing a genetically-encoded calcium indicator. **P-64**

Bouchet, Ana Modulation of the membrane properties by an alkyl-lipid derivate and its consequences in calcium entries and metastasis development. **P-65**

Brechard , Sabrina Regulation of neutrophil pro-inflammatory functions by Ca^{2+} signaling: Focus on cytokine secretion. **P-116**

Brohus , Malene Ca^{2+}-dependent calmodulin binding to cardiac ryanodine receptor (RyR2) calmodulin binding domains. **P-94**

Burgos, Miguel E152K STIM1 mutation deregulates Ca^{2+} signaling contributing to chronic pancreatitis **P-40/O-2.2**

Byts, Nadiya The Role of Transmembrane Prolyl-4-Hydroxylase (P4H-TM) In Calcium Signaling Regulation In Neural Cells. **P-10**

Calvo Rodriguez, Maria Aging and amyloid β oligomers enhance TLR4 expression and are required for LPS-induced Ca^{2+} responses and neuron cell death in rat hippocampal neurons. **P-5**

Calvo Rodriguez, Maria In vitro aging promotes ER-mitochondria Ca^{2+} cross talk and loss of store-operated Ca^{2+} entry in rat hippocampal neurons. **P-78**

Chantome, Aurelie Calcium sensing receptor controls SK3 channel expression and SK3-dependent cell migration in breast and prostate cancer cells. **P-63**

Chehab , Tala The sources, and characteristics, of calcium signals determine whether autophagy is up- or down-regulated. **P-75**

Corbacho , Isaac Study of calmodulin and beta-amyloid peptide binding to purified hPMCA4b-eYFP using fluorescence approaches. **P-45**

Corbacho , Isaac Expression and purification in yeasts of truncated and fluorescent human PMCA4b calcium pump. **P-46**

Czeredys , Magdalena Tetrahydrocarbazoles decrease elevated SOCE in a Huntington s disease model overexpressing huntingtin-associated protein 1 isoform A. **P-15**

Debant, Marjolaine CD5 controls B-CLL calcium constitutive entry by inducing TRPC1 expression **P-68**

Decrock, Elke Ca^{2+}, reactive oxygen species and connexin hemichannels modulate the radiation-induced bystander effect in brain microvascular endothelial cells. **P-26**

Del Re, Valeria Characterization of a calsequestrin-1 mutation identified in a patient affected by a vacuolar myopathy. **P-32**

Delprat, Benjamin ER-mitochondria crosstalk is regulated by WFS1/WIP1 interaction and is impaired in Wolfram syndrome. **P-77/O-5.1**

Dey , Kuntal SUMOylation of NCLX regulates mitochondrial localization. **P-86**

Docio , Inmaculada T-type calcium channels in rat carotid body chemoreceptor cells. Molecular and functional hallmarks. **P-24**

Dolga, Amalia The role of SK channels at the ER-mitochondria interface. **P-79**

Dupont, Genevieve Modelling the interplay between Ca^{2+} and β-amyloids dysregulations in the onset of Alzheimer's disease. **P-1**

Dyrda , Agnieszka Ion channel(s) gated by the new STIM1L isoform. **P-97**

Ellis-Dzvies, Graham BIST-2EGTA - a new caged calcium probe that is highly sensitive to 1P and 2P excitation. **P-99**

Escholten, Alexander Impaired calcium signaling of the neuronal calcium sensor GCAP1 associated with retinal distrophys cause aberrant regulation of retinal guanilate ciclase. **P-25**

Farfariello , Valerio TRPC3 regulates InsP3 receptor activity and controls senescence. **P-51**

Fliegert , Ralf Identification of a novel TRPM2 agonist. **P-117**

Fortalezas, Sofia Methyl-β-cyclodextrin impairs cytosolic calcium signaling via L-type calcium channels and NMDA receptors in cerebellar granule neurons in culture. **P-21**

Fourbon, Yann Ca^{2+} protein alpha 1D is a new mediator of colon cancer cells migration and invasion. **P-60**

Gailly, Phylippe Role of TRPV4 in pressure-induced inhibition of renin secretion by juxtaglomerular cells. **P-118**

Garcia , Martha Iveth Functional role of inositol 1,4,5-trisphosphate receptors in cardiac hypertrophy. **P-29**

Garcia Alvarado, Fernanda Repositioning of cholinergic medicines for neuroprotection against Ca^{2+}-dependent glutamate neurotoxicity. **P-22**

Garcia-Durillo , Monica Orai1 and Stim1 downregulation mediates the loss of store-operated Ca^{2+} entry in rat hippocampal neurons aged in vitro. **P-6**

Gargus , John Jay Autism susceptibility detected by high-throughput functional screen of selective calcium signaling deficit in subjects with typical sporadic autism spectrum disorder. **P-16**

Gazda , Kinga Amyloid precursor protein regulates cytosolic and endoplasmic reticulum Ca^{2+} levels, and STIM1 translocation to Orai1 channels. **P-4**

Genovese , Ilaria Mechanism of sorcin (Soluble Resistance-related Calcium binding proteIN)-dependent resistance to chemotherapeutic drugs in H1299 cancer cell line. **P-66**

Ghosh , Arijita LRRC8B: New associate of calcium signaling network. **P-82**

Gomis-Perez , Carolina Structural insights on how calcium regulates Kv7.2 channels through calmodulin **P-91**

Gonzalez , Lucia G. Inhibition of polyamine biosynthesis by DFMO reverts partially Ca^{2+} remodeling in colon cancer cells. **P-59**

Gruszczynska-Biegala , Joanna Relationship between AMPA receptors and STIM-dependent Store-Operated Calcium Entry in neurons. **P-20**

Gunaratne S., Gihan Targeting two-pore channels to inhibit MERS-Coronavirus cell entry. **P-42**

Gutierrez , Tomas Regulation of ER-mitochondria contacts and SERCA activity by the ER chaperones CNX and TMX1. **P-76/O-5.2**

Hernandez-Morales , Miriam The Warburg effect enhances store-operated Ca^{2+} entry in colon carcinoma cells by preventing inactivation of store-operated currents. **P-58**

Hogea Alexandra Store operated Ca^{2+} entry in sensory neurons. **P-17**

Honrath, Birgit SK channels regulate mitochondrial respiration and mitochondrial Ca^{2+} uptake. **P-12**

Hortua Triana, Miryam Two mechanisms of calcium entry in Toxoplasma gondii. **P-44**

Hotka , Matej Investigating the link between mitochondria, Ca^{2+} and epileptiform activity. **P-8/O-1.2**

Kaar, Aidan Evidence for Altered Calcium Homeostasis and Bcl-2 Expression in Cultured Hippocampal Neurons in Young (≤ 6 days) Rodent Models of Alzheimer's Disease (3xTg-AD Mouse & TgF344-AD Rat). **P-13**

Kalev-Zylinska , Maggie Glutamate-mediated calcium entry in megakaryocytic cells: evidence for a hijacking effect to support leukaemia growth. **P-71/O-4.2**

Kanatani , Shigeaki Cav3.2 mediates Neuronal Differentiation through Activating Caspase-3/7 **P-125**

Kanatani, Sachie Voltage-dependent calcium channel signaling mediates GABAA receptor-induced migratory activation of dendritic cells infected by Toxoplasma gondii. **P-126/O-9.2**

Kang , Jung Yun Mechanical stress-induced Ca^{2+} influx and RANKL expression in osteoblastic cells. **P-128**

Kelu , Jeffrey J. Characterizing the role of TPC2-mediated Ca^{2+} signaling during the development of slow muscle cells and primary motor neurons in zebrafish. **P-124**

Kim , Joon-Chul Shear stress induces transverse global Ca^{2+} waves via autocrine activation of P2X purinoceptors in rat atrial myocytes. **P-33**

Ko , Juyeon What happens if GCAMP is attached to TRPC channels **P-100**

Krizanova , Olga Sulforaphane-induced apoptosis involves the type 1 IP3 receptor. **P-67**

Krog Frandsen, Stine Cancer treatment using calcium electroporation induces tumor necrosis whilst sparing surrounding normal tissue. **P-69**

Kwak , Misun G α i-mediated TRPC4 activation by polycystin-1 contributes to the endothelial function via STAT1 activation. **P-49**

La Rovere , Rita Implications of ER-mitochondria Ca^{2+} signaling in cell death of malignant B cells. **P-72**

Lencesova , Lubomira Haloperidol causes translocation of the sigma 1/ type 1 IP3 receptor complex to the nucleus in differentiated NG108-15 cells. **P-87**

Leybaert, Luc Cx43 hemichannels contribute to smooth muscle Ca^{2+} dynamics and contractility. **P-112**

Leybaert, Luc Mitochondrial Cx43 forms hemichannels that contribute to calcium entry and cell death. **P-88**

Llopis , Juan Imaging calcium in cells and zebrafish with fluorescent-protein-Aequorin fusions. **P-107**

López Méndez, Maria-Cristina Ryanodine receptors of acidic Ca^{2+} stores generate a non-productive Ca^{2+} release in smooth muscle cells . **P-108/O-8.2**

Luo , Dali Calsequestrin-1 regulates store-operated Ca^{2+} entry and is involved in abnormal Ca^{2+}signaling in diabetic blood cells. **P-35**

Majewski , Lukasz STIM1 overexpression in transgenic mice brain leads to changes in synaptic plasticity. **P-3**

Mammano , Fabio ATP-induced ATP release is a key determinant of Ca^{2+} signaling in cochlear non-sensory: insight from a data driven computational model. **P-111**

Martinez Ramirez, Carmen Regulation by ER Ca^{2+} of the quantal catecholamine release in mouse chromaffin cells. **P-19**

Masoud , Said Can we use chemical induction of aging to develop a model of cardicac aging? **P-30**

Mata , Ana M. Shedding light on Tau involvement in Ca^{2+} dysregulation mediated by its interaction with PMCA. **P-9**

Matheou , Chrysanthi Mibefradil; a T-type calcium channel blocker as a potential anti-cancer agent. **P-70**

Mendez Lopez, Iago Altered mitochondrial ultrastructural changes precede the changes of Ca^{2+} and exocytotic signals in chromaffin cells of the SOD1G93A mouse model of amyotrophic lateral sclerosis. **P-11**

Methner, Axel SERCA inhibition as a novel treatment option in auto-inflammatory disease. **P-41**

Michalak, Marek Endoplasmic reticulum calcium dictates intracellular distribution of cholesterol. **P-83**

Miller , Andrew L The zebrafish scale as a possible model for studying Ca^{2+} homeostasis in vertebrates. **P-122**

Montenegro, Pablo Calcium dynamics in secretory granule subpopulations of neuroendocrine cells: An optical single-cell study. **P-105**

Moreno , Silvia A plastid Two Pore Channel and its role in organelle integrity and calcium signaling. **P-114**

Nanclares , Carmen Neurotransmission alterations related to the progression of Alzheimer´s disease in 3xTg-AD transgenic mice. **P-14**

Navas-Navarro , Paloma Endoplasmic reticulum calcium mobilization by physiological stimuli in the Langerhans pancreatic islets. **P-102**

Neant, Isabelle An interaction between TRPC1 and BMP-receptor type II regulates neural specification in Xenopus laevis. **P-130**

Overgaard , Michael A calmodulin mutation associated with LQT syndrome displays gain of function in modulating RyR2. **P-28**

Park , Eunice Yon June cAMP regulated PKD1L1 and PKD2L1 on the primary cilia. **P-38**

Pascual Caro, Carlos CRISPR/Cas-9 genome editing of STIM1 and ORAI1 in cancer cell lines. **P-119**

Peche, Georges Arielle Physiopathology of Tubular Aggregate Myopathy. **P-27**

Penny , Christopher J. Isolated pores from TPC2 reveal functional redundancy in the evolution of asymmetric ion channels. **P-92**

Perocchi , Fabiana From functional genomics to drug re-purposing: the mitochondrial uniporter case. **P-101/O-7.2**

Ponce Dawson, Silvina Changes in Ca^{2+} removal can mask the effects of geometry during IP3R mediated Ca^{2+} signals. **P-80**

Potier-Cartereau, Marie Lipid raft KCa/ Ca^{2+} channel complexes: novel targets to reduce tumor development by lipids. **P-47/O-3.1**

Pulli , Ilari Sphingosine kinase 1 regulates mitochondrial calcium. **P-84**

Rebas , Elzbieta The effect of Beta-estradiol, DHEA-sulfate and pregnenolone sulfate on activity of GABA-shunt enzymes under normal and impaired calcium homeostasis. **P-18**

Rentero Alfonso, Carles Annexin A6-mediated alanine uptake is necessary for liver regeneration in mice. **P-129/O-9.1**

Rietdorf , Katja How does fat cause heart diseases? Effects of epicardial adipocytes on cardiomyocyte signalling and contractility. **P-31/O-2.1**

Ring , Avi Novel adjunctive countermeasures to combat nerve agent poisoning and neurodegeneration. **P-23**

Rodríguez Prados, Macarena Calcium dynamics in the endoplasmic reticulum of astrocytes. Evidence for a CICR mechanism. **P-104**

Roest , Gemma The role of Ca^{2+} in ER stress-induced autophagy. **P-74**

Rojo Ruiz, Jonathan Calcium mobilization from the endoplasmic reticulum by the hypothalamic releasing factors in the adenohypophysis. **P-103**

Romain , Felix A new synthetic ether-lipid activator of TRPV2 promotes constitutive calcium entry and breast cancer cell migration. **P-62**

Rosado , Juan A Role of Filamin A as a modulator of store-operated calcium entry. **P-48**

Salido Ruiz, Gines M Role of Orai1 and Orai2 in the regulation of store-operated calcium entry, migration and FAK phosphorylation in HL60 cells. **P-55**

Savignac, Magali Cav1.2 and Cav1.3 calcium channels are critical during allergic asthma. **P-39**

Schenten , Veronique Secretion of the phosphorylated form of the alarmin S100A9 from neutrophils is essential for the pro-inflammatory functions of extracellular S100A8/A9. **P-52**

Schwab, Albrecht TRPC6 channels in neutrophil recruitment. **P-115**

Shao , Juan LETM1 forms a Ca^{2+} /H^{+} antiporter. **P-95**

Sharma , Kumar Gaurav Calcium phosphate crystals found in atherosclerotic plaques induce cytotoxic effects via loss of cellular calcium homeostasis. **P-34**

Sobradillo, Diego Contribution of Orai, Stim and TRP proteins to store-operated Ca^{2+} entry (SOCE) in human colonic mucosa normal and tumor cells. **P-57**

Stith , Bradley Lipid phosphatidic acid and an outwardly rotating disk of calcium release lead to the fertilization calcium wave. **P-131**

Sulmann, Stefan Differential Ca Ca^{2+} -sensing by GCAPs in rod and cone cells provide molecular basis of step-by-step regulation of retinal guanylate cyclase upon light activation. **P-110**

Tilunaite , Agne Cellular calcium signalling in the presence of single cell variability and dynamic stimuli. **P-109/O-8.1**

Tong , Chun Kit Benjamin Gamma-secretase cleaves stromal interaction molecule 1 induces capacitative calcium entry deficits in familial Alzheimer´s disease. **P-2**

Tong , Chun Kit Benjamin Nuclear Membrane Patch for Type 2 Ryanodine Receptor Channel. **P-89**

Van Petegem, Filip Crystallographic investigation of the calcium sensing apparatus of voltage-gated sodium and calcium channels: effect of arrhythmia-causing mutations. **P-90/O-6.1**

Varga, Karolina Expression of calcium pumps in different breast cancer cell lines during histone deacetylase inhibitor treatments. **P-61**

Vautrin , Alexia Type 3 Inositol (1,4,5)-Trisphosphate Receptor: a migratory modulator with a unique calcium signature in breast cancer cells. **P-53/O-3.2**

Vervloessem , Tamara Reciprocal sensitivity of B-cell cancer cells towards BH3 mimetics versus BH4- domain-targeting peptides. **P-73**

Wacquier, Benjamin Interplay between intracellular Ca^{2+} oscillations and Ca^{2+} -stimulated mitochondrial metabolism. **P-81**

Webb , Sarah E. Required role of store-operated Ca^{2+} entry (SOCE) for the successful completion of cytokinesis during the first two cell division cycles in zebrafish embryos. **P-123**

Wei , Zhang ALG-2 interacts with NFAT3 and inhibits transcriptional activation activity. **P-50**

Willegems, Katrien Ryanodine receptor in the open state with and without ryanodine. **P-93/O-6.2**

Wolters , Valerie NAD binding by human CD38 characterized by tryptophan fluorescence. **P-96**

Yates , Elizabeth Lysosomes, Ca^{2+} and LRRK2: A toxic relationship in Parkinson disease? **P-7/O-1.1**

Zuccolo , Estella Acetylcholine induces nitric oxide production by inducing intracellular Ca^{2+} oscillations in mouse brain endothelial cells. **P-113**

Zylinska, Ludmila Plasma membrane calcium pump in chemokine receptors-mediated calcium transient in differentiated PC12 cells. **P-121**

POSTER COMMUNICATION P-1
Calcium and Neurological Disorders
Selected for Flash Symposium Presentation

Modelling the interplay between Ca^{2+} and β-amyloids dysregulations in the onset of Alzheimer's disease.

Geneviève Dupont[1], Antonio Lobo-Antunes[1] and David Gall[2]

[1]Unit of Theoretical Chronobiology. [2]Laboratory of Physiology and Pharmacology. Université Libre de Bruxelles, Brussels, Belgium.

gdupont@ulb.ac.be

Ca^{2+} is a widespread intracellular messenger that mediates vital physiological functions in most cell types. A high level of spatio-temporal organisation of the stimulus-triggered Ca^{2+} increases ensures specificity in signalling. Modelling can be used to help understanding the molecular mechanisms responsible for these Ca^{2+} signals and to back-up experimental investigations[1]. Dysregulations of the Ca^{2+} signalling pathway are implicated in the development of neural diseases such as Alzheimer's disease[2]. In particular, the reported positive feedback loop between intra-neuronal Ca^{2+} and beta amyloids ($A\beta$) is thought to play a role in the onset of this disease[3,4]. By formalizing this loop in a minimal two variable model, we assessed the possible physiological consequences of this hypothesis and showed that it accounts for a variety of in vivo observations related to this disease[5]. Given the intricate pathways of electrical activity and Ca^{2+} entry in neurons, we next investigated if the dysregulations of Ca^{2+} homeostasis related with $A\beta$ accumulation are compatible with the reported alterations in electrical activity of hippocampal neurons. Using a prototypic model of electrical activity in this cell type[6,7], we focussed on $A\beta$-induced changes in activity of NMDA receptors and analysed the concomitant changes in electrical activity and in Ca^{2+} dynamics. Computational results confirm that Ca^{2+} and $A\beta$ both affect neuronal excitability in the same way. Interestingly, the model also provides some explanation for the reported opposite effect of Ca^{2+} on synaptic and extra-synaptic NMDA receptors.

(1) Wacquier B., Combettes L., Tran Van Nhieu G. and Dupont G. (2016) Scientific Reports 5, 19316.
(2) Berridge M. (2013) Prion 7, 2-13.
(3) Popugaeva E., Vlasova O. and Bezprozvanny I. (2015) Neurodegen. Dis. Man. 5, 395-398.
(4) Chakroborty S. and Stutzmann G. (2014) Eur. J. Pharmacol. 15, 83-95.
(5) De Caluwé J. and Dupont G. (2013) J. Theor. Biol. 331, 12-18.
(6) Roussel C., Erneux T., Schiffmann S. and Gall D. (2006) Cell Calcium 39, 455-466.
(7) Narayanan R. and Jonhston D. (2010) J. Neurophysiol. 104, 1020-1033.

POSTER COMMUNICATION P-2
Calcium and Neurological Disorders

Gamma-secretase cleaves stromal interaction molecule 1 induces capacitative calcium entry deficits in familial Alzheimer's disease.

Tong Chun-Kit[1], Lee Shuk-Kwan[1] and Cheung King-Ho[1,2]

[1]School of Biomedical Sciences, LKS Faculty of Medicine, University of Hong Kong, Hong Kong SAR, China.[2]HKU-Shenzhen Institute of Research and Innovation, Shenzhen, Guangdong, China.

ckben@hku.hk

Alzheimer's disease (AD) is the most common form of dementia and mounting evidence suggests calcium (Ca^{2+}) disruption is its proximal pathogenic origin. Ca^{2+} dysregulation observed in cells expressing familial Alzheimer's disease (FAD)-causing presenilins (PS) has been attributed to the exaggerated Ca^{2+} release and the attenuated store-operated Ca^{2+} entry (also known as capacitative Ca^{2+} entry, CCE). Several mechanisms have been proposed for the exaggerated Ca^{2+} release, yet the underlying molecular mechanisms for attenuated CCE remain elusive. In this study we employed Ca^{2+} imaging, FRET microscopy, in situ proximity ligation assay, in vitro γ-secretase cleavage assay and primary neuronal culture to delineate the mechanism for CCE attenuation and its linkage to AD pathology. We showed that the attenuation of CCE depends upon PS-associated γ-secretase activity. PS1 and STIM1 interact in human neuroblastoma SH-SY5Y cells, and mutant PS1 enhances γ-secretase cleavage of STIM1 in the transmembrane domain that has high similarity with amyloid precursor protein. Furthermore, FAD PS1-induced CCE attenuation destabilizes mature dendritic spines that are rescued by γ-secretase inhibition or overexpression of STIM1. Our results suggest a molecular mechanism of CCE deficits in which FAD-mutant PS1 enhances γ-secretase cleavage of STIM1, reducing recruitment of Orai1 that results in impaired CCE. These findings indicate a physiological role of PS1/γ-secretase in modulating the availability of STIM1 for CCE, and suggest that identification of STIM1 as a substrate of γ-secretase provides a novel therapeutic target for the treatment of AD.

Supported by the grants HKU764113M from the General Research Fund of Hong Kong and 31200883 from NSFC of China

POSTER COMMUNICATION P-3
Calcium and Neurological Disorders

STIM1 overexpression in transgenic mice brain leads to changes in synaptic plasticity.

Lukasz Majewski[1], Filip Maciag[1], Grzegorz Wiera[2], Tomasz Wójtowicz[2], Jerzy Mozrzymas[2] and Jacek Kuźnicki[1]

[1]International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Neurodegeneration, Poland; [2]Wroclaw Medical University, Department of Biophysics, Laboratory of Neuroscience. Poland.

jacek.kuznicki@iimcb.gov.pl

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder and still has no cure. Clinically, AD is characterized by progressive impairment of memory, mood and behavior changes. Altered calcium homeostasis in neurons is proposed to be one of the early events responsible for sporadic AD (SAD), the most widespread form of AD. Our group has shown that the cytoplasmic resting Ca^{2+} level in cultured neurons can be modulated by overexpression of STIM proteins, ER Ca^{2+} sensors involved in Store Operated Calcium Entry (SOCE) [J. Gruszczynska-Biegala, et al., 2011]. We also detected an enhanced magnitude of Ca^{2+} influx during SOCE in human lymphocytes from SAD patients [A. Jaworska, et al., 2013] and a decreased level of STIM2 protein in human lymphocytes from FAD patients in parallel to an attenuation of SOCE [L. Bojarski, et al., 2009]. One of the objectives of this project is to understand how elevated basal Ca^{2+} level in neurons contributes to neurodegeneration. We have generated transgenic mice lines overexpressing, specifically in brain neurons, key proteins of SOCE – STIM1, STIM2 and Orai1, and we make double transgenic mice by crossing them. We expect the STIM2/Orai1 line to have an increased basal Ca^{2+} level in neurons. We report the ongoing process of phenotyping, including LTP and LTD measurements in hippocampus and FURA 2AM calcium imaging in primary cultures from STIM1 and Orai1 transgenic lines.

Supported by funds from Maestro grant to JK from the National Science Centre (2011/02/A/NZ3/00144), Poland.

POSTER COMMUNICATION P-4
Calcium and Neurological Disorders

Amyloid precursor protein regulates cytosolic and endoplasmic reticulum Ca^{2+} levels, and STIM1 translocation to Orai1 channels.

Gazda K., Wegierski T. and Kuźnicki J.

Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology, Warsaw, Poland.

kgazda@iimcb.gov.pl

The development of Alzheimer's disease (AD) is characterized by the deposition of β-amyloid (Aβ) plaques, which are products of amyloid precursor protein (APP) regulated proteolysis by β–secretase and γ–secretase. It has been suggested that altered calcium homeostasis is one of early events responsible for disease development, but the exact role of APP in this process is obscure. Endoplasmic reticulum (ER) is the major intracellular Ca^{2+} store. ER Ca^{2+} levels are maintained by two opposite forces – Ca^{2+} pumping by sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and various Ca^{2+} release mechanisms, including passive leakage by ER "leak channels" system. Depletion of ER Ca^{2+} is sensed and counteracted by store-operated calcium entry (SOCE) machinery, composed of STIM1 ER-Ca^{2+} sensor and Orai1 plasma–membrane Ca^{2+} channel. We characterized a human cell line with high endogenous levels of APP, Orai1, STIM1 and SERCA2 proteins. The purpose of this study was to analyze how depletion of APP affects ER Ca^{2+} pools and signaling. We measured both cytoplasmic and ER Ca^{2+} levels with the use of Fura2 and CEPIA indicators. We complemented these data by quantitative co-localization analysis between endogenous STIM1 and Orai1 proteins. We show here that APP depletion affects both cytosolic and ER Ca^{2+} levels, and the rates of STIM1 translocation to Orai1 channels upon store depletion. Our data suggest that APP regulates ER Ca^{2+} homeostasis.

POSTER COMMUNICATION P-5
Calcium and Neurological Disorders

Aging and amyloid β oligomers enhance TLR4 expression and are required for LPS-induced Ca^{2+} responses and neuron cell death in rat hippocampal neurons.

María Calvo-Rodríguez[1,*], Carmen de la Fuente[1], Mónica García-Durillo[1], Carmen García-Rodríguez[1], Carlos Villalobos[1] and Lucía Núñez[1,2]

[1]Institute of Molecular Biology and Genetics (IBGM) and [2]Dept. of Biochemistry and Molecular Biology and Physiology, University of Valladolid and Spanish National Research Council (CSIC). Valladolid, Spain.

nunezl@ibgm.uva.es

Toll-like receptors (TLRs) are transmembrane pattern-recognition receptors of the innate immune system recognizing diverse pathogen-derived and tissue damage-related ligands, and evidences suggest that TLR signaling contributes to the pathogenesis of age-related neurodegenerative diseases, including Alzheimer's disease (AD). AD is associated to oligomers of the amyloid β peptide (Aβo) that cause intracellular Ca^{2+} dishomeostasis and neuron cell death in rat hippocampal neurons. Here we addressed the possible interplay between inflammation and Aβo in long-term cultures of rat hippocampal neurons, a model of in vitro aging. For this end, we used Ca^{2+} imaging and annexin V immunofluorescence staining in short-term and long-term cultures of rat hippocampal neurons to test the effects of the TLR4 agonist Lipopolysaccharide (LPS) and Aβo on cytosolic [Ca^{2+}] and apoptosis. Expression of TLR4 was tested using immunofluorescence. We found that LPS increases cytosolic [Ca^{2+}] and promotes apoptosis in long-term cultures of rat hippocampal neurons (considered aged neurons) but not in short-term cultures reflecting young neurons. Interestingly, the TLR4 antagonist CAY10614 inhibited the effects of LPS on cytosolic [Ca^{2+}] and on apoptosis. We also found that TLR4 is expressed in hippocampal neurons and the level of expression increases with age in culture. Treatment of aged neurons with Aβo further increases TLR4 expression, LPS-induced Ca^{2+} responses and neuron cell death. In summary, LPS increases cytosolic [Ca^{2+}] and promotes apoptosis in rat hippocampal neurons aged in vitro. This effect is associated to the age-dependent increase in TLR4 expression that is further enhanced by Aβo, the most likely neurotoxin in AD. We conclude that TLR4 and Aβo cross talk in neuron cell death related to aging and AD.

Supported by grants BFU2012-37146, BFU2015-70131R and SAF2013-44521R from Ministerio de Economía y Competitividad, Spain, VA145U13 and BIO/VA36/15 from Junta de Castilla y León (JCyL), Spain and RD12/0042/0026, RIC network from Instituto Carlos III, Spain. María Calvo-Rodríguez was supported by JCyL and the European Social fund. Present address for María Calvo-Rodríguez: Alzheimer Research Unit, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA.

POSTER COMMUNICATION P-6
Calcium and Neurological Disorders

Orai1 and Stim1 downregulation mediates the loss of store-operated Ca^{2+} entry in rat hippocampal neurons aged in vitro.

Mónica García-Durillo[1], María Calvo-Rodríguez[1,*], Carlos Villalobos[1] and Lucía Núñez[1,2]

[1]Institute of Molecular Biology and Genetics (IBGM) and [2]Dept. of Biochemistry and Molecular Biology and Physiology, University of Valladolid and Spanish National Research Council (CSIC) 47003 Valladolid, Spain.

monicagarciadurillo@gmail.com

Aging is associated to cognitive decline. Memory storage is linked to mushroom spines stability that depends on store-operated Ca^{2+} entry (SOCE), a pathway driven by the interaction of Stim1, a Ca^{2+} sensor at the endoplasmic reticulum and Orai1, a plasma membrane Ca^{2+} channel. The influence of aging on SOCE in hippocampal neurons remains poorly known. Here we have investigated changes in SOCE extent in rat hippocampal neurons aged in vitro and the possible contribution of molecular players involved in SOCE. For this end, we used calcium imaging and quantitative immunofluorescence in both short-term and long-term cultures of rat hippocampal neurons reflecting "young" and "aged" hippocampal neurons, respectively. Emptying of intracellular Ca^{2+} stores with SERCA pump blocker thapsigargin induces a Ca^{2+} entry pathway in identified hippocampal neurons that is prevented by SOCE antagonist Gd^{3+} and BTP2. SOCE was tested in the presence of a cocktail of ion channel antagonists to exclude contribution of voltage-gated Ca^{2+} channels. We found that SOCE in young hippocampal neurons is large and heterogeneous and tend to decrease significantly with age of culture, consistently with SOCE downregulation in aging. We also found that Orai1 and Stim1 the molecular players involved in SOCE are expressed in rat hippocampal neurons. Optical density quantification of immunofluorescence images obtained in young and aged cultures of rat hippocampal neurons using specific antibodies suggests that expression of Orai1 and Stim1 decreases significantly in aged neurons in culture. These results suggest that SOCE downregulation with aging is associated to changes in expression of molecular players involved in SOCE including Orai1 and Stim1 that may contribute to cognitive decline in the elderly.

Supported by grants BFU2012-37146, BFU2015-70131R from Ministerio de Economía y Competitividad, Spain and VA145U13 from Junta de Castilla y León (JCyL), Spain. MCR has been supported by JCyL and the European Social fund. Present address for María Calvo-Rodríguez: Alzheimer Research Unit, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA.

POSTER COMMUNICATION P-7
Calcium and Neurological Disorders
Selected for Oral Communication in Symposium #1

Lysosomes, Ca^{2+} and LRRK2: A toxic relationship in Parkinson disease?

Yates E[1], Kilpatrick BS[1], Chau D[2], Schapira AHV[2], Patel S[1]

[1]Department of Cell and Developmental Biology
[2]Department of Clinical Neuroscience, University College London, London, UK.

elizabeth.yates.13@ucl.ac.uk

The common G2019S mutation in leucine-rich repeat kinase 2 (LRRK2) is linked to familial and sporadic Parkinson disease (PD). Our previous work identified enlarged and clustered lysosomes in fibroblasts from patients with LRRK2 G2019S PD that were corrected by buffering local Ca^{2+} increases or inhibiting the endolysosomal Ca^{2+} release channel, TPC2. However, the consequences for stimulus-evoked Ca^{2+} signaling in PD remain unclear. Here we asked whether lysosome morphology is disturbed in a neuronal model of the disease and whether Ca^{2+} signaling is affected. In dopaminergic SH-SY5Y cells stably expressing LRRK2 G2019S, lysosome morphology was disrupted compared to wild type controls. Furthermore, these cells exhibited potentiated Ca^{2+} signals when stimulated with the cholinergic agonist, carbachol. Interestingly, there was no such difference in response to the peptide mediator, bradykinin. To assess the contribution of lysosomal Ca^{2+} to these signals, cells were pre-treated with the lysosomotropic agent, GPN or the novel TPC blocker, tetrandrine. Carbachol signals were inhibited but bradykinin signals were not. Our data associate defective lysosome morphology and agonist–evoked Ca^{2+} signals in LRRK2 G2019S neuronal cells with the release of Ca^{2+} from lysosomes. These findings may be an important consideration for the development of PD therapeutics.

POSTER COMMUNICATION P-8
Calcium and Neurological Disorders
Selected for Oral Communication in Symposium #1

Investigating the link between mitochondria, Ca^{2+} and epileptiform activity.

Matej Hotka[1], Michal Cagalinec[2] and Helmut Kubista[1]

[1] Department of Neurophysiology and Neuropharmacology, Center of Physiology and Pharmacology, Medical Univ. Vienna, Austria
[2] Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia.

matej.hotka@meduniwien.ac.at

In recent years a link between mitochondria and epilepsy has been proposed, which may at least partially be due to an increase of ROS production. Epileptogenesis is characterized by an abnormal electrical activity, termed the paroxysmal depolarization shift (PDS). PDS differ from normal action potentials by a prolonged depolarized shoulder lasting for several hundreds of milliseconds, mediated by an abnormal activity of the L-type calcium channels (LTCCs). LTCCs are sensitive to ROS, hence the possibility exists that ROS induced LTCC enhancement may play a role in PDS formation. Since PDS were suggested to be involved in the remodeling of neuronal networks, this may represent a crucial pathogenic mechanism in epileptogenesis. Here we investigated the link between mitochondria and epileptiform activity by studying the effect of paroxysmal depolarization shifts (induced by bicuculline + the LTCC agonist Bay K8644) on mitochondrial distribution, motility and morphology in neuronal networks formed from dissociated hippocampal cells. Likewise, the effect of experimentally induced changes in mitochondrial distribution (MGARP transfection), motility and morphology on epileptiform activity were studied. We found that PDS reversibly affected the mitochondria in the soma and the proximal neurites, by changing the mitochondrial morphology and inhibition of the movement up to 90%. We also found that neurons with disturbed mitochondrial distribution and morphology were more sensitive to PDS formation. These findings suggest the possible positive feedback mechanism in the epileptogenetic period which might be responsible for further neuronal pathology.

Funding: Austrian Science Fund (FWF, Project P-28179)

POSTER COMMUNICATION P-9
Calcium and Neurological Disorders

Shedding light on Tau involvement in Ca^{2+} dysregulation mediated by its interaction with PMCA.

María Berrocal, Isaac Corbacho and Ana M. Mata

Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura. Badajoz, Spain.

anam@unex.es

Alterations in calcium homeostasis are common hallmarks of neural aging and neurodegenerative pathologies. Among aging-related neuropathologies, Alzheimer's disease (AD) is one where both, the presence of increased levels of aberrant Tau protein and Ca^{2+} dysregulation have been associated to the neurodegenerative process. Although the relationship between the neurotoxic amyloid β-peptide, another biomarker of AD, and Ca^{2+} dyshomeostasis through Plasma Membrane Ca^{2+}-ATPase (PMCA) has been well documented, a functional connection between Ca^{2+} transporters and Tau is not fully understood. In this work we use kinetic assays to address this issue. First of all, we show that Tau inhibits PMCA activity in brain cell membranes and cell cultures. Besides, this effect seems to be age-dependent in healthy tissues but not in membranes from human brain affected by AD. This suggests that some of the molecular pathways involved in AD may change PMCA conformation to a different one with similar affinity for tau, independently of age. Besides, calmodulin was able to prevent the inhibitory effect of Tau. To characterize the effect of Tau on PMCA activity in terms of molecular interactions, kinetic assays were performed in truncated versions of PMCA. Tau did not have any effect in the variant lacking the whole C-terminal cytosolic domain. Furthermore, to get deep into the nature of Tau-PMCA interaction, effects of ionic strength were tested, showing that PMCA inhibition by Tau is inversely dependent on ionic strength. The physical Tau-PMCA interaction was also confirmed by overlay assays.

We acknowledge financial support from Spanish Ministerio de Economía y Competitividad (MINECO, BFU2014-53641-P) and Junta de Extremadura (GR15139).

POSTER COMMUNICATION P-10
Calcium and Neurological Disorders

The Role of Transmembrane Prolyl-4-Hydroxylase (P4H-TM) In Calcium Signaling Regulation In Neural Cells.

Nadiya Byts, Subodh Sharma, Jenny Laurila, Prodeep Paudel, Veli-Pekka Ronkainen, Joni Mäki, Peppi Koivunen and Johanna Myllyharju

Oulu Center for Cell-Matrix Research, Biocenter Oulu and Faculty of Biochemistry and Molecular Medicine, University of Oulu.

nadiya.byts@oulu.fi

HIF prolyl-4-hydroxylases (HIF-P4Hs 1-3) are the master regulators of hypoxia-inducible factors (HIFs). P4H-TM has also been reported to participate in HIF regulation, but its cellular effects and mechanisms are still largely unknown and it may also have other substrates. Our microarray data on P4H-TM knockout (KO) cortexes indicated expression changes in genes important for synaptic plasticity and ion/calcium transport. First, we studied the expression of calcium sequestering ATPases. Expression of the SERCA3 and SERCA2A2 isoforms as well as cellular ATPase activity were significantly up regulated in P4H-TM KO mouse astrocytes. Compensatory upregulation was observed in the expression of inositol 1,4,5-trisphosphate receptor (IP3R) type II and translocon subunits, which ensure passive leakage of calcium from endoplasmic reticulum (ER) to cytosol. To characterize calcium signaling in astrocytes we performed time-lapse imaging using Fluor-4 calcium indicator after application of different agonists. Our data revealed lower calcium response in P4H-TM KO cells after treatment with either ATP or the ionopore ionomycin, but higher response after application of the synthetic SERCA blocker Thapsigargin. This suggests lower calcium concentration inside the ER in P4H-TM KO cells. Next we analyzed consequences of the lowered calcium response to ATP. ATP induces gliotransmission in primary astrocytes, that is exocytosis of small vesicles containing gliotransmitters. We estimated vesicular exocytosis by TIRF live imaging of synaptobrevin (Syb2)-pHluorin transfected astrocytes. The number of both spontaneous fusion events and ATP-evoked events were lower in P4H-TM KO cells. To conclude P4H-TM is an important regulator of gliotransmission.

Supported by the Academy of Finland Center of Excellence 2012-2017 Grant 251314 (J.M.), by a CIMO fellowship (J.M.) and by FibroGen, Inc. (J.M.), Finland.

POSTER COMMUNICATION P-11
Calcium and Neurological Disorders

Altered mitochondrial ultrastructural changes precede the changes of Ca^{2+} and exocytotic signals in chromaffin cells of the $SOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis.

Iago Méndez-López, Antonio G. García and J. Fernando Padín

Instituto Anna Alvarez-Guaita[1] Teófilo Hernando y Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, Spain.

iagomendez@hotmail.es

Altered Ca^{2+} handling and synaptic disruption have been found in various neurodegenerative diseases. We have previously found that in adrenal chromaffin cells of APP/PS1 mouse model of Alzheimer´s disease (AD) the exocytotic fusion pore has a faster kinetics, that mediates smaller quantal size of single amperometric events (de Diego et al., Biochem Biophys Res Commun. 2012;428:482-6). In the $SOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis (ALS) we have found the opposite, namely a slower fusion pore kinetics with a higher quantal size (Calvo-Gallardo et al., Am J Physiol Cell Physiol 2015;308:C1-C19). In trying to find out a possible correlation between ultrastructural mitochondrial changes and functional changes, we here present a study done in CCs of $SOD1^{G93A}$ mice at early disease stages (P30 / P40 postnatal) and once the disease is established (P90/P120) to explore the ultrastructural changes undergone by mitochondria using confocal and electron microscopy. With respect to wildtype, $SOD1^{G93A}$ P30/P120 CCs exhibited the following mitochondrial changes: (1) higher number; (2) smaller size; (3) location at inner part of the cytosol; (4) altered morphology with vacuoles and cristae disruption; (5) more depolarized membrane potential. This contrast with functional studies that showed that P30/P40 CCs had no alterations in cell excitability, nicotinic, Na^+, and Ca^{2+} currents, $[Ca^{2+}]_c$ signals, and exocytosis. These functional parameters were certainly modified at advanced disease stages (P90/P120) namely lower cell excitability, diminished I_{ACh}, higher $[Ca^{2+}]_c$ transients, and slower kinetics of secretion. We conclude that in the $SOD1^{G93A}$ mouse model of ALS, the ultrastructural changes and distribution of mitochondria precede the functional changes linked to neurotransmitter release

Supported by SAF 2013-44108-P grant to AGG and FPI BES-2014-069005 to I. M, Ministerio de Economía y Competitividad, España. The continued support of Fundación Teófilo Hernando is also acknowledged.

POSTER COMMUNICATION P-12
Calcium and Neurological Disorders

SK channels regulate mitochondrial respiration and mitochondrial Ca^{2+} uptake.

Birgit Honrath[1], Lina Matschke[2], Tammo Meyer[3], Lena Magerhans[1], Fabiana Perocchi[4], Goutham Kumar Ganjam[1], Hans Zischka[5], Cornelius Krasel[1], Barbara M. Bakker[6], Moritz Bünemann[1], Stefan Strack[7], Niels Decher[2], Carsten Culmsee[1] and Amalia Dolga[1,3]

[1]Institute of Pharmacology and Clinical Pharmacy, University of Marburg, Germany; [2]Institute of Physiology and Pathophysiology, Vegetative Physiology, University of Marburg, Germany; [3]Faculty of Mathematics and Natural Sciences, Department of Molecular Pharmacology, University of Groningen, The Netherlands; [4]Department of Biochemistry, Ludwig-Maximilians-University Munich, Munich, Germany; [5]Institute of Molecular Toxicology and Pharmacology, Helmholtz Center Munich, German Research Center for Environmental Health GmbH, Neuherberg, Germany; [6]Center for Liver, Digestive and Metabolic Diseases, Department of Pediatrics & Systems Biology Center for Energy Metabolism and Ageing, University of Groningen, University Medical Center Groningen, The Netherlands; [7]Department of Pharmacology, University of Iowa Carver College of Medicine, Iowa City, USA.

honrathb@staff.uni-marburg.de

Small conductance calcium-activated potassium (SK) channels provide protection in different paradigms of neuronal cell death. Recently, these channels were identified at the inner mitochondrial membrane, however, their particular role in neuroprotection remained unclear. Here, we sought to investigate the distinct role of mitochondrial SK2 channels in protection against glutamate toxicity with particular focus on their involvement in the regulation of mitochondrial calcium uptake. We show that overexpression of mitochondria-targeted SK2 channels enhanced CyPPA-mediated mitochondrial resilience against glutamate toxicity, as evaluated through measurements of the mitochondrial membrane potential, mitochondrial ROS formation and cellular ATP levels in the neuronal HT22 cell line. Overexpression of a mitochondria-targeted dominant-negative SK2 channel mutant that suppresses the activity of endogenous SK2 channels located at the mitochondrial membranes inhibited CyPPA-mediated restoration of ATP levels. Analysis of respiration in isolated mitochondria revealed that SK2 channel activation, and overexpression of mitochondrial SK2 channels, attenuated basal and maximal mitochondrial respiration. Mitochondrial Ca^{2+} uptake was measured in real-time in neuronal HT22 cells via mitochondrial aequorin and in primary cortical neurons via a cameleon FRET sensor. In HT22 cells, SK channel activation attenuated mitochondrial Ca^{2+} uptake in response to extracellular ATP and carbachol stimulation, Further, FRET recordings in primary cortical neurons treated with extracellular ATP and glutamate revealed that SK channel activation reduced mitochondrial Ca^{2+} uptake not only initially but rather persistently during the time of stimulation. These findings strongly suggest that activation of mitochondrial SK2 channels mediated neuroprotection against glutamate toxicity by reducing mitochondrial respiration and mitochondrial calcium uptake.

Supported by a grant from the Deutsche Forschungsgemeinschaft, DFG (DO 1525/3-1), Germany.

POSTER COMMUNICATION P-13
Calcium and Neurological Disorders

Evidence for Altered Calcium Homeostasis and Bcl-2 Expression in Cultured Hippocampal Neurons in Young (≤ 6 days) Rodent Models of Alzheimer's Disease (3xTg-AD Mouse & TgF344-AD Rat).

A. Kaar[1], T. Vervliet[2], G. Bultynck[2] and M.G. Rae[1].

[1]University College Cork (UCC), Ireland and [2]KU Leuven, Belgium.

a.kaar@umail.ucc.ie

We are interested in the suggestion that neuronal calcium dysregulation may play a key role in Alzheimer's disease (AD) onset and progression, possibly through disruption of endoplasmic reticulum (ER) calcium handling. For the current study, cultured hippocampal neurons were prepared from control and transgenic 3xTg-AD mice and TgF344-AD rats between 3-6 days old. Using calcium imaging, group 1 metabotropic glutamate receptor (I-mGluR)-mediated somatic responses were measured under basal conditions and also under conditions where the ER was "preloaded" with calcium. In non-transgenic neurons from both models, I-mGluR activation combined with the loading stimulus evoked enhanced somatic Ca^{2+} signals relative to I-mGluR activation alone (3xTg-AD, 679±128%, n=47; TgF344-AD, 6948±1821%, n=46). In contrast, we did not observe enhanced responses in transgenic neurons (3xTg-AD, 21±14% reduction, n=36; TgF344-AD, P=0.6, n=28), suggesting a loss of this functional signaling. Secondly, we observed significantly larger I-mGluR responses under basal conditions in transgenic neurons compared with control neurons (3xTg-AD, P<0.01; n=36; TgF344-AD, P<0.01, n=28 unpaired t test), suggesting a pathological increase in ER calcium levels. Furthermore, molecular studies have revealed that the expression of B-cell lymphoma 2 (Bcl-2), which interacts with calcium release channels on the ER, is also altered relative to non-transgenic levels. The fact that such stark alterations in calcium homeostasis and signaling have been observed in neurons from rodent models of AD at such a young age (≤6 days), suggests that calcium dysregulation may occur at a much earlier stage in the disease progression than previously thought.

Supported by The Physiological Society (UK) (Seed Fund), UCC (Strategic Research Fund) and the Department of Physiology, UCC.

POSTER COMMUNICATION P-14
Calcium and Neurological Disorders

Neurotransmission alterations related to the progression of Alzheimer's disease in 3xTg-AD transgenic mice.

Carmen Nanclares, Inés Colmena, Juan Alberto Arranz-Tagarro, Antonio García García and Luis Gandía

Instituto Teófilo Hernando. Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain.

c.nanclares87@gmail.com

Alzheimer´s disease (AD) is the most common form of dementia. Alterations in several neurotransmitter systems have been reported. These alterations could be correlated with changes in the synthesis, storage or release of neurotransmitter. In this study we have used a triple transgenic murine model of AD (3xTg-AD). This animal model contains mutations in the gene encoding the amyloid precursor protein (βAPP_{Swe}), presenilin-1 ($PS1_{M146V}$) and tau_{P301L}, which determines a progressive development of the disease. We propose to study here the last steps of exocytosis in chromaffin cells of 3xTg-AD mice of different ages using the amperometric technique. We have found significant changes in mice of 6 and more than 12 months of age, where the pathology is already established and consolidated, respectively, when compared with prepathologic mice (2 months). These changes show an increase of the amperometric spikes during the development of the disease, although the quantal catecholamine content of each spike is lower. Kinetic analysis of secretory spikes shows that as the disease progresses amperometric spikes are faster and shorter in duration. Using patch-clamp technique we have measured different ionic currents implicated in catecholamine release, namely, nicotinic currents and Na^+, Ca^{2+} and K^+ currents. It is worth mentioning that an augmentation in Na^+, Ca^{2+} and K^+ currents has been observed as the disease progresses. These preliminary data indicate the existence of alterations in the neurosecretory process in chromaffin cell of 3xTg-AD mice, which could form the basis of the various neurotransmitter deficits that occur with the progression of AD.

Supported by SAF2013-44108-P and Fundación Eugenio Rodríguez Pascual, Spain.

POSTER COMMUNICATION P-15
Calcium and Neurological Disorders

Tetrahydrocarbazoles decrease elevated SOCE in a Huntington's disease model overexpressing huntingtin-associated protein 1 isoform A

Magdalena Czeredys[1], Filip Maciąg[1], Axel Methner[2] and Jacek Kuźnicki[1]

[1]Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology in Warsaw, Trojdena 4, 02-109 Warsaw, Poland. [2]Department of Neurology, University Medical Center and Focus Program Translational Neuroscience (FTN) of the Johannes Gutenberg-University Mainz, Langenbeckstr. 1, D-55131 Mainz, Germany.

mczeredys@iimcb.gov.pl

Store-operated Ca^{2+} entry (SOCE) is one of the mechanisms that regulate Ca^{2+} homeostasis and is enhanced in Huntington's disease (HD). However, it is still unknown how mutated huntingtin affects SOCE and there is no effective treatment of HD. We previously showed that huntingtin associated protein 1 (HAP1) is up-regulated in the striatum of HD model, YAC128 mice. We also found that selected tetrahydrocarbazoles stabilize the ER Ca^{2+} release in cellular Alzheimer's disease model. The aim of this work was to investigate the role of HAP1 protein in SOCE dysregulation and check the effect of tetrahydrocarbazoles on the ER Ca^{2+} release and SOCE as well as cell death in YAC128 medium spiny neurons (MSNs). Single cell Ca^{2+} imaging, gene silencing and overexpression as well as cell death and mitochondrial membrane potential assays were used for this purpose. We observed that HAP1 isoform A overexpression decreases ionomycin induced ER Ca^{2+} release and enhances SOCE, whereas its silencing attenuates SOCE and decreases ER Ca^{2+} release induced by DHPG, an $mGluR_{1/5}$ receptor agonist. In HD MSNs overexpressing HAP1A we found that certain tetrahydrocarbazoles have a stabilizing effect on elevated SOCE, however, no effect on ER Ca^{2+} release was observed. Moreover, we found that some of them increase mitochondrial membrane potential, but they are not able to stabilize glutamate induced cell death in HD model. We conclude that HAP1A increases SOCE in HD MSNs by IP3R activation and tetrahydrocarbazoles exhibit stabilizing effect on the disturbed Ca^{2+} homeostasis in HD model.

Supported by the European Regional Development Fund in the framework of the POMOST project no /2013-8/4 of Foundation for Polish Science for MCZ.

POSTER COMMUNICATION P-16
Calcium and Neurological Disorders

Autism susceptibility detected by high-throughput functional screen of selective calcium signaling deficit in subjects with typical sporadic autism spectrum disorder.

G. Schmunk[1,2], R. L. Nguyen[2], K. Kumar[2], Y.-H. Sung[1,2], D. L. Ferguson[1,2], I. Parker[1,2,3], and J. J. Gargus[1,2,4]

Institution/s: [1]Department of Physiology and Biophysics, School of Medicine, University of California, Irvine, California, USA [2] Center for Autism Research and Translation, (UCI-CART) University of California, Irvine, California, USA [3]Department of Neurobiology and Behavior, School of Biological Sciences, University of California, Irvine, California, USA [4]Division of Human Genetics & Genomics, Department of Pediatrics, School of Medicine, University of California, Irvine, California, USA.

jjgargus@uci.edu

Genetic evidence has improved our understanding of autism spectrum disorder (ASD) pathophysiology, identifying over 800 genes contributing to susceptibility, and supporting disrupted Ca^{2+}signaling in ASD etiology. Using "optical patch clamp" techniques we had discovered shared kinetic alterations in Ca^{2+}channel function of IP3Rs in fibroblasts from patients with three monogenic forms of ASD. Whereas there were no marked differences in Ca^{2+} store filling, unitary-event amplitudes, or latency to first opening elicited by photolysis of caged IP_3, all ASD cells showed a much shorter mean open channel time and a dramatic reduction in the apparent number of release sites. We have now developed a high-throughput screen to utilize this consistent signaling abnormality as a tractable biomarker and potential diagnostic, employing a custom, highly reproducible FLIPR assay for purinergic activation for this molecular difference between ASD cells and controls. We have extended our characterization to fibroblasts from deeply-phenotyped, genome-sequenced UCI-CART subjects with typical sporadic ASD, and confirmed the same signaling abnormality. We also now show that iPSC-derived human neuronal progenitors harbor the same IP_3 signaling deficit, suggesting its role in the neuronal function and pathophysiology of ASD. In view of the extreme heterogeneity of ASD, these preliminary results strongly suggest that dysregulated IP_3R signaling lies at a central node in a molecular pathway leading to core features of autism. Fibroblasts, routinely acquired as clinical specimens, may thus offer a promising technique in conjunction with behavioral testing for early detection of susceptibility to ASD, and for high-throughput screening of novel therapeutic agents.

Supported by The Thompson Family Foundation through a generous gift to UCI-CART, the UCI Center for Autism Research and Translation through The Center for Autism and Neurodevelopmental Disorders, an entrepreneurial public-private partnership (J.J.G.) and National Institutes of Health grant GM048071 (I.P.).

POSTER COMMUNICATION P-17
Calcium and Neurological Disorders

Store operated Ca^{2+}entry in sensory neurons.

Hogea Alexandra and Gamper Nikita.

Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom.

bsasg@leeds.ac.uk

Store-operated Ca^{2+} entry (SOCE) is necessary for maintaining intracellular Ca^{2+} signalling through the receptor-mediated release of Ca^{2+} from endoplasmic reticulum (ER). The molecular composition of SOCE in dorsal root ganglia (DRG), its regulation and role in inflammatory pain are poorly understood. Here we investigated functional expression of STIM and Orai proteins in DRG using immunohistochemistry and fluorescent imaging. We report that STIM1 is predominant in cytosol of small/medium diameter neurons while larger neurons predominantly express STIM2. Orai1 and Orai3 were found at the plasma membrane (PM) of neurons of all sizes but Orai1 was more abundant. Orai2 was found mostly in the nuclei. In the PM of large-diameter neurons Orai1 often co-localized with a PM-ER junctional protein junctophilin-4. SOCE inhibitor YM58483 reduced SOCE induced in by store depletion/ Ca^{2+} add-back paradigm DRG neurons, satellite glia (SGC) and HEK293 cells with IC_{50} of 0.39μM, 0.36μM and 0.41μM respectively. YM58483 and another SOCE inhibitor, Synta66, strongly suppressed sustained cytosolic Ca^{2+} entry generated by bradykinin in Fura-2AM loaded DRG neurons 93.04% ± 44.34% and 85.14% ± 12.49% respectively. Accordingly, ER store-refill after bradykinin-induced depletion in both SGC and bradykinin receptor expressing HEK293 cells (measured with ER Ca^{2+} indicator G-Cepia) was also abolished. Interestingly, YM58483 inhibited SOCE only after 1 hour pre-incubation but not when applied acutely during either Ca^{2+} add-back or store-depletion, suggesting that this compound is not a Ca^{2+} release-activated channel (CRAC) pore blocker. In sum, we characterized molecular composition and pharmacological properties of SOCE in DRG neurons and glia and demonstrated its importance for bradykinin signalling.

Supported by the University of Leeds, UK.

POSTER COMMUNICATION P-18
Calcium and Neurological Disorders

The effect of 17β-estradiol, DHEA-sulfate and pregnenolone sulfate on activity of GABA-shunt enzymes under normal and impaired calcium homeostasis.

Kinga Kusmirowska, Antoni Kowalski and Elzbieta Rebas.

Medical University of Lodz, Department of Molecular Neurochemistry, Poland.

elzbieta.rebas@umed.lodz.pl

GABA-shunt is involved in metabolism of two reversely acting neurotransmitters. This pathway comprises three enzymes: glutamate decarboxylase (existed in two isoforms GAD65 and GAD67), GABA-aminotransferase (GABA-T) and succinate semialdehyde dehydrogenase (SSADH). The aim of our study was to examine effect of selected steroid hormones on GABA-shunt enzymes activity. Because action of steroids can be affected by calcium ions, we performed experiments under normal and disrupted calcium homeostasis. We used stably transfected pseudoneuronal PC12 cells with reduced expression of neuron-specific isoforms of PMCA (2 or 3) and increased intracellular calcium concentration. In control cells, with normal calcium homeostasis estradiol stimulates activity of GAD, inhibits activity od GABA-T and does not change activity of SSADH. It can leads to increased level of GABA in cytoplasm. In the presence of fulvestrant, a selective inhibitor of estradiol receptors, effect of estradiol is a little stronger. We suggest that estradiol receptors, both membrane and intracellular, are not involved in this action. Additionally we observe, that expression of all GABA-shunt enzymes is not changes. In cells with disturbed calcium homeostasis effect of estradiol is quite different, it suggests that high calcium concentration modify estradiol effect on neurotransmitters metabolism. Interestingly, effect of two tested neurosteroids is similar to each other but different from estradiol. We conclude, metabolism of glutamate and γ-amino butyric acid depends on some steroid hormones in non genomic way and impaired calcium conditions puts out of order GABA-shunt work.

Supported by the Medical University of Lodz grants 502-03/6-086-02/502-64-086; 503/6-086-02/503-61-001

POSTER COMMUNICATION P-19
Calcium and Neurological Disorders

Regulation by ER Ca^{2+} of the quantal catecholamine release in mouse chromaffin cells.

Carmen Martínez-Ramírez, Iago Méndez López and Antonio G. García

Instituto Teófilo Hernando, Universidad Autónoma de Madrid, Spain.

mencita_1991@hotmail.com

We have recently found alterations in the kinetics of the exocytotic fusion pore in chromaffin cells (CCs) of an APP/PS1 mouse model of Alzheimer's disease (AD) (De Diego AM et al., Biochem Biophys Res commun, 2010; 428:482-6) and in CCs of a SOD1G93A mouse model of amiotrophic lateral sclerosis (ALS) (Calvo-Gallardo E et al., Am J Physiol Cell Physiol, 2015; 308:C1-C19). On the other hand, whether the handling of Ca^{2+} by the endoplasmic reticulum (ER) is involved in these altered secretory responses in these transgenic mice is unknown. As a first step of a project that focus on that question, here we have investigated how the inhibition by thapsigargin (Thap) and cyclopiazonic acid (CPA), two inhibitors of the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA), affected the quantal release of catecholamine monitored at the single-cell level with a carbon fibre microelectrode, in CCs from C57BL6J mice. Furthermore, they were repeatedly challenged with sequential pulses of acetylcholine (ACh), a condition reminding prolonged stress. In control cells we have found that the initial secretory burst of secretory spikes elicited by ACh (pulses of 100 µM, 3 s, given at 15 s intervals) decayed with time. However, upon Thap (0.5 µM) or CPA (5 µM) application the secretory responses to ACh were maintained about 50% above the control. We conclude that Ca^{2+} handling by the ER is necessary to reload with new vesicles the secretory machinery and maintain a healthy secretory response to repeated pulses of ACh at 37ºC. Future experiments will explore whether the ER is similarly influencing the quantal release of catecholamine in the SOD1G93A mouse model of familiar ALS.

Supported by grants SAF-44108-P 2013 from Ministerio de Economía y Competitividad, Spain.

POSTER COMMUNICATION P-20
Calcium and Neurological Disorders

Relationship between AMPA receptors and STIM-dependent, Store-Operated Calcium Entry in neurons.

Joanna Gruszczynska-Biegala, Maria Sladowska and Jacek Kuznicki

International Institute of Molecular and Cell Biology, Warsaw, Poland.

joannag@iimcb.gov.pl

Store-Operated Calcium Entry (SOCE) is a process, which leads to refilling of endoplasmic reticulum (ER) with calcium ions (Ca^{2+}) after their release to the cytoplasm. The interaction between (ER)-located proteins (STIM1, STIM2) and plasma membrane (PM)-located Ca^{2+} channel-forming protein (ORAI1) mediates the formation of complexes and underlies SOCE in non-excitable cells. Recent studies have recognized the importance of SOCE also in neurons and found complex relationship between STIM proteins and neuronal calcium channels, but its molecular mechanism in neurons requires more detailed investigation. Our previous data indicated that both STIMs are involved in Ca^{2+} homeostasis in neurons, form complexes with endogenous ORAI1, but play a distinct role in SOCE. In contrast to non-excitable cells, Ca^{2+} influx in neurons is modulated mainly by voltage-gated Ca^{2+} channels and ionotropic receptor-operated Ca^{2+} channels. Here we report, that SOCE inhibitor ML-9 reduces AMPA-induced Ca^{2+} influx to 20%. To assess the role of AMPA receptors (AMPAR) in SOCE, they were inactivated in cortical neurons by their specific inhibitors. As estimated by FURA-2AM single-cell Ca^{2+} measurements in the presence of CNQX or NBQX thapsigargin-induced Ca^{2+} influx was decreased 2.2 or 3.7 times, respectively. These results suggest that during SOCE, calcium ions can enter neurons also through AMPA receptors. In addition, we found by co-immunoprecipitation assays, when Ca^{2+} level is low in neuronal ER, a physical association of endogenous STIM proteins with endogenous GluA1 or GluA2 subunits of AMPAR occurs. Taken together, these data suggest an involvement of AMPAR in SOCE and its link with STIM proteins.

Supported by funds from a National Science Centre (2011/01/D/NZ3/02051, JGB).

POSTER COMMUNICATION P-21
Calcium and Neurological Disorders

Methyl-β-cyclodextrin impairs cytosolic calcium signaling via L-type calcium channels and NMDA receptors in cerebellar granule neurons in culture.

Sofia Fortalezas, Dorinda Marques-da-Silva and Carlos Gutierrez-Merino

Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura. Badajoz, Spain.

salmeidar@almunos.unex.es

In previous works we have shown that the control of cytosolic calcium in mature cerebellar granule neurons (CGN) within the concentration range required for neuron survival is largely dependent on the activity of plasma membrane calcium transport systems that are largely clustered within caveolin-1-rich lipid rafts nanodomains [Marques-da-Silva and Gutierrez-Merino (2014) Cell Calcium 56, 108-123]. In this work, we have studied the effects of treatments of CGN with methyl-β-cyclodextrin (MβCD), which disrupts lipid rafts in biological membranes, on the steady-state cytosolic calcium, on the calcium entry through L- type calcium channels (LTCC's) and on the rise of cytosolic calcium induced by L-glutamate through NMDA receptors activation. To this end, CGN were loaded with Fura-2AM and ratio 340/380 images were acquired as indicated in previous works. The results showed that treatment of CGN with MβCD largely attenuated the calcium entry through LTCC's and also through NMDA receptors, before a significant loss of cell viability was observed. As a result MβCD lowered the steady-state cytosolic concentration of CGN in the survival 25 mM KCl medium to values close to those measured in the pro-apoptotic 5 mM KCl medium. This effect correlated with the decrease of the phosphorylation level of the β_2 subunit of LTCC's and of NMDA receptors from the high levels measured in 25 mM KCl medium to those measured in 5 mM KCl medium. Thus, our results highlight the relevance of lipid rafts for the control of cytosolic calcium homeostasis within the survival range in mature CGN in culture.

Supported by Grant BFU2014-53641-P of the Spanish Ministerio de Economía y Competitividad and by Grant GR15139 of the Junta de Extremadura to research group BBB008]. Sofia Fortalezas is supported by predoctoral Fellowship FRH/BD/84543/2012 of the Fundação para a Ciência e Tecnologia, Portugal.

POSTER COMMUNICATION P-22
Calcium and Neurological Disorders

Repositioning of cholinergic medicines for neuroprotection against Ca^{2+}-dependent glutamate neurotoxicity.

Fernanda García-Alvarado[1], Estefanía Alonso-Gómez[1], Paloma Ania[1], Laia Montell[2], Josep Vergés[2] and Antonio G. García[1].

[1]*Instituto Teófilo Hernando, Universidad Autónoma de Madrid, Spain*
[2]*Bioibérica Laboratories, Barcelona, Spain.*

fernanda0788@hotmail.com

Excess synaptic glutamate concentration causes Ca^{2+} overload and apoptotic neuronal death. The process includes mitochondrial Ca^{2+} overload, opening of the permeability transition pore, release of cytochrome c, and the activation of the apoptotic cascade. This cell death pathway is also associated to redox imbalance, with overproduction of reactive oxygen species (ROS) that may also contribute to neuronal damage. This pathway has been implicated in the neuronal damage chronically occurring in Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS), as well as in brain and spinal cord trauma, and in stroke (De Diego, et al. In "Therapeutic Targets: Modulation, Inhibition and Activation". L. Botana and M. Loza (eds). Wiley & Sons Inc. pp. 123-200, 2012). As a strategy to find novel neuroprotective agents with potential therapeutic applications in those diseases, we have built an experimental platform to test clinically used medicines to find a repositioning action in neuroprotection. In rat embryo primary cortical neurons, we have defined the conditions to elicit about 40% neuronal death with a 30 min glutamate pulse; such Ca^{2+}-dependent neurotoxic effect is fully antogonised by MK801, indicating the involvement of Ca^{2+}-permeable NMDA receptors in the cell death process. We have selected 30 medicines with clinical applications in CNS and peripheral processes, with mechanisms of action linked to muscarinic and nicotinic receptors or to inhibition of acetylcholinesterase (AChE). We have found neuroprotective effects of some muscarinic ligands as well as nicotinic receptor ligands that may potentially have a repositioning receptor collateral indication in CNS diseases above mentioned.

Supported by CABICYC UAM-Bioibérica and Fundación Teófilo Hernando, Spain.

POSTER COMMUNICATION P-23
Calcium and Neurological Disorders

Novel adjunctive countermeasures to combat nerve agent poisoning and neurodegeneration.

Avi **Ring[1], Anders Ziegler[1], Øystein Rusten[1,2], Marthe Fredheim Fjelldal[1,2], Rita Tansø[1] and Ragnhild E. Paulsen[2]**

Norwegian Deefence Research Est., Norway;
Dept. of Pharmacy, University of Oslo, Norway.

avi.ring@ffi.no

CNS effects of nerve agent (na) poisoning inactivates acetylcholinesterase (AChE) resulting in unrestrained acetylcholine (ACh) stimulation of nerve cells which stimlates increased release of glutamate and overstimulation of calcium permeable NMDA receptors (NMDAR), present in virtually every nerve cell in the brain. The efficiency of the standard treatment of the epilepsia and excitotoxicity that develops is limited because a) different nerve agents require different AChE reactivators and b) after 20-30 min both antimuscarinics and GABAergics have limited effect. The epilepsia is then autonomously driven by the released glutamate causing lethal convulsions and neurodegeneration. Glutamate receptor inhibitors are therefore potential adjunctives to the standard treatment but their clinical applications have largely failed. Nevertheless, NMDAR inhibitors are potent anticonvulsive and neuroprotective agents and we developed an experimental model using primary cultures of forebrain neurons from 10 day old chicken embryos to screen NMDAR inhibitors. The in-vitro model is simple, economical and Reduces/Replaces animal experiments. We used calcium responses to compare potencies and evaluate the benefit of inhibitors. We compared potencies on different molecular targets for their potential use to inhibit epilepsia and calcium overloading. Two groups of inhibitors were considered a) Inhibitors specific to the NR2B subtype NMDAR's, abundant in brain regions where seizures develop and b) omega-3 fatty acids which inhibit different types of ion channels and, specifically, also NMDAR's. We propose that these strategies will aid to control seizure development/stop epilepsia after na poisoning with significantly less toxic side effects than other NMDAR inhibitors.

POSTER COMMUNICATION P-24
Calcium and Neurological Disorders

T-type calcium channels in rat carotid body chemoreceptor cells. Molecular and functional hallmarks.

Inmaculada Docio, María Ramírez, Ana Isabel Cáceres, Elena Olea, Teresa Gallego and Asunción Rocher

Departamento de Bioquímica, Biología Molecular y Fisiología. Instituto de Biología y Genética Molecular (IBGM). Universidad de Valladolid.
CIBERES, ISCiii. Valladolid, Spain.

inma.docio@ibgm.uva.es

An important path of extracellular calcium influx in carotid body chemoreceptor cells (CBCC) during hypoxic activation and neurotransmitter release is through voltage activated calcium channels of the plasma membrane. Both high (HVA) and low voltage-activated (LVA) Ca^{2+} channels are present in CBCC, yet no much is known about the relevance of the LVA T-type channels. Three different genes codify for T-type channels, Cav3.1, Cav3.2 and Cav3.3, but only the Cav3.2 isoform has been found in carotid body. It has been reported that chronic hypoxia (CH) up-regulates Cav3.2 channel expression in PC12, chromaffin and pulmonary artery smooth muscle cells and, more recently, in CBCC. In the present study, we investigate the expression and the role of T-type Ca^{2+} channels in rat CB responses to acute and chronic hypoxia using a combination of pharmacological and molecular approaches. By immunocytochemistry and RT-PCR experiments we provide molecular evidence for a main presence of Cav3.1 and minor Cav3.2 expression, but not Cav3.3, in CBCC. Rats exposure to CH during 7 days up-regulates both, Cav3.1 and Cav3.2 channels, but mainly the former. Hypoxia stimulated CB response can be 50% reduced by specific T-type inhibitors: low concentration of mibefradil, Ni^{2+} and TTA-A2 and TTA-P2 blockers. We demonstrated augmented responses in CH treated rat CB which also are 50% sensitive to Ni^{2+} and mibefradil. We conclude that in rat CBCC, Cav3.1 is the predominant T-type calcium channel contributing to basal and CH augmented CBCC excitability and secretory response.

Supported by grants BFU2015-70616R from MINECO and ISCiii CIBER CB06/06/0050.

POSTER COMMUNICATION P-25
Calcium and Neurological Disorders

Impaired Ca^{2+}-signaling of the neuronal Ca^{2+}-sensor GCAP1 associated with retinal dystrophies cause aberrant regulation of retinal guanylate cyclase.

Valerio Marino[b], Alexander Scholten[a], Karl-Wilhelm Koch[a] and Daniele Dell'Orco[b]

[a] Dept. of Neurosciences, Biochemistry Group, University of Oldenburg, 26111 Oldenburg, Germany
[b] Dept. of Neurosciences, Biomedicine and Movement Sciences, Sect. of Biological Chemistry, University of Verona, 37134 Verona, Italy.

alexander.scholten@uni-oldenburg.de

Guanylate cyclase-activating proteins (GCAPs) are retina specific neuronal Ca^{2+} sensor proteins (NCS proteins) that regulate the activity of sensory membrane bound guanylate cyclases (GCs). GCAPs switch conformation and function upon binding of divalent cations: from GC-inhibitor (Ca^{2+} bound) to GC-activator (Mg^{2+} bound). Strict control of GC-activity is crucial for the interplay between Ca^{2+} and cGMP homeostasis in photoreceptor cells. In healthy cells this interplay is important for shaping the kinetics of photoresponse and for light adaptation. Point mutations in GUCA1A, the gene encoding GCAP1, cause inherited retinal degenerative diseases due to an imbalance of second messenger homeostasis in photoreceptor cells. Most GCAP1 mutations lead to autosomal dominant cone (COD) or cone-rod (CORD) dystrophies. Here we investigated two GCAP1 mutants (L84F and I107T), which exhibit additional symptoms of macular dystrophies. We therefore investigated biochemical and biophysical properties of the two mutants to see, whether they share common molecular features with other retinal dystrophy variants of GCAP1. Similar to other GCAP1-mutants described before GC-activity assays revealed a shift in Ca^{2+}-sensitivity towards higher Ca^{2+}-concentrations for both mutants, resulting in constitutive active GCs in affected photoreceptor cells. Interestingly, when tested with a Ca^{2+}-chelating reagent, only I107T exhibited a decreased Ca^{2+}-affinity, whereas L84F binds Ca^{2+} with similar constants as wildtype-GCAP1. When we compared structural aspects of both mutants with wildtype-GCAP1, employing circular dichroism spectroscopy and Ca^{2+}-titration monitored by tryptophan fluorescence, I107T showed high similarities with wildtype-GCAP1, whereas L84F exhibited greater differences, specifically in the tertiary structure. Our results suggest that these two novel GCAP1-mutants affect GC-regulation via different processes.

POSTER COMMUNICATION P-26
Calcium and Neurological Disorders

Ca^{2+}, reactive oxygen species and connexin hemichannels modulate the radiation-induced bystander effect in brain microvascular endothelial cells.

Elke Decrock[1], Delphine Hoorelbeke[1], Marijke De Bock[1], Tinneke Delvaeye[1,2], Valérie Van Haver[1], Hubert Thierens[3], Dmitri V Krysko[2], Benedicte Descamps[4], Christian Vanhove[4] and Luc Leybaert[1]

[1]Physiology Group – Dept. Basic Medical Sciences, Ghent University; [2]Inflammation Research Center, VIB/Ghent University, Ghent; [3]Medical Physics – Dept. Basic Medical Sciences, Ghent University; [4]INFINITY-MEDISIP-iMINDS, Ghent University. Ghent, Belgium.

Elke.Decrock@gmail.com

The effectiveness of brain tumor radiotherapy is frequently limited by the sensitivity of normal brain tissue to ionizing radiation. Evidence highlights a critical contribution of microvascular endothelial cell (mEC) dysfunction to radiation-induced normal tissue damage. The latter can be amplified by a process termed the radiation-induced bystander effect (RIBE) which consists of the intercellular propagation of radiobiological effects from irradiated to non-irradiated neighboring cells. Reactive oxygen species (ROS) are hypothesized to play a key role in coordinating the RIBE but the mechanism of distant action of these short-lived molecules remains unclear. We here aimed to explore the role of intercellular Ca^{2+} signaling via connexin (Cx) gap junction channels (direct cell-cell coupling) and hemichannels (paracrine release/uptake pathway), as a feed-forward propagation mechanism of ROS production underlying the RIBE in brain mECs. We optimized an in vitro model in which X-rays (1 and 20 Gy) are applied to a well-delineated zone of a brain mEC monolayer. The presence of radiation-induced DNA double-strand breaks (γ-H2AX foci) was detected in both the irradiated and adjacent non-irradiated bystander zone, with the number of γ-H2AX-positive cells being reduced by Cx43 knockout strategies and hemichannel targeting peptides. Extracellular ATP release via Cx43 hemichannels was detected 5 min post-irradiation and purinergic receptor blockers reduced the RIBE. Application of Ca^{2+}/ROS scavengers and Ca^{2+} imaging further revealed a contribution of both Ca^{2+} and ROS to RIBE. Our results thus suggest a novel mechanism for radiation-induced brain mEC damage which involves Cx43 hemichannels, ATP release and the Ca^{2+}/ROS signaling axis.

Fund for Scientific Research – Flanders (FWO) –Belgium.

POSTER COMMUNICATION P-27
Good and bad sides of Ca^{2+} signalling

Physiopathology of tubular aggregate myopathy.

G. Arielle Peche[1,2,3], Aymen Rabai[1,2,3], Johann Bohm[1,3,4], and Jocelyn Laporte[1,2,3]

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), [2]CNRS, [3]Université de Strasbourg, [4]INSERM, France.

peche@igbmc.fr

Abnormal calcium (Ca^{2+}) homeostasis is observed in various muscle disorders, including tubular aggregate myopathy (TAM). TAM are progressive muscle diseases characterized by abnormal accumulation of membrane tubules in muscle fibers.We identified STIM1 as the first TAM gene. STIM1 regulates store-operated calcium entry (SOCE) through activation of the calcium entry channel ORAI1. We demonstrated that STIM1 mutations impair Ca^{2+} homeostasis in TAM myoblasts, but the physiopathology of TAM and the sequence of events starting from STIM1 mutations, leading to muscle dysfunction need to be unraveled. This works aims to address those questions using immunocytochemistry, correlative light and electron microscopy, and fluorescence calcium signaling. We found that unlike WT STIM1, mutant STIM1 constitutively clusters, recruits the WT STIM1 and ORAI1 at the puncta. Moreover, overexpression of mutant but not WT STIM1 induces the formation of membrane stacks in our cellular model. In addition, mutant STIM1 triggers a higher increase in cytosolic Ca^{2+} level after addition of exogenous Ca^{2+} compare to WT STIM1. To analyze downstream effects of the cytoplasmic Ca^{2+} increase, we focused on the NFAT pathway. NFAT is a transcription factor which translocates from the cytoplasm to the nucleus following cytosolic Ca^{2+} elevation. In the cells transfected with mutant STIM1, we observed a dramatic increase in NFAT nuclear translocation compared to cells expressing WT STIM1. Our data demonstrate that the STIM1 mutations trigger a constitutive activation of SOCE, leading to a deregulation of the NFAT pathway controlling the expression of various skeletal muscle genes.

Supported by Région Alsace, AFM Téléthon, MDA.

POSTER COMMUNICATION P-28
Good and bad sides of Ca^{2+} signalling

A calmodulin mutation associated with LQT syndrome displays gain of function in modulating RyR2.

h T. Søndergaard[1,2], Kamilla T. Larsen[1], Filip van Petegem[3], Michael Fill[4], S.R. Wayne Chen[2] and Michael T. Overgaard[1]

[1]Aalborg University, Department of Chemistry and Bioscience, Aalborg, Denmark; [2]University of Calgary, Department of Physiology and Pharmacology, Calgary, Canada; [3]University of British Columbia, Department of Biochemistry and Molecular Biology, Vancouver, Canada; [4]Rush University, Department of Molecular Biophysics and Physiology, Chicago, IL.

mto@bio.aau.dk

Since our initial discovery of a calmodulin (CaM) mutation in a family with cardiac arrhythmia and sudden cardiac death, several mutations have been identified in all three genes encoding identical CaM proteins. All CaM mutations identified are associated with severe ventricular arrhythmias, predominantly CPVT and LQTS. In cardiomyocytes, CaM regulates a number ion-channels, including the SR-Ca^{2+} release channel RyR2. Mutations in RyR2 cause CPVT by increasing the propensity for spontaneous Ca^{2+}-release, leading to triggered activities and arrhythmias. CPVT-associated CaM mutations (N53I and N97S), but also CaM mutations causing LQTS (N97S, D95V, D129G), severely impair RyR2 function. Here we investigated the LQTS-causing F141L CaM mutation and its regulation of RyR2 mediated Ca^{2+}-release in RyR2-expressing HEK cells. In contrast to other CaM mutations, no increase in Ca^{2+}-release was induced by F141L mutation compared to WT CaM. We further determined the activity of single RyR2 channels from rat ventricular SR microsomes. WT CaM significantly decreases the open probability (P_O) compared to no-CaM. In marked contrast, D95V, N97S, D129G and N53I CaM mutations did not lower RyR2 P_O. Even more strikingly, adding F141L CaM markedly decreased RyR2 P_O even below that observed for WT CaM. Hence, the F141L CaM mutation is a gain-of-function mutation in terms of inhibiting RyR2 Ca^{2+} release, despite the loss-of-function of CaM C-domain Ca^{2+} binding. Understanding of the underlying molecular details of these results may provide insights into promoting the inhibition of RyR2 by CaM, a potential therapeutic strategy for treating arrhythmia, heart failure and neurodegenerative diseases.

Supported by grants from the Lundbeck Foundation, the Novo Nordisk Foundation, the Obelske Family foundation, and grant DFF-4181-00447 from the Danish Council for Independent Research.

POSTER COMMUNICATION P-29
Good and bad sides of Ca^{2+} signalling

Functional role of inositol 1,4,5-trisphosphate receptors in cardiac hypertrophy.

M. Iveth Garcia, Anja Karlstaedt, Heinrich Taegtmeyer and Darren Boehning

University of Texas Medical Branch at Galveston (UTMB), TX, USA. University of Texas Health Science Center Houston, TX, USA.

mivgarci@UTMB.edu

Calcium is a second messenger that is essential for the control of a variety of cellular functions. In the heart, calcium plays an integral role in many cellular processes including muscle contraction, gene expression, and cell death. The inositol 1,4,5-trisphosphate receptor (IP_3R) is a calcium channel that is ubiquitously expressed throughout the body. There are three IP_3R isoforms encoded by separate genes. In the heart, the IP_3R-2 isoform is reported to being most predominant with regards to both expression levels and functional significance. Several groups have shown that perinuclear IP3R-2 may play a key role in the progression of hypertrophy by mediating nuclear calcium release in response to endothelin-1 (ET-1) and angiotensin II. However, the functional roles of IP_3R-1 and IP_3R-3 in the heart are essentially unexplored despite measureable expression levels. We hypothesize that all three IP_3R isoforms can contribute to cytosolic calcium transients and cardiac stress induced by ET-1. As our model we used dissociated neonatal and adult rat ventricular cardiomyocytes. We found readily detectable levels of expression of all three IP_3Rs in both neonatal and adult rat ventricular cardiomyocytes. We found that in addition to the perinuclear area, all three IP_3R isoforms were expressed throughout the cardiomyocyte including the sarcoplasmic reticulum. Using isoform specific siRNA, we found that knocking down individual IP_3Rs did not alter ET-1 induced calcium responses in neonatal cardiomyocytes. However, knocking down all three isoforms abolished the effects of ET-1 on calcium homeostasis. We next tested whether IP_3Rs mediate nuclear-specific calcium elevations. Using a genetically encoded calcium indicator targeted to the nucleus we were able to specifically discriminate nuclear versus cytosolic calcium transients after ET-1 treatment. Contrary to previous reports, we found no evidence that IP_3R-mediated calcium release is confined to the nucleus. Our findings that IP_3R isoforms are functionally redundant in the heart have significant implications for animal models of hypertrophy and human disease

Supported by grants 5R01GM081685 and R01GM081685S1 from National Institute of General Medical Sciences, USA.

POSTER COMMUNICATION P-30
Good and bad sides of Ca^{2+} signalling

Can we use chemical-induction of ageing to develop a model of cardiac ageing?

Said Masoud[1], Martin D. Bootman[1], Fraser McDonald[2] and Katja Rietdorf[1].

[1]The Open University, Dept. of Life, Health and Chemical Sciences, Walton Hall, Milton Keynes, MK7 6AA. [2]Kings College London, Department of Orthodontics, craniofacial Development and Stem Cell Biology, 310 Tower Wing, Guy's, London, SE1 9RT.

Said.Masoud@open.ac.uk

Cardiovascular diseases are the leading cause of deaths worldwide, with age being one of the biggest risk factors. Cardiovascular ageing is characterised by a decline in cardiac function attributed to changes in ultrastructure and calcium homeostasis. Typically, celular ageing is studied longitudinally by comparing young and old animals. These studies are effective, but expensive and time consuming. Recent work has shown that neuronal ageing can be acutely studied by adding hydroxyurea (HU) or D-galactose to isolated cells, or giving it to animals. We want to test whether this chemically-induced ageing model can also be used to study ageing in cardiomyocytes. In this study, we induced ageing of neonatal rat ventricular myocytes (NRVM) by addition of 50 or 500 µM HU for 7 days. Using calcium imaging, we showed that HU treatment increased the frequency of spontaneous calcium transients after 2 Hz electrical field stimulation. Similarly, HU treatment decreased the ability of cardiomyocytes to follow electrical pacing and increased the generation of alternans; two known pro-arrhythmic behaviours. We also found an increase in the levels of autophagy, and a disruption of mitochondria after HU addition. EM analysis revealed that HU treatment increased the appearance of autophagosomes, rough sarcoplasmic reticulum and gangliosides. Our results indicate that prolonged incubation with HU evoked changes in the ultrastructure and calcium homeostasis of cardiomyocytes similar to those observed in naturally aged cells. We propose that chemically induced ageing provides a relevant model of cellular ageing, which can be applied acutely and inexpensively.

Supported by The Open University and King's College London.

POSTER COMMUNICATION P-31
Good and bad sides of Ca^{2+} signalling
Selected for Oral Communication in Symposium #2

How does fat cause heart diseases? Effects of epicardial adipocytes on cardiomyocyte signalling and contractility.

Said Masoud[1], Martin D. Bootman[1], Fraser McDonald[2], Hilary MacQueen[1] and Katja Rietdorf[1]

[1]The Open University, Department of Life, Health and Chemical Sciences, Walton Hall, Milton Keynes, MK7 6AA. [2]Kings College London, Department of Orthodontics, Craniofacial Development and Stem Cell Biology, 310 Tower Wing, Guy's, London, SE1 9RT.

katja.rietdorf@open.ac.uk

The heart is surrounded by a layer of fat known as epicardial adipose tissue (EAT). EAT thickness is a predictor for several cardiac diseases. Adipocytes within the EAT cause harmful effects via secretion of adipokines and cytokines, which can act on neighbouring cardiomyocytes in a paracrine manner. However, EAT also has beneficial effects, and is critical to the heart's function by providing energy and offering physical protection. We found that adipocytes caused negative chronotropic (0.51 ± 0.01 vs. 0.32 ± 0.04 Hz contraction frequency) and negative inotropic responses (6.9 ± 0.3 vs. 3.8 ± 0.5 µm contraction amplitude) after 3 days of co-culture with neonatal rat ventricular cardiomyocytes. Using signalling arrays we found a significant up-regulation of cardiomyocyte pro-apoptic signalling pathways by the adipocytokines TNF-α and Activin A. In contrast, adiponectin reduced ERK and mTOR activity, in line with its proposed cardioprotective effects. These data illustrate that adipose-derived adipocytokines have complex effects on both contractility and signaling pathways in cardiomyocytes. Because mature adipocytes do not attach to cell culture surfaces it is impossible to study the paracrine interaction between adipocytes and cardiomyocytes for prolonged periods using conventional 2-dimensional co-cultures. Addition of adipocyte-conditioned medium is often used, but cannot replicate the paracrine interactions occurring between cells being in physical contact. To study the effects of adipocytes on adjacent cardiomyocytes, and vice versa, over prolonged periods of time we are establishing a 3-dimensional co-culture. This approach allows cellular phenotypes, calcium signalling and contraction to be measured within the cultures.

Supported by The Open University.

POSTER COMMUNICATION P-32
Good and bad sides of Ca^{2+} signalling

Characterization of a calsequestrin-1 mutation identified in a patient affected by a vacuolar myopathy.

Valeria Del Re, Virginia Barone, Alessandra Gamberucci, Simone Spinozzi, Valentina Polverino and Vincenzo Sorrentino.

Section of Molecular Medicine, Department of Molecular and Developmental Medicine, University of Siena, Italy.

valeriadelre@libero.it

Calsequestrin (CASQ1) is the major protein of the sarcoplasmic reticulum of striated muscle that binds Ca^{2+} with high capacity and moderate affinity. CASQ1 exists as monomer and polymer. CASQ1 polymerization depends on Ca^{2+} concentrations and on the ionic strength of the environment. Polymerization starts with a front-to-front interaction between two adjacent monomers. After, back-to-back interactions lead to the formation of polymers further promoting Ca^{2+} binding. The CASQ1 polymer is stable and anchored to the sarcoplasmic reticulum membrane thanks to its interactions with triadin and junctin, allowing the modulation of the calcium release channel RYR1. We found a mutation in the CASQ1 gene in patients with a vacuolar myopathy. The CASQ1 mutation (CASQ1D244G) affects one of the high-affinity Ca^{2+}-binding sites of the protein and alters the kinetics of Ca^{2+} release in muscle fibers from patients. Expression of the CASQ1D244G in myotubes and in mouse fibers causes the appearance of sarcoplasmic reticulum vacuoles containing aggregates of the mutant CASQ1 protein that resemble those observed in patients. CASQ1WT and CASQ1D244G were expressed in bacteria, purified and analysed for their ability to polymerize at increasing Ca^{2+} concentrations. Turbidity measurements indicated that the CASQ1D244G protein aggregates at lower Ca^{2+} levels and more rapidly than CASQ1WT. CASQ1D244G conformational changes were analysed by trypsin proteolysis in presence of increasing Ca^{2+} concentration. Results indicated that there are no differences with respect to CASQ1WT. These results suggest that the CASQ1D244G mutation interferes with the correct process of Ca^{2+}-dependent protein polymerization probably causing the formation of protein aggregates.

Supported by grants AFM–Telethon grant 18822 to VS.

POSTER COMMUNICATION P-33
Good and bad sides of Ca^{2+} signalling

Shear stress induces transverse global Ca^{2+} waves via autocrine activation of P2X purinoceptors in rat atrial myocytes.

Joon-Chul Kim and Sun-Hee Woo

College of Pharmacy, Chungnam National University, 99 Daehak-ro, Daejeon 305-764, South Korea.

jc7418@naver.com

Atrial myocytes are exposed to high shear stress during blood regurgitation and high intra-atrial pressure due to valve diseases and heart failure, since such disturbances disrupt endocardium. We have previously reported that shear stress induces two types of global Ca^{2+} waves in atrial myocytes, longitudinal and transverse Ca^{2+} waves (T-waves) (Biophys J 2012;102(3, Suppl 1);227a), and that the longitudinal wave is triggered by Ca^{2+} release via $P2Y_1$ purinoceptor-inositol 1,4,5-trisphosphate receptor signaling (J Physiol 2015;593:5091-5109). Here, we investigated cellular mechanism for the generation of T-wave in atrial cells under shear stress. Shear stress of ~16 dyn/cm^2 was applied onto single myocytes using micro fluid-jet, and two-dimensional confocal Ca^{2+} imaging was performed. Shear stress-induced T-waves were observed repetitively under 3-4 min intervals between the stimuli, and occurred at ~1 event per 10 s. They were eliminated by inhibition of the voltage-gated Na^+ or Ca^{2+} channels, or ryanodine receptors, suggesting that the T-wave is mediated by action potential-triggered Ca^{2+} release. Blockades of key stretch signaling molecules, stretch-activated channel, Na^+—Ca^{2+} exchange, and NADPH oxidase, did not suppress T-wave generation by shear. However, shear-induced T-wave generation was abolished by pre-incubation of cells with external ATP-metabolizing enzyme apyrase, the gap junction blocker carbenoxolone, or with P2X purinoceptor antagonist iso-PPADS. Inhibition of $P2Y_1$ purinergic signaling that mediates the longitudinal Ca^{2+} wave under shear did not attenuate the occurrence of T-waves. Our data suggest that shear stress induces activation of P2X purinoceptors via gap junction-mediated ATP release, thereby triggering action potential with subsequent T-wave in atrial myocytes.

Supported by National Research Foundation of Korea (NRF) grant funded by the Korean Government (NRF-2015R1A2A2A01002625).

Name of presenting author: Joon-Chul Kim

POSTER COMMUNICATION P-34
Good and bad sides of Ca^{2+} signalling

Shear stress enhances Ca^{2+} spark occurrence via mitochondrial NADPH oxidase and nitrogen oxide synthase in rat ventricular myocytes.

Sun-Hee Woo, Jun Wang and Joon-Chul Kim

College of Pharmacy, Chungnam National University, 99 Daehak-ro, Daejeon 305-764, South Korea.

shwoo@cnu.ac.kr

It has been reported that shear stress enhances Ca^{2+}-induced Ca^{2+} release during depolarization in ventricular myocytes. To know molecular basis for the increase in global Ca^{2+} releases we assessed the effects of shear stress (16 dyn/cm2) on the frequency of Ca^{2+} sparks and underlying mechanism for the shear-mediated Ca^{2+} spark regulation using confocal Ca^{2+} imaging in rat ventricular myocytes. The frequency of Ca^{2+} sparks was immediately (within 1 s) increased by shear stress by 80%, and further increased by 150% by prolonged (20 s) shear exposure. Inhibition of nitric oxide synthase (NOS) and interference of cytoskeletal integrity using L-NAME and colchicine, respectively, partially attenuated the prolonged shear-mediated enhancement in spark frequency. Blockade of Na^+/Ca^{2+} exchanger and L-type Ca^{2+} channel did not alter the shear effect on spark occurrence. Pretreatment of reducing agent significantly reduced the shear-mediated spark enhancement. Inhibitor of NADPH oxidase (NOX) diphenyleneiodonium and mitochondrially targeted antioxidant mito-TEMPO suppressed shear-mediated spark enhancements. Measurement of intracellular reactive oxygen species (ROS) revealed increase in ROS level by shear stress. Sarcoplasmic reticulum (SR) Ca^{2+} content was not altered immediately after shear application, but significantly increased after 20-s long shear exposure. Our data suggest that shear stress enhances the frequency of Ca^{2+} sparks partly by producing ROS via mitochondrial NOX, and that prolonged enhancement in spark frequency by shear stress may be also mediated by increase in the SR Ca^{2+} loading and by NOS. These mechanisms may explain the shear-mediated enhancement in Ca^{2+} transient in ventricular myocytes.

Supported by National Research Foundation of Korea (NRF) grant funded by the Korean Government (NRF-2015R1A2A2A01002625).

POSTER COMMUNICATION P-35
Good and bad sides of Ca^{2+} signalling

Calcium phosphate crystals found in atherosclerotic plaques induce cytotoxic effects via loss of cellular calcium homeostasis.

Kumar Gaurav Sharma[1], Katja Rietdorf[1], Diane Proudfoot[2], Fraser McDonald[3], and Martin D. Bootman[1]

[1]The Open University, Department of Life, Health and Chemical Sciences, Walton Hall, Milton Keynes, MK7 6AA, United Kingdom. [2]The Babraham Institute, Babraham Hall, Babraham, Cambridgeshire, CB22 3AT, United Kingdom. [3]Department of Orthodontics, Floor 22, Tower Wing, St Thomas St., King's College London Dental Institute, London, SE1 9RT, United Kingdom.

kumar.sharma@open.ac.uk

Smooth muscle cells (SMCs) help to prevent atherosclerotic plaques from rupturing by synthesizing collagen that surrounds, and protects, the fibrous plaque cap. SMC death occurs within atherosclerotic plaques, contributing to plaque instability. Previous observations (1) suggest that calcium phosphate (CaP) crystals formed within a plaque are cytotoxic to SMCs and cause rapid necrosis that coincides with a loss of calcium homeostasis. In our study, A7r5 cells were exposed to 12.5 µg/ml of CaP crystals, and changes in intracellular calcium homeostasis were recorded for 45 minutes using the calcium indicator Fura-2. We observed that CaP crystals induced calcium oscillations followed by an irreversible calcium rise that culminated in cell death. Reducing sarco/endoplasmic reticulum calcium release by expression of a 5'-phosphatase enzyme, which rapidly metabolises IP_3, significantly reduced the cytotoxicity of CaP crystals. Similarly, adding the SERCA pump inhibitor cyclopiazonic acid, preventing store-operated calcium release via addition of gadolinium, incubation with the lysosomal calcium release antagonist bafilomycin A1, or depolarising the mitochondria membrane potential with antimycin/oligomycin, also significantly reduced the cytotoxicity of CaP crystals. Expression of annexins, a familiy of cell membrane repair proteins, protected SMCs from CaP-induced cell death. Our data supports the hypothesis whereby endocytosis of CaP crystals, or CaP induced cell membrane rupture, leads to a rapid loss of calcium homeostasis and cell death. Attenuating calcium signalling, or enhancing cell membrane repair, can protect cells from the cytotoxic effects of CaP crystals.

1) Ewence et al (2008) Circ Res. 29;103(5):e28-34.

Supported by The Open University, King's college London, UK.

POSTER COMMUNICATION P-36
Good and bad sides of Ca^{2+} signalling

Decreased PMCA4b levels in the red blood cells of healthy individuals is connected to a minor haplotype in the ATP2B4 gene.

Boglárka Zámbó[1], György Várady[1], Rita Padányi[2], Edit Szabó[1], Adrienn Németh[1], Ágnes Enyedi[2] and Balázs Sarkadi[1,3]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary, [2]2nd Department of Pathology, Faculty of Medicine, Semmelweis University, Budapest, Hungary. 3MTA-SE Molecular Biophysics Research Group, Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary.

zambo.boglarka@ttk.mta.hu

We have developed a flow cytometry method to measure quantitatively the levels of several membrane proteins in human red blood cells (RBCs). When screening the PMCA4b protein levels in a large number of healthy volunteers we observed a significant heterogeneity in the expression levels. In subjects with low PMCA4b expression, Western-blot analysis revealed that the molecular mass of the PMCA4b protein was unchanged, and no compensatory increase in other PMCA4 variants were observed. We confirmed that the reduced PMCA4b levels resulted in an impaired calcium pump function in Fluo-4 loaded RBCs. In order to explore the genetic background of the reduced expression, we sequenced the ATP2B4 gene in individuals with low PMCA4b levels. While no alterations in the coding regions were observed, the reduced PMCA4b protein levels correlated with a minor variant of a haplotype (n=176, $p<0.001$; MAF=0.1; CT 25%, TT 50% reduction). This haplotype is overlapping the second promoter region of the ATP2B4 gene, and the decreased protein expression is probably related to alterations in the binding of transcription factors in this region. Interestingly, while we found these alterations in healthy volunteers, recent GWA studies indicate that SNPs of this haplotype result in a decreased mean corpuscular hemoglobin concentration (MCHC) in the RBCs, and may be protective against malaria infection.

This work has been supported by OTKA K115375.

POSTER COMMUNICATION P-37
Good and bad sides of Ca^{2+} signalling

Ca^{2+} reducing therapy for symptomatic treatment of sickle cell disease: first results of a phase IIa-b trial.

Anna Bogdanova, Asya Makhro, Elena Seiler, Nynke Moelijker, Inga Hegemann and Jeroen Goede

Red Cell Research Group, Institute of Vet. Physiology, Vetsuisse Faculty, University of Zurich; Division of Hematology, University Hospital Zurich; Division Oncology and Hematology, Cantonal Hospital Winterthur.

annab@access.uzh.ch

Abnormally high intracellular Ca^{2+} in red blood cells (RBCs) of patients with sickle cell disease (SCD) is a cause of facilitated dehydration, oxidative stress and proteolysis as well as a decrease in hemoglobin oxygen affinity. All these factors contribute to induction of polymerization of deoxy-hemoglobin S that in turn triggers thrombosis and hemolysis manifesting in pain and hemolytic crises. Earlier on we have shown that Ca^{2+} overload in RBCs was associated with abnormally high abundance and activity of N-methyl D-aspartate(NMDA) receptors which are involved in Ca^{2+} uptake. We are currently testing the novel Ca^{2+} reducing therapeutic approach in a small group of adult patients with SCD that receive a daily 20 mg dose of memantin-Mepha (ClinicalTrials.gov Identifier: NCT02615847). Oral administration of memantine resulted in a rapid drop in the intraerythrocytic Ca^{2+} followed by reduction in the NMDA receptor abundance at the RBC membranes. We could confirm that reduction of Ca^{2+} levels in RBCs in vivo results in re-hydration, reduction in oxidative stress and stabilization of plasma membrane of RBCs of SCD patients. RBCs showed less adherence when passing through the "artificial spleen". Suppression of Ca^{2+} influx into RBCs mediated by the NMDA receptors has changes metabolic profile of RBCs. Furthermore, increase in O2 affinity of hemoglobin further contributed to the substantial decrease in the levels of pain killers and improvement in the quality of life of 6 patients that are undergoing treatment at present. The total number of patients with this rare hereditary anemia foreseen for the trial is 10-12.

Supported by the European Union's Seventh Framework Programme for research, technological development and demonstration (CoMMiTMenT grant agreement N° 602121) and Horizon 2020.

POSTER COMMUNICATION P-38
Good and bad sides of Ca^{2+} signalling

cAMP regulated PKD1L1 and PKD2L1 on the primary cilia.

Kotdaji Ha, Eunice YJ Park and Insuk So

Department of Physiology, Seoul National University, College of Medicine, Seoul 110-799, Republic of Korea.

eunice421@snu.ac.kr

Primary cilia are solitary organelles that extend from the basal body of the apical surface into the extra cellular matrix of most eukaryotic cells. Dysfunctions of primary cilia underlie a multitude of human disorders called autosomal dominant polycystin kidney disease (ADPKD). PKD1L1 and PKD2L1 were reported to form a heterodimeric calcium channel that regulates ciliary calcium concentration, and thereby ciliary signaling. However, the trafficking mechanism of PKD1L1 and PKD2L1 still remains unveiled. PKD1L1 displays a similar homology compared to PKD1 whereas the function of PKD1L1 is distinct. Since PKD1 is considered as adhesion G-Protein-Coupled-Receptor with a common feature of GPCR-autoproteolysis-inducing (GAIN) domain and cleavage of the extracellular amino-terminus at a GPS site, PKD1L1 is thought to be activated via autocleavage at GPS site. However, a crucial amino acid sequence, HLT for autocleavage does not exist in PKD1L1. Autocleavage is an inevitable step for PKD1 to translocate in primary cilia and it remains uncovered how PKD1L1 reaches to the cilia without autocleavage. To unveil the trafficking mechanism of PKD1L1, we identified the expression of PKD1L1 to primary cilia of mIMCD-3 cells using confocal microscopy. We also detected the expression level of PKD1L1 to find its full and cleaved fragment. Since ADPKD patients show abnormal sensory cilia function, we try to identify a novel ciliary trafficking determinant in PKD1L1 and the unknown role of PKD1L1 in calcium regulation in cilia.

POSTER COMMUNICATION P-39
Good and bad sides of Ca^{2+} signalling
Selected for Flash Symposium Presentation

Cav1.2 and Cav1.3 calcium channels are critical during allergic asthma.

Magali Savignac[1], Marion Mars[1], Claire Cenac[1], Jean-Charles Guery[1] and Lucette Pelletier[1]

[1]INSERM U1043, CNRS U5282, University Paul Sabatier, Center of Physiopathology Toulouse Purpan, France.

magali.savignac@inserm.fr

Besides CRAC/ORAI, other calcium channels such as voltage-gated calcium channels (Cav1) contribute also to the calcium influx in activated T lymphocytes. Cav1 channels are formed by the ion forming-pore α 1 (encoded by Cav1.1-Cav1.4) and auxiliary subunits including Cav β and a2d. We previously demonstrated that mouse Th2-cells selectively express Cav1.2 and Cav1.3 α 1 channels. Knocking down these channels with Cav1 specific antisense oligonucleotides (Cav1AS) impairs TCR-driven Ca^{2+} responses and cytokine production by mouse and human Th2-cells. Moreover, intranasal administration of Cav1AS suppressed airway inflammation and hyperreactivity in an active model of asthma. Using newly created cell type-specific Cav1.2-deficient mice (specifically in T lymphocytes) and bone marrow chimera experiments with Cav1.3-deficient mice, we study that the selective deletion of Cav1.2 or Cav1.3 in CD4+ T cells in several models of experimental allergic asthma.

POSTER COMMUNICATION P-40
Good and bad sides of Ca^{2+} signalling
Selected Oral Communication for Symposium #2

E152K STIM1 mutation deregulates Ca^{2+} signaling contributing to chronic pancreatitis.

Miguel Burgos[1,2], Fabrice Antigny[3], Pauline Dubar[1], Reginald Philippe[1], Peter Stathopoulos[4,5], Mitsuhiko Ikura[5], Wesley Brooks[6], Juan Llopis[2], Beatriz Domingo[2], Jian-Min Chen[1], Thierry Capiod[7], Claude Ferec[1] and Olivier Mignen[1]

[1]INSERM U1078 Génétique, Génomique fonctionnelle et biotechnologies, Faculté de Médecine et des Sciences de la Santé, Université de Bretagne Occidentale, Brest, France; [2]Centro Regional de Investigaciones Biomédicas (CRIB) and Facultad de Medicina de Albacete, Universidad Castilla-La Mancha, Albacete, Spain; [3]Dept of Basic Neurosciences, 1, Rue Michel Servet, 1211 Geneva 4, Switzerland; [4]Princess Margaret Cancer Centre and Dept of Medical Biophysics, University of Toronto, Canada; [5] Dept of Physiology and Pharmacology, University of Western Ontario, Canada; [6] Dept of Chemistry, University of South Florida, Tampa, FL USA; [7] INSERM U1151 – Institut Necker Enfants Malades (INEM) Faculté de Médecine Paris Descartes, Paris, France.

Miguel.burgos@uclm.es

Pancreatic acinar cell damage observed in pancreatitis can result from an abnormal regulation of $[Ca^{2+}]i$ leading to enhanced intracellular trypsin activation. We identified different mutations in the Stromal Interaction Molecule 1 (STIM1) gene in 3 cohorts of patients suffering from chronic pancreatitis. A mutation in the STIM1 EF-SAM domain (E152K) induced an increase in endoplasmic reticulum (ER) Ca^{2+} release in fibroblasts obtained from patients and STIM1-E152K transfected HEK293 cells. This increase resulted from higher calcium levels in the ER due to enhanced SERCA pump activity. Differences in STIM1-SERCA interactions between wild type and mutated STIM1 may explain this change in SERCA activity caused by modifications of STIM1 biophysical characteristics. In AR42J cells expressing STIM1-E152K, perturbations in Ca^{2+} signaling is correlated with defects in trypsin activation and secretion and also an increased cytotoxicity after cell stimulation with cholecystokinin. Altogether, STIM1-E152K induces deregulation of Ca^{2+} homeostasis that could lead to cell damage in acinar cells and contribute to the development of chronic pancreatitis in patients bearing the mutation.

We acknowledge MINECO/FEDER grants, reference BFU2012-39548-C02-01 and BFU2015-69874-R, and group grant GI20163512 from Vicerrectorado de Investigación y Política Científica, UCLM. We also acknowledge the Cystic Fibrosis association "Gaetan Saleun", Brest, France.

POSTER COMMUNICATION P-41
Good and bad sides of Ca^{2+} signalling

SERCA inhibition as a novel treatment option in auto-inflammatory disease.

Ann-Kathrin Herrmann, Florian Kurschus, Peter Vangheluwe and Axel Methner

University Medical Center Mainz, Germany; KU Leuven, Belgium.

axel.methner@gmail.com

Dimethyl fumarate (DMF) is a novel oral therapeutic agent used in Multiple Sclerosis (MS). It reduces disease activity and progression in patients with relapsing-remitting MS and has a similar effect in the mouse model of MS, experimental autoimmune encephalomyelitis. We observed that DMF causes significant changes in Ca^{2+} homeostasis in mice and man. DMF increases the cytosolic Ca^{2+} concentration and decreases the store content in line with an inhibitory effect on SERCA, the Ca^{2+} pump that refills the endoplasmic reticulum after receptor activation. Store-operated Ca^{2+} entry in contrast was unaltered. DMF was also able to directly suppress SERCA function in rabbit microsomes. As correct Ca^{2+} handling is paramount for the correct functioning of the immune system, we assume that SERCA inhibition constitutes the yet-unknown mechanism of action of DMF and constitutes a novel principle in the treatment of auto-inflammatory diseases. It is well known that DMF elicits short-lived oxidative stress by scavenging glutathione, the major cellular antioxidant. It is also known that oxidation of SERCA2b at cysteine 674 attenuates its function. We are therefore following the hypothesis that DMF alters cysteine 674 of SERCA2b, thereby inhibiting its function.

POSTER COMMUNICATION P-42
Good and bad sides of Ca^{2+} signalling
Selected for Flash Symposium Presentation

Targeting two-pore channels to inhibit MERS-Coronavirus cell entry.

Gihan Gunaratne and Jonathan Marchant

University of Minnesota Medical School, Department of Pharmacology, MN, USA.

gunar005@d.umn.edu

The Middle East respiratory syndrome coronavirus (MERS-CoV) is a deadly virus with no currently approved medical treatment. As an enveloped virus, MERS-CoV exploits the host cell's intracellular trafficking machinery to gain access to endosomal compartments which facilitate viral translocation and infection. Recent studies have revealed a role of nicotinic acid adenine dinucleotide phosphate (NAADP)-regulated two-pore channels (TPC1, TPC2) in intracellular trafficking events and Ebola virus infectivity. Here, we have investigated the contribution of TPC activity to the cellular translocation of MERS-CoV. Knockdown of endogenous TPCs (TPC1 or TPC2) significantly inhibited MERS-pseudovirus infectivity, while manipulation of the lysosomal cation channel, mucolipin-1 had no effect. Pharmacological exploration of bizbenzylisoquiniline compounds related to the previously identified TPC-blocker tetrandrine, illuminated structure-activity relationships for inhibition of MERS-pseudovirus translocation. Inhibitors of MERS-pseudovirus infectivity displayed a positive correlation as inhibitors of NAADP-evoked Ca^{2+} release. In contrast, no correlation was found between MERS inhibition and IP_3- or cADPR-evoked Ca^{2+} release. We conclude that inhibition of TPCs significantly reduces MERS infectivity, and propose that large-scale screening for NAADP antagonists and TPC blockers may yield effective MERS-CoV therapeutics.

Supported by grant GM088790 from NIH

POSTER COMMUNICATION P-43
Good and bad sides of Ca^{2+} signalling
Selected for Flash Symposium Presentation

The intracellular Ca^{2+} regulation as a rational therapeutic target for new drugs against trypanosomatid agents of Chagas Disease and Leishmaniasis.

Gustavo Benaim, Andrea Pinto, Veronica García, Yetsenia de Gouveia and Jessica Rodríguez.

Instituto de Estudios Avanzados (IDEA), and Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela.

gbenaim@idea.gob.ve

Trypanosoma cruzi and Leishmania mexicana are the causative agents of Chagas disease and leishmaniasis respectively. At the present there is not an efficient cure against these human infections. The intracellular Ca^{2+} regulation in these parasites, albeit similar in some aspects to that present in human cells, are distinct in many relevant aspects. This fact could be rationally exploited on the look for new therapies against these diseases. We have found that many of the new drugs postulated as candidates for the cure of these illness, acts in part by disrupting the Intracellular Ca^{2+} concentration of the parasite, without affecting the human counterpart. Amiodarone and dronedarone, commonly used human antiarrythmics, both possess a dramatic antiparasitic effect, inducing a rapid Ca^{2+} release from the single giant mitochondria present in these parasites, by collapsing the H^+ electrochemical potential. Similarly, these drugs, induces the release of Ca^{2+} from the acidocalcisomes, a particular organelle present trypanosomatids, involved in the bioenergetic of the parasite, which is able to accumulate large amounts of Ca^{2+} together with pyrophosphate, an energetic coin alternative to ATP present in these parasites. The Ca^{2+} release from these organelles is traduced in an intracellular Ca^{2+} increase in Trypanosoma cruzi as well as in Leishmania mexicana. On the other hand, we have demonstrated that miltefosine, a drug of common use against Leishmaniasis, iinduces a rapid increase in the intracellular Ca^{2+} concentration, by the opening of Ca^{2+} channel similar to the L-type VGCC present in human cells, but which is sensitive to sphingosine in trypanosomatids.

Supported by the Fondo Nacional de Ciencias, Tecnología e Investigación, Venezuela (FONACIT; Grant 2011000884 to GB) and by the Consejo de Desarrollo Científico y Humanístico-Universidad Central de Venezuela (CDCH-UCV; Grant PG-03-8728-2013 to GB)

POSTER COMMUNICATION P-44
Good and bad sides of Ca^{2+} signalling
Selected for Flash Symposium Presentation

Two mechanisms of Ca^{2+} entry in Toxoplasma gondii.

Miryam A. Hortua Triana, Karla Marques Nogueras and Silvia N.J. Moreno.

Center of Tropical and Emerging Global Diseases (CTEGD), University of Georgia. Athens, GA USA.

mahortua@uga.edu

Toxoplasma gondii is a protist parasite that belongs to the phylum Apicomplexa and is an important cause of congenital disease and infection in immunocompromised patients. As an obligate intracellular parasite, active invasion of host cells with subsequent replication, egress and reinvasion into new cells are essential pathogenic features. Ca^{2+} signaling forms part of the pathways activating effectors involved in the stimulation of essential pathogenic features like invasion, replication, and egress. While the parasite is extracellular it is exposed to a high concentration of Ca^{2+} and our hypothesis is that it uses this extracellular Ca^{2+} influx to enhance Ca^{2+}-dependent invasion processes as well as to replenish intracellular Ca^{2+} stores. We propose the presence of two mechanisms of calcium entry: a voltage-gated pathway which responds to changes in the surrounding ionic composition and a second mechanism activated by a signaling cascade involving calcium, a phospholipase C and a protein kinase G (PKG). A TRP-like channel would be the final player of this second pathway. The voltage-gated Ca^{2+} entry pathway is inhibited by nifedipine and activated by Bay K and Calcium itself. The second mechanism is activated by cGMP, which activates a protein kinase G. We used zaprinast, an analogue of Viagra, to inhibit the phosphodiesterase that hydrolyzes cGMP. Inhibition of PKG abrogates the stimulation of Calcium entry by Zaprinast. We used Fura-2AM loaded-parasites and GCAMP6 expressing parasites and inhibitors for PIPLC, PKG and TRP channels. We also created genetic mutants for key players like a PIPLC, a TRP-like channel and mutant PKG.

Supported by NIH

POSTER COMMUNICATION P-45
Targeting protein-protein interactions

Study of calmodulin and β-amyloid peptide binding to purified hPMCA4b-eYFP using fluorescence approaches.

Isaac Corbacho, María Berrocal, Sofia Fortalezas, Ana M. Mata and Carlos Gutierrez-Merino.

Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura. Badajoz, Spain.

icorbacho@unex.es

Calmodulin is a relevant physiological activator of the plasma membrane calcium pump (PMCA). In previous works, we have shown that calmodulin antagonized the inhibition of the Ca^{2+}-ATPase activity of PMCA by amyloid-β peptide (Aβ). However, in this cross-modulation two issues should be addressed: first, the binding sites of calmodulin and Aβ in PMCA still remain unclear; and second, if calmodulin protection against inhibition by Aβ could be due to direct competition of partially overlapping binding sites. Since PMCA4 is the most enriched isoform in brain we have expressed the human PMCA4b tagged with enhanced yellow fluorescent protein (hPMCA4b-eYFP) in Saccharomyces cerevisiae and we have studied calmodulin and Aβ binding to purified hPMCA4b-eYFP using fluorescence approaches. The titration of the hPMCA4b-eYFP fluorescence with calmodulin and Aβ showed that both elicited over 40% quenching of the fluorescence at saturation, yielding dissociation constants (K_d) in the nM scale range. To locate the binding sites of calmodulin and Aβ with respect to the eYFP-tag of hPMCA4b we have performed FRET experiments using Badan-calmodulin (kindly supplied by Dr. Katalin Török) and HiLyte Fluor 555 Aβ fluorescent derivatives that form donor-acceptor pairs with eYFP fluorescence. The analysis of these results allowed us to calculate values of K_d for the two binding sites of calmodulin in the hPMCA4b, the K_d of Aβ from PMCA4, distances between calmodulin and Aβ binding sites with respect to the eYFP-tag and experimentally assess competition between these two proteins for binding to hPMCA4b.

Supported by Grant BFU2014-53641-P from the Spanish Ministerio de Economía y Competitividad and by Grant GR15139 from Junta de Extremadura to research group BBB008].

POSTER COMMUNICATION P-46
Targeting protein-protein interactions

Expression and purification in yeasts of truncated and fluorescent human PMCA4b calcium pump.

Isaac Corbacho, María Berrocal and Ana M. Mata

Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura. Badajoz, Spain.

icorbacho@unex.es

Plasma membrane Ca^{2+}-ATPase (PMCA) is one of the major regulators of intracellular Ca^{2+} levels. PMCA functional analysis has been carried out traditionally with totally/partially purified PMCA protein from different sources like pig brain, where our group developed a method for PMCA purification. More recently, our group has reported a new and improved method to express and purify native human PMCA4b isoform (hPMCA4b) using yeasts, based in our previous method for pig brain. That method showed higher yield and lower costs compared with previous yeast-based methods. Purified protein was characterized, showing similar functional regulation than that obtained from other sources. Although this protein is extremely useful for functional analysis we have designed two new variants to further analyze the involvement of structural domains in hPMCA regulation. In this work we show the cloning and expression in yeasts of a truncated form of hPMCA4b, whose autoinhibitory domain has been replaced by GST-tag, and a second variant where full length hPMCA4b has been fused to yellow fluorescent protein (YFP). Both proteins have been successfully purified by affinity chromatography, showing high yields comparable to those obtained previously for the native form. Preliminary analysis of purified proteins has shown that, together with the native form, the truncated protein is an excellent candidate for functional analysis of hPMCA4b in the absence of its autoinhibitoy domain. In addition to this, the fluorescent protein hPMCA4b-YFP broadens horizons for using FRET to go in depth on revealing and fine tuning the mechanisms of PMCA interactions with other molecules.

Supported by Ministerio de Economía y Competitividad (BFU2011-23313 and BFU2014-53641-P); Junta de Extremadura (GR10108 and GR15139), Fundación Caja de Extremadura and FEDER.

POSTER COMMUNICATION P-47
Targeting protein-protein interactions
Selected Oral Communication for Symposium #3

Lipid raft KCa/Ca^{2+} channel complexes: novel targets to reduce tumor development by lipids.

M. Guéguinou[1,9], T. Harnois[2,9], D. Crottes[3], A. Uguen[6,7], N. Deliot[2,9], A. Gambade[1], A. Chantôme[1,9], J.P. Haelters[4,9], P.A. Jaffrès[4,9], M.L. Jourdan[1,10], G. Weber[1], O. Soriani[8], P. Bougnoux[1,9, 10], O. Mignen[6,9], N. Bourmeyster[2,9], B. Constantin[2,9], T. Lecomte[5,9,10], C. Vandier[1,9*] and M. Potier-Cartereau[1,9*‡].

[1]INSERM UMR 1069, Université de Tours, France. [2]Equipe ERL 7368 CNRS Université de Poitiers, France. [3]Department of Physiology, University of California, San Francisco. [4]CNRS-UMR 6521-Université de Brest, France. [5]GICC– UMR 7292 Université de Tours, France. [6]INSERM- UMR 1078 Université de Brest, France. [7] CHRU Brest, Service d'anatomie et cytologie pathologiques Brest, France. [8] CNRS UMR 7299, INSERM-UMR 1099, Université de Nice Sophia-Antipolis, France. [9] Ion channels Network and Cancer-Cancéropôle Grand Ouest, (IC-CGO). [10]CHRU Tours, Tours, France.

marie.potier-cartereau@univ-tours.fr

Membrane lipid rafts are distinct plasma membrane nanodomains that are enriched with cholesterol, sphingolipids and gangliosides, with occasional presence of saturated fatty acids and phospholipids containing saturated acyl chains. It is well known that they organize receptors (such as Epithelial Growth Factor Receptor), ion channels and their downstream acting molecules to regulate intracellular signaling pathways. Among them are Ca^{2+} signaling pathways, which are modified in tumor cells and inhibited upon membrane raft disruption. In addition to protein components, lipids from rafts also contribute to the organization and function of Ca^{2+} signaling microdomains. Here we focus on the lipid raft KCa/Ca^{2+} channel complexes that regulate Ca^{2+} in cancer cells, and discusses the potential modification of these complexes by lipids as a novel therapeutic approach in tumor development.

Supported by the Ligue Nationale contre le cancer, SNFGE, Région Centre Val de Loire, Cancéropole Grand Ouest, Ion channel network of Cancéropole Grand Ouest, INCa, ANR

POSTER COMMUNICATION P-48
Targeting protein-protein interactions
Selected for Flash Symposium Presentation

Role of Filamin A as a modulator of store-operated Ca^{2+} entry.

José J. López, Letizia Albarrán, Félix Gutierrez-Gallego, Ginés M. Salido and Juan A. Rosado.

Ficell research group, Department of Physiology, University of Extremadura, Cáceres, Spain.

jarosado@unex.es

Since the identification of STIM1 as an essential protein involved in the activation of store-operated Ca^{2+} entry (SOCE), several studies have been focused on the identification of modulators that regulate the interaction of STIM1 with capacitative channels. In this study, the combination of immunoprecipitation, silver staining of proteins and MALDITOF/TOF mass spectrometry demonstrates the interaction of Filamin A (FLNA), an actin-binding protein, with STIM1 after Ca^{2+} store depletion in human platelets. Previous studies have shown that FLNA, via Ca^{2+}-dependent phosphorylation at Ser^{2152}, regulates and integrates different intracellular signal pathways and functions. According to this, we have found that FLNA/STIM1 interaction is dependent on: 1) rises in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$), as demonstrated in cells loaded with dimethyl BAPTA, an intracellular Ca^{2+} chelator that prevented TG-evoked elevation in $[Ca^{2+}]_c$, and 2) phosphorylation of FLNA at Ser^{2152}, as demonstrated in cells treated with KT-5720, an inhibitor of FLNA phosphorylation, or Brefeldin A, an inhibitor of FLNA dephosphorylation. To investigate the physiological role of this interaction on SOCE, we monitored the changes in $[Ca^{2+}]_c$ in fura-2 loaded platelets treated with KT-5720 or Brefeldin A. Our results demonstrate that the interaction of FLNA phosphorylated at ser^{2152} and STIM1 reduces TG-induced SOCE. Taken together, these findings support a regulatory role of Filamin A in the modulation of SOCE mediated by its interaction with STIM1.

Supported by MINECO (BFU2013-45564-C2-1-P) and Junta de Extremadura-FEDER (GR15029). JJL was supported by Juan de la Cierva Program (JCI-2012-12934).

POSTER COMMUNICATION P-49
Targeting protein-protein interactions

$G\alpha_i$-mediated TRPC4 activation by polycystin-1 contributes to the endothelial function via STAT1 activation.

Misun Kwak, Chansik Hong, Jongyun Myeong, Kotdaji Ha and Insuk So

Department of Physiology, Seoul National University College of Medicine, Seoul, Republic of Korea.

misunkwak@snu.ac.kr

Polycystin-1 (PC1) is a candidate protein for polycystic kidney diseases and regulates a number of cellular processes, for example, heterotrimeric G protein and transcription factor. We have previously reported that TRPC4/C5 channel can be activated by $G\alpha_i$-coupled receptors. We assumed that PC1 might act as a GPCR, so there might be interaction between PC1 and TRPC4 via $G\alpha_i$ based on the phenotypes, that is, aneurysm in PKD and endothelial dysfunction in TRPC4 $^{-/-}$ mice. Here, we identified that PC1 dominantly interacts with $G\alpha_{i3}$. We recorded the activity of TRPC4 heterologously co-expressed with PC1 in HEK293 cells. PC1 activated TRPC4 β channel by modulating G-protein signaling without change in TRPC4 translocation. Dominant negative $G\alpha_{i3}$ mutant inhibited PC1-activated TRPC4 current. Using Fura-2 indicator, we observed intracellular Ca^{2+} increase by PC1 through TRPC4. We, next, investigated whether PC1/TRPC4 induces activation of STAT proteins, leading to cell proliferation or death. We observed that STAT1 and STAT3, but not STAT6 activation. When PC1 co-expressed with TRPC4, STAT1 activation was further increased compared to each sole expression, causing cystic cell death. To determine the role of PC1 with TRPC4 activation in endothelial cell migration, we performed a wound-healing assay in HUVECs. The downregulation of PC1 and TRPC4 activity by the PC1 knockdown or TRPC4 antagonist inhibited the migration of HUVECs. Our findings indicated an important function between PC1 and TRPC4/C5 in modulation of intracellular Ca^{2+} signaling and provided a new potential therapeutic approach targeting TRPC4/C5 channel in polycystic kidney disease, especially intracranial aneurysms.

Supported by grants from the National Research Foundation of Korea, which is funded by the Ministry of Science, ICT (Information & Communication Technology) and Future Planning (MSIP) (2015R1A2A1A05001756 to I. So) of the Korean government.

POSTER COMMUNICATION P-50
Targeting protein-protein interactions

ALG-2 interacts with NFAT3 and inhibits transcriptional activation activity.

Wei Zhang, Takuya Achiha, Xuanbo Bao, Rina Matsuo, Terunao Takahara, Hideki Shibata and Masatoshi Maki.

Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.

zhang.wei@i.mbox.nagoya-u.ac.jp

ALG-2 (Apoptosis-Linked Gene 2, gene name: PDCD6) is a Ca^{2+}-binding protein containing five serially repeated EF-hand motifs. By interacting with various intracellular proteins in a Ca^{2+}-dependent manner, ALG-2 is involved in apoptosis, cancer development, signal transduction, membrane trafficking, etc. Although ALG-2 is present both in the cytoplasm and in the nucleus, most known ALG-2-interacting proteins are cytoplasmically localized and little is known about its nuclear function. New insights of Ca^{2+}-dependent gene transcription regulation may be provided by searching for novel nuclear ALG-2-interacting proteins. Since most ALG-2-interacting proteins are known to contain proline-rich regions, we focused on NFAT3 (NFATc4, Nuclear factor of activated T-cells, cytoplasmic 4), which has proline-rich regions in both N-terminal and C-terminal domains, and investigated whether it functions as a new ALG-2-interacting protein. In vitro binding assays including GST-ALG-2 pulldown, co-immunoprecipitation and Far Western were performed, and the obtained results indicated that ALG-2 directly interacted with NFAT3 Ca^{2+}-dependently at multiple sites in NFAT3. Furthermore, Nano-Glo Dual-luciferase reporter assay was carried out to investigate the effects of ALG-2 on transcriptional activation activity of NFAT. A striking inhibitory effect of ALG-2 was observed on Nluc-RE/IL-2 reporter (Nluc-gene fused with NFAT-response element of IL-2 gene promoter) upon ionomycin/PMA stimulation, but not on Nluc-RE/AP1 reporter (Nluc-gene fused with canonical AP1 response element), indicating that ALG-2 inhibits the transactivation activity of NFAT3 specifically. Our findings suggest that ALG-2 modulates Ca^{2+} signal responses by the regulation of Ca^{2+}-dependent gene expression.

Supported by JSPS Grant-In-Aid for Scientific Research B (Project No. 26292050)

POSTER COMMUNICATION P-51
Targeting protein-protein interactions

TRPC3 regulates InsP3 receptor activity and controls senescence.

Valerio Farfariello, Dmitri Gordienko, Emmanuelle Germain, Ingrid Fliniaux, Emilie Desruelles, Oksana Iamshanova, Christian Slomianny, Michela Bernardini, Pascal Mariot, George Shapovalov, Loïc Lemonnier, Alexandre Bokhobza and Natalia Prevarskaya.

University Lille, Inserm, U1003 - PHYCEL - Physiologie Cellulaire, F-59000 Lille, France.

valerio.farfariello@inserm.fr

Cellular senescence is a stable long-term loss of proliferative capacity with preserved cellular viability and metabolic activity and acts as mechanism to arrest cell growth when cells are subjected to stressful conditions. For example, replicative senescence is reached due to telomeres shortening, whereas premature senescence may be induced by UV radiation, oxidative stress, DNA damage and even oncogenic stress. Calcium is an important modulator of virtually all the basic cellular behaviors, including proliferation, differentiation, and apoptosis. However, little is known about the relationship between calcium homeostasis and senescence. Therefore, the aim of this work is to explore the role of calcium-permeable channels in the regulation of premature senescence. Fluorescence Ca^{2+} and confocal imaging, patch-clamp electrophysiology, quantitative PCR, western-blotting, gene silencing and transfection, cell proliferation and survival assays along with the assessment of markers of senescence were used for this purpose. Among several channels, we found that TRPC3 was downregulated in senescent cells. TRPC3 silencing was capable to induce a senescent-like phenotype as confirmed by reduced proliferation and increase of β-galactosidase activity. TRPC3 was found to interact with $InsP_3$ receptors and Ca^{2+} imaging experiments showed significant difference in terms of basal and $InsP_3$-induced Ca^{2+} release in cells with reduced TRPC3 expression, suggesting that the regulation of Ca^{2+} flux via $InsP_3$ by this channel can modify the cell homeostasis and eventually the mitochondrial bioenergetics.

Supported by fellowships and grants from the Fondation pour la Récherche Médicale (SPF20130526749) and the Fondation de France (00057928).

POSTER COMMUNICATION P-52
Targeting protein-protein interactions

Secretion of the phosphorylated form of the alarmin S100A9 from neutrophils is essential for the pro-inflammatory functions of extracellular S100A8/A9.

Veronique Schenten, Fabrice Tolle, Sabrina Bréchard, Sébastien Plançon and Eric J. Tschirhart

Calcium Signalling and Inflammation Group, Life Sciences Research Unit, University of Luxembourg, Luxembourg.

veronique.schenten@uni.lu

S100A8 and S100A9 are members of the S100 family of cytoplasmic EF-hand Ca^{2+}-binding proteins and are abundantly expressed, mostly under heterodimeric form, in the cytosol of neutrophils. In addition to various intracellular roles, S100A8/S100A9 can be secreted in the extracellular environment and be considered as alarmins modifying the inflammatory response. The intracellular activity of S100A8/A9 was shown to be regulated by S100A9 phosphorylation, but the importance of this phosphorylation on the extracellular activity of S100A8/A9 has not yet been extensively studied. Therefore, we focused our work on the impact of the phosphorylation of secreted S100A9 on the pro-inflammatory function of neutrophils. First of all, we characterized the secretion of S100A8/A9 under different stimulatory conditions and investigated the phosphorylation state of secreted S100A9. Our results on neutrophil-like differentiated HL-60 cells (dHL-60) and purified human neutrophils show a time-dependent secretion of S100A8/A9 when induced by PMA and importantly, secreted S100A9 was found in a phosphorylated form. Then we studied the impact of this phosphorylation on pro-inflammatory cytokine expression and secretion in dHL-60 cells. In this aim, time course experiments with unphosphorylated or phosphorylated S100A8/A9 were performed and the expression and secretion levels of IL1α, IL1ß, IL6, TNFα, CCL2, CCL3, CCL4 and CXCL8 were measured by real-time PCR and cytometry bead array respectively. Our results clearly show that only the phosphorylated form of the complex induces pro-inflammatory cytokine expression and secretion. Finally, we were able to show that S100A8/PhosphoS100A9 is inducing cytokine secretion through TLR4 signaling.

Supported by the grant F1R-LSC-PUL-13DAMP from the University of Luxembourg, Luxembourg

POSTER COMMUNICATION P-53
Targeting protein-protein interactions
Selected Oral Communication for Symposium #3

Type 3 Inositol (1,4,5)-trisphosphate receptor: a migratory modulator with a unique calcium signature in breast cancer cells.

Alexia Vautrin, Abdallah Mound, Arthur Foulon, Béatrice Botia, Frederic Hague, Halima Ouadid-Ahidouch, Lise Rodat-Despoix and Fabrice Matifat.

Laboratory of Cellular and Molecular Physiology (EA-4667), "Ion channels in breast cancer", University of Amiens, 33 Rue Saint-Leu, 80039 Amiens France.

alexia.vautrin@u-picardie.fr

As the most common lethal cancer in women worldwide, breast cancer remains a research priority. Though the involvement of ion channels in cancer cell signaling pathways is now well established, the role of inositol (1,4,5)-trisphosphate (IP_3) receptors (IP_3Rs) remains enigmatic. In this context, we investigated the involvement of the three IP_3Rs (IP_3R1, 2 & 3) in migration processes of human breast cancer cells. Migratory behavior of cancer cells was studied on three breast cancer cell lines: the low-migrating MCF-7 cell line and the highly migrating and invasive MDA-MB-231 and MDA-MB-435s cell lines. We demonstrated that a higher IP_3R3 expression at both mRNA and protein levels is correlated to a stronger migration capacity. Gene silencing of IP_3R3 leads to a significant decrease of their migration abilities without changing their proliferation rate. We then investigated the IP_3R3 calcium signature in these three cell lines. Calcium imaging assays reveal an increasing calcium resting ratio according to cellular migration capacities. IP_3R3 silencing caused a drastic modification of the temporal feature of ATP (5 µM)-induced Ca^{2+} signaling, displaying a pattern of sinusoidal Ca^{2+} oscillations instead of a plateau phase. We hypothesized that the migration capacity of breast cancer cells could be related to the temporal feature of the IP_3R3 dependent Ca^{2+} signal. Altogether, our results demonstrate that IP_3R3 is a key target in human breast cancer migration processes. It modulates the migration capacity of cells by affecting the calcium signaling profile.

Supported by the "Région Picardie" and "Fonds Européen de Développement Economique et Regional" (FEDER).

POSTER COMMUNICATION P-54
Calcium Remodeling in Cancer Selected Oral Communication for Symposium #4

Assessment of cytosolic free calcium levels in GCaMP6m expressing MDA-MB-231 breast cancer cells undergoing cell death using time-lapse high-content imaging.

John Bassett, Amelia Peters, Sarah Roberts-Thomson and Gregory Monteith.

School of Pharmacy, The University of Queensland, Brisbane, Australia; Mater Research, The University of Queensland, Brisbane, Australia.

j.bassett@uq.edu.au

Processes important in cancer including cell death and migration occur over long periods of time making the measurement of Ca^{2+} signaling during such events suited to the use of genetically encoded calcium indicators. This study assessed alterations in cytoplasmic free Ca^{2+} ($[Ca^{2+}]_{CYT}$) during cell death using an MDA-MB-231 triple negative breast cancer cell line stably expressing the genetically encoded calcium indicator GCaMP6m. Following treatment with ceramide (100 µM) or staurosporine (1 µM) relative changes in $[Ca^{2+}]_{CYT}$ were investigated. This was achieved by employing time-lapse high-content imaging over 6 hours in low-fluorescence FluoroBrite™ DMEM media using an ImageXpress Micro (Molecular Devices) system, in the presence of propidium iodide 1 µg/mL as an indicator of cell death. Ceramide treatment invoked a significant increase in $[Ca^{2+}]_{CYT}$. The time to reach peak $[Ca^{2+}]_{CYT}$ showed considerable variance between individual cells, from immediate to 5 hours. Peak relative $[Ca^{2+}]_{CYT}$ was greater in the subset of cells that underwent cell death (~10%). Moreover, increases in $[Ca^{2+}]_{CYT}$ always preceded increases in propidium iodide permeability (cell death). Current studies are assessing the consequences of silencing plasma membrane Ca^{2+} ATPase isoforms on ceramide-induced $[Ca^{2+}]_{CYT}$ increases and the relationship with propidium iodide permeability. Staurosoporine treatment induced an initial rapid increase in $[Ca^{2+}]_{CYT}$ and morphological changes followed by sustained oscillations in $[Ca^{2+}]_{CYT}$. Assessment of the mechanism of $[Ca^{2+}]_{CYT}$ oscillations and their association with cell death pathways may provide new insight into the relationship between calcium signaling and cell death in triple negative breast cancer cells.

Supported by National Health and Medical Research Council of Australia Project Grants (1079671, 1079672), Australian Postgraduate Award.

POSTER COMMUNICATION P-55
Calcium Remodeling in Cancer

Role of Orai1 and Orai2 in the regulation of store-operated Ca^{2+} entry, migration and FAK phosphorylation in HL60 cells.

Ginés M. Salido, Raquel Diez, Isaac Jardin and Juan A. Rosado.

Department of Physiology (Cellular Physiology Research Group), University of Extremadura, 10003 Caceres, Spain.

gsalido@unex.es

The adequate modulation of intracellular Ca^{2+} homeostasis is vital for a number of physiological processes. Store-operated calcium entry (SOCE) mainly fine-tunes such events through the Orai and TRP plasma membrane channel families. Deregulations in SOCE, together with modified expression levels in the proteins involved has been postulated to play a critical role in the evolution or progression of several cancer hallmarks, including uncontrolled proliferation, migration, invasion or resistance to apoptosis. Here we demonstrate that, in the acute myeloid leukemia cell line HL60, Orai2 is highly expressed both at the mRNA and protein level when compared to the chronic myeloid leukemia cell line MEG01 or human platelets, whereas Orai1 expression was reduced. In addition, the TRPC1, TRPC3 and TRPC6 channels were almost absent in HL60 cells. Furthermore, by using fluorescence Ca^{2+} imaging, we show that silencing both Orai1 and Orai2 significantly abated TG-induced SOCE, hence implying a critical role of these proteins in SOCE in HL60 cells. While knockdown of Orai1 and Orai2 did not have any effect in cell proliferation, their expression silencing did significantly impair the ability of HL60 to migrate in vitro, as determined by using the transwell migration assay, presumably due to diminishment of FAK tyrosine phosphorylation. In summary, our findings support a role for Orai1 and Orai2 in SOCE and migration in the human HL60 promyeloblastic cell line.

Supported by grants BFU2013-45564-C2-1-P and Junta de Extremadura-FEDER GR15029. IJ and RD from Ministerio de Economía y Competitividad, Spain.

POSTER COMMUNICATION P-56
Calcium Remodeling in Cancer

STIM1 and Orai1 modulate the migration and proliferation of human thyroid cancer ML-1 cells.

Asghar M.Y.[1], Magnuson M.[1], Lassila T.[1], Kemppainen K.[1] and Törnquist K.[1,2]

[1]Cell Biology, Faculty of Science and Engineering, Åbo Akademi University, 20520 Turku, Finland. [2]Cellular Physiology, Minerva Foundation Institute of Medical Reasearch, Biomedicum Helsinki 00270, Helsinki, Finland.

myasghar@abo.fi

Calcium signaling is pivotal in regulation of many cellular processes. Despite of the ubiquitous expression of STIM1 and Orai in human cells, the function of these calcium channels in thyroid cells remained elusive. In the present investigation, we examined the role of STIM1 and Orai1 channels in thyroid cancer ML-1 cell migration and proliferation. For this purpose, we generated stable ML-1 shNT STIM1 and shNT Orai1 knock-down cells. Our results showed that the STIM1 and Orai1 expression in respective knock-down cells was considerably downregulated on both the mRNA (≈90%) and protein (≈50%) levels. In both STIM1 and Orai knockdown cells, SOCE was decreased and there was a significant decrease in the serum-evoked invasion, as compared to mock-transfected control cells (MOCK cells). Furthermore, the S1P induced increase in invasion was abolished in both the STIM1 and Orai1 knockdown cells, as compared with MOCK cells. This decrease in S1P-evoked migration was due to the downregulation of pro-migratory S1P1/S1P3 and VEGFR2 receptors in these cells. In addition, proliferation assay showed a significant and sustained decrease in the proliferation rate of both STIM1 and Orai1 knock-down cells due to a prolonged G1 and decreased S phases of the cell cycle, compared to MOCK cells. In knock-down cells, there was a significant increase in the expression of the cyclin-dependent kinase inhibitors p-21 and p-27. We conclude that in human follicular thyroid ML-1 cancer cells, STIM1 and Orai1 calcium channels modulate the invasion and proliferation through calcium dependent mechanism and the expression of pro-migratory receptors.

Supported by the grants from Sigrid Juselius Foundation and Finnsih-Norwagian Medical Foundation which are gratefully acknowledged.

POSTER COMMUNICATION P-57
Calcium Remodeling in Cancer

Contribution of Orai, Stim and TRP proteins to store-operated Ca^{2+} entry (SOCE) in human colonic mucosa normal and tumor cells.

Diego Sobradillo[1], Daniel Ubierna[1], Miriam Hernández-Morales[1], Lucía Núñez[1,2] and Carlos Villalobos[1].

[1]Institute of Molecular Biology and Genetics (IBGM) and [2]Dept. of Biochemistry and Molecular Biology and Physiology, University of Valladolid and Spanish National Research Council (CSIC) 47003 Valladolid, Spain.

ubierna@ibgm.uva.es

Ca^{2+} is involved in critical cancer hallmarks including enhanced cell proliferation, invasion and survival. We have shown recently that store-operated Ca^{2+} entry (SOCE) and store-operated currents (SOCs) are increased in human colon carcinoma cells relative to normal human mucosa cells. However, the molecular players involved in SOCE in normal and tumor cells have not been fully elucidated. Here we investigated expression and contribution of some of these channels to SOCE in normal and tumor cells. Normal human colonic mucosa cells (NCM460 cells) express molecular players involved in SOCE including Orai1, Orai2 and Orai3, Stim1 and Stim2. In addition, they also express TRP channels that have been related to SOCE including TRPC1, TRPC4, TRPV4, TRPV6 and TRPM8. In contrast, human colon carcinoma cells (HT29 cells) express all Orai and Stim proteins as well as TRPC1 and TRPC4 but no TRPV4, TRPV6 or TRPM8. Silencing of either Orai1, Stim1 or Stim2 decreases SOCE in normal mucosa cells while the removal of either Orai2, Orai3, alone or in combination, has no effect on SOCE. In tumor cells, silencing of either Orai1 or Stim1 inhibits SOCE in tumor cells while removal of Stim2 has no effect. Interestingly, silencing of both Stim1 and Stim2 in tumor cells reduced SOCE to a larger extent than silencing Stim1 alone. Finally, removal of Orai2 and Orai3, either alone or in combination, has no effect on SOCE. When taken together, these and our previous results indicate that, in normal colonic mucosa cells, SOCE is driven by Orai1, Stim1 and Stim2. In contrast, in colon cancer cells, SOCE depends on Orai1, Stim1 and TRPC1, but it does not depend on Stim2 unless Stim1 is absent. Thus, the calcium channel complex involved in SOCE and SOCs in colonic mucosa cells switches Stim2 by TRPc1 in colon cancer.

Supported by grants BFU2012-37146, BFU2015-70131R and BIO/VA46/14 from Ministerio de Economía y Competitividad and Junta de Castilla y León, Spain. DU was supported by pre-doctoral fellowship from Junta de Castilla y León, Spain and the European Social Fund. DS was supported by a JAE predoctoral fellowship for Spanish National Research Council (CSIC).

POSTER COMMUNICATION P-58
Calcium Remodeling in Cancer

The Warburg effect enhances store-operated Ca^{2+} entry in colon carcinoma cells by preventing inactivation of store-operated currents.

Diego Sobradillo[1], Miriam Hernández-Morales[1,*], David del Bosque[1], Lucía Núñez[1,2] and Carlos Villalobos[1].

[1]Institute of Molecular Biology and Genetics (IBGM), [2]Dept. of Biochemistry and Molecular Biology and Physiology, University of Valladolid and Spanish National Research Council (CSIC). Valladolid, Spain.

d.sobradillo@ibgm.uva.es

We have shown recently that human colon carcinoma cells are characterized by increased store-operated Ca^{2+} entry (SOCE) and store-operated currents (SOCs) that contribute to colon cancer cell hallmarks (Sobradillo et al., 2014). It has been also shown that SOCE and SOCs depend on mitochondrial Ca^{2+} uptake to prevent Ca^{2+}-dependent inactivation. This modulation may have important implications as several non-steroideal anti-inflammatory drugs (NSAIDs) that prevent colon cancer might inhibit SOCE and SOCs acting on mitochondrial control of SOCE. Accordingly, we decided to investigate mitochondrial control of SOCE and SOCs in normal and colon carcinoma cells. We found that mitochondrial Ca^{2+} uptake is larger in tumor cells than in normal cells regardless the activating mechanism. Expression of the mitochondrial Ca^{2+} uniporter and regulatory proteins MCU1 and MCU2 is similar in normal and tumor cells. However, mitochondrial potential, the driving force for mitochondrial Ca^{2+} uptake is much larger in tumor than in normal cells, probably due to the Warburg effect. We investigated also mitochondrial control of SOCs in normal and tumor cells using planar patch clamp electrophysiology. In normal cells, low Ca^{2+} buffering decreased SOC activation and this effect was prevented by enhancing respiration with a mitochondrial cocktail. In tumor cells, low Ca^{2+} buffering decreased SOC activation and promoted its inactivation and this latter effect was reversed by mitochondrial cocktail. Consistently, FCCP decreased SOC activation in normal cells and promoted SOC inactivation in tumor cells. These results suggest that mitochondria are essential for SOC activation in normal cells. However, in tumor cells, mitochondria are required to prevent SOC inactivation. Mitochondrial depolarization with NSAIDs including salicylate, sulindac and R-flurbiprofen decreased SOC in tumor cells and these effects are abolished in high Ca^{2+} buffering. We conclude that the Warburg effect in colon carcinoma cells enhances mitochondrial potential, thus favouring mitochondrial Ca^{2+} uptake that prevents SOC inactivation for a stronger, sustained SOCE.

Supported by grants BFU2012-37146 and BFU2015-70131R from Ministerio de Economía y Competitividad, Spain and BIO/VA46/14 from Junta de Castilla y León, Spain. DS was supported by a JAE predoctoral fellowship for Spanish National Research Council (CSIC). DS and MHM contributed equally to this work.

POSTER COMMUNICATION P-59
Calcium Remodeling in Cancer

Inhibition of polyamine biosynthesis by DFMO reverts partially Ca^{2+} remodeling in colon cancer cells.

Lucía G. Gutiérrez, Miriam Hernández-Morales, Diego Sobradillo, Lucía Núñez and Carlos Villalobos

Institute of Molecular Biology and Genetics (IBGM), University of Valladolid and Spanish National Research Council (CSIC) 47003 Valladolid, Spain.

lgonzalezg@ibgm.uva.es

Recently we have shown that colon cancer cells undergo remodeling of intracellular Ca^{2+} homeostasis including changes in store-operated Ca^{2+} entry (SOCE), store-operated currents and Ca^{2+} store content associated to changes in molecular players involved in SOCE (Sobradillo et al., 2014). Reversing this remodeling could contribute to protection against cancer and cancer chemoprevention. Difluoromethylornithine (DFMO) is a suicide inhibitor of ornithine decarboxylase (ODC), the limiting step in the synthesis of polyamines that is considered one of the best chemopreventive compounds against colon cancer. Here we tested the effects of DFMO treatment on SOCE, SOCs, Ca^{2+} store content, the molecular players involved and resistance to cell death, a critical cancer hallmark. We found that ODC was largely overexpressed in colon cancer cells suggesting increased synthesis of polyamines in colon cancer cells. Short-term treatment with DFMO (500 µM, 12 h) decreased significantly SOCE and store-operated currents in colon cancer cells. DFMO had no effect on Icrac but prevented selectively the appearance of the outward component of store-operated current likely mediated by TRPC1. DFMO also increased Ca^{2+} store content and the fraction of cells undergoing early apoptosis induced by H_2O_2. At the molecular level, we found DFMO tend to decrease all molecular players involved in SOCE except Stim2 but the effects were statistically significant only for TRPC1 mRNA. In summary, inhibition of polyamine synthesis decreases SOCE and SOCs in human colon cancer cells acting probably on expression of TRPC1 and tends to increase Ca^{2+} store content and susceptibility to apoptosis in human colon cancer cells.

Supported by grants BFU2012-37146 and BFU2015-70131R from Ministerio de Economía y Competitividad and BIO/VA46/14 Junta de Castilla y León, Spain. LGG and DS were supported by University of Valladolid and CSIC. DS was supported by a JAE predoctoral fellowship from Spanish Nationa Research Council (CSIC).

POSTER COMMUNICATION P-60
Calcium Remodeling in Cancer

Ca^{2+} protein alpha 1D is a new mediator of colon cancer cells migration and invasion.

Yann Fourbon[1], Maxime Guéguinou[1], Romain Felix[1], Bruno Constantin[2], Arnaud Hugen[3], Laurie Lajoie[4], Thierry Lecomte[4], Gaëlle Fromont[1], Aurélien Chatelier[2], Olivier Mignen[3], Marie Potier-Cartereau[1], Aurélie Chantôme[1], Patrick Bois[2] and Christophe Vandier[1]

[1]Inserm UMR 1069, Université de Tours-France. [2]Equipe ERL 7368 CNRS, Université de Poitiers-France. [3]Inserm UMR 1078 IFR148, Université de Bretagne Occidentale-France. [4]GICC– UMR 7292, Université de Tours, France.

yann.fourbon@etu.univ-tours.fr

It is generally accepted that voltage-gated calcium channels, CaV, and the CaV1.3 regulate calcium homeostasis in excitable cells following plasma membrane depolarization. Nevertheless, the role of the CaV1.3 in the biology of colon cancer cells is unknown. Here we investigated the role of the calcium protein alpha 1D, the protein that forms the pore of CaV1.3 channel, in the biology of the non-excitable and epithelial cancer cells HCT116. Our studies show that alpha 1D protein is overexpressed in colorectal cancer biopsies compared to normal tissues. Verapamil, a pharmacological inhibitor of CaV1.3 and gene silencing experiments targeting alpha 1D protein both reduced the migration and the invasion of HCT116 colon cancer cells. In addition, verapamil and gene silencing experiments reduced the basal cytosolic Ca^{2+} concentration and the frequency of cytosolic Ca^{2+} calcium oscillations induced by the sodium-calcium exchanger working in its reverse mode. However, membrane depolarization does not induce an increase in intracellular calcium and no CaV currents could be recorded in resting or migrated cells using whole cell configurations of both broken and perforated patch-clamp. Finally, flow cytometry studies showed that only 10% of alpha 1D protein is expressed at the plasma membrane of HTC116 cells. All these data showed that alpha 1D protein of CaV1.3 is involved in the regulation of calcium homeostasis and migration/invasion of HCT116 cells. Nevertheless, the mechanism remains to be determined but that does not depend on its plasma membrane canonical function.

Supported by the "University of Tours", "INSERM", the "Région Centre" of France, the Canceropole Grand Ouest, the "Ligue Nationale Contre le Cancer" the Association "CANCEN" and Tours' Hospital oncology association ACORT." Yann Fourbon and Maxime Guéguinou held fellowship from the "Région Centre". We thank the « Local tumor tissue biobank BB-0033-00037 ("CRB Santé/Tumorothèque de Brest") and Aurore Douaud-Lecaille, Morgan Aubry and Isabelle Domingo for technical assistance and Catherine Leroy for secretarial support.

POSTER COMMUNICATION P-61
Calcium Remodeling in Cancer

Expression of calcium pumps in different breast cancer cell lines during histone deacetylase inhibitor treatments.

Karolina Varga[1,2], Rita Padányi[1], Luca Hegedűs[3], Katalin Pászty[4], Béla Papp[5] and Agnes Enyedi[1,6]

[1]2nd Department of Pathology, Semmelweis University, Budapest, Hungary; [2]MTA-SE-NAP Brain Metastasis Research Group of the Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary; [3]Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria; [4]Molecular Biophysics Research Group of the Hungarian Academy of Sciences and Department of Biophysics, Semmelweis University, Budapest, Hungary; [5]U978, Institut National de la Santé et de la Recherche Médicale and Université Paris-13, PRES Sorbonne Paris-Cité, Bobigny, France; [6]Molecular Oncology Research Group of the Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary.

varga.karolina@med.semmelweis-univ.hu

Ca^{2+} homeostasis remodeling is one of the main hallmarks of cancer. According to the literature, Ca^{2+} pump expressions (plasma membrane Ca^{2+} ATPases, PMCAs and/or sarco/endoplasmic reticulum Ca^{2+} ATPases, SERCAs) are altered in cancer cells and tissue samples. Our previous results show low expression of the PMCA4b isoform in luminal-type MCF-7 breast cancer cells. Treatment of these cells with histone deacetylase inhibitors (HDACis) resulted in a marked upregulation of PMCA4b and enhanced Ca^{2+} clearance. Based on these findings we examined how HDACis such as valproic acid and suberoylanilide hydroxamic acid (SAHA) affect Ca^{2+} pump expression in breast cancer cell lines with different genetic backgrounds. These epigenetic drugs are in clinical trials or are already used in the treatment of selected cancers. We found low PMCA4b expression in luminal-type cells and a marked upregulation of this pump upon HDACi-treatments. In contrast, PMCA4b expression was relatively high in the basal subtypes and in the non-tumorigenic MCF-10A cell line, and HDACi treatments increased PMCA4b expression only marginally. Immunocytochemical analysis revealed that PMCA4b was located mostly in the plasma membrane in luminal cells. However, in basal cells the plasma membrane expression of PMCA4b was much less pronounced. SERCA3 was expressed in all luminal subtypes and HER2 overexpressing cells, while it was totally absent in basal cells and in MCF-10A. Our results show, that Ca^{2+} homeostasis is remodeled in breast cancer in a subtype specific manner, and that the pharmacological manipulation of histone modifications has profound consequences on the Ca^{2+} homeostasis of breast carcinoma cells.

Supported by the Hungarian Scientific Research Funds OTKA K101064, ANN110922 and the ARC, France.

POSTER COMMUNICATION P-62
Calcium Remodeling in Cancer

A new synthetic ether-lipid activator of TRPV2 promotes constitutive calcium entry and breast cancer cell migration.

Maxime Guéguinou[1], Romain Félix[1], Aubin Penna[2], Séverine Marionneau-Lambot[7], Thibauld Ouiller[7], François Carreaux[8], Audrey Gambade[1], Y Fourbon[1], Christophe Arnoult[5], Thomas Harnois[3], Aurélie Chantôme[1], Gaëlle Simon[6], Jean-Pierre Haelters[6], Paul-Alain Jaffrès[6], Günther Weber[1], Bruno Constantin[3], Thierry Lecomte[4], Olivier Mignen[4], Christophe Vandier[1] and Marie Potier-Cartereau[1]

[1]Inserm UMR 1069, Université de Tours-France. [2]IRSET Inserm U1085, Université de Rennes1-France. [3]Equipe ERL 7368 CNRS, Université de Poitiers-France. [4]Inserm UMR 1078 IFR148, Université de Bretagne Occidentale-France. [5]GICC— UMR 7292, Université de Tours-France. [6]CNRS UMR 652, Université de Brest-France. [7]Cancéropôle Grand-Ouest, Nantes-France. [8]UMR CNRS 6226, Université de Rennes1-France.

romain.felix@univ-tours.fr

TRPV2 channel (Transient receptor potential vanilloid) was found to regulate glioblastoma cell proliferation and to be implicated in the progression of prostate and bladder cancers to more invasive phenotype. Lipids such as LPC (LysophosPhatidylCholine) have been shown to activate TRPV2 channels but surprisingly the role of ether-lipid is not known. In this study we compared the effects of LPC and of the synthetic ether-lipid GS1197 on its capacity to modulate TRPV2 channel and migration of two breast cancer cell lines: MDA-MB-435s and MDA-MB-231 cells. We found that LPC and GS1197 enhanced a constitutive calcium entry by inducing the translocation of TRPV2 protein to the plasma membrane through Golgi stimulation. Patch clamp experiments revealed that TRPV2 channels recruited, following LPC or GS1197 stimulation, are functional at the plasma membrane. In addition, these lipids induced a cytoskeletal reorganization and thus increase the migration of MDA-MB-231 and MDA-MB-435s cells. These two effects of GS1197 are dependent of PI3K/AKT/Rac signalling pathway. This study that demonstrates a role of TRPV2 channels in breast cancer cell lines migration and, more importantly, we highlight the potential use of synthetic ether-lipid as a novel modulator of TRPV2 channel in cancer.

Supported by the "University of Tours", "INSERM", the "Région Centre" of France, the Cancéropôle Grand Ouest, the "Ligue Nationale Contre le Cancer" the Association "CANCEN" and Tours' Hospital oncology association ACORT. Maxime Guéguinou held fellowship from the "Région Centre". We thank Aurore Douaud-Lecaille, Morgan Aubry and Isabelle Domingo for technical assistance and Catherine Leroy for secretarial support.

POSTER COMMUNICATION P-63
Calcium Remodeling in Cancer

Calcium sensing receptor controls SK3 channel expression and SK3-dependent cell migration in breast and prostate cancer cells.

Aurélie Chantôme[1], Lucie Clarysse[1], Marie Roy[1], Emeline Bon[1], Grégoire Vandecasteele[2], Stephan Chevalier[1], Marie Potier-Cartereau[1] and Christophe Vandier[1].

[1]Inserm UMR 1069, Nutrition croissance et cancer, Université de Tours, France. [2]UMR-S769, Signalisation et Physiopathologie Cardiaque, Faculté de Pharmacie Université Paris-Sud, Châtenay-Malabry, France.

aurelie.chantome@univ-tours.fr

The abnormal expression of the SK3 channel (a member of small conductance calcium-activated potassium channels) in breast and prostate cancer cells promotes calcium-dependent cell migration through its association with the Orai1 channel. This complex led to calcium entry, calpain activity and cell migration. Interestingly, we showed that SK3 channel, in breast cancer cells, promotes bone metastases development, this having a direct link between the activation of SK3 by calcium and the high calcium concentrations found in the bone environment. Since several studies suggest that the calcium sensing receptor (CaSR) facilitate bone metastases of breast and prostate cells we have explored the role of CaSR in SK3 expression and thus on SK3-dependent cell migration of breast MDA-MB-435s and prostate PC3 cancer cells. Western-blotting, qPCR, gene reporter assays, gene silencing/sur-expressing and cell migration experiments were performed. We found that an increase of external [Ca^{2+}] up-regulated SK3 proteins and transcripts through the control of KCNN3 gene transcription and this led to an increase of SK3-dependent cell migration. CaSR gene silencing reduced KCNN3 transcription that became insensitive to external [Ca^{2+}] variations. In contrast CaSR over-expression increased KCNN3 gene transcription. All these data demonstrate that CaSR controls the expression of SK3 channel and cancer cell migration probably through the recruitment of the $G\alpha i$ by the CaSR since we already demonstrated that activation of the adenylate cyclase by forskolin treatment decreased SK3 expression. As a next step we propose to explore, by immunohistochemistry, whether a correlation exists between CaSR and SK3 expression on bone metastases tissues.

Supported by the "University of Tours", "INSERM", the "Ligue Nationale Contre le Cancer", Ion Channel and Cancer network of « Cancéropôle Grand Ouest », the Association "CANCEN" and Tours' Hospital oncology association ACORT. Lucie Clarysse held fellowship from the "INSERM" and "Région Centre". Emeline Bon held fellowship from the "Institut Universitaire de France".

POSTER COMMUNICATION P-64
Calcium Remodeling in Cancer

Changes in calcium signaling as a consequence of doxorubicin treatment in MDA-MB-231 breast cancer cells expressing a genetically-encoded calcium indicator.

Alice H.L. Bong[1], John J. Bassett[1], Sarah J. Roberts-Thomson[1] and Gregory R. Monteith[1,2].

[1]School of Pharmacy, University of Queensland. Brisbane, Australia. [2]Mater Research, The University of Queensland. Brisbane, Australia.

h.bong@uq.edu.au

Doxorubicin is the most commonly used chemotherapy agent to treat triple-negative breast cancer; however, its therapeutic efficacy is limited in later stages of disease. Recent evidence suggests that some chemotherapeutic agents alter Ca^{2+} signaling; however, the effects of doxorubicin on Ca^{2+} signaling in triple-negative breast cancer cells are still poorly understood. Here, we characterized doxorubicin-induced changes in intracellular Ca^{2+} signaling in MDA-MB-231 triple-negative breast cancer cells stably expressing the GCaMP6m genetically-encoded Ca^{2+} sensor. Using live cell imaging and confluency measurements, the concentration-response effects of doxorubicin on the proliferation of GCaMP6m-expressing MDA-MB-231 cells were defined. High-content imaging was used to measure intracellular Ca^{2+} changes after a 24-hour pulse-treatment with doxorubicin. The effect of doxorubicin on agonist-mediated increases in intracellular free Ca^{2+} entry were assessed by the addition of ATP at 1 µM and 100 µM. No significant changes in the amplitude and time taken to elicit ATP-induced Ca^{2+} transients were observed, however, a significantly higher proportion of cells treated with doxorubicin (1 µM) responded to ATP (1 µM) compared to control cells. Doxorubicin (1 µM) treatment also significantly prolonged the time at which cytoplasmic Ca^{2+} recovered to 50% of peak levels reached after store-operated Ca^{2+} entry (SOCE). In conclusion, doxorubicin treatment in GCaMP6m-expressing MDA-MB-231 cells appears to be associated with changes in components of SOCE and alteration in sensitivity to sub-maximal ATP stimulation. Further studies are now warranted assessing other aspects of Ca^{2+} signaling in MDA-MB-231 cells treated with doxorubicin and the mechanisms responsible for changes in ATP responses and SOCE.

Supported by an Australian Postgraduate Award.

POSTER COMMUNICATION P-65
Calcium Remodeling in Cancer

Modulation of the membrane properties by an alkyl-lipid derivate and its consequences in calcium entries and metastasis development.

Ana Bouchet[1,3], Fernando Herrera[4], Paul-Alain Jaffres[2,3], Aurélie Chantôme[1,3], Marie Potier-Cartereau[1,3], Jose Requejo-Isidro[5] and Christophe Vandier[1,3]

[1]INSERM UMR 1069, Université de Tours, France. [2]CEMCA UMR CNRS 6521, équipe Phosphore & Vectorisation, Université de Brest, France. [3]Network and cancer-Canceropole Grand Ouest, (IC-CGO),[4]Physics Dept, Universidad del Litoral, Santa Fe, Argentina. [5]Unidad de Biofisica (CSIC-UPV/EHU), Leioa, Spain.

anamaria.bouchet@univ-tours.fr

Specific properties of lipid organisation (fluidity, phase separation, thickness) regulate the structure and activity of membrane proteins, including ion channels. Their role in the regulation of several diseases has been pointed out during the last decade. Our previous data have shown that the SK3 channel (a calcium-activated potassium channel) is involved in calcium entries and the migration of various cancer cells. Interestingly an alkyl-lipid derivate named Ohmline modulates the activity of this cannel (1). The decrease in channel activity by Ohmline is associated with a reduction of cancer cell migrations but does not affect the migration of non-cancer cells (2). Many antecedents indicate that during cell migration a change in membrane fluidity occurs in the plasma membrane of cancer cells providing evidence of a pivotal role of biophysical adaptation in this biological process that ultimately lead to metastatic development. In the present work, we characterise the physicochemical properties of Ohmline in various lipid environments. Molecular dynamic simulations suggest that Ohmline molecules organize differently depending on the lipid bilayer nature. Fluorescence spectroscopy on model lipid membranes confirm that Ohmline modulates the bilayer order. Imaging the lipid-packing state upon treatment of live cells with Ohmline supports the model in-situ. The consequences of these observations will be discussed in terms of therapeutic prospectives in tumour development and more particularly in metastasis development. (1) Jaffres et al. (2016) Pharmacol Ther.. (2) Girault A, et al. (2011) Current Cancer Drug Target, 11, 1111.

Supported by University of Tours, PRESTIGE-European FP7, Region Centre (LIPIDS project of ARD2020-Biomédicaments), la Ligue Contre le Cancer, INSERM, CNRS, Cancéropôle Grand Ouest, the association "CANCEN" and Tours' Hospital oncology association ACORT". Ana Bouchet holds a post-doc mobility grant from PRESTIGE-European FP7 / University of Tours.

POSTER COMMUNICATION P-66
Calcium Remodeling in Cancer

Mechanism of sorcin (Soluble Resistance-related Calcium binding proteIN)-dependent resistance to chemotherapeutic drugs in H1299 cancer cell line.

[1]Ilaria Genovese, [2]Silvia Masciarelli, [3]Andrea Ilari Andrea, [1]Annarita Fiorillo, [2]Francesco Fazi and [3]Gianni Colotti

[1]Department of Biochemistry "A. Rossi Fanelli" Sapienza University of Rome; [2] Department of Anatomical, Histological, Forensic & Orthopaedic Sciences, Section of Histology & Medical Embryology; [3] CNR-National Research Council of Italy, Institute of Molecular Biology and Pathology.

ilaria.genovese@uniroma1.it

Sorcin is overexpressed in several human tumors, is a marker of Multi-Drug Resistance (MDR), is highly expressed in chemoresistant cell lines, and confers MDR when overexpressed. Sorcin gene is in the same amplicon of glycoprotein P (mdr1) and its silencing increases cancer cell sensitivity to chemotherapeutic drugs (Colotti et al., 2014). Sorcin is involved in mitosis and cytokinesis and dynamically localizes at nuclear, cell, vesicles membranes, midbody and endoplasmic reticulum (ER). Sorcin regulates ER calcium channels, enabling calcium accumulation inside ER, preventing ER stress and cell death (Lalioti et al., 2014). Recently we solved the crystal structure of sorcin in the apo and calcium-bound forms. Upon calcium binding, a large conformational change occurs, with the exposure of hydrophobic surfaces, that allows sorcin interaction with molecular targets (Ilari et al., 2015). To elucidate sorcin-dependent mechanisms of chemoresistance we used H1299 lung cancer cells, which express high amount of sorcin; We silenced sorcin expression through siRNA; We treated cells with doxorubicin to elucidate changes in the uptake process and in the biological response; We tested sorcin affinity for doxorubicin through Surface Plasmon Resonance and fluorescence experiments. Our data show that: i) Sorcin binds doxorubicin directly and with high affinity. ii) Upon sorcin silencing, an increase in cell death can be observed upon treatment with doxorubicin, with respect to control H1299 cells. iii) After silencing, cells exhibits increased drug accumulation upon treatment with doxorubicin, compared to control cells. iv) Sorcin silencing decreases MDR1 expression and reduces the efflux of drug from cells.

Supported by qPMO, CNCCS, Ricerca Finalizzata Salute, Flagship Project "Nadine"

POSTER COMMUNICATION P-67
Calcium Remodeling in Cancer

Sulforaphane-induced apoptosis involves the type 1 IP_3 receptor.

Sona Hudecova, Lucia Csaderova, Tibor Stracina[1], Paulina Gronesova, Marie Novakova[1], Silvia Pastorekova, Jan Sedlak and Olga Krizanova

Biomedical Research Center, SAS, Bratislava, Slovakia; [1]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

olga.krizanova@savba.sk

In this study we show that anti-tumor effect of sulforaphane (SFN) is partially realized through the type 1 inositol 1,4,5-trisphosphate receptor (IP_3R1). This effect was verified in vitro on three different stable cell lines and also in vivo on the model of nude mice with developed tumors. Early response (6 hours) of A2780 ovarian carcinoma cells to SFN treatment involves generation of mitochondrial ROS and increased transcription of NRF2 and its downstream regulated genes including heme oxygenase 1, NAD(P)H:quinine oxidoreductase 1, and KLF9. Prolonged SFN treatment (24 hours) upregulated expression of NRF2 and IP_3R1. SFN induces a time-dependent phosphorylation wave of HSP27. Use of IP3R inhibitor Xestospongin C (Xest) attenuates both SFN-induced apoptosis and the level of NRF2 protein expression. In addition, Xest partially attenuates anti-tumor effect of SFN in vivo. SFN-induced apoptosis is completely inhibited by silencing of IP_3R1 gene but only partially blocked by silencing of NRF2; silencing of IP_3R2 and IP_3R3 had no effect on these cells. Xest inhibitor does not significantly modify SFN-induced increase in the rapid activity of ARE and AP1 responsive elements. We found that Xest effectively reverses the SFN-dependent increase of nuclear content and decrease of reticular calcium content. Moreover, immunofluorescent staining with IP_3R1 antibody revealed that SFN treatment induces translocation of IP_3R1 to the nucleus. Our results clearly show that IP3R1 is involved in SFN-induced apoptosis through the depletion of reticular calcium and modulation of transcription factors through nuclear calcium up-regulation.

Supported with following grant schemes: VEGA 2/0082/16, VEGA 2/0177/11, APVV-0045-11 and CEMAN.

POSTER COMMUNICATION P-68
Calcium Remodeling in Cancer

CD5 controls B-CLL Ca^{2+} constitutive entry by inducing TRPC1 expression.

Marjolaine Debant [1,2,4], Paul Buscaglia [1,4], Patrice Hemon [1,2], Christelle le Dantec [1], Miguel Burgos [3], Yves Renaudineau [2,4] and Olivier Mignen [1,4]

[1]Inserm U1078, CSC Team, Brest, France ; [2]LiPaB, E12216, ESPRI-ERI29, Brest, France ; [3]CRIB and Facultad de Medicina, Albacete, Spain [4] Canceropole Grand Ouest, France.

marjolaine.debant@etudiant.univ-brest.fr

Chronic Lymphocytic Leukemia (CLL) is the most common type of leukemia in Western countries, resulting of the accumulation of mature monoclonal B cells that express the T cell marker CD5. The aim of the study was to characterize the molecular actors involved in the constitutive activation of Ca^{2+} dependent surviving factors such as pERK, NFAT2 and the interleukin 10. CLL patients were subdivided in two groups (high and low) based on the amplitude of the constitutive Ca^{2+} entry (CaCE) measured in unstimulated CLL B cells using Ca^{2+} fluorescence imaging. Patients presenting an elevated CaCE present a more aggressive disease (Binet score B/C, and shorter Progression Free Survival). The analysis of Orai1 and TRPC1 expression using flow cytometry revealed a higher expression of these proteins in patients with elevated CaCE. Experiments using siRNA targeting CD5 in CLL B cells indicated that CD5 regulates TRPC1 mRNA expression in CLL B cells. Finally, to further demonstrate the role of CD5 in CaCE regulation, the human B cell line Jok-1 was transfected with CD5 revealing that CD5 expression in B cells was effective to induce TRPC1 expression, that in turns increases the CaCE and downstream Erk1/2 phosphorylation, and IL-10 secretion. In conclusion, our results highlight a novel Ca^{2+} signaling pathway in CLL B cells implicated in CLL progression and thus appearing as a potential therapeutic target.

This work is granted by Région Bretagne and Ligue Contre le Cancer, France

POSTER COMMUNICATION P-69
Calcium Remodeling in Cancer

Cancer treatment using calcium electroporation induces tumor necrosis whilst sparing surrounding normal tissue.

Stine Krog Frandsen[1,3], Mie Barthold Krüger[1], Trine Tramm[2], Ivana Novak[3], and Julie Gehl[1]

[1]Center for Experimental Drug and Gene Electrotransfer, Department of Oncology, Herlev and Gentofte Hospital, University of Copenhagen, Denmark. [2]Department of Experimental Clinical Oncology, Aarhus University Hospital, Denmark. [3]Department of Biology, Section for Cell Biology and Physiology, University of Copenhagen, Denmark.

Stine.Krog.Frandsen@regionh.dk

Background. Calcium electroporation is an experimental novel anti-cancer therapy where application of short, high voltage pulses causes transient permeabilization (electroporation) of the cell membrane allowing high calcium influx into the cytosol. Clinical studies are ongoing.This study aimed at investigating sensitivity to calcium electroporation in different human tumor models and the surrounding normal tissue as well as estimating the intracellular calcium content and the plasma membrane Ca^{2+}-ATPase (PMCA) protein expression.
Results. Calcium electroporation induced necrosis in all four tested tumor types (bladder (SW780), breast (MDA-MB231), colon (HT29), and small cell lung cancer (H69)) with difference in sensitivity (36-88% necrosis two days after treatment). Calcium electroporation induced tumor necrosis when using calcium concentrations of 100-500 mM and injection volumes from 20-80% of tumor volume ($p<0.0001$ vs calcium injection only). Only limited effects were seen in normal surrounding skin and muscle tissue. Intracellular free calcium concentration measured using Fluo-4 increased significantly in vitro after calcium electroporation. Total intracellular calcium content increased 3-fold ($p<0.0001$) in tumor and skin but decreased significantly ($p<0.05$) in skin after treatment, however not in tumor. This might be explained by the 5-23 fold lower expression ($p<0.0001$) of PMCA in the cancer cell lines compared with human primary normal fibroblasts (HDF-n).
Conclusion. Taken together, our findings show that calcium electroporation induced tumor necrosis while sparing normal surrounding tissue. Among other factors, this might be due to a lower PMCA expression in cancer cells.

Funding: S.K. Frandsen (University of Copenhagen), I. Novak (the Danish Council for Independent Research Natural Sciences; 4002-00162 and FP7 Marie Curie ITN "IonTraC"; 289648), and J. Gehl (The Danish Cancer Society, R110-A6996).

POSTER COMMUNICATION P-70
Calcium Remodeling in Cancer

Mibefradil; a T-type calcium channel blocker as a potential anti-cancer agent.

Chrysanthi Matheou[1], Stephen Chandler[1], Francesco Crea[1], Nuno Faria[2], Jonathan Powell[2], Fraser McDonald[3], Katja Rietdorf[1] and Martin D Bootman[1]

[1]The Open University, Department of Life, Health and Chemical Sciences, Milton Keynes, UK; [2]MRC Human Nutrition Reserach, Cambridge, UK; [3]Kings College London, Department of Orthodontics, Craniofacial Development and Stem Cell Biology, London, SE1 9RT; UK.

Chrysanthi.matheou@open.ac.uk

T-type calcium channels are known to be involved in critical physiological functions such as neuronal excitability and cardiac pacemaking. Additionally, their involvement has been suggested for the proliferation and/or differentiation of cancer cells. Indeed, overexpression of T-type calcium channels has been demonstrated for several cancer cell types, as well as other rapidly proliferating cells. A clinical trial is currently underway to establish whether T-type calcium channel blockers could potentially be used as therapeutic anti-cancer agents. This project examined putative cytotoxicity/cytostatic effects of the T-type calcium channel blocker mibefradil, and the L-type calcium channel blocker verapamil, on a number of rapidly proliferating cell lines: PC3 (prostate cancer), HSC3 (skin cancer), HeLa (cervical cancer) and HaCaT (human keratinocyte). Cell viability was assessed using MTT assays performed 24 h after drug addition. We found that mibefradil caused a concentration-dependent inhibition of proliferation in all four cell lines with different sensitivities: IC_{50} HaCaT 12.9 μM < HSC3 19.9 μM < PC3 21.3 μM < HeLa 37.7 μM. In contrast, verapamil only showed a very modest effect on the proliferation of HaCaT cells when applied at 200 μM, and had no significant effect on the other cell types at any concentration used. These results show that selectively targeting T-type calcium channels can inhibit cell proliferation. Future experiments need to show whether this is a cytotoxic or a cytostatic effect, whether it is selective to fast proliferating cell lines like cancer cells, and how inhibition of T-type calcium channels leads decreased cell proliferation.

Supported by The Open University, MRC Human Nutrition Research, Kings College London, UK.

POSTER COMMUNICATION P-71
Calcium Remodeling in Cancer Selected Oral Communication for Symposium #4

Glutamate-mediated calcium entry in megakaryocytic cells: evidence for a hijacking effect to support leukaemia growth.

Tania Kamal[1]*, James I. Hearn[1]*, Yohanes Nursalim[1], Taryn N. Green[1], Marie-Christine Morel-Kopp[2], Christopher M. Ward[2], Matthew J. During[3], Emma C. Josefsson[4] and Maggie L. Kalev-Zylinska[1,5]

*[1]The University of Auckland, Auckland, New Zealand; [2]The University of Sydney and Royal North Shore Hospital, Sydney, Australia; [3]The Ohio State University, Columbus, OH, USA; [4]The Walter and Eliza Hall Institute of Medical Research and University of Melbourne, Melbourne, VIC, Australia; [5] LabPlus Haematology, Auckland Academic Health Alliance, Auckland, New Zealand. *contributed equally to this work.*

m.kalev@auckland.ac.nz

We have previously reported a novel mechanism for calcium (Ca^{2+}) entry into leukaemic megakaryoblasts that increases cell proliferation. This mechanism engages N-methyl-D-aspartate receptors (NMDARs) that are glutamate-gated calcium ion channels located in the plasma cell membrane. Intriguingly, we saw that NMDAR inhibition reduces proliferation of leukaemic cells, suggesting a novel way to interfere with megakaryocytic cancers. Here, we continued to interrogate NMDAR functionality and its contribution to the growth of normal and leukaemic megakaryocytes. Meg-01, Set-2 and K-562 cells were used as models of leukaemic megakaryoblasts. NMDAR effects in cultured cells were tested using well-established NMDAR agonists and antagonists. The role of NMDARs in normal megakaryocytes was examined using a conditional knock-out mouse model (Pf4-Grin1$^{-/-}$ mice) and in ex vivo cultures of primary mouse megakaryocytes. The NMDAR-mediated Ca^{2+} fluxes were detected in megakaryocytic cells loaded with Fluo-4-AM. When cultured in the presence of NMDAR inhibitors, leukaemic cells underwent differentiation and acquired cytoplasmic vacuoles. This pro-differentiation effect was associated with mild induction of apoptosis but more evidence for autophagy. In contrast, NMDAR antagonists reduced megakaryocytic differentiation of normal lineage-negative mouse progenitors and diminished proplatelet formation ex vivo. Further, Pf4-Grin1$^{-/-}$ mice had lower platelet counts. In conclusion, glutamate-mediated Ca^{2+} entry appears hijacked in leukaemic cells away from supporting differentiation towards supporting proliferation. We are now using a CRISPR/Cas9 system to confirm effects of pharmacological modulators in cultured cells and characterising mechanisms through which Pf4-Grin1$^{-/-}$ mice develop thrombocytopaenia. Elucidation of these effects may help design new strategies to modulate human megakaryopoiesis.

Supported by Child Cancer Foundation (project 12/17); Leukaemia and Blood Cancer New Zealand and donation from Anne, David and Victoria Norman.

POSTER COMMUNICATION P-72
ER-mitochondrial Ca^{2+} Signaling

Implications of ER-mitochondria Ca^{2+} signaling in cell death of malignant B cells.

R. La Rovere[1], M. Kerkhofs[1], H. Akl[2], M. Bittremieux[1], T. Vervloessem[1], T. Luyten[1], K. Welkenhuyzen[1], L. Missiaen[1], H. De Smedt[1], J.B. Parys[1], and G. Bultynck[1]

[1]KU Leuven, Laboratory of Molecular and Cellular Signaling, 3000 Leuven, Belgium; [2]Lebanese University, Dept. of Biology, Hadath, Lebanon.

rita.larovere@kuleuven.be

The survival of malignant B cells is mainly due to the anti-apoptotic effects of Bcl-2 proteins at the endoplasmic reticulum (ER). The BH4 domain of Bcl-2 is responsible for the cancer cell survival via inositol 1,4,5-trisphosphate (IP3) receptor inhibition. A Bcl-2 suppressor (Bcl-2/IP3R Disrupter – 2, BIRD-2) targeting specifically BH4 domain has been recently developed to counteract IP3 signaling. BIRD-2 induces pro-apoptotic Ca^{2+}-signaling events in the cytosol of primary chronic lymphocytic leukemia cells and a subset of diffuse large B-cell lymphoma (DL-BCL) cell lines. So far, the mechanism by which BIRD-2-induced Ca^{2+} overload leads in these cancer cells to cell death is unknown. Here we focus on the impact of BIRD-2 on mitochondria Ca^{2+} uptake using rhoamine-based probes. We found that BIRD-2 exposure leads to a rapid and sustained mitochondrial Ca^{2+} overload in BIRD-2-sensitive DL-BCL cells (like SU-DHL-4), but not in BIRD-2-resistant DL-BCL cells (like OCI-LY-1). As a consequence, in SU-DHL-4 cells, but not in OCI-LY-1 cells, BIRD-2 triggers the production of mitochondrial reactive oxygen species (ROS), measured after loading the cells with MitoSOX red, and the loss of the mitochondrial potential, measured after loading the cells with Tetramethylrhodamine, Methyl Ester (TMRM). These events are followed by the opening of the mitochondrial permeability transition pore (mPTP), measured via a calcein-cobalt-quenching assay, and the activation of caspase 3. Although further work is necessary to dissect the molecular mechanisms involved in mitochondrial Ca^{2+} transport in malignant B cells we provide evidence that BIRD-2 is able to induce a mitochondrial Ca^{2+} overload causing mPTP opening and ensuing cell death.

Supported by grants from the Fund for Scientific Research – Flanders (FWO) (grants G.0571.12N, G.0634.13N, G.0C91.14N, G.0927.15N and G.0A34.16N) and the Research Council – KU Leuven (OT14/101).

POSTER COMMUNICATION P-73
ER-mitochondrial Ca^{2+} Signaling

Reciprocal sensitivity of B-cell cancer cells towards BH3 mimetics versus BH4-domain-targeting peptides.

Tamara Vervloessem[1], Haidar Akl[1], Thomas Tousseyn[2], Humbert De Smedt[1], Jan B. Parys[1] and Geert Bultynck[1]

[1]Laboratory of Molecular and Cellular Signaling and [2]Translational Cell & Tissue Research, KU Leuven, Leuven, Belgium.

tamara.vervloessem@kuleuven.be

Bcl-2 is often upregulated in B-cell malignancies to neutralize the BH3-only protein Bim at the mitochondria. BH3 mimetics (e.g. ABT-199) kill cancers by targeting Bcl-2's hydrophobic cleft and disrupting Bcl-2/Bim complexes. Some cancers with elevated Bcl-2 levels display poor responses towards BH3 mimetics, indicating an additional function for anti-apoptotic Bcl-2. Indeed, Bcl-2 via its BH4 domain prevents cytotoxic Ca^{2+} release from the endoplasmic reticulum (ER) by directly inhibiting the inositol 1,4,5-trisphosphate receptor (IP_3R). Recently, a cell-permeable peptide, the Bcl-2/IP_3R disruptor-2 (BIRD-2), was developed, that can kill Bcl-2-dependent cancers by targeting Bcl-2's BH4 domain, thereby unleashing pro-apoptotic Ca^{2+}-release events. Here, we compared eight "primed to death" diffuse large B-cell lymphoma cell lines (DL-BCL) for their apoptotic sensitivity towards BIRD-2 and ABT-199. By determining their IC_{50} for both compounds using cytometric cell-death analysis, we discovered a reciprocal sensitivity of DL-BCL cells towards ABT-199 versus BIRD-2, indicating that cells sensitive to ABT-199 are resistant to BIRD-2 and vice versa. Using immunoblotting, we quantified the expression levels of IP_3R2 and Bim, revealing that BIRD-2 sensitivity correlated with IP_3R2 levels but not with Bim levels. Hence, our findings indicate that some cancer cells require Bcl-2 at the mitochondria to prevent Bax activation, an action mediated by Bcl-2's hydrophobic cleft, while others require Bcl-2 at the ER to prevent IP_3R2 hyperactivity, an action mediated by Bcl-2's BH4 domain. Thus, cancer cells can exploit these different anti-apoptotic actions of Bcl-2 for their survival, dictating their relative apoptotic sensitivity to BH3 mimetics and BH4-domain targeting compounds.

POSTER COMMUNICATION P-74
ER-mitochondrial Ca^{2+} Signaling

The role of Ca^{2+} in ER stress-induced autophagy.

Gemma Roest, Evelien Hesemans, Geert Bultynck and Jan B. Parys

Laboratory of Molecular and Cellular Signaling, KU Leuven, Leuven, Belgium.

gemma.roest@kuleuven.be

Endoplasmic reticulum (ER) stress is caused by an accumulation of proteins in the ER, activating the unfolded protein response (UPR). Depending on the intensity and duration of the stress, this can result in autophagy and/or apoptosis. However, it is not known whether ER stress-induced autophagy is also Ca^{2+} dependent. We investigated the effect of glucosamine, the proline analogue L-azetidine carboxylic acid (AZC) and the glucosidase inhibitor castanospermine (cas) on autophagy, which, unlike e.g. thapsigargin, do not directly modify intracellular Ca^{2+} homeostasis. As ER stress markers we used the protein levels of Binding immunoglobulin protein, the phosphorylation status of Eukaryotic translation initiation factor 2α and the extent of splicing of X-box protein (XBP) 1 mRNA. For autophagy, we determined the lipidation status of microtubule-associated protein light chain (LC) 3 as well as the expression levels of p62 and beclin 1. Treatment of HeLa cells with 5mM glucosamine, 5mM AZC or 1mM cas induced ER stress, but autophagy was only induced after at least 6h of AZC treatment or 20h of glucosamine treatment, while none of the compounds induced apoptosis as assessed by Parp cleavage. 6h or 20h treatment with 5mM AZC induced XBP1 splicing, indicating induction of the IRE1α branch of the UPR. The effect of 5mM AZC on LC3 lipidation did not appear to be affected by addition of 10μM of the Ca^{2+} chelator BAPTA-AM for 6h. Further work will determine whether tighter modulation of intracellular Ca^{2+} could affect ER stress-induced autophagy or whether Ca^{2+} does not play a role.

Supported by the Research Foundation Flanders (FWO)

POSTER COMMUNICATION P-75
ER-mitochondrial Ca^{2+} Signaling

The sources and characteristics of calcium signals determine whether autophagy is up- or down-regulated.

Tala Chehab[1], Katja Rietdorf[1], Jan B. Parys[2], Geert Bultynck[2] and Martin D. Bootman[1]

[1]Department of Life, Health and Chemical Sciences, The Open University, UK
[2]Laboratory of Molecular and Cellular Signaling, KU Leuven, Belgium.

tala.chehab@open.ac.uk

Autophagy is a catabolic process important for cell survival and homeostasis that involves degradation of dysfunctional cellular components. Disruption of autophagy is associated with many disorders. Calcium has been proposed to be both pro- and anti-autophagic. To understand how calcium can have these opposing effects, we investigated how particular sources of calcium, and the characteristics of calcium signals, impact on autophagy. It has been proposed that calcium transfer from $InsP_3Rs$ to the mitochondrial matrix stimulates ATP production, and thereby prevents the induction of autophagy. Consistent with this hypothesis, we found that inhibiting calcium release from $InsP_3Rs$, or inhibiting mitochondrial respiration, stimulated autophagy in HEK and HeLa cells. These data suggest that the absence of cellular calcium signals is pro-autophagic. However, evoking cytosolic calcium signals by inhibiting SERCA with cyclopiazonic acid (CPA) also stimulated autophagy in the presence of extracellular calcium, suggesting that a sustained calcium influx signal was needed. Inhibition of calcium/calmodulin-dependent kinase kinase (CaM-KK) prevented CPA-induced autophagy, suggesting that cytosolic calcium elevation induces autophagy via CaM-KK-mediated phosphorylation. Finally, we find that BAPTA inhibited autophagy evoked by either $InsP_3R$ inhibition, CPA, rapamycin or nutrient starvation, again highlighting the importance of calcium signals in autophagy induction. The results illustrate the dual effect of calcium on autophagy; both the absence of calcium signals (following $InsP_3R$ inhibition) and the presence of calcium signals (following SERCA inhibition) stimulate autophagic flux. Clamping calcium signals with BAPTA inhibits autophagy induction by all treatments used, consistent with a calcium-dependent step in the initiation of autophagosome formation.

Supported by The Open University, UK and KU Leuven, Belgium.

POSTER COMMUNICATION P-76
ER-mitochondrial Ca^{2+} Signaling Selected Oral Communication for Symosium #5

Regulation of ER-mitochondria contacts and SERCA activity by the ER chaperones CNX and TMX1.

Tomás Gutiérrez, Arun Raturi, Carolina Ortiz-Sandoval, Gabriel Chan and Thomas Simmen

Department of Cell Biology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada.

gutirrez@ualberta.ca

The endoplasmic reticulum (ER) and mitochondria lie in close apposition forming physical contacts in a specialized membrane domain, where a flux of Ca^{2+} between these two organelles takes place. This ion-based signaling regulates several cell functions, including mitochondrial metabolism and apoptosis. We have identified two transmembrane ER chaperones that regulate this signaling mechanism, calnexin (CNX) and TMX1. CNX KO cells have increased ER-mitochondria contacts as well as increased Ca^{2+} transfer to mitochondria, resulting in increased mitochondrial metabolism and ATP levels. This function is accompanied by reduced ER Ca^{2+} content and reduced Ca^{2+} clearance from the cytosol compared to WT cells, suggesting that CNX activates SERCA. Accordingly, CNX co-immunoprecipitates with SERCA, suggesting a direct regulation. In contrast, TMX1 has an opposite effect. TMX1 also co-immunoprecipitates with SERCA, but TMX1 KO cells show increased ER Ca^{2+} levels and their cytosolic Ca^{2+} clearance is faster compared to WT cells, suggesting that TMX1 inhibits SERCA. TMX1 KO cells also show reduced ER-mitochondria contacts and reduced Ca^{2+} transfer to the mitochondria, resulting in reduced ATP levels. Finally, TMX1 KO cells have increased CNX-SERCA interaction, indicating that CNX and TMX1 competitively interact with SERCA exerting opposite effects on its activity.

Supported by CCSRI grant 2010-700306 and CRS grant 18325. Tomás Gutiérrez was supported by an Alberta Innovates Health Solutions fellowship.

POSTER COMMUNICATION P-77
ER-mitochondrial Ca^{2+} Signaling Selected Oral Communication for Symosium #5

ER-mitochondria crosstalk is regulated by WFS1/WIP1 interaction and is impaired in Wolfram syndrome.

Benjamin Delprat[1,2], Claire Angebault[1,2], Jérémy Fauconnier[3], Jennifer Rieusset[4], Jolanta Jagodzinska[1,2], Corentin A. Affortit[1,2], Camille Mégy[1,2], Chantal Cazevieille[1,2], Julia Korchagina[1,2], Delphine Bonnet- Wersinger[1,2], Dan Milea[5,6], Alain Lacampagne[3], Christian Hamel[1,2,7] and Cécile Delettre[1,2]

[1]NSERM U1051, Institute of Neurosciences of Montpellier, 34090 Montpellier, France; [2]University of Montpellier, 34090 Montpellier, France; [3]INSERM U1046, UMR CNRS 9214, University of Montpellier, 34295 Montpellier, France; [4]INSERM U1060, UMR INRA 1397, CarMeN Laboratory, Lyon 1 University, F-69003 Lyon, France; [5]Department of Ophthalmology, Angers University Hospital, 43933 Angers, France; [6]Singapore National Eye Centre, Singapore; Singapore Eye Research Institute, Singapore; Duke-NUS, Neuroscience and Behavioral Diseases, 169857 Singapore; [7]CHRU Montpellier, Centre of Reference for Genetic Sensory Diseases, CHU, Gui de Chauliac Hospital, 34090 Montpellier, France.

benjamin.delprat@inserm.fr

Communication between endoplasmic reticulum (ER) and mitochondria plays a pivotal role in calcium (Ca^{2+}) signalling, energy metabolism and cell survival. Dysfunctions of this crosstalk lead to metabolic and neurodegenerative diseases. Wolfram syndrome (WS) is a fatal neurodegenerative disease due to mutations of the ER resident protein WFS1. However, clinical phenotype of WS resemble mitochondrial disorders. Here, we show that WFS1 forms a complex with WIP1, inositol 1,4,5-trisphosphate receptor (ITPR) and voltage-dependent anion channel 1 (VDAC1) to promote ER-mitochondrial Ca^{2+} transfer in response to stimuli that generate inositol-1,4,5-trisphosphate. Moreover, we demonstrate that WFS1 associates with WIP1 to prevent its degradation by the proteasome. Finally, we show that WIP1 regulates VDAC expression and mitochondrial respiratory chain. Our results describe an unexpected key role of WFS1 and WIP1 in ER-mitochondria crosstalk and reconcile the ER expression of WFS1 with the mitochondrial phenotype, underlining a novel pathogenic mechanism for WS and opening new insights into the biogenesis of other neurodegenerative diseases.

Supported by grants from the Agence Nationale pour la Recherche (ANR-12-JSV1-0008-01), France.

POSTER COMMUNICATION P-78
ER-mitochondrial Ca^{2+} Signaling

In vitro aging promotes ER-mitochondria Ca^{2+} cross talk and loss of store-operated Ca^{2+} entry in rat hippocampal neurons.

María Calvo-Rodríguez*, Mónica García-Durillo, Carlos Villalobos and Lucía Núñez

Instituto de Biología y Genética Molecular (IBGM). Consejo Superior de Investigaciones Científicas (CSIC) y Universidad de Valladolid. Valladolid, Spain.

MCALVORODRIGUEZ@mgh.harvard.edu

Aging is associated to cognitive decline and susceptibility to neuron death, two processes related to subcellular Ca^{2+} homeostasis. In fact, memory storage is linked to mushroom spines stability that has been proposed to depend on store-operated Ca^{2+} entry (SOCE). In addition, Ca^{2+} transfer from endoplasmic reticulum (ER) into mitochondria is required to sustain the Krebs cycle and energy production, although transfer excess may lead to mitochondrial Ca^{2+} overload and apoptosis. Here we asked whether SOCE and ER-mitochondria Ca^{2+} transfer are influenced by aging in rat hippocampal neurons. For this end, we use fluorescence and bioluminescence imaging of cytosolic and mitochondrial Ca^{2+} in short-term and long-term cultures of rat hippocampal neurons reflecting young and aged neurons, respectively. Susceptibility to neuron cell death and expression of molecular players involved in Ca^{2+} remodelling were tested using quantitative immunofluorescence. We found that short-term culture neurons show large SOCE, low Ca^{2+} store content and no functional coupling between ER and mitochondria. In contrast, in long-term cultures reflecting aging neurons, SOCE is essentially lost, Ca^{2+} stores are overloaded, Ca^{2+} release is enhanced and most Ca^{2+} released from the ER is transferred to mitochondria. This remodelling is mediated by changes in the expression of molecular players involved in SOCE and mitochondrial Ca^{2+} uptake. These results suggest that neuronal aging is associated to increased ER-mitochondrial cross talking and loss of SOCE. Ca^{2+} remodelling might contribute to cognitive decline in the elderly at the expense of increased susceptibility to neuron cell.

Supported by grants BFU2012-37146 and BFU2015-7013R from Ministerio de Economía y Competitividad, Spain and VA145U13 from Junta de Castilla y León (JCyL), Spain. MCR was supported by JCyL and the European Social fund.

Present address for María Calvo-Rodríguez: Alzheimer Research Unit, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA.

POSTER COMMUNICATION P-79
ER-mitochondrial Ca^{2+} Signaling
Selected for Flash Symposium Presentation

The role of SK channels at the ER-mitochondria interface.

Birgit Honrath[1,2], Inge E. Krabbendam[1], Valentina Pegoretti[1], Carsten Culmsee[2] and Amalia M. Dolga[1]

[1]Department of Molecular Pharmacology, University of Groningen, The Netherlands, [2]Institute of Pharmacology and Clinical Pharmacy, University of Marburg, Germany.

a.m.dolga@rug.nl

Multiple research lines emphasize that the etiology of several neurodegenerative diseases is strongly associated with dysregulation of Ca^{2+} homeostasis. Mitochondrial K^+ channels are increasingly gaining interest due to their neuroprotective properties. In particular, the small conductance calcium-activated K^+ (SK) channel was indicated to provide protection against glutamate-induced toxicity. One of the features of oxidative stress is the increased production of reactive oxygen species (ROS) which has been linked with dysfunction in mitochondrial metabolism, and lipid peroxidation. Interestingly, interaction between endoplasmic reticulum (ER) and mitochondria is thought to coordinate Ca^{2+} transfer between the two organelles, that communicate via mitochondria-associated endoplasmic reticulum membranes (MAMs). In our study, we hypothesize that strengthening ER-mitochondria connection will increase mitochondrial calcium ($[Ca^{2+}]_m$) uptake thereby leading to increased cell vulnerability under oxidative stress. By using drug-inducible fluorescent interorganelle linkers in immortalized hippocampal HT-22 cells, we strengthened MAMs formation that could be visualized by live cell imaging. A detailed analysis of $[Ca^{2+}]_m$ homeostasis using multiple techniques in neuronal cells, including Ca^{2+}-binding mitochondrial-targeted aequorin, FACS-based measurements, and FRET-based specific $[Ca^{2+}]_m$ fluorescent probes showed a strong decrease of $[Ca^{2+}]_m$ uptake upon SK channel activation. Positive modulation of SK channels showed a protective role against oxidative stress even in conditions of increased ER-mitochondria interaction, as indicated by xCELLigence cell impedance-based system. Hence, interaction between ER and mitochondria facilitates mitochondrial Ca^{2+} fluxes that can be prevented by SK channels. Our results will provide further knowledge in the underlying mechanisms of apoptosis and the role of SK channels as potential neuroprotective targets.

Supported by a grant from the Deutsche Forschungsgemeinschaft, DFG (DO 1525/3-1). AMD is the recipient of a Rosalind Franklin Fellowship from the University of Groningen.

POSTER COMMUNICATION P-80
ER-mitochondrial Ca^{2+} Signaling

Changes in Ca^{2+} removal can mask the effects of geometry during IP_3R mediated Ca^{2+} signals.

Estefanía Piegari, Cecilia Villarruel and Silvina Ponce Dawson

Departamento de Física, FCEN-UBA e IFIBA (CONICET, UBA). Buenos Aires, Argentina.

silvina@df.uba.ar

The variety of cytosolic Ca^{2+} spatio-temporal distributions is key for the versatility of Ca^{2+} signals, particularly of those which involve Ca^{2+} release from the endoplasmic reticulum through Inositol 1,4,5-Trisphosphate Receptors (IP_3R). IP_3Rs open probability depends on $[Ca^{2+}]$. Individual IP3R openings are then coupled through Calcium Induced Calcium Release (CICR). The clustered organization of IP_3Rs leads to a hierarchy of coupling levels and a variety of signals that go from local to global ones. Here we combine experiments in untreated and in progesterone-treated Xenopus Laevis oocytes with mathematical models to study the interplay between Ca^{2+} dynamics and different IP_3R spatial distributions. We show that the rate of cytosolic Ca^{2+} removal affects the coupling between Ca^{2+} release sites in such a way that the underlying non-uniform IP_3R spatial distribution can be masked.

Supported by grants PICT 2013-1301 (Agencia Nacional de Promoción Científica y Tecnológica) and UBACYT 20020130100480BA (Universidad de Buenos Aires) of Argentina.

POSTER COMMUNICATION P-81
ER-mitochondrial Ca^{2+} Signaling

Interplay between intracellular Ca^{2+} oscillations and Ca^{2+}-stimulated mitochondrial metabolism.

Benjamin Wacquier[1], Laurant Combettes[2], Guy Tran Van Nhieu[3] and Geneviève Dupont [1]

[1]Unité de Chronobiologie Théorique, Université Libre de Bruxelles, CP231, Boulevard du Triomphe, 1050, Brussels, Belgium. [2]Université Paris Sud, UMRS1174, Orsay F-91405, France. [3]Equipe Communication Intercellulaire et Infections Microbiennes, Centre de Recherche Interdisciplinaire en Biologie (CIRB), Collège de France, 11 Place Marcelin Berthelot, Paris 75005, France.

benjamin.wacquier@gmail.com

Oscillations of cytosolic Ca^{2+} concentration are a widespread mode of signalling. Oscillatory spikes rely on repetitive exchanges of Ca^{2+} between the endoplasmic reticulum (ER) and the cytosol, due to the regulation of inositol 1,4,5-trisphosphate receptors. Mitochondria also sequester and release Ca^{2+}, thus affecting Ca^{2+} signalling. Mitochondrial Ca^{2+} activates key enzymes involved in ATP synthesis. We propose a new integrative model for Ca^{2+} signalling and mitochondrial metabolism in electrically non-excitable cells. The model accounts for (1) the phase relationship of the Ca^{2+} changes in the cytosol, the ER and mitochondria, (2) the dynamics of mitochondrial metabolites in response to cytosolic Ca^{2+} changes, and (3) the impac ts of cytosol/mitochondria Ca^{2+} exchanges and of mitochondrial metabolism on Ca^{2+} oscillations. Simulations predict that as expected, oscillations are slowed down by decreasing the rate of Ca^{2+} efflux from mitochondria, but also by decreasing the rate of Ca^{2+} influx through the mitochondrial Ca^{2+} uniporter (MCU). These predictions were experimentally validated by inhibiting MCU expression. Despite the highly non-linear character of Ca^{2+} dynamics and mitochondrial metabolism, bioenergetics were found to be robust with respect to changes in frequency and amplitude of Ca^{2+} oscillations.

POSTER COMMUNICATION P-82
ER-mitochondrial Ca^{2+} Signaling
Selected for Flash Symposium Presentation

LRRC8B: New associate of calcium signaling network.

Arijita Ghosh[1], Nitin Khandelwal[2], Arvind Kumar[2] and Amal Kanti Bera[1]

[1]Department of Biotechnology, Indian Institute of Technology Madras, Chennai, India, [2]CSIR Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India.

arijitag@gmail.com

Molecular basis of the calcium signaling network has been incessantly riveting. Versatility of the calcium signaling network dictates essential cellular processes like cell death, gene expression, cell proliferation etc. where the membrane bound Ca^{2+} permeable channel proteins contribute substantially. In this study, we identify a new member, Leucine-rich repeat-containing 8 B (LRRC8B), involved in Ca^{2+} leak from endoplasmic reticulum (ER). LRRC8 proteins have been proposed to emerge from the fusion of a pannexin on N ter side and a LRR domain on the C ter end. LRRC8 proteins consist of four putative trans-membrane helices wherein they share maximum sequence and structural similarity with pannexins. Despite being linked to essential physiological processes like B cell maturation, adipocyte differentiation and swelling activated volume-regulated anion channel (VRAC) formation, the molecular function and biology of LRRC8 proteins remain debatable. The present study exemplifies that overexpression of LRRC8B in HEK293 cells attenuated both IP_3 and ATP-induced intracellular calcium rise. siRNA mediated knockdown or overexpression of LRRC8B enhanced or reduced thapsigargin (TG)-releasable Ca^{2+} pool in the endoplasmic reticulum (ER), respectively. Confocal microscopy and sub-cellular fractionation followed by western blot analysis revealed the localization of the protein in ER. Moreover, Ca^{2+} imaging in ER displayed faster or slower Ca^{2+} leak rate in LRRC8B overexpressed or knockdown HEK293 cells respectively. Our study provides evidence for the participation of LRRC8B in intracellular Ca^{2+} homeostasis by acting as an ER leak channel.

Supported by grant BIO/AMAL/14-15/DSTX from Department of Science and Technology (DST), Govt. of India.

POSTER COMMUNICATION P-83
ER-mitochondrial Ca^{2+} Signaling
Selected for Flash Symposium Presentation

Endoplasmic reticulum calcium dictates intracellular distribution of membrane cholesterol.

Marek Michalak[1], Wen-An Wang[1] and Luis B. Agellon[2]

[1]Department of Biochemistry, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada and [2]School of Dietetics and Human Nutrition, McGill University, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada.

marek.michalak@ualberta.ca

The Endoplasmic Reticulum (ER) is a major Ca^{2+} store and a site for regulating folding quality of nascent glycoproteins. It is crucial for the ER to maintain homeostasis to ensure proper cell functioning and disruptions of ER homeostasis have been linked to many pathological and physiological disorders. Calreticulin is a Ca^{2+} buffering chaperone that resides in the ER lumen. Calreticulin deficiency ($Calr^{-/-}$) in mice is embryonic lethal due to abnormal cardiac development but can be rescued with cardiac-specific expression of activated calcineurin. The rescue mice have a functional heart but display metabolic problems like growth retardation, hypoglycemia and elevated blood cholesterol and triglycerides suggesting that either calreticulin or endoplasmic reticulum Ca^{2+} plays a role in lipid homeostasis. We discovered that inhibition or elimination of calreticulin gene expression in mammalian cells and in C. elegans caused increased lipid biosynthesis due to enhanced processing of sterol-response element-binding proteins (SREBP). We discovered that the SREBP processing pathway in both wild-type and calreticulin-deficient cells was responsive to changes in cellular cholesterol status but differed in basal sensitivity. In particular, the reduction of ER Ca^{2+} content altered the distribution of ER membrane cholesterol that signals the activation of SREBPs. These findings establishes the role of Ca^{2+} in determining the reference set-point for controlling cellular cholesterol and lipid homeostasis.

Supported by the Canadian Institute of Health Research

POSTER COMMUNICATION P-84
ER-mitochondrial Ca^{2+} Signaling

Sphingosine kinase 1 regulates mitochondrial calcium.

Pulli I.[a], Löf C.[b], Kemppainen K.[a], Bäck N.[c], Dufour E.[d], Cooper H.M.[a], Sanz A.[e] and Törnquist K.[af].

[a] Åbo Akademi University, Tykistökatu 6A, 20520 Turku, Finland. [b] University Of Turku, Department of Physiology, Institute of Biomedicine, Kiinamyllynkatu 10, 20520 Turku, Finland. [c] Institute of Biomedicine, Anatomy, University of Helsinki
[d] University of Tampere, 33520 Tampere, Finland. [e] Newcastle University Institute for Ageing, Newcastle upon Tyne NE4 5PL. [f] Minerva Foundation Institute For Medical Research, Biomedicum Helsinki, 00270 Helsinki, Finland.

ilari.pulli@abo.fi

Sphingosine kinase 1 (SK1) converts sphingosine to the lipid signaling molecule sphingosine 1-phosphate (S1P) which acts through five G-protein coupled receptors (S1P1-5). Sphingosine and its precursor, ceramide, are generally considered as pro-apoptotic lipids whereas S1P is known to promote cell migration and proliferation. Thus, aberrations in SK1 levels may affect cell fate, and overexpression of SK1 has been linked to oncogenesis. SK1/S1P signaling is known to regulate intracellular calcium, which is achieved mainly through the auto/paracrine actions of S1P. Altered calcium signaling is a key factor in many pathologies, including cancer. Moreover, mitochondrial calcium levels regulate central cellular functions such as energy production and initiation of apoptosis. We have previously shown that SK1/S1P signaling specifically modulates calcium levels at the caveolar sub-compartments of the plasma membrane without significantly affecting cytosolic calcium. In this study, we elucidate the role of SK1 in mitochondrial and endoplasmic reticulum (ER) calcium signaling by employing the genetically targeted calcium indicator, aequorin. We show that SK1 modulates the inositol trisphosphate-induced release of calcium from the ER calcium stores and that the altered release of ER calcium is sensed by the mitochondria. Interestingly, SK1 does not affect the calcium uptake capacity of mitochondria in permeabilized cells. In addition, we demonstrate that SK1 affects cellular respiration, possibly through mitochondrial calcium handling. This alteration in the cellular energy metabolism may be relevant in understanding the oncogenic actions of SK1.

Supported by The Swedish Cultural Foundation in Finland, Magnus Ehrnrooth Foundation, Sigrid Juselius Foundation, Suomalais-Norjalainen Lääketieteen Säätiö Foundation, K. Albin Johansson Foundation.

POSTER COMMUNICATION P-85
ER-mitochondrial Ca^{2+} Signaling

Functional roles of MICU1 and MICU2 in mitochondrial Ca^{2+} uptake.

Jessica Arias-del-Val, Jessica Matesanz-Isabel, Pilar Alvarez-Illera, Rosalba I Fonteriz, Mayte Montero and Javier Alvarez

Instituto de Biología y Genética Molecular (IBGM), Dpt. De Bioquímica y Biología Molecular y Fisiología, Facultad de Medicina, Universidad de Valladolid and Consejo Superior de Investigaciones Científicas (CSIC), Valladolid, Spain.

jessica.arias@ibgm.uva.es

MICU1 and MICU2 are the main regulators of the mitochondrial Ca^{2+}-uniporter (MCU), but their precise functional role is still under debate. We have studied here the effect of silencing MICU1, MICU2 or both on the kinetics of mitochondrial Ca^{2+} uptake in HeLa cells. We show that silencing of any of them activates mitochondrial Ca^{2+} uptake at low cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$), but with important kinetic differences. MICU2 behaves as a pure inhibitor of MCU at low $[Ca^{2+}]_c$, but its effects disappear above 7μM. Regarding MICU1, studying its effects is more difficult because knockdown of MICU1 leads also to loss of MICU2. However, while knockdown of MICU2 induces only a persistent increase in mitochondrial Ca^{2+} uptake, knockdown of MICU1 induces also a peculiar use-dependent transient activation of MCU that cannot be attributed to the parallel loss of MICU2. Therefore, MICU1 is endowed with a specific inhibitory effect on MCU at low $[Ca^{2+}]_c$, separate and kinetically different from that of MICU2. On the other hand, we and others have shown previously that MICU1 activates MCU at $[Ca^{2+}]_c$ above 2.5μM. Therefore, MICU2 is a genuine inhibitor of MCU, though only at low $[Ca^{2+}]_c$, and MICU1 has a double role, inhibitory at low $[Ca^{2+}]_c$ and activatory at high $[Ca^{2+}]_c$.

Supported by grants from the spanish Ministerio de Ciencia e Innovación [BFU2011-25763] and Ministerio de Economía y Competitividad [BFU2014-55731-R].

POSTER COMMUNICATION P-86
ER-mitochondrial Ca^{2+} Signaling

SUMOylation of NCLX regulates mitochondrial localization.

Kuntal Dey and Israel Sekler.

Department of physiology and cell biology, Faculty of health sciences Ben-Gurion University of the Negev, Beer Sheba, Israel

kd.bcku@gmail.com

Mitochondrial Na^+/Ca^{2+} exchanger (NCLX), an inner mitochondrial membrane protein, is responsible for Ca^{2+}extrusion from mitochondria. Proteins can be post-translationally modified at its lysine residue via conjugation of the Small Ubiquitin-like MOdifier (SUMO) known as SUMOylation. The present study was undertaken to investigate whether NCLX is a target for SUMOylation. "SUMOplot" analysis of human NCLX showed several SUMOylation sites (ΨKXE, Ψ is any hydrophobic amino acid). In western blot study, along with the regular one, a higher molecular weight band of NCLX was apparent when cells were treated with N-ethymelamide (NEM, 25mM) or transiently transfected with SUMO1. The higher molecular weight band was also detected by anti-SUMO1 antibody. Moreover, SUMO1 was found to be co-precipitated with NCLX. Taken together, these results confirmed that NCLX is SUMOylated by SUMO1. Among the three potential SUMOylation sites in NCLX (Lys 519, 331 and 328), only mutation at Lys519 effectively prevented NCLX SUMOylation. Importantly, decimating this SUMOylation site reduced the expression of NCLX in mitochondria and decreased mitochondrial Ca^{2+} efflux. Interestingly, impaired localization of NCLX leads to mitochondrial damage as evidenced by an increase in cytochrome C release. Impairment of SUMOylation resulted in ubiquitination of NCLX rather its sorting to plasma membrane. Altogether, the current study reveals that SUMOylation of NCLX at Lys519 is required for its stable mitochondrial expression.

POSTER COMMUNICATION P-87
ER-mitochondrial Ca^{2+} Signaling

Haloperidol causes translocation of the sigma 1/type 1 IP_3 receptor complex to the nucleus in differentiated NG108-15 cells.

Lubomira Lencesova[1], Jana Kubickova[1], Lucia Csaderova[2], Tibor Stracina[3], Sona Hudecova[1], Petr Babula[3], Marie Novakova[3] and Olga Krizanova[1,3]

[1]Institute for Clinical and Translational Research, Biomedical Research Center, SAS, Bratislava, Slovakia; [2]Institute of Virology, Biomedical Research Center SAS, Bratislava, Slovakia; [3]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

lubomira.lencesova@savba.sk

Haloperidol is a prototypic ligand of sigma 1 receptors (σ1Rs), which have been shown to interact with inositol 1,4,5-trisphosphate receptors (IP_3Rs). We investigated possible changes in IP_3Rs and σ1Rs in differentiated NG108-15 cells resulting from haloperidol treatment and proposed physiological consequences for those changes. NG108-15 cell differentiation was performed by the dibutyryl cAMP (dbcAMP). We studied changes in expression, calcium levels and cell plasticity after haloperidol treatment. To study co-localization of IP_3R1 and σ1R we used immunoprecipitation and immunofluorescence. Haloperidol treatment resulted in up-regulation of both type 1 IP_3Rs (IP_3R1s) and σ1Rs at mRNA and protein levels. IP_3R1s are bound to σ1Rs, and translocation of this complex from endoplasmic reticulum (ER) to nucleus occurs in the group of cells treated with haloperidol and is followed by an increase in nuclear calcium levels. Haloperidol-induced changes in cytosolic, reticular and nuclear calcium levels were similar, when specific σ1 blocker – BD 1047 was used. Haloperidol increases cytosolic calcium, primarily through calcium release from ER mediated by IP_3R1s. Furthermore, IP_3R1/σ1R complex translocates to nucleus, where it likely modulates gene expression through increase in intranuclear calcium levels. Changes in calcium levels in nucleus, ER and cytoplasm might be responsible for alterations in cellular plasticity because length of neurites increased and number of neurites decreased in haloperidol-treated, differentiated cells. IP_3R1/σ1R complex might be a major player in this process.

We acknowledge the following financial support for this project: APVV 51-0045-11, VEGA 2/0082/16, and MUNI/A/1326/2014.

POSTER COMMUNICATION P-88
ER-mitochondrial Ca^{2+} Signaling

Mitochondrial Cx43 forms hemichannels that contribute to calcium entry and cell death.

Luc Leybaert[1], Ashish Kumar Gadicherla[1], Nan Wang[1], Marco Bulic[2], Amadou Camara[3], Klaus-Dieter Schlüter[2], Rainer Schulz[2] and Wai-Meng Kwok[3]

[1]Physiology Group, Dept. Basic Medical Sciences, Faculty of Medicine & Health Sciences, Ghent University, Belgium; [2]Physiologisches Institut, Justus-Liebig-Univiersität Giessen, Germany; [3]Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, USA.

Luc.Leybaert@ugent.be

Mitochondrial connexin 43 (Cx43) plays a key role in cytoprotection caused by repeated exposure to short periods of non-lethal ischemia/reperfusion, a condition known as ischemic preconditioning. However, connexins form channels that are calcium (Ca^{2+}) permeable and may also potentially lead to mitochondrial Ca^{2+} overload and cell death. Here, we studied the role of Cx43 in facilitating mitochondrial Ca^{2+} entry and investigated its downstream consequences. To that purpose, we used various connexin targeting peptides interacting with extracellular (Gap26) and intracellular (Gap19, RRNYRRNY) Cx43 domains and tested their effect on mitochondrial dye- and Ca^{2+}-uptake, electrophysiological properties of plasmalemmal and mitochondrial Cx43 channels, and cell injury/cell death. Our results in isolated mice cardiac subsarcolemmal mitochondria indicate that Cx43 forms hemichannels that contribute to Ca^{2+} entry and may trigger permeability transition and cell injury/death. RRNYRRNY displayed the strongest effects in all assays and inhibited plasma membrane as well as mitochondrial Cx43 hemichannels. RRNYRRNY also strongly reduced the infarct size in ex vivo cardiac ischemia-reperfusion studies. These results indicate that Cx43 contributes to mitochondrial Ca^{2+} homeostasis and is involved in triggering cell injury/death pathways.

Supported by Ghent University (BOF fund) and the Interuniversity Attraction Poles Program [P6/31 and P7/10 to L.L.].

POSTER COMMUNICATION P-89
ER-mitochondrial Ca^{2+} Signaling
Selected for Flash Presentation Symposium

Nuclear membrane patch for type 2 ryanodine receptor channel.

Tong Chun-Kit[1], Lee Shuk-Kwan[1] and Cheung King-Ho[1,2]

[1]School of Biomedical Sciences and [2]Research Centre of Heart, Brain, Hormone and Healthy Aging, LKS Faculty of Medicine, University of Hong Kong, Hong Kong, China.

ckben@hku.hk

Mobilization of intracellular calcium (Ca^{2+}) by second messengers, including the inositol-trisphate ($InsP_3$), cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), triggers signaling cascade that controls life and death. It is well-established $InsP_3$ binds to $InsP_3$ receptor to release Ca^{2+} from the endoplasmic reticulum (ER). Recently nuclear membrane electrophysiology demonstrated NAADP activates Ca^{2+} release from the two-pore channel (TPC). Although cADPR has been suggested to mobilize ER Ca^{2+} through the activation of ryanodine receptor (RyR), direct evidence showing the activation of RyR by cADPR in native ER membrane is lacking. In this study, we applied nuclear membrane electrophysiology on a tetracycline-induced stable mouse RyR2 receptor expressing HEK293 cell. We characterized the electrophysiological properties and the ligand regulations of RyR2 single channel using the nuclear membrane patch-clamp technique. Using 140 mM potassium ion (K^+) as the charge carrier, we demonstrated that both caffeine and cADPR activate RyR2 single channel from isolate nuclei with conductance of about 650 pS. The detected RyR2 single channel traces show a linear current-voltage relationship in symmetrical K^+ solutions and channel open probability displays [cADPR] denpendency that peaked at 100 nM. Furthermore, in saturating [cADPR], RyR2 channel activities display cytoplasmic [Ca^{2+}] dependence. Taken together, the nuclear membrane electrophysiological approach provides an alternate approach to characterize the biophysical properties of RyR and both cADPR and Ca^{2+} are physiological ligands that regulate RyR channel activities.

Supported by the grants HKU17104514M and HKU764113M from the General Research Fund of Hong Kong.

POSTER COMMUNICATION P-90
Structures of Ca^{2+} Ion Channels Selected Oral Communication for Symposium #6

Crystallographic investigation of the calcium sensing apparatus of voltage-gated sodium and calcium channels: effect of arrhythmia-causing mutations.

Kaiqian Wang, Jocelyn Liu, Bernd Gardill, Ching-Chieh Tung, and Filip Van Petegem

University of British Columbia, Dept. of Biochemistry and Molecular Biology, Canada.

filip.vanpetegem@gmail.com

Voltage-gated sodium (NaV) and calcium (CaV) channels are membrane proteins that allow for the selective passage of Na^+ or Ca^{2+} ions across the plasma membrane. Interestingly, both have the ability to sense cytosolic Ca^{2+} levels. In several CaV channels, Ca^{2+} entering through the pore can result in accelerated inactivation, a process known as 'Calcium dependent Inactivation'. In some cases it can also increase ionic currents through the process of 'calcium dependent facilitation'. In NaV channels, cytosolic Ca^{2+} has been shown to affect channel inactivation, and to interfere with the so-called 'late sodium current'. These are essential electrophysiological properties of the NaV and CaV channels, shaping the action potentials in many excitable cells. Any interference can result in devastating conditions, such as cardiac arrhythmias and epilepsy. In both channels, the calcium-dependent feedback mechanisms have been shown to be mediated by Calmodulin. The cytosolic components that interact with Calmodulin, as well as Calmodulin itself, are targets for a multitude of disease-causing mutations. In the cardiac variants, these are known to result in Long-QT and Brugada syndromes, two types of arrhythmias. Here we present high-resolution crystal structures of these regions in both their WT and disease mutant forms, along with an electrophysiological analysis. The results show that different disease mutations are able to affect the calcium-dependent feedback mechanisms in different ways. Intriguingly, some mutations in calmodulin affect the function of the Ryanodine Receptor, whereas others selectively affect CaV channels. Comparing structures of these disease mutant forms helps explain these differential effects.

Supported by the Canadian Institutes of Health Research (CIHR)

POSTER COMMUNICATION P-91
Structures of Ca^{2+} Ion Channels

Structural insights on how calcium regulates Kv7.2 channels through calmodulin.

Carolina Gomis-Pérez, Ganeko Bernardo-Seisdedos, Eider Nuñez-Viadero, Covadonga Malo, Pilar Areso, Oscar Millet and Alvaro Villarroel.

Instituto Biofisika (IBF). Consejo Superior de Investigaciones Científicas (CSIC) y Universidad del País Vasco y CiC Biogune. Leioa y Zamudio, Spain.

karolgomis@gmail.com

Calmodulin (CaM) is an EF-hand protein that relays calcium signals to a vast number of different targets. CaM binding can be classified in two major groups: a.- when it interacts in the absence of Ca^{2+} (apocalmodulin). b.- when the interaction takes place when CaM is loaded with this cation (holocalmodulin). Both modes are observed when the targets are Kv7.2 channels, which are one of the main components of the non-inactivating K^+ M-current, a key controller of neuronal excitability. CaM binds to helices A and B in the C-terminal domain and regulates channel trafficking and function. To get insights on how Ca^{2+} gates Kv7.2 channels through CaM, we have studied the conformational changes prompted by this cation using FRET and NMR spectroscopy of the AB-CaM complex. The movements detected by these complementary techniques are small compared to those described in other targets. The atomic level impact on channelopathic mutations as this site and possible gating mechanism compatible with the structural data will be discussed.

Supported by grants BFU2015-66910-R and BG 2015 from Ministerio de Economía y Competitividad and Basque Government, Spain.

POSTER COMMUNICATION P-92
Structures of Ca^{2+} Ion Channels
Selected for Flash Symposium Presentation

Isolated pores from TPC2 reveal functional redundancy in the evolution of asymmetric ion channels.

Christopher J Penny[1,3], Taufiq Rahman[2], Altin Sula[3], Andrew J Miles[3], BA Wallace[3] and Sandip Patel[1]

[1]Department of Cell and Developmental Biology, University College London, London, UK, WC1E 6BT; [2]Department of Pharmacology, University of Cambridge, Cambridge, UK, CB2 1PD; [3]Institute of Structural and Molecular Biology, Birkbeck College, University of London, London, UK, WC1E 7HX.

c.penny.12@ucl.ac.uk

Four-domain voltage-gated ion channels appear to have evolved through sequential rounds of intragenic duplication from a primordial one-domain precursor. Modularity within symmetrical one-domain channels is established through the functionality of isolated 'pore only' proteins. However, little is known about the roles of individual regions within more complex, asymmetric channels where the domains have undergone substantial divergence. Here we isolated, expressed and functionally characterised both of the pore regions from human TPC2, a two-domain channel that holds a key intermediate position in the evolution of voltage-gated ion channels. In HeLa cells, each pore localised to and depleted the ER of Ca^{2+}, consistent with constitutive activity. Additionally, one of these pores expressed at high levels in E. coli, and formed stable, folded, tetrameric channels that supported Ca^{2+} and Na^+ flux. Therefore, despite extensive domain divergence, we demonstrate that either pore forms a structurally and functionally competent channel in the absence of the other. These findings reveal striking functional redundancy during evolution of asymmetric ion channel pores and provide a tractable framework for probing complex human ion channel pores.

Supported by BBSRC grants BB/J014567 (CJP), BB/L006790 (BAW), BB/J019135 (BAW), BB/N01524X (SP) and BB/K000942 (SP). TR was supported by Royal Society grants RG69132 and RG65196.

POSTER COMMUNICATION P-93
Structures of Ca^{2+} Ion Channels Selected Oral Communication for Symposium #6

Ryanodine receptor in the open state with and without ryanodine.

Katrien Willegems and Rouslan Efremov

Structural Biology Research Center, Vrije Universiteit Brussel (VUB), Vlaams instituut biotechnologie (VIB), Pleinlaan 2, 1030 Brussels. Belgium.

kwillege@vub.ac.be

Ryanodine receptors (RyRs) are calcium channels in the sarcoplasmic and endoplasmic reticulum crucial in the excitation-contaction mechanism in both skeletal and cardiac muscles. Recently, high-resolution structures of skeletal RyR1 in the closed state were obtained using single-particle cryo-electron microscopy (SP cryo-EM). Here we present the structures of skeletal RyR1 in the open state and in the open state bound to one of its many ligands, ryanodine.

Supported by a IWT sbo fellowship from agentschap innoveren en ondernemen, Flanders, Belgium.

POSTER COMMUNICATION P-94
Structures of Ca^{2+} Ion Channels

Ca^{2+}-dependent calmodulin binding to cardiac ryanodine receptor (RyR2) calmodulin binding domains.

Malene Brohus[a], Mads T. Søndergaard[a,b], S. R. Wayne Chen[b], Filip van Petegem[c], and Michael T. Overgaard[a]

[a]Dept. of Chemistry and Bioscience, Aalborg University, Denmark; [b]Libin Cardiovascular Institute of Alberta, Dept. of Physiology and Pharmacology and Dept. of Biochemistry and Molecular Biology, University of Calgary, Canada; [c]Dept. of Biochemistry and Molecular Biology, University of British Columbia, Canada.

mbrohus@bio.aau.dk

The ryanodine receptor 2 (RyR2) is the key gatekeeper of Ca^{2+} release from the sarcoplasmic reticulum in cardiomyocytes, and this release causes the heart to contract. Consequently, precise regulation of RyR2 opening and closing is critical for proper cardiac excitation-contraction cycling. A pivotal regulator of RyR2 activity is the cytosolic Ca^{2+}-sensing protein calmodulin (CaM) which inhibits RyR2 Ca^{2+} release both at diastolic (100 nM) and systolic (200 μM) $[Ca^{2+}]_{cyt}$. The Ca^{2+}-dependent regulation of RyR2 through its interaction with CaM is not fully understood, but several CaM-binding-domains (CaMBD) in RyR2 are thought to be essential for channel function. The increasing number of arrhythmogenic CaM mutations, which cause abnormal RyR2 regulation, emphasize the necessity to understand the CaM-RyR2 interaction in more detail. We investigated the Ca^{2+}-dependency of the interaction between CaM, CaM N- and C-domains, and three RyR2 CaMBDs, under conditions that span below diastolic and above systolic Ca^{2+}-levels. CaM binds to $RyR_{3581-3607}$ and $RyR_{4246-4276}$ with >100-fold higher affinity than to $RyR_{1941-1965}$ at all $[Ca^{2+}]$ tested. Our data suggest that $RyR_{3581-3607}$ is the primary interaction site under physiological $[Ca^{2+}]$, and CaM proves more sensitive to Ca^{2+} when complexed to $RyR_{3581-3607}$ compared to $RyR_{4246-4276}$. We further find domain-specific binding to the CaMBDs, with the C-domain anchored to $RyR_{3581-3607}$ at all $[Ca^{2+}]$, leaving the CaM N-domain to interact with e.g. $RyR_{4246-4276}$ at increased $[Ca^{2+}]$. Understanding of the mechanism underlying CaM regulation of RyR2 may support the CaM-RyR2 complex as a potential target for modulating Ca^{2+}-release in cardiac arrhythmias and heart failure.

Supported by grants from the Lundbeck Foundation, the Novo Nordisk Foundation, the Obelske Family foundation, and grant DFF-4181-00447 from the Danish Council for Independent Research.

POSTER COMMUNICATION P-95
Structures of Ca^{2+} Ion Channels Selected for Flash Symposium Presentation

LETM1 forms a Ca^{2+}/H^{+} antiporter.

Juan Shao[1,2], Zhenglin Fu[3,4], Yanli Ji[1,2], Xiangchen Guan[1], Shang Guo[1], Zhanyu Ding[3,4], Xue Yang[1], Yao Cong[3,4] and Yuequan Shen[1,2]

[1]State Key Laboratory of Medicinal Chemical Biology, [2]College of Life Sciences, Nankai University, 94 Weijin Road, Tianjin 300071, China; [3]National Center for Protein Science Shanghai, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, [4]Shanghai Science Research Center, Chinese Academy of Sciences, Shanghai 201204, China.

yshen@nankai.edu.cn

Leucine zipper-EF-hand-containing transmembrane protein1 (LETM1) is located in the mitochondrial inner membrane and is defective in Wolf-Hirschhorn syndrome. LETM1 contains only one transmembrane helix, but it behaves as a putative transporter. Our data supports that LETM1 is a Ca^{2+}/H^{+} antiporter. Purified LETM1 exhibits Ca^{2+}/H^{+} anti-transport activity, and the activity is enhanced as the proton gradient is increased. Moreover, the residue Glu221 of mouse LETM1, which is responsible for Ca^{2+} flux, was identified. The mutation of Glu221 to glutamine abolishes the Ca^{2+}-transport activity of LETM1 in cells. More importantly, using negative staining electron microscopy, we revealed that the purified LETM1 oligomer has a central pore that is suitable for ion conducting, and also, exists high-conductance and low-conductance conformational states under alkaline and acidic conditions, respectively. Our results provide important information regarding the unique working mechanism of the LETM1 protein.

Supported by the Ministry of Science and Technology China 973 program and the National Natural Science Foundation of China.

POSTER COMMUNICATION P-96
Structures of Ca^{2+} Ion Channels

NAD binding by human CD38 characterized by tryptophan fluorescence.

Valerie Wolters, Anette Rosche, Andreas Bauche, Angelika Harneit, Ralf Fliegert and Andreas H. Guse

The Calcium Signaling Group, Department of Biochemistry and Molecular Cell Biology, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, Germany.

v.wolters@uke.de

The ADP-ribosyl cyclase CD38 catalyzes the synthesis of the important Ca^{2+} messengers ADP-ribose (ADPR) and cyclic ADP-ribose (cADPR) (Lee HC, 2006, Mol Med; Lee HC, 2001, Annu Rev Pharmacol Toxicol). Binding of NAD to a soluble fragment of human CD38 and its catalytically inactive mutant was investigated by fluorimetry. CD38 shows endogenous tryptophan fluorescence allowing the detection of conformational changes in the protein (Lacapère JJ, 2003, Biochim Biophys Acta). Addition of 100 µM NAD resulted in a pronounced decrease in CD38(44-300) fluorescence of about 15% due to nucleotide binding. Amplitude of the fluorescence decrease was amplified by increasing NAD concentrations allowing determination of a dissociation constant for NAD (K_D: 29 µM). After about 38 s fluorescence recovered to initial values at 37 ° C. At that time 30% of NAD had been metabolized as shown be HPLC analysis. Kinetics of recovery of fluorescence is slowed down with decreasing temperature and CD38 concentration and increasing NAD concentration demonstrating that the recovery in fluorescence is proportional to the enzymatic activity of CD38. The catalytically inactive CD38 mutant showed a decrease in fluorescence of 20% after addition of 100 µM NAD and a K_D of 37 µM indicating that binding of NAD is not significantly affected by the mutation. Importantly, recovery in fluorescence was not observed with this mutant suggesting that tryptophan fluorescence mirrors initial substrate binding. Taken together, tryptophan fluorescence of CD38 is a useful read out for binding and metabolism of NAD.

Research Funding Scheme of the Freie and Hansestadt Hamburg (Research group "ReAd Me! Regulatorische Adeninnukleotide auf Membranoberflächen")

POSTER COMMUNICATION P-97
Structures of Ca^{2+} Ion Channels

Ion channel(s) gated by the new STIM1L isoform.

Agnieszka Dyrda, Sophie Saüc, Nicolas Demaurex and Maud Frieden

University of Geneva, Department of Cell Physiology and Metabolism, 1, rue Michel Servet, 1211 Geneva 4, Switzerland.

agnieszka.dyrda@unige.ch

Depletion of the endoplasmic reticulum Ca^{2+} store leads to a Ca^{2+} entry called store-operated Ca^{2+} entry (SOCE), which is due to the activation of Orai1 channel gated by STIM1. We identified recently a new splice variant of STIM1, called STIM1L (long) that has an extra 106 aa in the C-term part. STIM1L is as efficient as STIM1 in eliciting SOCE, but so far, nothing is known about the channel(s) gated by STIM1L. In 50% of STIM1L/Orai1 expressing HEK cells a linear current developed upon store depletion. In the other 50% of recordings, we measured a current similar to I_{CRAC}, but with a small amplitude. Removing external Na^+ strongly reduced the inward STIM1L-induced linear current, demonstrating the non-selective nature of this current. We postulated that this linear current could be due either to STIM1L gating of other channels than Orai1 (i.e. endogenous TRPC), or the gating of Orai1 by STIM1L changes its ionic selectivity. To test this hypothesis we expressed the Orai1V102C mutant and tested if STIM1L is able to restore the Ca^{2+} selectivity of Orai1V102C, showing its ability to gate the channel. Indeed, we obtained a CRAC-like current with STIM1L, even if the Ca^{2+} selectivity was not as high as with STIM1. Overall, our data are in favor of a change of Orai1 Ca^{2+} selectivity upon STIM1L gating. The variation between Ca^{2+} selective and non-selective current might be due to the ratio of STIM1L and Orai1, which remains to be established

Supported by the Swiss National Foundation (Grant 310030_166313), FSRMM, Foundation Marcel Levaillant

POSTER COMMUNICATION P-98
New tools for Ca^{2+} Imaging Selected Oral Communication for Symposium #7

Genetically engineered calcium indicators for visualization of calcium signals; from single cells to tissues.

Tünde Berecz[1], Kornélia Szebényi[1], András Füredi[1], László Homolya[1], Tamás Orbán[1], János Réthelyi[2], Balázs Sarkadi[1] and Ágota Apáti[1]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences,Budapest, Hungary. [2]National Brain Research Project (NAP) Molecular Psychiatry Research Group, Hungarian Academy of Sciences, Budapest, Hungary.

apati@biomembrane.hu

The cell permeable small molecular calcium sensitive dyes are commonly used in calcium signaling studies, while their applications have numerous drawbacks, especially in sensitive cell types such as human pluripotent stem cells (hPSC) and in their differentiated offsprings. The recently developed variants of genetically encoded Ca^{2+} indicators (GECIs) serve as alternatives for calcium imaging. To reveal the applicability of GECIs, we have generated hPSCs which stably express the GCaMP2 calcium indicator and tested the effects of various ligands in hPSCs and their differentiated derivatives (cardiomyocytes, neural and mesenchymal cell types) in parallel with similar cells loaded with Fluo4. We found no significant changes in ligand induced Ca^{2+}-signals in GCaMP2-expressing cells as compared to signaling in Fluo4-loaded hPSCs. To extend our knowledge towards neural tissues we have also generated GCaMP6fast-expressing neural cell cultures from hPSC-derived neural progenitor cells. We found that spontaneous activity and connectivity of neural tissues could be efficiently studied both in GCaMP6fast-expressing and Fluo4-loaded cells. We have also established a transgenic rat strain with one copy of the GCaMP2 transgene per allele with a defined insertion pattern, without major genetic or phenotypic alterations. Calcium signals in primary cell cultures established from the heart and kidney of transgenic rats and the proximal tubular cells in in vivo experiments were studied. From our data we concluded that this system provides a new model for studying in vitro or in vivo cellular calcium signalling and opens new possibilities for physiological and pharmacological investigations.

This work was supported by grants Hungarian Brain Research Program [KTIA VKSZ_12, NAP-A-1.10] and by the National Research, Development and Innovation Office [KTIA_AIK_12-1-2012-0025, KMR_12-1-2012-0112].

POSTER COMMUNICATION P-99
New tools for Ca^{2+} Imaging
Selected for Flash Symposium Presentation

BIST-2EGTA - a new caged calcium probe that is highly sensitive to 1P and 2P excitation.

G. C. R. Ellis-Davies

Dept of Neuroscience, Mount Sinai School of Medicine, New York, NY, USA.

i.davies@mssm.edu

Optical probes for measurement and manipulation of Ca^{2+} have proved fundamental in enabling the study of Ca^{2+} signaling. My lab has developed a series of caged Ca^{2+} compounds that have been the most widely used over the past 25 years. For one-photon (1P) manipulation of Ca^{2+} we use light in the 350-405 nm region, and 2P uncaging requires high energy doses at 720 nm. My lab has developed a new caged Ca probe that is uniquely sensitive to blue light. Further, this probe has 2P cross-section that is 100s of times larger than any other Ca cage. We call this new molecule "BIST-2EGTA". BIST is a dinitro derivative of bisstyrylthiophene that absorbs light very effectively, having an extinction coefficient at 440 nm 66,000/M/cm, and two-photon cross section of 350 GM at 775 nm. BIST-2EGTA is a Ca-selective chelator that binds Ca with high affinity (84 nM pH 7.2). The photoproducts have 20,000-fold lower affinity. 2P flash photolysis revealed that Ca is released in less 0.2 ms. Importantly BIST-2EGTA is photolyzed with good quantum yield (0.23). Using the patch-clamp method BIST-2EGTA, loaded with Ca, was delivered into acutely isolated mouse cardiac myocytes, where either one- or two-photon uncaging of Ca^{2+} induced highly local or cell-wide physiological Ca^{2+} signaling events. These data show that BIST-2EGTA has a uniquely powerful set of photochemical properties that may prove very useful for cell physiology.

Supported by NIH.

POSTER COMMUNICATION P-100
New tools for Ca^{2+} Imaging

What happens if GCAMP is attached to TRPC channels.

Juyeon Ko, Jongyun Myeong and Insuk so

Department of physiology, College of medicine, Seoul National University, Seoul, Korea.

juuyeon@snu.ac.kr

Canonical transient receptor potential (TRPC) channels are nonselective Ca^{2+}-permeable cation channels. These channels contribute to receptor-induced Ca^{2+} entry directly or indirectly via depolarization of voltage-gated Ca^{2+} channels. We show that TRPC4-mediated Ca^{2+} influx, measured using a genetically encoded Ca^{2+} indicator (GCaMP6s) attached directly to TRPC4. At low calcium concentration change, GCaMP is more sensitive than other Ca^{2+} fluorescence dyes. So we used GCaMP to measure the calcium entry. When stimulate TRPC4 and GCaMP6s co-expressed cells by Englerin A, inward current was recorded but calcium influx was not recorded by GCaMP6s. To measure the local calcium influx by TRPC4 channels, we tagged GCaMP6s at N-terminus of TRPC4 channel (TRPC4-GCaMP6s). By TRPC4-GCaMP6s, we could see the change of current and calcium concentration at a time. However, expressing TRPC4-GCaMP6s in HEK293 cell alones, it expressed in cytosol rather than plasma membrane. Through co-expressing TRPC4-CFP, we could make it possible for TRPC4-GCaMP6s to working in the membrane. We think it is because four TRPC4 subunits form tetrameric channel. To identify correlation between time course of inward current and calcium influx of TRPC4, imaging and whole-cell patch clamp were executed simultaneously in TRPC4-GCaMP6s expressed cells. Channels was stimulated by EnglerinA(-) (EA) and Carbachol (Cch). We observed interesting thing that some calcium influx trace and current trace shapes are not similar. So we first thought that TRPC4 has different time course of inward current and calcium influx. And also each cation would have different time course. But we have a question, is there a difference, really.

POSTER COMMUNICATION P-101
New tools for Ca^{2+} Imaging Selected Oral Communication for Symposium #7

From functional genomics to drug re-purposing: the mitochondrial uniporter case.

Fabiana Perocchi[1,2]

[1]Gene Center Munich and Department of Biochemistry, Ludwig-Maximilians-Universität, 81377 München, Germany. [2]Institute of Human Genetics, Helmholtz Zentrum München, 85764 Neuherberg, Germany.

Perocchi@genzentrum.lmu.de

Mitochondria are essential hubs of calcium-mediated signaling networks. The organelle can take up, buffer, and release calcium ions to effectively shape intracellular calcium transients, stimulate ATP production and regulate cell death. Although, the basic mechanisms of mitochondrial calcium homeostasis have been firmly established for decades, the molecular identity of the mitochondrial calcium signaling toolkit has evaded classical bottom-up approaches. Our previous studies [1,2,3] have provided a compelling example of the power of systems approaches applied to mitochondrial calcium signaling to discover hitherto unknown molecular components of the calcium uniporter. We developed computational and experimental frameworks for a systematic reconstruction of calcium-dependent signal transduction cascades in mitochondria. By combining evolutionary genomics and loss-of-function genetic and chemical screens, our systems approach holds the potential to shed light on yet unanswered questions in the field of mitochondrial calcium signaling.

1. Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliansky V, Mootha VK (2011). Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature. 476(7360):341-5.
2. Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, Mootha VK (2010). MICU1 encodes a mitochondrial EF hand protein required for calcium uptake. Nature. 467(7313):291-6.
3. Cheng Y, Perocchi F (2015). ProtPhylo: identification of protein-phenotype and protein-protein functional associations via phylogenetic profiling. NAR. 43(W1):W160-8.

POSTER COMMUNICATION P-102
New tools for Ca^{2+} Imaging

Endoplasmic reticulum calcium mobilization by physiological stimuli in the Langerhans pancreatic islets.

Paloma Navas-Navarro[1], Jonathan Rojo[1], Beatriz Merino[2], Ivan Quesada[2], Angel Nadal[2], Maria Teresa Alonso[1] and Javier García-Sancho[1]

[1]Instituto de Biología y Genética Molecular (IBGM), Universidad de Valladolid y Consejo Superior de Investigaciones Científicas (CSIC), Valladolid, Spain and [2]Instituto de Bioingeniería, Universidad Miguel Hernández, Elche, Spain.

palnavnav@ibgm.uva.es

Exocytic secretion is regulated by cytosolic Ca^{2+} signals ($[Ca^{2+}]_C$), which can be generated by Ca^{2+} entry through the plasma membrane or by Ca^{2+} release from the endoplasmic reticulum (ER). In addition, Ca^{2+} entry signals can eventually be amplified by ER release via calcium-induced calcium release (CICR). Insulin secretion in pancreatic β-cells is doubly regulated, metabolically via blood glucose levels, and by vagal cholinergic innervation via peripheral nervous system. We have studied the contribution of the luminal Ca^{2+} to these physiological stimuli in intact islets of Langerhans. Changes of ER $[Ca^{2+}]$ ($[Ca^{2+}]_{ER}$) were imaged in islets isolated from transgenic mice expressing the ER-targeted fluorescent protein sensor GAP3[1]. Simultaneous measurements of $[Ca^{2+}]_{ER}$ with erGAP and $[Ca^{2+}]_C$ with fura-2 were performed. High glucose stimulation resulted in $[Ca^{2+}]_C$ oscillations associated with in phase-$[Ca^{2+}]_{ER}$ oscillations, suggesting that ER Ca^{2+} uptake was secondary to the stimulation of the sarco-endoplasmic reticulum Ca^{2+}-ATPase by the increased $[Ca^{2+}]_C$, with no detectable CICR. Direct stimulation of Ca^{2+} entry by depolarization with high-K^+ increased $[Ca^{2+}]_{ER}$, confirming that CICR is not present in these cells. By contrast, acetylcholine stimulation elicited a decrease of $[Ca^{2+}]_{ER}$ with a concomitant rise in $[Ca^{2+}]_C$, demonstrating that the source of the $[Ca^{2+}]_C$ peak is the ER. Our results indicate that β-cells have two different regulation mechanisms, one metabolic and one nervous, with little cross-talk between them. This dissociation had also been suggested by functional studies.

[1]Navas-Navarro et al. (2016) Cell Chem Biol. 23:1-8

Supported by grants from MINECO (BFU2014-53469P) and ISC III (TerCel; RD12/0019/0036 and RD16/0011/0003)

POSTER COMMUNICATION P-103
New tools for Ca^{2+} Imaging

Calcium mobilization from the endoplasmic reticulum by the hypothalamic releasing factors in the adenohypophysis.

Jonathan Rojo-Ruiz, Paloma Navas-Navarro, Lucía Núñez, Maria Teresa Alonso and Javier García-Sancho.

Instituto de Biología y Genética Molecular (IBGM), Universidad de Valladolid y Consejo Superior de Investigaciones Científicas (CSIC), Valladolid, Spain

jonathan.rojo@ibgm.uva.es

The adenohypophysis contains 5 cell types (somatotropes, lactotropes, thyrotropes and corticotropes and gonadotropes), each one secreting a different hormone, and controlled by 4 hypothalamic releasing hormones (HRHs). Exocytic secretion is regulated by cytosolic Ca^{2+} signals ($[Ca^{2+}]_C$), which can be generated by Ca^{2+} entry through the plasma membrane or by Ca^{2+} release from the endoplasmic reticulum (ER). In addition, Ca^{2+} entry signals can eventually be amplified by ER release via calcium-induced calcium release (CICR). We have investigated the contribution of ER Ca^{2+} release to the action mechanism of the physiological agonists in pituitary gland. Changes of $[Ca^{2+}]$ in the ER ($[Ca^{2+}]_{ER}$) were measured with the recently described low-affinity Ca^{2+} sensor GAP3[1] targeted to the ER. We employed a transgenic mouse strain that expressed erGAP3 driven by a ubiquous promoter. 79% of the total pituitary cells were positive for the sensor. Intact pituitary glands or acute slices from the transgenic mouse were used to perform measurements of $[Ca^{2+}]_{ER}$. $[Ca^{2+}]_C$ was measured simultaeously with rhod-2. A large fraction of cells responded to luteinizing hormone-releasing hormone (LHRH) with a decrease of $[Ca^{2+}]_{ER}$ a concomitant rise of $[Ca^{2+}]_C$. A smaller fraction of cells responded to thyrotropin (TRH). By contrast, depolarization with high K^+ triggered a rise of $[Ca^{2+}]_C$ without a decrease of $[Ca^{2+}]_{ER}$, indicating that CICR is not present in these cells. Our results suggest that ER contributes to generate the $[Ca^{2+}]_C$ signals produced by LHRH and TRH1.

[1]Navas-Navarro et al. (2016) Cell Chem Biol. 23:1-8

Supported by grants from MINECO (BFU2014-53469P) and ISC III (TerCel; RD12/0019/0036 and RD16/0011/0003)

POSTER COMMUNICATION P-104
New tools for Ca^{2+} Imaging

Calcium dynamics in the endoplasmic reticulum of astrocytes. Evidence for a CICR mechanism.

Macarena Rodríguez-Prados, Jonathan Rojo-Ruiz, Paloma Navas-Navarro, Javier García-Sancho and María Teresa Alonso

Instituto de Biología y Genética Molecular (IBGM). Consejo Superior de Investigaciones Científicas (CSIC) y Universidad de Valladolid. Valladolid, Spain.

mrodriguez@ibgm.uva.es

Astroglia Ca^{2+} signalling is controlled by Ca^{2+} entry through plasma membrane and Ca^{2+} release from organelles, mostly from the endoplasmic reticulum (ER). Astrocytic ER possesses inositol 1,4,5-trisphosphate receptors ($InsP_3R$) which can be activated upon stimulation through a variety of metabotropic G-protein coupled receptors, including ATP and glutamate. We have recently developed the GAP family of Ca^{2+} sensors that can be used either as a fluorescent sensor to image Ca^{2+}, or as a bioluminescent sensor when it is reconstituted with coelenterazine. Low affinity variants (GAP1-3)[1] were generated to conform to the high Ca^{2+} concentrations found in the ER. Ca^{2+} dynamics were analysed in virally delivered astrocytes or in cells isolated from transgenic mice expressing the sensor. Simultaneous imaging of ER- and cytosolic-Ca^{2+} were performed with erGAPs and rhod-2, respectively. Resting $[Ca^{2+}]_{ER}$ averaged 400 μM and maximal stimulation with ATP provoked a complete and reversible ER discharge. Coordinated ER- and cytosolic Ca^{2+} oscillations were imaged, both spontaneously and challenged by various stimuli. Ca^{2+} entry stimulated by high K^+ depolarization elicited a fast ER Ca^{2+} release and a rise in cytosolic Ca^{2+}, which were dose-dependent and fully abolished by external Ca^{2+} removal or by addition of Ni^{2+}. Our results demonstrate the presence of Calcium-Induced Calcium Release (CICR) in astrocytes. These results were validated both in neuron-glia mixed cultures and in organotypic cortical slices cultures.

1Navas-Navarro et al. (2016) Cell Chem Biol. 23:1-8

Supported by grants from MINECO (BFU2014-53469P) and ISC III (TerCel; RD12/0019/0036 and RD16/0011/0003).

POSTER COMMUNICATION P-105
New tools for Ca^{2+} Imaging

Calcium dynamics in secretory granule subpopulations of neuroendocrine cells: An optical single-cell study.

Pablo Montenegro, Carmen D. Lobatón, Javier Álvarez and Alfredo Moreno.

Dpto. de Bioquímica y Biología Molecular y Fisiología, Instituto de Biología y Genética Molecular (IBGM) Facultad de Medicina. Universidad de Valladolid y Consejo Superior de Investigaciones científicas (CSIC), Valladolid, Spain.

monty@ibgm.uva.es

Although the second intracellular messenger Ca^{2+} is the main signal that triggers exocytosis, the secretory granules of neuroendocrine cells are one of the most important and least understood subcellular compartments in terms of calcium dynamics. Their study is therefore of great interest to understand the neurosecretory machinery. In this work, we have performed single-cell measurements of intragranular [Ca^{2+}] in different cell regions by confocal microscopy. We have developed chimeras with the pH-resistant ratiometric Ca^{2+} biosensor GEM-GECO1 targeted to secretory granules by localization sequences VAMP and NPY. Stimulation with ATP or high K^+ depolarization of neuroendocrine cell lines PC12 and INS-1 induced increases in intragranular [Ca^{2+}], triggering calcium peaks. These calcium peaks were reduced in the presence of calcium-pump-inhibitor tert-Butylhydroquinone (BHQ). This inhibitor also significantly reduced the calcium uptake of secretory granules in cells previously emptied of Ca^{2+}. A progressive decrease in the level of vesicular calcium uptake and a slower kinetic of the process is observed as the selected regions of vesicular subpopulations are located farther away from the plasma membrane. This decrease was more prominent in calcium records with VAMP-GEMGECO1 than in those performed with NPY-GEMGECO1. Although further research is required, the use of fluorescent calcium-sensors in single-cell studies of intragranular calcium dynamics, as described in this work, provides an interesting starting point to elucidate the role of vesicular calcium among different subpopulations of secretory granules and their contribution to exocytosis process.

Supported by grant from Ministerio de Ciencia e Innovación (BFU2011-25763). Pablo Montenegro holds a JAEpredoc fellowship from the Consejo Superior de Investigaciones científicas (CSIC), Spain.

POSTER COMMUNICATION P-106
New tools for Ca^{2+} Imaging

Long-term monitoring of Ca^{2+} dynamics in C. elegans pharynx: an in vivo energy balance sensor.

Pilar Alvarez-Illera[1], Adolfo Sanchez-Blanco[2], Paloma García-Casas, Silvia Lopez-Burillo[1], Rosalba I Fonteriz[1], Javier Alvarez[1] and Mayte Montero[1]

[1]Instituto de Biología y Genética Molecular (IBGM), Dpt. Bioquímica y Biología Molecular y Fisiología, Facultad de Medicina, Universidad de Valladolid and Consejo Superior de Investigaciones Científicas (CSIC), Valladolid, Spain [2]Deprt of Biology, University of Hartford, West Hartford, Connecticut 06117, USA.

pilar_alvill@hotmail.com

Ca^{2+} is a key signal transducer for muscle contraction. Continuous in vivo monitoring of intracellular Ca^{2+}-dynamics in C. elegans pharynx muscle revealed surprisingly complex Ca^{2+} patterns. Despite the age-dependent decline of pharynx pumping, we observed unaltered fast Ca^{2+} oscillations both in young and old worms. In addition, sporadic prolonged Ca^{2+} increases lasting many seconds or minutes were often observed in between periods of fast Ca^{2+} oscillations. We attribute them to the inhibition of ATP-dependent Ca^{2+}-pumps upon energy depletion. Accordingly, food deprivation largely augmented the frequency of prolonged [Ca^{2+}] increases. However, paradoxically, prolonged [Ca^{2+}] increases were more frequently observed in young worms than in older ones, and less frequently observed in energy-deficient mitochondrial respiratory chain nuo-6 mutants than in wild-type controls. We hypothesize that young animals are more susceptible to energy depletion due to their faster energy consumption rate, while nuo-6 mutants may keep better the energy balance by slowing energy consumption. Our data therefore suggest that the metabolic state of the pharynx during feeding stimulation depends mainly on the delicate balance between the instant rates of energy production and consumption. Thus, in vivo monitoring of muscle Ca^{2+} dynamics can be used as a novel tool to study cellular energy availability.

Supported by a grant from Ministerio de Economía y Competitividad, Spain [BFU2014-55731-R].

POSTER COMMUNICATION P-107
New tools for Ca^{2+} Imaging

Imaging calcium in cells and zebrafish with fluorescent-protein-Aequorin fusions.

Beatriz Domingo, Adil Bakayan, Cecilia F. Vaquero and Juan Llopis

Facultad de Medicina de Albacete and Centro Regional de Investigaciones Biomédicas (CRIB), Universidad de Castilla La Mancha, Albacete, Spain.

juan.llopis@uclm.es

Aequorin (Aeq) is a genetically-encoded Ca^{2+} indicator (GECI), a photoprotein which emits blue light in contact with free Ca^{2+}. It has been widely used to monitor Ca^{2+} in cell populations by luminometry, but it is hard to use in single-cell or in vivo studies owing to its low quantum yield and blue emission. To facilitate imaging of Ca^{2+} from deep tissues of animals, we have attempted to shift Aeq emission to the red, since red light is much less attenuated than blue or green light by blood and other tissues. We generated molecular fusions of Aeq with red fluorescent proteins (RFP), in which there is bioluminescence resonance energy transfer (BRET) from Aeq to the RFP, resulting in robust red emission. A variant named Redquorin was obtained with more than 80% of emitted light above 575 nm. Using Redquorin we were able to image spontaneous Ca^{2+} oscillations in single HEK-293 cells. We expressed Redquorin and other Aeq variants in developing zebrafish embryos, and demonstrated Ca^{2+} transients occurring during the segmentation period. In addition, Redquorin expression was directed to specific organelles using appropriate targeting signals. Its co-expression with green-emitting Aeq allows simultaneous Ca^{2+} measurements in two cellular compartments of the same cells.

Supported by MINECO/FEDER grants, reference BFU2012-39548-C02-01 and BFU2015-69874-R, and group grant GI20163512 from Vicerrectorado de Investigación y Política Científica, UCLM.

POSTER COMMUNICATION P-108
Ca^{2+} signalling Selected Oral Communication for Symposium #8

Ryanodine receptors of acidic Ca^{2+} stores generate a non-productive Ca^{2+} release in smooth muscle cells.

Mª Cristina López-Méndez, Ubaldo García and Agustín Guerrero-Hernández

Centro de Investigación y de Estudios Avanzados del IPN (CINVESTAV-IPN Unidad Zacatenco), México.

crismas2005@hotmail.com

Ryanodine receptors (RyRs) in the sarcoplasmic reticulum produce $[Ca^{2+}]_i$ transients of various sizes, small as sparks or large as global Ca^{2+} waves. The former activate BKCa channels in smooth muscle cells and are associated with relaxation, while the latter are associated with contraction. We have shown that RyRs are expressed in three different intracellular compartments, perinuclear, cytoplasmic and superficial; however, SERCA pump is present only in the perinuclear and superficial compartments. To study the role of cytoplasmic RyRs that are devoid of SERCA pump, we have carried out simultaneous recordings of changes in the cytoplasmic (with Fura-red) and intraluminal (with Mag-fluo-4) $[Ca^{2+}]$ in freshly isolated smooth muscle cells from guinea pig urinary bladder with confocal microscopy. Transient applications of caffeine produced reproducible $[Ca^{2+}]_i$ responses both in perinuclear and cytoplasmic compartments, that were inhibited by ryanodine or thapsigargin (inhibitor of SERCA pump). However, the $[Ca^{2+}]_i$ response in cytoplasmic region was of smaller amplitude and delayed with respect to perinuclear one. The luminal $[Ca^{2+}]$ responses required external Ca^{2+} for recovery and were inhibited by thapsigargin but only in the perinuclear region, while those in the cytoplasmic region were inhibited by bafilomycin (inhibitor of V-type H^+ ATPase). These data suggest that RyRs in the perinuclear region produce the $[Ca^{2+}]_i$ response that travels to the cell surface, while RyRs in cytoplasmic region are in an acidic compartment that produces basically no $[Ca^{2+}]_i$ response to maximal activation by caffeine either because the Ca^{2+} response is short-lived or comes from a small capacity acidic Ca^{2+} store.

Supported by grant 377848 from Consejo Nacional de Ciencia y Tecnologia (MX).

POSTER COMMUNICATION P-109
Ca^{2+} signalling Selected Oral Communication for Symposium #8

Cellular calcium signalling in the presence of single cell variability and dynamic stimuli.

[1]Agne Tilūnaite, [2]Wayne Croft, [3]Noah Russell, [2]Tomas C. Bellamy and [1]Rüdiger Thul

[1]School of Mathematical Sciences, University of Nottingham, Nottingham, UK; [2]School of Life Sciences, University of Nottingham, Nottingham, UK; [3]Department of Electrical and Electronic Engineering, University of Nottingham, Nottingham, UK.

pmxat6@nottingham.ac.uk

Under physiological conditions, cells often experience time dependent stimuli such as transient changes in neurotransmitter or hormone concentrations, but it remains an open question how cells transduce such dynamic stimuli. We exposed HEK293 cells and astrocytes to dynamically varying time courses of carbachol and ATP, respectively, and investigated the corresponding cellular calcium spike sequences. While single cells generally fail to follow the applied stimulation due to their intrinsic stochasticity and heterogeneity, faithful signal reconstruction is observed at the population level. We provide a simple transfer function that explains how dynamic stimulation is encoded into ensemble calcium spike rates. When dynamically stimulated, different cells often experience diverse stimuli time courses. Furthermore, cell populations may differ in the number of cells or exhibit various spatial distributions. In order to understand how these conditions affect population responses, we compute the single cell response to a given dynamic stimulus. Single cell variability and the small number of calcium spikes per cell pose a significant modelling challenge, but we demonstrate that Gaussian processes can successfully describe calcium spike rates in these circumstances and outperform standard tools such as peri-stimulus time histograms and kernel smoothing. Having the single cell response model will allow us to compare responses of various sets of cells to the observed population response and consequently obtain insight into tissue-wide calcium oscillations for heterogeneous cell populations.

Supported by Leverhulme Trust (RPG-2012-628).

POSTER COMMUNICATION P-110
Ca^{2+} signalling

Differential Ca^{2+}-sensing by GCAPs in rod and cone cells provide molecular basis of step-by-step regulation of retinal guanylate cyclase upon light activation.

Stefan Sulmann[§], Amanda Kussrow[‡], Darryl J. Bornhop[‡] and Karl-Wilhelm Koch[§]

§ Biochemistry Group, Department of Neuroscience, University Of Oldenburg, 26111-Oldenburg, Germany. ‡ Department of Chemistry, The Vanderbilt Institute for Chemical Biology, Vanderbilt University, Nashville, TN 37235, USA.

Stefan.sulmann@uni-oldenburg.de

Guanylate cyclases (GC) are key enzymes in many signaling pathways. In retinal phototransduction membrane bound GCs are regulated by small calcium sensors named Guanylate Cyclase-Activating Proteins (GCAPs). Upon light stimuli and fluctuating intracellular Ca^{2+}-levels, the GC-GCAP complexes regulate the cellular cGMP homeostasis thereby contributing to photoresponse recovery and light adaptation in photoreceptor cells. The Ca^{2+}-dependent trigger of activity regulation in the GC-GCAP multiprotein complex is not understood at the molecular level. In fact, different experimental approaches yielded inconclusive results concerning the binding interface of GCAPs and target GCs (Peshenko et al, J Biol Chem., 2015). In the present study we investigated GCAP-GC interactions using a novel technique called Backscattering interferometry (BSI), which allows label- and immobilization-free interaction analysis at extremely high sensitivity (Bornhop et al., PNAS, 2016). Here we show that GCAP1 and GCAP2 bind to its transmembrane target GC in a Ca^{2+}-independent manner. Furthermore, it will be demonstrated that the differential Ca^{2+}-sensing properties of GCAP1 and GCAP2 are reflected in two separate binding sites in the target GC. GCAP1 binds to the kinase homology domain near the trans-membrane region of the GC (i.e. juxtamembrane domain). Whereas GCAP2 did not interact with the GC in this region, an observation consistent with a model where GCAP1 and GCAP2 are permanently bound to GC at distinctly separated binding sites in vivo. Finally, BSI proved to be an invaluable tool for the study of these previously intractable membrane targets, without the need of fluorescent labels and/or isolation of the target enzyme.

SS and KWK gratefully acknowledge funding from the DFG (GRK1885/1) and AK and DJB acknowledge funding from NSF Grant CHE 1307899.

POSTER COMMUNICATION P-111
Ca^{2+} signalling

ATP-induced ATP release is a key determinant of Ca^{2+} signaling in cochlear non-sensory: insight from a data driven computational model.

Federico Ceriani[a,b,c,1], Tullio Pozzan [d,e,] and Fabio Mammano [a,b,c,f,2]

[a]Dept of Biomedical Sciences, Institute of Cell Biology and Neurobiology, Italian National Research Council, 00015 Monterotondo (RM), Italy. [b]Dept of Physics and Astronomy, University of Padua, 35131 Padua, Italy. [c]Venetian Institute of Molecular Medicine, Foundation for Advanced Biomedical Research, 35129 Padua, Italy. [d]Dept of Biomedical Sciences, Institute of Neuroscience (Padua Section), Italian National Research Council, 35121 Padua, Italy. [e]University of Padua, Dept of Biomedical Sciences, 35121 Padua, Italy. [f]ShanghaiTech University, Shanghai Institute for Advanced Immunochemical Studies, Shanghai 201210, China; [1]Present address: University of Sheffield, Dept of Biomedical Science, Sheffield, S10 2TN, UK.

fabio.mammano@cnr.it

In the sensory epithelium of the mammalian cochlea, elevation of extracellular adenosine trisphophate concentration ($[ATP]_e$) trigger oscillatory cytosolic free calcium concentration ($[Ca^{2+}]_c$) signals and propagation of intercellular inositol 1,4,5-trisphosphate (IP_3)-dependent Ca^{2+} waves. Phosphatidylinositol phosphate kinase type 1_ (PIPKI_) is the enzyme which is primarily responsible for the synthesis of the IP_3 precursor PIP_2 in the cochlea. Connexins participate in this sequence of events as gap junction channels, composed of connexin26 and connexin30, allow IP_3 movement between coupled cells of the organ of Corti. At the same time, connexin hemichannels in the cell plasma membrane open in response to raised level of the $[Ca^{2+}]_c$ and release intracellular ATP to the extracellular milieu, whereas ATP degradation by ectonucleotidases terminates signaling. What remains uncertain is the relative contribution of gap junction channels and connexin hemichannels to these fundamental mechanisms, whose defects impair hearing acquisition. To address this question, we performed carefully designed Ca^{2+} imaging experiments and constructed a computational model in strict and quantitative adherence to experimental data. The simulations show that Hopf-type bifurcations govern the occurrence of $[Ca^{2+}]_c$ oscillations within the experimental range of $[ATP]_e$. The model replicates accurately the spatial extent and propagation speed of intercellular Ca^{2+} waves and predicts that ATP–induced ATP release is the primary mechanism underlying propagation of Ca^{2+} signals in the developing mouse cochlea. The approach presented here overcomes major limitations due to lack of specific connexin channel inhibitors and can be readily adapted to other coupled cellular systems.

Supported by the Fondazione Telethon (grant GGP13114 to FM) and the CNR (Project DSB.AD009.001.004/INVECCHIAMENTO IBCN to F.M). F.C. was supported by a post-doctoral fellowship from Padova University (grant CPDR132235 to FM).

POSTER COMMUNICATION P-112
Ca^{2+} signalling

Cx43 hemichannels contribute to smooth muscle Ca^{2+} dynamics and contractility.

Luc Leybaert[1], Mélissa Bol[1], Nan Wang[1], Marijke De Bock[1], Benjamin Wacquier[2], Elke Decrock[1], Geert Bultynck[3] and Geneviève Dupont[2].

[1]Physiology Group, Dept. Basic Medical Sciences, Faculty of Medicine & Health Sciences, Ghent University, Belgium; [2]Unité de Chronobiologie Théorique, Faculté des Sciences, Université Libre de Bruxelles (ULB), Belgium. [3]Laboratory of Molecular and Cellular Signalling, Dept. Cellular & Molecular Medicine, KULeuven, Belgium.

Luc.Leybaert@ugent.be

Connexins form gap-junctions (GJs) that directly connect cells, thereby coordinating vascular function and controlling blood vessel diameter. Connexins also form hemichannels that, when open, lead to the entry/loss of ions and the escape of ATP. Here, we hypothesized that hemichannel Ca^{2+} entry/ATP release contributes to smooth muscle cell (SMC) Ca^{2+} dynamics and blood vessel contractility. We applied several peptide hemichannel modulators and inhibitors of Ca^{2+} and ATP signaling to investigate their influence on SMC Ca^{2+} dynamics and blood vessel contraction. Confocal Ca^{2+} imaging studies on freshly isolated rat small mesenteric arteries (SMAs) demonstrated that norepinephrine-induced SMC Ca^{2+} oscillations were inhibited by blocking IP_3-receptors with xestospongin-C and by blocking Cx43 hemichannels with TAT-L2 peptider but also by promoting hemichannel opening with TAT-CT9. Evidence for SMC hemichannel opening was supported by the fact that TAT-CT9 significantly increased resting cytoplasmic Ca^{2+} concentration in SMCs, indicating it facilitated Ca^{2+} entry, and by the observation that blood vessel norepinephrine-induced ATP release was blocked by TAT-L2. Contraction experiments on isolated SMAs showed significant inhibition of norepinephrine-triggered contractility by the ATP receptor antagonist suramin, but the most strong effect was observed with TAT-L2 that gave ~80% inhibition at 37° C. TAT-L2 inhibition of blood vessel contraction was significantly reduced in conditional Cx43 KO animals, indicating the effect was Cx43-dependent. These results indicate that Cx43 hemichannels contribute to SMC Ca^{2+} dynamics and contractility, by facilitating Ca^{2+} entry, ATP release and purinergic signaling. Computational modeling indicates the opening of a single hemichannel per cell is sufficient to explain the results.

Supported by the Fund for Scientific Research Flanders, Belgium [G.0140.08, 3G.0134.09, G.0298.11N, G.0571.12N, G.0A54.13N and G.0A82.13N to L.L. and G.0298.11N to G.B.], and the Interuniversity Attraction Poles Program [P6/31 and P7/10 to L.L. and P7/13 to G.B.]

POSTER COMMUNICATION P-113
Calcium Signaling

Acetylcholine induces nitric oxide production by inducing intracellular Ca^{2+} oscillations in mouse brain endothelial cells.

Estella Zuccolo, Dmitry Lim, Egidio D'Angelo, Germano Guerra, Francesco Moccia.

Laboratory of General Physiology. Department of Biology and Biotechnology "L. Spallanzani". University of Pavia. Pavia, Italy.

estella.zuccolo@unipv.it

Neocortical vasodilation elicited by basal forebrain stimulation is mediated by local release of acetylcholine (Ach), which leads to increased nitric oxide (NO) synthesis in brain microvessel endothelial cells (ECs). Surprisingly, the mechanism by which Ach induces NO synthesis in brain ECs is still unknown. An increase in intracellular Ca^{2+} concentration recruits a multitude of endothelial Ca^{2+}-dependent pathways, such as Ca^{2+}/Calmodulin endothelial NO synthase (eNOS). Herein, we investigated the role of intracellular Ca^{2+} signaling in Ach-induced NO production in bEnd5 cells, an established model of mouse brain microvascular ECs. We found that Ach induced dose-dependent asynchronous Ca^{2+} oscillations in bEnd5 cells. Ach-evoked Ca^{2+} oscillations didn't arise in the absence of external Ca^{2+} but rapidly resumed on Ca^{2+} restitution. Pharmacological manipulation indeed revealed that Ach stimulates bEnd5 cells by inducing a burst of intracellular Ca^{2+} spikes which is patterned by the interplay between ER-dependent Ca^{2+} mobilization and SOCE. Consistently, the depletion of the ER Ca^{2+} store with cyclopiazonic acid, a selective inhibitor of SERCA, revealed the expression of a BTP2- and La^{3+}-sensitive SOCE. Moreover, real-time polymerase chain reaction revealed that most of the components of the Ca^{2+} toolkit are present in bEnd5 cells at transcript level. Next, we found that Ach-induced NO production was hindered by L-NAME, a selective NOS inhibitor, and BAPTA, a membrane permeable intracellular Ca^{2+} buffer. Moreover, Ach-elicited NO synthesis was blocked by the pharmacological abrogation of the accompanying Ca^{2+} spikes. Ach-elicited Ca^{2+} spikes result in NO production and are predicted to control local CBF in mouse brain.

POSTER COMMUNICATION P-114
Ca^{2+} signalling

A plastid Two Pore Channel and its role in organelle integrity and calcium signaling.

Zhu-hong Li[1], Stephen Vella[1], Thayer P. King[1], Lawrence Ayong[1] Nathan Chasen[1] and Silvia N.J. Moreno[1]

[1]Center for Tropical and Emerging Global Diseases and Dept of Cellular Biology, University of Georgia, Athens, GA, 30602, USA.

smoreno@uga.edu

Toxoplasma gondii is an intracellular pathogen of humans and animals. These parasites harbor a vestigial plastid organelle termed the apicoplast, which is non-photosynthetic and is present in most Apicomplexan parasites. Because of previous evidences of a large acidic Ca^{2+} pool in T. gondii, we searched for homologues of two-pore channels (TPCs), which mediate Ca^{2+} release from acidic organelles. A gene for a TPC (TgTPC) was found in the database. To investigate the localization of TgTPC, a HA-tag was inserted at the 3' end of the gene. TgTPC localized to the apicoplast, as demonstrated by co-localization studies with apicoplast markers. The apicoplast is a unique chloroplast-like organelle, which is essential for parasite growth and pathogenesis. Almost nothing is known about the role of this organelle (or its relative, the chloroplast) in Ca^{2+} signaling or homeostasis. We inserted a tet-regulatable promoter upstream the gene coding region to modulate its expression with tetracycline. TgTPC1 expression was ablated 4 days with Tet treatment. TgTPC1 was essential for T. gondii growth and for all steps of its lytic cycle. The apicoplast was lost with decreased TgTPC expression. Growth defects were rescued by complementation with TgTPC gene. Amino acid mutations in both pore domains of the channel generated molecules unable to rescue growth attesting to the channel function of TgTPC. Acidic Ca^{2+} stores were altered in mutant parasites. We expressed GCAMp6 in the apicoplast to study the role of the organelle in Ca^{2+} signaling. Interest findings show that the apicoplast takes up Ca^{2+} from other organelles like the PLV or the ER.

POSTER COMMUNICATION P-115
Ca^{2+} signalling

TRPC6 channels control CXCR2-induced recruitment of neutrophils.

Albrecht Schwab[1], Jan Rossaint[2], Mike Wälte[1], Alexander Zarbock[2] and Otto Lindemann[1]

[1]Institute of Physiology II, University of Münster, Germany
[2]Department of Anaesthesiology, Intensive Care and Pain Medicine, Münster, Germany.

aschwab@uni-muenster.de

The inflammatory recruitment of neutrophils includes activation, adhesion to the endothelium and transendothelial migration as well as chemotaxis to the site of inflammation. Most of the underlying signal transduction pathways utilize Ca^{2+} transients which are often triggered by activation of G-protein coupled receptors. To identify the involved Ca^{2+} entry channels we investigated the role of TRPC6, a member of the transient receptor potential (TRP) channel family that is involved in receptor-operated Ca^{2+} entry. In vivo recruitment of murine neutrophils was investigated in a renal ischemia/reperfusion model. Neutrophil adhesion, arrest and transmigration under stimulation with fMLP (end-target chemoattractant) or KC/CXCL1 (intermediary chemoattractant) were analyzed in intravital microscopy. Chemotaxis of murine neutrophils in 3-dimensional matrices in direction of FMLP or KC/CXCL1 was studied with time-lapse videomicroscopy. Intracellular Ca^{2+} concentration was analyzed with the fluorescent Ca^{2+}-indicator Fura-2 and neutrophil adhesion on endothelial cells was studied with Atomic Force Microscopy (AFM). Renal inflammation and damage after ischemia related to neutrophil recruitment and serum creatinine was attenuated in $TRPC6^{-/-}$ mice. Adhesion, arrest and transmigration of $TRPC6^{-/-}$ neutrophils were diminished in vivo when applying KC. In contrast, recruitment of $TRPC6^{-/-}$ neutrophils was not affected after application of fMLP. In AFM experiments the loss of TRPC6 strongly reduced the adhesion forces between neutrophils and endothelial cells. Chemotaxis in gradients of KC was impaired, while fMLP-mediated chemotaxis was unaffected in $TRPC6^{-/-}$ neutrophils. The chemotaxis and adhesion defect of $TRPC6^{-/-}$ neutrophils in response to KC were accompanied by diminished Ca^{2+} influx and decreased CXCR2 signaling. Diminished Ca^{2+} transients after KC stimulation in $TRPC6^{-/-}$ neutrophils were likely responsible for reduced F-actin formation and integrin activation/adhesion. Our findings indicate that TRPC6 channels are important regulators in neutrophil recruitment. TRPC6 is a component of intermediary chemoattractant-triggered adhesion and actin polymerization but has no influence on end-target chemoattractant-mediated recruitment of neutrophils.

POSTER COMMUNICATION P-116
Ca^{2+} signalling

Regulation of neutrophil pro-inflammatory functions by Ca^{2+} signaling: Focus on cytokine secretion.

Sabrina Bréchard, Véronique Schenten, Sébastien Plançon, Nicolas Jung and Eric. J. Tschirhart

Life Sciences Research Unit, Calcium Signalling and Inflammation group, University of Luxembourg, Luxembourg.

sabrina.brechard@uni.lu

Ca^{2+} signalling is critical for the regulation of diverse neutrophil pro-inflammatory functions which ensure an effective killing of infecting microorganisms. In this view, Ca^{2+} mobilization has been largely described to be involved in the regulation of the NADPH oxidase, a multi-component enzyme responsible of the production of reactive oxygen species which can be involved in the development of chronic inflammatory diseases. In neutrophils, Ca^{2+} concentration ($[Ca^{2+}]_c$) changes are predominantly associated with receptor-mediated release of Ca^{2+} from intracellular stores and subsequent activation of Ca^{2+} channels in the plasma membrane during the process referred to as store-operated Ca^{2+} entry (SOCE). Over the past years, we presented a hypothetical model for the Ca^{2+}-dependent NADPH oxidase regulation involving TRPC channels and Orai1/STIM1. Currently, we question on the mechanistic basis for Ca^{2+}-dependent cytokine secretion by human neutrophils. RT-PCR and cytometric bead array analysis revealed that IL-8, CCL-2, CCL-3, CCL-4, CCL-5, IL-8, IL-6, TNF-α, IL-1 α and IL-1β RNA expression was increased following fMLF stimulation whereas only IL-8 and CCL-4 secretion was affected by such treatment.Incubation of cells in the absence of extracellular Ca^{2+} or with BAPTA prevented fMLF-induced cytokine secretion. In the same view, pharmacological inhibitors and knockdown of SOCE constituents attenuated the release of IL-8 and CCL-4 in differentiated HL-60 cells. These results indicate that extracellular Ca^{2+} entry and SOCE-related channels regulate fMLF-stimulated IL-8 and CCL-4 secretion in neutrophils.

This work was supported by the University of Luxembourg.

POSTER COMMUNICATION P-117
Ca^{2+} signalling

Identification of a novel TRPM2 agonist.

Ralf Fliegert[1], Monika Rozewitz[1], Joanna Swarbrick[2], Tanja Kirchberger[1], Christelle Moreau[2], Barry V.L. Potter[3] and Andreas H. Guse[1].

[1]The Calcium Signaling Group, Department of Biochemistry and Molecular Cell Biology, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, Germany. [2]Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK. [3]Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT, UK.

r.fliegert@uke.de

TRPM2 is a non-selective, Ca^{2+}-permeable cation channel that can be activated by binding of adenosine 5'-diphosphoribose (ADPR) to its cytoplasmic C-terminus. Since the ADPR concentration for TRPM2 activation is in the upper micromolar range, TRPM2 has initially been mainly perceived as a mediator of cell death following exposure of cells to reactive oxygen species and other genotoxic noxes. Recently it has become clear that TRPM2 also participates in physiological processes like chemotaxis, cytokine and insulin secretion, but it remains unknown how the channel is activated under these circumstances and whether this also involves ADPR. To probe the structural requirements for the activation of TRPM2 by ADPR we determined the agonist activity of ADPR analogues that we had previously tested as TRPM2 antagonists (Moreau 2013 ref). While most of the analogues had no, or negligible, agonist activity, we identified one novel TRPM2 agonist with surprising properties. This compound not only activates TRPM2 with an efficiency comparable to ADPR but interestingly induces 8.6-fold higher currents in whole cell patch-clamp experiments. To obtain more insight into this unsuspected behavior, we compared activation of TRPM2 by the agonists in excised inside-out patches from TRPM2 expressing HEK293 cells. These experiments show that the slope conductance from single channels does not depend on the agonist, while activation by the newly identified agonist results in significantly higher average open probability and slower inactivation rate.
References: Moreau, et al. (2013) J Med Chem. 56(24):10079-102.

Supported by Wellcome Trust Project Grant 084068 (to BVLP and AHG) and Programme Grant 082837 (to BVLP).

POSTER COMMUNICATION P-118
Ca^{2+} signalling Selected for Flash Symposium Presentation

Role of TRPV4 in pressure-induced inhibition of renin secretion by juxtaglomerular cells.

François Seghers, Xavier Yerna and Philippe Gailly

University of Louvain, Laboratory of Cell Physiology, 1200 Brussels, Belgium.

philippe.gailly@uclouvain.be

The renin – angiotensin system is a crucial blood pressure regulation system in which therate-limiting enzyme is renin, which is secreted into circulation by renal juxtaglomerular (JG) cells in response to low pressure in the renal afferent arteriole. In contrast, an increase in blood pressure in the afferent arteriole results in a decrease in renin secretion. This is accompanied by an increase in cytosolic calcium concentration ($[Ca^{2+}]_i$) of JG cells. The inverse relationship between $[Ca^{2+}]_i$ and renin secretion has been called the "calcium paradox" and is due to a Ca^{2+}-dependent inhibition of adenylate cyclase. How increase in pressure induces a $[Ca^{2+}]_i$ transient is however unknown. We observed that $[Ca^{2+}]_i$ transients induced by mechanical stimuli in JG As4.1 cells were inhibited by Gd^{3+} and ruthenium red, two non specific inhibitors of mechano-sensitive channels. More specifically, the response was reduced by siRNA-mediated repression of TRPV4 expression, but not after repression of TRPV2 or Piezo1 that are also expressed in As4.1 cells. As expected, Ca^{2+} response was inhibited by HC067047 and RN1734, two inhibitors of TRPV4. Interestingly, the stimulation of renin secretion by the AC activator forskolin was blunted by GSK1016790A and 4α—PDD, two activators of TRPV4. Moreover, in isolated perfused kidneys from $Trpv4^{-/-}$ mice, the pressure - renin relationship was significantly altered. In vivo, $Trpv4^{-/-}$ mice presented an increased reninemia compared to wild-type mice. Altogether, our results suggest that TRPV4 is involved in pressure-induced entry of Ca^{2+} in JG cells, which inhibits renin release and allows the negative feedback regulation on blood pressure.

POSTER COMMUNICATION P-119
Ca^{2+} signalling

CRISPR/Cas9 genome editing of STIM1 and ORAI1 in cancer cell lines.

[1]Carlos Pascual-Caro, [1]Aida M. Lopez-Guerrero, [2]Thomas Macartney, [2]Dario R. Alessi, [3]Eulalia Pozo-Guisado and [1]Francisco Javier Martin-Romero.

[1]Departamento de Bioquímica y Biología Molecular. Facultad de Ciencias. [3]Departamento de Biología Celular. Facultad de Medicina. Universidad de Extremadura. Badajoz, Spain. [2]MRC-Phosphorylation and Ubiquitylation Unit. College of Life Sciences. University of Dundee. Dundee, Scotland, UK.

carlospc@unex.es; fjmartin@unex.es

Store operated Ca^{2+} entry (SOCE) is a major Ca^{2+} influx pathway that controls diverse cellular events, including proliferation, migration, gene expression, etc. This pathway is regulated by the endoplasmic reticulum (ER) protein STIM1, an intraluminal Ca^{2+} sensor that activates the plasma membrane Ca^{2+} channel ORAI1 upon partial depletion of Ca^{2+} concentration within the ER. To study the role of this Ca^{2+} influx pathway in cancer cell lines we have generated STIM1 and ORAI1 knock out (KO) cell lines using a CRISPR/Cas9 genome editing approach. In contrast to recent available protocols we have used a mutant version of the nuclease, Cas9 D10A (nickase), in order to enhance the genome editing specificity and decrease off-target modifications. With this technique we were able to generate genomic modifications in U2OS, PC3, and SH-SY5Y cell lines to fully abolish STIM1 or ORAI1 gene expression. Here we describe the strategy for the double nicking by RNA-guided editing, and the subsequent results of genome sequencing in the modified clones. These results show a diversity of genomic indels that generate frameshifting and premature stop codons in all cell lines. Protein expression as well as functional assays to measure SOCE in KO cells were also assessed to fully validate the technique. As expected, STIM1-KO and ORAI1-KO cells did show a large decrease in Ca^{2+} entry levels in response to thapsigargin, making these KO cells an appropriate tool to study the role of SOCE in cancer cell signaling and physiology.

Supported by grants BFU2014-52401-P (Spanish Ministerio de Economía y Competitividad) and GR15077 (Junta de Extremadura).

POSTER COMMUNICATION P-120
Ca^{2+} signalling

Long lasting steady-state secretory responses in ouabain- treated bovine chromaffin cells.

Andrés Mateo Baraibar[1], Ricardo De Pascual[1,2], Inés Colmena[1,2], Lucía Ruiz-Pascual[1], Javier Egea[1,2], Martín Pérez Ciria[1], Ignacio Polo Vaquero[1], Luis Gandía[1,2] and Antonio G. García[1,2,3].

[1]Instituto Teófilo Hernando, [2]Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, C/ Arzobispo Morcillo, 4; 28029 Madrid, Spain. Servicio de Farmacología Clínica,Instituto de Investigación Sanitaria, Hospital Universitario de la Princesa, Universidad Autónoma de Madrid, c/ Diego de León, 62; 28006 Madrid, Spain.

mateobiologia@outlook.es

It is known that the sustained depolarisation of adrenal medullary bovine chromaffin cells (BCCs) with high K^+ concentrations produces an initial sharp catecholamine release that subsequently fades off in spite depolarisation persists. Here we have explored the secretory responses in BCCs exposed to the Na^+/K^+ ATPase blocker ouabain, to elicit their sustained depolarisation for an hour. They were perifused with nominal $0Ca^{2+}$ solution and secretion responses were elicited by intermittent application of short $2Ca^{2+}$ pulses (Krebs-HEPES containing 2 mM Ca^{2+}). These pulses elicited a biphasic secretory pattern with an initial 30-min period with secretoryresponses of increasing amplitude and a second 30-min period with steady-state, non-inactivating responses. The initial phase was not due to gradual depolarisation neither to gradual increases of the cytosolic calcium transients ($[Ca^{2+}]_c$) elicited by $2Ca^{2+}$ pulses in they were triggered by Ca^{2+} entry through non-inactivating L-type calcium channels. Nifedipine also blocked the $[Ca^{2+}]_c$ transients elicited by $2Ca^{2+}$ pulses. Furthermore, the plasmalemmal Na^+/Ca^{2+} exchanger (NCX) blocker SEA0400 caused a mild inhibition followed by a large rebound increase of the steady-state secretory responses. We conclude that these two phases of secretion are mostly contributed by Ca^{2+} entry through L calcium channels, with a minor contribution of Ca^{2+} entry through the reverse mode of the NCX.

Supported by SAF2013-344108-P and SAF2013-344108-P

POSTER COMMUNICATION P-121
Ca^{2+} signalling

Plasma membrane calcium pump in chemokine receptors-mediated calcium transient in differentiated PC12 cells.

Tomasz Radzik[1], Malwina Lisek[1], Bozena Ferenc[1], Tomasz Boczek[1,2] and Ludmila Zylinska[1]

[1]Department of Molecular Neurochemistry, Medical University of Lodz, Poland
[2]Boston Children's Hospital and Harvard Medical School, Boston, MA, USA.

ludmila.zylinska@umed.lodz.pl

There is a strong evidence that in the CNS chemokines may induce neurodegeneration directly via activation of their receptors on the surface of neurons. Some chemokines may induce calcium release from intracellular stores, due to activation of phospholipase C. In the cells plasma membrane calcium pump (PMCA), as the most sensitive Ca^{2+} detector, participates in Ca^{2+} clearance, and the efficiency of this process depends on abundance and location of both, chemokine receptors and PMCA isoforms. Using the developed model of differentiated PC12 cells with reduced expression of PMCA2 or PMCA3 we assayed the role of selected chemokine receptors – Ccr1, Ccr3 and Ccr5 - in calcium transients generation under stress conditions. By using Western blot and confocal imaging, we first identified the presence of these receptors in PC12 cells, but also some differences in their amount and distribution between control and PMCA-reduced lines. Co-immunoprecipitation assay using specific chemokine receptors antibodies and 5F10 antibody, recognizing all PMCA isoforms, revealed an altered formation of Ccr/PMCA complexes. All three lines responded to chemokine CCL5, and it indicated existence of functional receptors in the cell membranes. The shape of responses was similar between PC12 lines. There was an initial large Ca^{2+}spike followed by a sustained oscillatory phase with increasing spike amplitudes and periodicity, which was the highest in control cells. Our results suggest that both proteins could be functionally linked, and it could be a new, yet non-described mechanism potentially engaged in regulation of cellular calcium homeostasis.

Supported by the grants 502-03/6- 086-02/502-64-086 and 503/6-086-02/503-01 from Medical University of Lodz.

POSTER COMMUNICATION P-122
Ca^{2+}, stem cells and regeneration

The zebrafish scale as a possible model for studying Ca^{2+} homeostasis in vertebrates.

Jacky T.S. Hung[1], Alessandro Rubinacci[2], Joseph Kunkel[3], Alan M. Shipley[4], Paola Pajevic-Devieti[5], Sarah E. Webb[1] and Andrew L. Miller[1,6]

[1]Division of Life Science & State Key Laboratory of Molecular Neuroscience, HKUST, Clear Water Bay, Hong Kong, PRC. [2]Bone Metabolism Unit, San Raffaele Hospital, Milan, Italy; [3]University of New England, Marine Science Center, Biddeford, ME 04005, USA; [4]Applicable Electronics Inc., New Haven, CT 06520-7072, USA; [5]Massachusetts General Hospital, Endocrine Unit THR 1101, Boston, MA 02114, USA; and [6]Marine Biological Laboratory, Woods Hole, MA 02543, USA.

almiller@ust.hk

It has been proposed that elasmoid fish scales might be a useful complementary model to study human bone development, function, disease, injury, and regeneration. Such scales are composed of many of the essential cell types and structures found in mammalian bone, such as: osteoclasts, osteoblasts, matrix proteins, hydroxyapatite crystals, and collagen fibers. We are currently investigating whether fish scales also show a similar short-term physiological reaction as mammalian bone, when responding to calcemic challenges. Our preliminary data suggest that the scales of zebrafish do possess a short-term Ca^{2+}-response mechanism. Via the use of a non-invasive, extracellular scanning ion-selective electrode technique (SIET), we showed that Ca^{2+} fluxes generated by zebrafish scales ex vivo, are dependent on the extracellular [Ca^{2+}], where hypocalcemic and hypercalcemic conditions lead to an outward and inward Ca^{2+} flux, respectively. In addition, immunolabeling revealed the presence of both cathepsin K-positive osteoclasts and Zns5-positive osteoblasts on scales. With regards to hormonal regulation of scale-mediated Ca^{2+} exchange, we showed via RT-PCR that the calcitonin receptor-like a and b (Calcrla, Calcrlb), estrogen receptor isoforms (Esr1, Esr2a, and Esr2b), and parathyroid hormone 1 receptor A (Pth1ra) are all expressed in scales. Furthermore, treatment with calcitonin or parathyroid hormone (1-34) modified the Ca^{2+} flux behavior of zebrafish scales ex vivo, as predicted. Our data indicate, therefore, that the fish scale might provide an alternative and more accessible model to study Ca^{2+} homeostasis than vertebrate bone.

Supported by HK RGC-GRF awards 662113, 16101714, 16100115; ANR/RGC award A-HKUST601/13, HK-TRS award T13-706/11-1, and HK-ITC (ITCPD/17-9).

POSTER COMMUNICATION P-123
Ca^{2+}, stem cells and regeneration

Required role of store-operated Ca^{2+} entry (SOCE) for the successful completion of cytokinesis during the first two cell division cycles in zebrafish embryos.

Sarah E. Webb[1], Ching Man Chan[1], Yiyun Chen[1], Jacqueline T.M. Aw[1], Tin Shing Hung[1], and Andrew L. Miller[1,2]

[1]Division of Life Science & State Key Laboratory of Molecular Neuroscience, HKUST, Clear Water Bay, Hong Kong, PRC. [2]MBL, Woods Hole, MA 02543, USA.

barnie@ust.hk

During the first few cell division cycles in zebrafish, distinct Ca^{2+} transients are localized to the early embryonic cleavage furrows, where they are required for furrow positioning, propagation, deepening and daughter cell apposition. The ER has previously been shown to act as the primary store for the Ca^{2+} generating these transients, via release through IP_3Rs. However, the elevated levels of intracellular Ca^{2+} required for furrow deepening/apposition were hypothesized to result in a depletion of the available Ca^{2+} in this store. Thus, the role of SOCE was examined. Newly fertilized, dechorionated embryos were incubated with various SOCE inhibitors, and their effect on mitosis, furrow positioning, propagation, deepening and apposition, as well as the generation of the cytokinetic Ca^{2+} transients during the first cell division cycle was determined. Treatment with 2-APB or SKF 96365 had no obvious effect on mitosis, furrow positioning or propagation, but furrow deepening was inhibited, resulting in cleavage furrow regression. These inhibitors also blocked the furrowing Ca^{2+} transient, with SKF 96365 having a more profound inhibitory effect than 2-APB. Components of SOCE (STIM1, Orai1 and TRPC1) were immunolabelled in cleaving embryos and were shown to be localized to the furrow, mainly during deepening and apposition. Together, these data suggest that during the early embryonic cell division cycles, SOCE appears to be essential for maintaining the elevated levels of $[Ca^{2+}]_i$ for the extended periods required for successful furrow deepening and daughter cell apposition.

HK RGC-GRF awards 662113, 16101714, 16100115; ANR/RGC award A-HKUST601/13, HK-TRS award T13-706/11-1, and HK-ITC (ITCPD/17-9).

POSTER COMMUNICATION P-124
Ca^{2+}, stem cells and regeneration

Characterizing the role of TPC2-mediated Ca^{2+} signaling during the development of slow muscle cells and primary motor neurons in zebrafish.

Jeffrey J. Kelu[1], Sarah E. Webb[1], Margarida Ruas[2], John Parrington[2], Antony Galione[2] and Andrew L. Miller[1,3]

[1]Division of Life Science & State Key Laboratory of Molecular Neuroscience, HKUST, Clear Water Bay, Hong Kong, PRC. [2]Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, UK. [3]Marine Biological Laboratory, Woods Hole, MA 02543, USA.

jjkelu@connect.ust.hk

Ca^{2+} signaling is reported to play a key role in the regulatory networks that control differentiation in many cell types, including vertebrate skeletal muscle and neurons. We are using transgenic lines of zebrafish that express: 1) the bioluminescent Ca^{2+} reporter, apoaequorin, specifically in the skeletal muscle; and 2) the fluorescent Ca^{2+} reporter, GCaMP7, specifically in the primary motor neurons. Using the apo-aequorin-expressing fish, we showed that two pore channel 2 (TPC2) plays a crucial role in generating Ca^{2+} signals during slow muscle cell (SMC) myogenesis in zebrafish. Morpholino (MO)-based knock-down of TPC2 resulted in a dramatic attenuation of the Ca^{2+} signals, whereas co-injection of TPCN2 mRNA or treatment with IP_3/BM partially rescued the Ca^{2+} signaling signature in SMCs. Injection of the TPCN2-MO into embryos of the GCaMP7 transgenic fish resulted in an inhibition of the alternate firing of the contralateral caudal primary motor neurons (CaPs). This suggests a possible regulatory role for TPC2-mediated Ca^{2+} signaling during CaP activation. To validate the MO-generated data, we generated a TPC2 mutant line using CRISPR/Cas9 gene editing. Our results indicate that there are similarities between the MO-generated TPC2 knock-down and CRISPR/Cas9-generated TPC2 knock-out embryos obtained. Together, our data provide evidence to suggest that spontaneous TPC2-mediated Ca^{2+} release might be essential for regulating the development and function of SMCs in zebrafish via modulating the upstream neurogenic signals.

Funding: HK RGC-GRF awards 662113, 16101714, 16100115; ANR/RGC award A-HKUST601/13, HK-TRS award T13-706/11-1, and HK-ITC (ITCPD/17-9).

POSTER COMMUNICATION P-125
Ca^{2+}, stem cells and regeneration

Cav3.2 mediates Neuronal Differentiation through Activating Caspase-3/7.

Paola Rebellato †, Dagmara Kaczynska †, Carlos Villaescusa, Erik Norberg, Anna Falk, Ernest Arenas, Shigeaki Kanatani* and Per Uhlén*

Karolinska Institutet, SE-171 77 Stockholm, Sweden

shigeaki.kanatani@ki.se

Here we report that the voltage dependent T-type calcium (Ca^{2+}) channel Cav3.2 (α1h) regulates Caspase-3/7 activity and mediates neuronal differentiation by activating Caspase-3/7. At the onset of neuronal differentiation neural progenitor cells exhibit low frequency Ca^{2+} activity initiated by the low voltage activated (LVA) Ca^{2+} channel α1h. Interestingly, cells exhibiting strong spontaneous Ca^{2+} activity had Caspase-3/7 activity, without inducing significant apoptosis. Inhibition of the Ca^{2+} activity by LVA inhibitors and viral knock down of Cav3.2 resulted in decreased Caspase-3/7 activity followed by suppressed neurogenesis. When Cav3.2 was overexpressed increased neurogenesis was detected. Cav3.2 knockout mice showed a decrease of ventricular zone thickness at E16.5 when crossed with 129X1/SvJ strain in which Caspase 3 knockout mice showed brain malformation during development. In summary, we demonstrate a novel relationship between Cav3.2 and Caspase signaling that affects neurogenesis in the developing brain.

Supported by Swedish Research Council Grants 2005-6682, 2005-7204, 2007-5977, 2009-3364, 2010-4392, and 2013-3189 (P.U.), Swedish Foundation for Strategic Research (P.U.), Linnaeus Center in Developmental Biology for Regenerative Medicine (DBRM) (P.U.), the Knut and Alice Wallenberg Foundation Grant to Center of Live Imaging of Cells at Karolinska (CLICK) Institutet (P.U.), the Royal Swedish Academy of Sciences (P.U.), Hjärnfonden Grant FO2011-0300 (P.U.), the Wenner-Gren Foundation (S.K.), and Japan Society for the Promotion of Science (S.K.).

POSTER COMMUNICATION P-126
Ca^{2+}, stem cells and regeneration Selected Oral Communication for Symposium #9

Voltage-dependent calcium channel signaling mediates GABAA receptor-induced migratory activation of dendritic cells infected by Toxoplasma gondii.

Sachie Kanatani[1,2], Jonas M. Fuks[1,2], Linda Westermark[1,2] Manuel Varas-Godoy[3], Per Uhlén[3] and Antonio Barragán[1,2]

[1]Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, 106 09 Stockholm, Sweden, [2]Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, 141 86 Stockholm, Sweden, [3] Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden.

Sachie.kanatani@su.se

The obligate intracellular parasite Toxoplasma gondii exploits cells of the immune system to disseminate. Upon T. gondii-infection, γ-aminobutyric acid (GABA)/GABAA receptor signaling triggers a hypermigratory phenotype in dendritic cells (DCs) by unknown signal transduction pathways. Here, we demonstrate that calcium (Ca^{2+}) signaling in DCs is indispensable for T. gondii-induced DC hypermotility and transmigration in vitro. We report that Toxoplasma infection modulates the expression of the GABAA receptor regulator NKCC1 with an impact on Ca^{2+} signaling and hypermotility. We found that GABA induces calcium entry in DCs. We report that murine bone marrow-derived DCs preferentially express the L-type voltage-dependent Ca^{2+} channel (VDCC) subtype CaV1.3. Silencing of CaV1.3 by short hairpin RNA or pharmacological antagonism of VDCCs abolished the hypermigratory phenotype. The present data establish that Ca^{2+} is a second messenger to GABAergic signaling in DCs and that T. gondii-induced migration of DCs requires signaling via VDCC subtype CaV1.3. The findings define a novel motility-related signaling axis in DCs. The findings also unveil that interneurons and immune cells share common GABAergic motogenic pathways and that T. gondii employs non-canonical pathways to induce host cell migration.

Supported by grants from the Swedish Research Council and the Hedlund Foundation.

POSTER COMMUNICATION P-127
Ca^{2+}, stem cells and regeneration

Remodeling of mitochondrial Ca^{2+} uptake capacity during the transition proliferation to quiescence of glioblastoma stem-like cells.

Francisco Aulestia[1], Isabelle Néant[1], Jacques Haiech[2], Marie Claude Kilhoffer[2], Marc Moreau[1] and Catherine Leclerc[1]

[1]Centre de Biologie du Développement CNRS UMR5547, Université Toulouse 3, 118 route de Narbonne, F31062 Toulouse, Cedex 04, France. [2]Laboratoire d'Innovation Thérapeutique, Université de Strasbourg, UMR CNRS 7200, 74 route du Rhin, 67401 Illkirch, France.

Francisco.aulestia-araya@univ-tlse3.fr

Glioblastoma multiforme (GBM) are the most common and aggressive primary brain tumors with a survival period rarely exceeding 2 years after initial diagnosis. GBMs display significant heterogeneity within the tumor mass, among which a small sub-population of tumor cells with stem-like properties (GSCs) is responsible for tumor growth, resistance to therapies and tumor recurrence. Within the tumor mass GSCs localized to hypoxic and acidic microenvironments have been found to be in a quiescent state and to represent the most aggressive form of GSCs. Although cellular quiescence is one option for cancer stem-like cells to evade killing, the functional characterization of quiescent GSCs remain poorly understood. Here, using the bioluminescent Ca^{2+} reporter EGFP-aequorin targeted to the mitochondria or the cytosol we explored how proliferating and quiescent GSCs maintain Ca^{2+} homeostasis. Quiescence of GSCs was obtained by lowering the extracellular pH. We showed that the transition from proliferation to quiescence is characterized by (1) the reversible remodeling of mitochondrial morphology from a tubular to a donut shape, (2) an increased capacity of mitochondria from quiescent GSCs to capture Ca^{2+}, (3) the down-regulation of MICU2 and of MCUb mRNAs, two negative regulators of the mitochondrial Ca^{2+} uniporter MCU and (4) the modification of the kinetic of Ca^{2+} influx through store-operated channels (SOC). Our data suggest that the remodeling of the Ca^{2+} homeostasis and the reshaping of mitochondria during the transition proliferation to quiescence constitute a protective mechanism that favors cancer stem-like cells survival and its aggressiveness in GBM.

Supported in France by the Centre National de la Recherche Scientifique (CNRS), Université Toulouse 3, Université de Strasbourg and by a joint grant from the Agence Nationale de la Recherche (ANR) given between France and Hong Kong to CL, JH and MM (CalciumGlioStem ANR-13-ISV1-0004).

POSTER COMMUNICATION P-128
Ca^{2+}, stem cells and regeneration

Mechanical stress-induced Ca^{2+} influx and RANKL expression in osteoblastic cells

Jung Yun Kang, Aran Son, Yu-Mi Yang and Dong Min Shin.

Departament of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul, Korea.

hannahkang77@gmail.com

Mechanical stress plays an important role in bone remodeling. However, the intracellular mechanisms of mechanical stress, especially hypotonic stress-mediated bone remodeling are not well understood. In this study, we investigated signaling pathway of hypotonic stress-induced bone remodeling, mainly focused on the expression of the bone remodeling factors, including receptor activator of nuclear factor-kappa B ligand (RANKL) in osteoblastic cells. Hypotonic stress increased the mRNA expression of RANKL but not OPG. It also augmented intracellular calcium concentration ($[Ca^{2+}]i$). Extracellular Ca^{2+} depletion completely inhibited hypotonic stress-induced increases in $[Ca^{2+}]i$. In addition, non-specific plasma membrane Ca^{2+} channel blockers, gadolinium (Gd3+) and lanthanum (La3+), abolished both hypotonic stress-induced increases in $[Ca^{2+}]i$ and RANKL mRNA expression suggesting a critical role of $[Ca^{2+}]i$. We identified the expression and activation of transient receptor potential vaniloid 4 (TRPV4) and melastatin 3 (TRPM3) in osteoblasts. 4α-phorbol didecanoate (4α-PDD) and pregnenolone sulfate (PS), which are activators of TRPV4 and TRPM3, respectively induced increases of RANKL expression and HC067047 and ononetin, which are inhibitors of TRPV4 and TRPM3, respectively reversed the increase of RANKL expression. Both pharmacological (HC067047 and ononetin) and genetic (small interfering RNA [siRNA]) inhibitors of TRPV4 and TRPM3 reduced hypotonic stress-mediated increases of $[Ca^{2+}]i$ in osteoblasts. Our study shows that hypotonic stress induced RANKL mRNA expression through TRPV4- and TRPM3-mediated increases in $[Ca^{2+}]i$. This signaling pathway in osteoblastic cells may play an essential role in hypotonic stress-mediated bone remodeling.

Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (2015R1A2A1A15054157) and (MOE) (2015R1D1A1A01057277).

POSTER COMMUNICATION P-129
Ca^{2+}, stem cells and regeneration Selected Oral Communication for Symposium #9

Annexin A6-mediated alanine uptake is necessary for liver regeneration in mice.

Anna Alvarez-Guaita[1], Elsa Meneses-Salas[1], Thomas Grewal[2], Carlos Enrich[1] and Carles Rentero[1].

[1]Departament de Biomedicina, Universitat de Barcelona, Barcelona, 08036, Spain; [2]Faculty of Pharmacy, University of Sydney, Sydney, NSW, 2006, Australia.

carles.rentero@ub.edu

Annexin A6 (AnxA6) belongs to a conserved family of Ca^{2+}- and phospholipid-binding proteins which interact with membranes in a calcium-dependent manner. Although AnxA6 is highly abundant in the liver (0.25% total protein), its function in the physiology of this organ remains unknown. Thus, we aimed to investigate the in vivo liver function of AnxA6 using the AnxA6 null-mutant ($AnxA6^{-/-}$) mouse model. $AnxA6^{-/-}$ mice show a highly reduced survival rate after two-thirds partial hepatectomy (PHx) associated to a prolonged hypoglycemia. A comprehensive analysis of glucose metabolism pointed to an impairment in liver gluconeogenesis in $AnxA6^{-/-}$ mice, revealing a new function for AnxA6 in liver glucose production during the regeneration process and fasting. $AnxA6^{-/-}$ hepatocytes are incapable of performing gluconeogenesis specifically from alanine, which is the main gluconeogenic substrate during liver regeneration and starvation. Interestingly, we found that AnxA6 deficiency cause an impairment of alanine uptake in hepatocytes. The results here presented suggest that AnxA6 regulates the intracellular trafficking of SNAT2, the major liver alanine transporter, to the hepatocyte sinusoidal plasma membrane. Notably, the exogenous administration of glucose rescued $AnxA6^{-/-}$ mice survival after surgery, supporting the lack of glucose production in this strain as the cause of the reduced survival after partial PHx. This study demonstrates that AnxA6 is a new regulator of hepatic gluconeogenesis and critical for mice survival after PHx. A novel role for AnxA6 in alanine uptake and as a liver gluconeogenesis regulator is described, being essential for maintaining blood glucose levels during both liver regeneration and fasting.

Supported by the Ministerio de Economía y Competitividad (MINECO) [grant numbers BFU2012-36272 and CSD2009-00016] and Fundació Marató TV3 [grant number PI042182] (Spain) to CE and The University of Sydney, Australia [grant numbers 2010-02681, U7007 and U7042] (Australia) to TG.

POSTER COMMUNICATION P-130
Ca^{2+}, stem cells and regeneration

An interaction between TRPC1 and BMP-receptor type II regulates neural specification in Xenopus laevis.

Isabelle Néant[1], Ho Chi Leung[2], Sarah E. Webb[2], Andrew L. Miller[2], Marc Moreau[1] and Catherine Leclerc[1]

[1]Centre de Biologie du Développement CNRS UMR5547, Université Toulouse 3, 118 route de Narbonne, F31062 Toulouse, Cedex 04, France. [2]Division of Life Science and State Key Laboratory of Molecular Neuroscience, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, PR China.

isabelle.neant@univ-tlse3.fr

It is well established that in vertebrates, Ca^{2+} signalling and the inhibition of the bone morphogenetic protein (BMP) pathway are both involved in neural induction, which is the first step in the specification of the embryonic nervous system. We have previously demonstrated that in Xenopus, treatment with noggin, a BMP signalling antagonist, triggers an influx of Ca^{2+} through Ca_v1 Ca^{2+} channels. In addition, we showed that this influx constitutes a necessary and sufficient signal to trigger neural induction. However, up until recently, the molecular mechanism(s) that link the inhibition of BMP signalling and Ca^{2+} entry were unknown. Here, we show that the transient receptor potential cation channel, TRPC1, binds to BMP receptor type II (BMPRII) via its C-terminal intracellular domain. This therefore establishes a link between the BMP pathway and the activation of the Ca_v1 channel. We also show that TRPC1 is required both for the noggin-induced Ca^{2+} influx and for the expression of Zic3, an early neural specifier gene. These results therefore provide the first evidence of a functional link between TRPC1 and BMPRII in the regulation of Ca^{2+} entry through Ca_v1 channels.

Supported in France by the Centre National de la Recherche Scientifique (CNRS), Université Toulouse 3 and LIA "Rocade" from CNRS (grant to M.M.); in Hong Kong by RGC awards: HKUST662113,16101714 and 16100115; an ANR/RGC award: A-HKUST601/13, and TRS award: T13-706/11-1

POSTER COMMUNICATION P-131
Ca^{2+}, stem cells and regeneration
Selected for Flash Symposium Presentation

Lipid phosphatidic acid and an outwardly spiraling disk of calcium release leads to the fertilization calcium wave.

Bradley J. Stith.

Department of Integrative Biology, University of Colorado Denver. CO. USA

brad.stith@ucdenver.edu

Working with the Xenopus model system, we have shown that sperm activate egg phospholipase D1b (PLD1b) to increase phosphatidic acid (PA) mass, and that PA then binds and stimulates Src (leading to phospholipase C-gamma (PLC γ) activation, IP3 and intracellular $[Ca]_i$ increases). As compared to 14 other lipids, PA strongly bound Xenopus Src but not PLC γ. Inhibition of PLD by two different inhibitors prevented the increase in PA mass at fertilization, Src and PLC γ activation and reduced ~87% of the $[Ca]_i$ release during fertilization. PA addition to eggs activated Src and PLC γ, doubled the amount of PLC γ in rafts, and elevated IP_3 mass to levels equivalent to that induced by sperm but twice that achieved by Ca^{+2} ionophore (Ca^{+2} buffers did not block)(see 2 Dev. Bio. papers: Bates et al., 2014; and Stith, 2015). As 5 different tyrosine kinase inhibitors only partially inhibited (~40%), we suggest that there are three paths to $[Ca]_i$ release at fertilization: PA can activate an unknown PLC isoform independently without Src (52% of total $[Ca]_i$ release, a slow activation), PA acts through Src (35%, fast), and an unknown PA-independent pathway (13%, slow). In an important breakthrough on the fertilization $[Ca]_i$ wave, videos of intracellular $[Ca]_i$ show that the wave is due to an outwardly spiraling disk of elevated $[Ca]_i$ (~70 µm diameter, moving clockwise). The acceleration of the fertilization $[Ca]_i$ wave at 30 s-1min appears to be due to the appearance of additional rotating disks that arise at this time (moving clockwise, or counterclockwise).

Supported by NIH grant 1R15HD065661-01.

LIST OF AUTHORS

LIST OF ATTENDANTS

Albinsson, Sebastian.
Lund University, Sweden. sebastian.albinsson@lu.se
Almeida Fortalezas, Sofia.
Universidad Extremadura. Spain. sofia_furta@hotmail.com
Alonso, María Teresa.
Universidad de Valladolid, Spain. talonso@ibgm.uva.es
Alvarez, Javier.
Universidad de Valladolid, Spain. jalvarez@ibgm.uva.es
Álvarez-Illera, Pilar.
Universidad de Valladolid, Spain. pilar_alvill@hotmail.com
Ames, James.
University of California, USA. jbames@ucdavis.edu
Apáti, Ágota.
Research Centre of Natural Sciences, Hungary. apati@biomembrane.hu
Arias Del Val, Jésica.
Universidad de Valladolid, Spain. jessica.arias@ibgm.uva.es
Asghar, Muhammad Yasir.
Åbo Akademi University, Finland. myasghar@abo.fi
Aulestia, Francisco.
CNRS, Toulouse, France. francisco.aulestia-araya@univ-tlse3.fr
Baraibar, Andres M.
Universidad Autónoma Madrid, Spain. mateobiologia@outlook.es
Bassett, John.
University of Queensland, Australia. j.bassett@uq.edu.au
Benaim, Gustavo.
Universidad Central de Venezuela, Venezuela. gbenaim@gmail.com
Bird, Gary.
NIEHS, NIH, USA. Bird@niehs.nih.gov
Boehning, Darren.
University of Texas, USA. Darren.F.Boehning@uth.tmc.edu
Bogdanova, Anna.
University of Zurich, Switzerland. annab@access.uzh.ch
Boglarka, Zambo.
Hungarian Academy of Sciences, Hungary. Zambo.boglarka@ttk.mat.hu
Bolotina, Victoria.
Boston University, USA. bolotina@bu.edu
Bong, Alice Hui Li.
University of Queensland, Australia. h.bong@uq.edu.au
Bootman, Martin B.
The Open University, UK. martin.bootman@open.ac.uk
Bouchet, Ana M.
Université de Tours, France. anamaria.bouchet@univ-tours.fr
Brechard, Sabrina.
University of Luxembourg, Luxembourg. sabrina.brechard@uni.lu
Brohus, Malene.
Aalborg University, Denmark. mbrohus@bio.aau.dk
Bultynck, Geert.
KU Leuven, Belgium. geert.bultynck@med.kuleuven.be
Burgos, Miguel.
University of Castilla-La Mancha, Spain. Miguel.burgos@uclm.es
Byts, Nadiya.
University of Oulu, Finland. nadiya.byts@oulu.fi
Calvo-Rodríguez, María.
University Valladolid, Spain. mariacalvorodriguez@gmail.com
Cano-Abad, María.
Universidad Autónoma Madrid. Madrid. Spain. maria.cano@uam.es
Castrillo, David.
Hamamatsu Photonics, Spain. dcastrillo@hamamatsu.es
Chantòme, Aurélie.
Université de Tours, France. aurelie.chantome@univ-tours.fr
Chazin, Walter.
Vanderbilt Ctr. Structural Biology, USA. walter.chazin@vanderbilt.edu
Chehab, Tala.
The Open University, UK. tala.chehab@open.ac.uk
Chun Kit, Tong.
University of Hong Kong, Hong Kong SAR. ckben@hku.hk
Corbacho, Isaac.
Universidad de Extremadura, Spain. icorbacho@unex.es
Czeredys, Magdalena.
Int. Institute Molecular & Cell Biology, Poland. mczeredys@iimcb.gov.pl
Debant, Marjolaine.
INSERM, France. debantm@gmail.com
Decrock, Elke.
Ghent University, Belgium. elke.decrock@gmail.com
Del Bosque, David.
National Research Council of Spain (CSIC), Spain. d.bosque@csic.es
Del Re, Valeria.
University of Siena, Italy. valeriadelre@libero.it
Del Río, Alba.
Universidad de Valladolid, Spain. alba.rio@ibgm.uva.es
Delettre-Cribaillet, Cécile.
INSERM, France. cecile.delettre@inserm.fr
Delprat, Benjamin.
INSERM, France. benjamin.delprat@inserm.fr
Dey, Kuntal.
Ben-Gurion University, Israel. kd.bcku@gmail.com
Docio, Inmaculada.
Universidad de Valladolid, Spain. inmadocio@hotmail.com
Dolga, Amalia.
University of Groningen, Netherlands. a.m.dolga@rug.nl
Draeguer, Anette.
Universitat Bern, Switzerland. annette.draeger@ana.unibe.ch
Duchen, Michael R.
University College London, UK. m.duchen@ucl.ac.uk
Dupont, Geneviève.
Université Libre de Bruxelles, Belgium. gdupont@ulb.ac.be
Dyrda, Agnieszka.
University of Geneva, Switzerland. agnieszka.dyrda@unige.ch
Ehrlich, Barbara.
Yale University, USA. barbara.ehrlich@yale.edu
Ellis-Davies, Graham.
Mount Sinai School of Medicine, USA. graham.davies@mssm.edu
Enrich, Carlos.
Universitat de Barcelona, Spain. enrich@ub.edu
Falcke, Martin.
Max Delbrück Center, Germany. martin.falcke@mdc-berlin.de
Farfariello, Valerio.
University Lille/INSERM, France. yann.fourbon@etu.univ-tours.fr
Félix, Romain.
Université de Tours, France. romain.felix@univ-tours.fr
Fliegert, Ralf.
University Medical Center Hamburg-Eppendorf, Germany. r.fliegert@uke.de

Fonteriz, Rosalba.
Universidad de Valladolid, Spain. rfonteri@ibgm.uva.es
Fourbon, Yann.
Université de Tours, France. yann.fourbon@etu.univ-tours.fr
Frandsen, Stine.
Herlev Hospital, Denmark. stine.krog.frandsen@regionh.dk
Gailly, Philippe.
University of Louvain, Belgium. philippe.gailly@uclouvain.be
García, Antonio G.
Universidad Autónoma de Madrid, Spain. agg@uam.es
García, Martha Iveth.
University Texas Medical Branch, USA. martha.ivethgarcia@gmail.com
García-Alvarado, Fernanda.
Univ. Autónoma de Madrid, Spain. fernanda0788@hotmail.com
García-Casas, Paloma.
Universidad de Valladolid, Spain. palomabio21@gmail.com
García-Durillo, Mónica.
National Research Council of Spain (CSIC). monicagd@ibgm.uva.es
García-Rodríguez, C.
National Research Council of Spain (CSIC), Spain. cgarcia@ibgm.uva.es
García-Sancho, Javier.
Universidad de Valladolid, Spain. jgsancho@ibgm.uva.es
Gargus, John J.
University of California, Irvine, USA. jjgargus@uci.edu
Gataulin, Diana.
Weizmann Institute of Science, Israel. dianaga@weizmann.ac.il
Gazda, Kinga.
International Institute Molecular and Cell Biology, Poland. kgazda@iimcb.gov.pl
Genovese, Ilaria.
AR Fanelli Sapienza University of Rome, Italy. ilaria.genovese@uniroma1.it
Gerke, Volker.
Center Molecular Biology of Inflammation, Germany. gerke@uni-muenster.de
Ghosh, Arijita.
Indian Institute of Technology Madras, India. arijitag@gmail.com
Gómez-Riesgo, Enrique.
Universidad de Valladolid, Spain.
Gomis-Pérez, Carolina.
Insituto Biofisika (CSIC, UPV/EHU). Spain. karolgomis@gmail.com
González-Gutiérrez, Lucía.
National Research Council of Spain (CSIC). lgonzalezg@ibgm.uva.es
Griesbeck, Oliver.
Max Planck Institute for Neurobiology, Germany. griesbeck@neuro.mpg.de
Gruszczynska-Biegala, Joanna.
Int. Institute of Mol. & Cell Biol, Poland. joannag@iimcb.gov.pl
Gunaratne, Gihan.
University of Minnesota, USA. gunar005@d.umn.edu
Guse, Andreas H.
University Medical Center Hamburg, Germany. guse@uke.de
Gutiérrez, Tomás.
University of Alberta, Canada. gutirrez@ualberta.ca
Gutiérrez-Merino, Carlos.
Universidad de Extremadura, Spain. carlosgm@unex.es
Haiech, Jacques.
University of Strasbourg, France. haiech@unistra.fr
Heizmann, Claus.
University of Zurich, Switzerland. claus.heizmann@bluewin.ch
Hoenderop, Joost.
Radboud University, Netherlands. Joost.Hoenderop@radboudumc.nl
Hogea, Alexandra.
University of Leeds, UK. bsasg@leeds.ac.uk
Honrath, Birgit.
University of Marburg, Germany. honrathb@staff.uni-marburg.de
Hotka, Matej.
Medical University Viena, Austria. matej.hotka@mediuniwien.ac.at
Iino, Matsumino.
The University of Tokyo, Japan. iino@m.u-tokyo.ac.jp
Ikura, Mitsu.
University of Western Ontario, Canada. mikura@uhnresearch.ca
Jang, Inok.
Seoul National University, South Korea. insuk@snu.ac.kr
Jung, Nicolas.
University of Luxembourg, Luxembourg.
Kaar, Aidan.
University College Cork, Ireland. a.kaar@umail.ucc.ie
Kalev-Zylinska, Maggie.
The University of Auckland, New Zealand. m.kalev@auckland.ac.nz
Kallay, Enikö.
Medical University of Vienna, Austria. enikoe.kallay@meduniwien.ac.at
Kanatani, Sachie.
Sweden. sachie.kanatani@su.se
Kanatani, Shigeaki.
Karolinska Institutet, Sweden. shigeaki.kanatani@ki.se
Kang, Jung Yun.
Yonsei Univ. College of Dentistry, South Korea. hannahkang77@gmail.com
Kelu, Jeffrey Jenkin.
HKUST, Hong Kong. jjkelu@ust.hk
Kim, Joon-Chul.
Chungnam National University, South Korea. jc7418@naver.com
Klee, Claude B.
National Institutes of Health (NIH), USA. ckl@helix.nih.gov
Ko, Juyeon.
Seoul National University, South Korea. juuyeon@snu.ac.kr
Koch, Karl-Wilhelm.
University of Oldenburg, Germany. karl.w.koch@uni-oldenburg.de
Kouba, Sana.
Université de Tours, France. sana.kouba@hotmail.com
Krebs, Joachim.
Max Planck Institute, Germany, jkrebs@nmr.mpibpc.mpg.de
Krizanova, Olga.
Biomedical Research Center, Slovakia. olga.krizanova@savba.sk
Krog Frandsen, Stine.
University of Copenhagen, Denmark. Krog.Frandsen@regionh.dk
Kuznicki, Jacek.
International Institute Molecular & Cell Biol, Poland. jkuznicki@iimcb.gov.pl
Kwak, Misun.
Seoul National University, South Korea. misunkwak@snu.ac.kr
La Cour, Jonas.
Denmark. jonaslacour@gmail.com
La Rovere, Rita.
KU Leuven, Belgium. rita.larovere@kuleuven.be
Leclerc, Catherine.
Université de Toulouse, France. catherine.leclerc@univ-tlse3.fr

Lencesova, Lubomira.
Biomedical Research Center, Slovakia. lubomira.lencesova@savba.sk
Leybaert, Luc.
Ghent University, Belgium. Luc.Leybaert@UGent.be
Li, Na.
Baylor College of Medicine, USA. nal@bcm.edu
Liu, Qun.
Brookhaven National Laboratory, USA. qunliu@bnl.gov
Llopis, Juan F.
University of Castilla-La Mancha, Spain. Juan.Llopis@uclm.es
López-Méndez, Maria.
CINVESTAV-IPN, Mexico. Méxicocrismas2005@hotmail.com
Luo, Dali.
Capital Medical University, China. luodl@ccmu.edu.cn
Luyten, Tomas.
KU Leuven, Belgium. tomas.luyten@kuleuven.be
Maki, Masatoshi.
Nagoya University, Japan. mmaki@agr.nagoya-u.ac.jp
Mammano, Fabio.
Italian National Research Council, Italy. fabio.mammano@cnr.it
Marchant, Jonathan.
University of Minnesota, USA. march029@umn.edu
Martínez-Ramírez, Carmen.
Univ. Autónoma de Madrid, Spain. mencita_1991@hotmail.com
Martín-Romero, Francisco Javier.
Universidad de Extremadura, Spain. fjmartin@unex.es
Masoud, Said.
The Open University, UK. Said.Masoud@open.ac.uk
Mata, Ana M.
Universidad de Extremadura, Spain. anam@unex.es
Matheou, Chrysanthi.
The Open University, UK. chrysanthi.matheou@open.ac.uk
Méndez López, Iago.
Universidad Autónoma de Madrid, Spain. iagomendez@hotmail.com
Meshcheriakova, Anna.
Israel. a.meshcher@gmail.com
Methner, Axel.
Johannes Gutenberg-University, Germany. axel.methner@gmail.com
Michalak, Marek.
University of Alberta, Canada. marek.michalak@ualberta.ca
Miller, Andrew.
Hong Kong University of Science and Technology, Hong Kong. almiller@ust.hk
Mitoshiba, Katsuhiko.
University of Tokio and Riken, Japan. mikosiba@brain.riken.jp
Monteith, Gregory.
University of Queensland, Australia. gregm@uq.edu.au
Montenegro, Pablo.
Universidad de Valladolid, Spain. monty@ibgm.uva.es
Montero, Mayte.
Universidad de Valladolid, Spain. mmontero@ibgm.uva.es
Moreau, Marc.
University of Toulouse, France. marc.moreau@univ-tlse3.fr
Moreno, Silvia N.
University of Georgia, USA. smoreno@uga.edu
Nanclares, Carmen.
Universidad Autónoma de Madrid, Spain. c.nanclares87@gmail.com
Nathanson, Michael.
Yale University, USA. michael.nathanson@yale.edu
Navas-Navarro, Paloma.
Universidad de Valladolid, Spain. palnavnav@ibgm.uva.es
Néant, Isabelle.
CNRS, Toulouse, France. isabelle.neant@univ-tlse3.fr
Neher, Erwin.
Max Planck Institute for Biophysical Chemistry, Germany. eneher@gwdg.de
Núñez, Lucía.
Universidad de Valladolid, Spain. nunezl@ibgm.uva.es
Overgaard, Michael.
Aalborg University, Denmark. mto@bio.aau.dk
Park, Yon June.
Seoul National University, South Korea. eunice421@snu.ac.kr
Parys, Jan B.
KU Leuven, Belgium. jan.parys@med.kuleuven.be
Pascual-Caro, Carlos.
Universidad de Extremadura, Spain. carlospc@unex.es
Patel, Sandip.
University College London, UK. patel.s@ucl.ac.uk
Peche, Georges A.
CERBM-GIE, France. peche@igbmc.fr
Penny, Christopher.
University College London, UK. cjpenny90@gmail.com
Perocchi, Fabiana.
Ludwig-Maximilians-Universität, Germany. perocchi@genzentrum.lmu.de
Pinton, Paolo.
University of Ferrara, Italy. pnp@unife.it
Plançon, Sébastien.
University of Luxembourg, Luxembourg.
Pochet, Roland.
Université Libre de Bruxelles, Belgium. rpochet@ulb.ac.be
Ponce Dawson, Silvina.
CONICET-Univer. Buenos Aires, Argentina. silvinadfubaar@gmail.com
Potier-Cartereau, Marie.
Université de Tours, France. marie.potier-cartereau@univ-tours.fr
Prevarskaya, Natalia.
University Lille, France. natacha.prevarskaya@univ-lille1.fr
Pulli, Ilari.
Åbo Akademi University, Finland. ilari.pulli@abo.fi
Putney, James W Jr.
NIEHS-NIH, USA. putney@niehs.nih.gov
Rae, Mark.
University College Cork, Ireland. m.rae@ucc.ie
Rebas, Elzbieta.
Medical University of Lodz, Poland. elzbieta.rebas@umed.lodz.pl
Reddy, G. Prem.
Wayne State University, USA. PREDDY1@hfhs.org
Rentero, Carles.
Universitat de Barcelona, Spain. carles.rentero@ub.edu
Rietdorf, Katja.
The Open University, UK. katja.rietdorf@open.ac.uk
Rieusset, Jennifer.
Université de Lyon, France. jennifer.rieusset@univ-lyon1.fr
Ring, Avi.
Norwegian Defence Research Est./ Oslo University, Norway. Avi.Ring@ffi.no

Rodat-Despoix, Lise.
Université dAmiens, France. lise.despoix@u-picardie.fr
Rodríguez Prados, Macarena.
Universidad de Valladolid, Spain. maqui200@hotmail.com
Roest, Gemma.
KU Leuven, Belgium. gemma.roest@kuleuven.be
Rojo-Ruiz, Jonathan.
Universidad de Valladolid, Spain. jonathan.rojo@ibgm.uva.es
Rosado, Juan A.
Universidad de Extremadura, Spain. jarosado@unex.es
Roufogalis, Basil.
University of Western Sydney, Australia. basil.roufogalis@sydney.edu.au
Sacks, David.
NIH, USA. sacksdb@mail.nih.gov
Salido, Ginés M.
Universidad de Extremadura, Spain. gsalido@unex.es
Sánchez, Ana.
Universidad de Valladolid, Spain. asanchez@ibgm.uva.es
Sarkadi, Balazs.
Res. Cent. Hungarian Acad. Sci., Hungary. sarkadi@biomembrane.hu
Savignac, Magali.
INSERM, Toulouse, France. magali.savignac@inserm.fr
Schenten, Veronique.
University of Luxembourg, Luxembourg. veronique.schenten@uni.lu
Scholten, Alexander.
Univ. of Oldenburg, Germany. alexander.scholten@uni-oldenburg.de
Schwab, Albretch.
University of Münster, Germany. aschwab@uni-muenster.de
Sekler, Israel.
Ben Gurion University, Israel. Israel.sekler@gmail.com
Serysheva, Irina.
University of Texas Medical School, USA. irina.i.serysheva@uth.tm
Sharma, Kumar.
The Open University, UK. kumar.sharma@open.ac.uk
Shen, Yuequan.
Nankai University, China. YSHEN@NANKAI.EDU.CN
Shoshan-Barmatz, Varda.
Ben-Gurion University, Israel. vardasb@bgu.ac.il
So, Insuk.
Seoul National University, South Korea. insuk@snu.ac.kr
Sobradillo, Diego.
National Research Council of Spain (CSIC), Spain. d.sobradillo@ibgm.uva.es
Stathopulos, Peter.
University Western Ontario, Canada. peter.stathopulos@schulich.uwo.ca
Stith, Bradley.
Colorado University, USA. brad.stith@ucdenver.edu
Sulmann, Stefan.
University of Oldenburg, Germany. stefan.sulmann@uni-oldenburg.de
Taylor, Colin.
University of Cambridge, UK. cwt1000@cam.ac.uk
Tilunaite, Agne.
University of Nottingham, UK. pmxat6@nottingham.ac.uk
Törnquist, Kid.
Åbo Akademi University, Finland. ktornqvi@abo.fi
Ubierna, Daniel.
National Research Council of Spain (CSIC) d.ubierna@gmail.com
Ullah, Ghanim.
University of South Florida, USA. gullah@usf.edu
Van Petegem, Filip.
University of British Columbia, Canada. filip.vanpetegem@gmail.com
Varga, Karolina.
Semmelweis University, Hungary. varga.karolina@med.semmelweis-univ.hu
Vautrin, Alexia.
University of Amiens, France. alexia.vautrin@u-picardie.fr
Vervloessem, Tamara.
KU Leuven, Belgium. tamara.vervloessem@kuleuven.be
Villalobo, Antonio.
National Research Council of Spain (CSIC). antonio.villalobo@iib.uam.es
Villalobos, Carlos.
National Research Council of Spain (CSIC), Spain. carlosv@ibgm.uva.es
Villarroel, Álvaro.
Insituto Biofisika, CSIC, UPV/EHU, Spain. alvaro.villarroel@csic.es
Wacquier, Benjamin.
Université Libre de Bruxelles, Belgium. benjamin.wacquier@gmail.com
Webb, Sarah.
HKUST. Hong Kong. barnie@ust.hk
Wei, Zhang.
Nagoya University, Japan.zhang.wei@i.mbox.nagoya-u.ac.jp
Willegems, Katrien.
Vrije Universiteit Brussel (VUB), Belgium. kwillege@vub.ac.be
Wolters, Valerie.
Universitätsklinikum Hamburg-Eppendorf, Germany. v.wolters@uke.de
Woo, Sun-Hee.
Chungnam National University, South Korea. shwoo@cnu.ac.kr
Yates, Elizabeth.
University college London, UK. elizabeth.yates.13@ucl.ac.uk
Yue, Yianbo.
City University of Hong Kong, China. jianbyue@cityu.edu.hk
Yule, David.
University of Rochester Medical Center, USA. David_Yule@urmc.rochester.edu
Zambo, Boglarka.
Research Centre for Natural Sciences, Hungary. zambo.boglarka@gmail.com
Zuccolo, Estella.
University of Pavia, Italy. estella.zuccolo@unipv.it
Zylinska, Ludmila.
Medical University of Lodz, Poland. ludmila.zylinska@umed.lodz.pl