16th International Meeting of the European Calcium Society

(ECS2022)

21st to 25th August 2022

Devere Hall, Áras na Mac Léinn, University College Cork

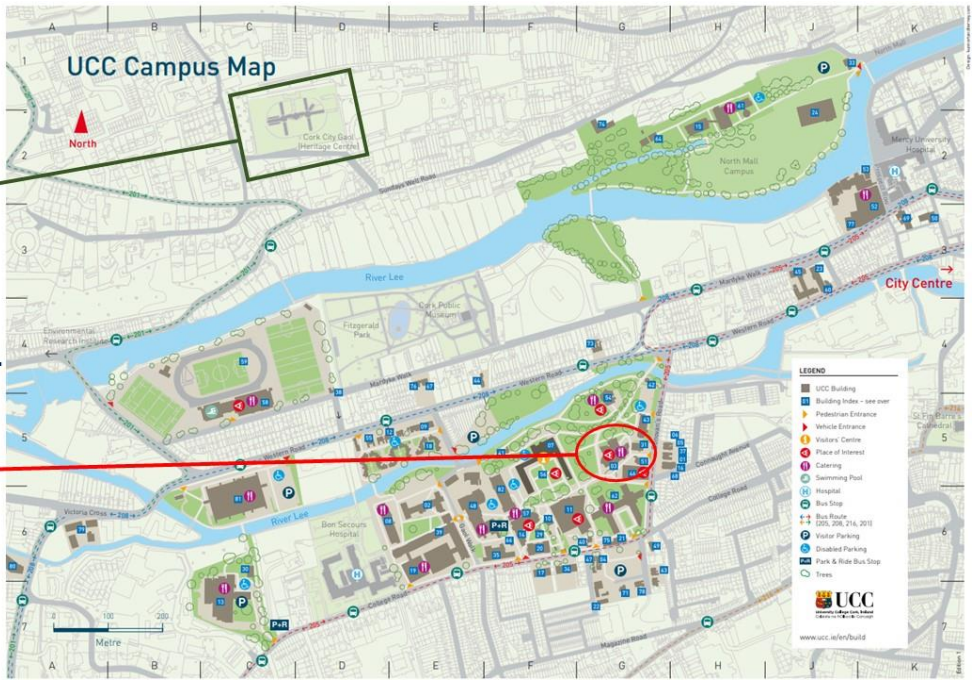
(Junior ECS2022, 20th August 2022)

Conference Venue

Cork City Gaol
(Venue for the Gala Dinner, 7pm on 24th August. 1.5 km from Devere Hall)

The Kingsley Hotel
(1.5 km from Devere Hall)

Devere Hall, Áras na Mac Léinn ("Student Centre")



All of the program apart from the Gala Dinner will be at Devere Hall, Áras na Mac Léinn, University College Cork, College Road, University College Cork (UCC), Cork, T12 XR6F.

Devere Hall is on the first floor of this building, accessible by stairs and by a lift. The Registration Desk is in the atrium adjoining the Hall.

Directions

For accessible route options: please contact John Mackrill (j.mackrill@ucc.ie) or arasoperations@ucc.ie.

On foot or bicycle: approximately 20 minutes walking from Cork city centre (St. Patrick's Street, about 1.5 km in total). At the southwestern end of St. Patrick's Street, turn right onto Washington Street and carry on walking past the Old Court House (to the right) and the River Lee Hotel. At the end of this street, you will come to the UCC main gates. Walk up the hill, past one set of steps and you will come to a large set of steps. Walk up these and another one minute straight ahead. Áras na Mac Léinn is to the left.

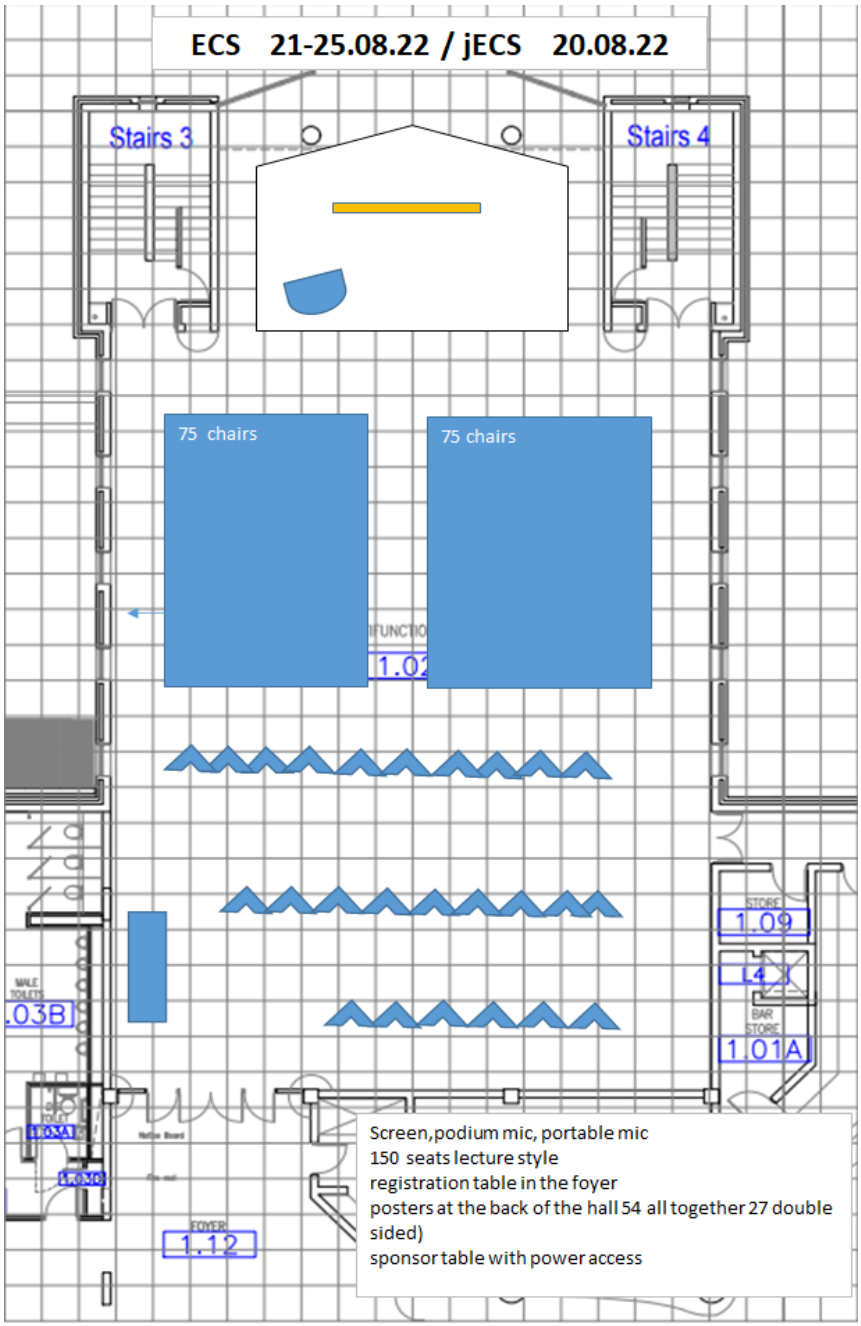
Bus and coach: both the 205 (towards 'CIT/Rossa Avenue') and 208 (towards 'Bishopstown' or 'CUH') city buses can be boarded at Parnell Square bus station, or on St. Patrick's Street (near to Marks & Spencer). Both stop close to the UCC main gates. Intercity buses stop either at Parnell Square or across the River Lee from it on St. Patrick's key (in the case of some coaches to Dublin airport). These bus stops are all within 200 m of St. Patrick's Street

Railway: Kent Station has regular connections to Dublin Heuston (2.5-3 hour journey time). Cork city bus 205 can be boarded at Kent railway station, for UCC.

Air travel: Cork airport is about 8 km from the city centre. A taxi from the airport to the centre costs around €20. Buses 225 and 226 travel from the airport to Parnell Square bus station (from where buses 205 or 208 can connect you to UCC).

Car: There are limited paid public carparking spaces at UCC. At the main UCC gates, turn left onto O'Donovan's Road and turn right on to College Road. UCC's visitor car park is immediately on your left (entrance from Perrott Avenue). For the Western Gateway Building parking (600 m from venue), continue down Western Road. The entrance is on your left. Outside of UCC, street parking spots require "parking discs", which cost €2 and are only valid for 2 hours.

Devere Hall


ECS 21-25.08.22 / jECS 20.08.22
Stairs 3
Stairs 4
75 chairs
75 chairs
1.02
STORE
1.09
1.4
BAR STORE
1.01A
.03B
FOYER
1.12
Screen,podium mic, portable mic
150 seats lecture style
registration table in the foyer
posters at the back of the hall 54 all together 27 double sided)
sponsor table with power access

Welcome

On behalf of the Organizing Team and of the Scientific Committee, I am extremely pleased to welcome you to the 16th International Meeting of the European Calcium Society, University College Cork (UCC). This conference has been a long time in planning: I had the pleasure of hosting a completely different ECS Board for our ECS2020 meeting bid, on two hot days in June 2018. Since then, we have all faced immense worldwide challenges and changes to our lives, one of which resulted in the postponement of ECS2020.

It is great to see that thanks to you, the delegates, ECS2022 will go ahead with a vibrant and exciting programme. I anticipate that you will have a very pleasant stay in Cork: the UCC Campus is probably the loveliest in Ireland; the city is relatively small, friendly, easy to navigate and in many ways distinct from other European centres. The highlight of the social programme will be the Gala Dinner, at Cork City Gaol. This will be much more pleasant than it might first appear and you will offered the chance to participate in a Céilí (traditional Celtic music and dancing).

In terms of the drive, planning and shared stress of this meeting, I am very thankful to Geert Bultynck and to Jan Parys. I am also grateful to the Junior ECS Board, in particular Malene Brohus, for their organization of the jECS satellite meeting, to be held on Saturday 20th August 2022. The jECS represents the future of the Society and the future looks bright.

We are excited about welcoming you all to UCC and are looking forward to learning even more about calcium research and the people that contribute to it.

Beir bua agus beannacht ("take a victory & a blessing")

Acknowlegements

Platinum Sponsor

NIH National Institute of Environmental Health Sciences

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Silver Sponsors

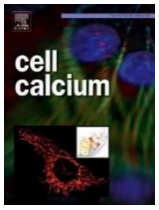
HAMAMATSU PHOTON IS OUR BUSINESS

CoolLED Simply Better Control

BBA Molecular Cell Research

Contact

breakthrough CANCER RESEARCH



John Mackrill

cells an Open Access Journal by MDPI

We would also like to thank Prof. Ken O'Halloran (Head of Dept. of Physiology, UCC), Prof. Paula O'Leary (Head of School of Medicine, UCC), Prof. Sandip Patel (UCL, UK), Prof. Carlos Villalobos (IBGM, CSIC, Spain), Prof. Andreas Guse (UKE, Germany), Dr. Gary Bird (NIEHS, USA), and Dr. Martin Bootman and Dr. Katja Reidorf (Open University, UK) for continued support.

Organization

Local organizing team

John Mackrill (Chair)

Michael Vaughan (jECS Local Organizer)

Greg Jasionek (web page design & administration)

Natalia Pinfield (Med. School Accounting)

Jackie Maguire (Administration)

Nicola Redmond (Administration)

Mark Rae (UCC, Vice-Chair)

Anne Cahill (Cork Convention Bureau)

Evelyn O'Sullivan (Cork Convention Bureau)

Letitia Wade (Fàilte Ireland)

Karen O'Neill (Cork City Gaol)

Maria Gryzlak (Devere Hall)

David Jones (Devere Hall)

Sian James (Conference UCC)

Jean O'Callaghan (Conference UCC)

Michael Hurley (Conference UCC)

John Coughlan (The Kingsley Hotel)

Indu Nair (Registration, UCC)

Emma Condon (Registration, UCC)

Ciara Sherlock (Registration, UCC)

Barbara Doyle Prestwich (UCC)

Maurice Ryan (Digital Legacy, UCC)

Scientific committee

Geert Bultynck (Chair)

John Mackrill (Vice-chair)

Andreas Guse

Felicity Davis

Paola Pizzo

Sandip Patel

Khaled Machaca

Amalia Dolga

Mark Rae

Carlos Villalobos

Marc Moreau

Gregory Monteith

Jose Naranjo

Doron Shkolnik

The ECS Board

The jECS Board

Instructions for Speakers and Chairs

It is the responsibility of the Chair of each session to ensure stringent timekeeping. Please allow 5 minutes for questions as part of the total time allocated to each speaker.

Chairs should ensure that all presentations are loaded onto the Devere Hall computer prior to the session (no later than the break before your session; John Mackrill and local support will be available to assist with this process). Devere Hall opens at 8:00 each day, in advance of the first session.

Presentations will be in English. The computer at Devere Hall uses MS Windows and PowerPoint. Mac users should check for compatibility in advance of their presentation.

At the Flash Symposium, abstracts will be selected for a brief oral presentation. This should aim to attract delegates to your poster. Further details will follow from the Chair of this session.

Instructions for Poster Presenters

Please note. In cases where a delegate has been selected to give an oral presentation, the same presentation should not be given in poster form. However, where a delegate is presenting author and has submitted two abstracts, one can be given as an oral presentation and the other as a poster. Both oral presentations (O) and poster presentations (P) are listed in this book of abstracts.

Posters will be in A0 portrait format (vertical, 110 cm wide x 200 cm tall). Pins will be provided and the poster board material is felt. Each poster board has been assigned a number (P1-P52) and posters should be affixed to the board of the same number, ensuring that the poster number is also visible.

Poster mounting and dismounting.

All posters will be on continuous display from 16:40 on Monday 22nd August until 14:15 on Wednesday 24th August. Boards will be available from 08:30 on Saturday 20th August (for jECS Satellite Meeting). Please dismount posters as soon as possible after the second poster session (14:15 Wednesday 24th August). Poster boards will also be available at the jECS satellite meeting (20th August 2022) and posters can be left on these for the main meeting.

Presenting authors are expected to accompany their posters at the following times:

Odd Numbers: 16:40-17:40 on Monday; 13:00-14:15 on Wednesday

Even Numbers: 17:40-18:40 on Monday; 12:15-13:00 on Wednesday

This will allow interaction with other delegates and judging of the posters.

Practical Information

Official Language

English is the official language of the conference. (Much of the signage in Ireland is bilingual and Irish (Gaeilge) is compulsory in schools. Gaeltacht are districts in Ireland where Gaeilge is the predominant vernacular. There are also Irish radio and television channels).

Badges

Conference badges will be supplied on registration and should be worn at all ECS2022 events. Letters of attendance will also be supplied.

Poster printing

Posters can be printed at several local commercial printing services, including Snap printing (https://www.snap.ie/contact-a-centre/snap-cork/; cork@snap.ie); Hacketts printing (https://www.hackettsprint.ie/); and Quality Print (https://qualityprint.ie/)

Time zone and climate

Cork is in the same time zone as London, UK (CET-1). The weather in August is likely to be warm and possibly dry, but visitors often experience “four seasons in one day”.

Covid-19 Updates

Irish guidelines on Covid-19: https://www.gov.ie/en/news/7e0924-latest-updates-on-covid-19-coronavirus/. Covid-19 related travel advice to and from Ireland: https://www.gov.ie/en/campaigns/75d92-covid-19-travel-advice/

Modelling indicates that infection rates will be at a low level in Ireland during the dates of the meeting: COVID-19 (healthdata.org)

Mask wearing is advised but is not mandatory.

Business hours and shopping

Most shops open 09:00-17:30 Mon-Sat with late opening until 21:00 on Thursdays & Fridays. Sunday opening tends to be from 12:00-17:00.

Different restaurants set their own opening hours: it is worth checking on-line.

Pubs open at various times in the afternoon and stay open until 23:30 Mon-Thurs;00:30 on Fri & Sat; and 23:00 on Sundays.

Tourist information

Cork Tourist Information Centre, 125 St. Patrick's Street, Cork. T12 AE81

Phone: 1800 230 330. Websites: https://www.discoverireland.ie/cork & https://purecork.ie/

Taxi companies

There are many taxi companies in Cork City. Recommended companies include Cork Taxi Co-op: (021)-427-2222; Satellite Taxis: (021)-480-8080; and ABC Taxis: 1800-855-688. Cars with the word "TAXI" on top can be hailed from the curb side or requested at taxi ranks (at St. Patrick's Street, Kent Rail Station, the Airport, Parnell Square Bus Station and other City locations).

WiFi at venue

WiFi access will be available to ECS2022 delegates at Devere Hall and across the UCC Campus, using the following login details:

Username: **ecs2022**

Password: **E7jbqyws**

Sustainability

We will not supply printed programmes unless requested. Chilled water dispensers are available across the campus, for filling of re-useable drinking vessels. The Local Organizing Team has already requested information on meal attendance, to minimize food waste. Transport-for-Ireland bicycles can be hired at automated stations around the City (https://www.bikeshare.ie/cork.html).

Cancellations & Force majeure

Cancellations with full refunds will be possible **up until 24th July 2022**. The organizers are not liable for any claims for damage or loss if the entire conference is cancelled due to a force majeure incident.

Disclaimer

The organizers are not liable for any kind of damage, injury or loss to conference delegates and accompanying persons. By registering, delegates have given permission for photographs and video recordings to be taken. However, oral presentations will not be recorded without obtaining the speaker's consent.

Programme ECS2022

Sunday August 21st 2022

12:00-16:00	Registration Desk Open, entrance to Devere Hall, Áras na Mac Léinn
14:00-15:30	ECS Board Meeting (for Board Members only), Muscra Meeting Room, Áras na MacLéinn
16:00-16:20	**Welcome by the Chair, Local Organisers and ECS President**
16:20-17:10	**O1: BBA-Molecular Cell Research Opening Lecture** Volker Gerke: "The Ca^{2+} regulated plasticity of vascular endothelial cells"

17:10-19:00	**Mechanically activated Ca^{2+}-permeable ion channels** ***Chair: Felicity Davis***
17:10	O2: David Beech (University of Leeds, UK): "Piezo1 force sensor in cardiovascular health and disease"
17:35	O3: Medha Pathak (UC Irvine, USA): "Molecular Choreography of Piezo1"
18:00	O4: Swetha Murthy (Vollum Institute, OHSU, Sydney, Australia): "Examining the role of TMEM63s in mechanosensation"
18:25	O5: Soléne Barbeau (University of Bordeaux, France): "TRPV4 channel activity in response to hypoxia is affected by cell density"
18:40	O6: Valeriya Gushchina (Medical University of Vienna, Austria): "The role of the calcium-sensing receptor in the activation of the intestinal prostaglandin E2 pathway"
18:55	O7: Geert Bultynck (KU Leuven, Belgium. Sponsored by Hamamatsu): "The FDSS µCELL instrument for live calcium imaging in high-throughput"
19:00	Reception**:** barbeque and soft drinks. The bar will also be open for other drinks, but these will not be free (payment by delegates). Note that Devere Hall will close at 21:30 precisely.
21:30	Free evening

Monday 22nd August 2022

Note changes: 9:45 finish for this session, due to cancellation

Flash symposium lasts 30 min

8:30-10:15	**Mitochondrial calcium signalling & cell functions** ***Chair: Paola Pizzo***
8:30	O8: Riccardo Filadi (Neuroscience Institute, National Research Council, CNR, Italy): "Ca^{2+}signals as modulators of ER-mitochondria contacts in health and disease"
8:55	O9: Araceli Del Arco (Universidad Autónoma de Madrid & Universidad de Castilla-La Mancha, Spain): "Regulation by Calcium of neuronal metabolism. Role of MCU and Aralar-Malate-Aspartate Shuttle"
9:20	O10: Julio Cesar Cárdenas (Center for Integrative Biology, Universidad Mayor, Chile): "Keeping zombies alive: the reticulum-to-mitochondria Ca^{2+} transfer in senescent cells" CANCELLED. Announcement will be made at start of this session
9:45	O11: Arijita Ghosh (Thomas Jefferson University, USA): " Anti-apoptotic Bcl-xL controls inositol 1,4,5 triphosphate (IP_3) receptor mediated ER-mitochondria Ca^{2+} transfer"
10:00	O12: Fernanda Lemos (KU Leuven, Belgium): "Regulation of intracellular Ca^{2+} signaling: a novel function for the metabolic enzyme pyruvate kinase M2 (PKM2)"
10:15-10:40	Coffee break
10:40-12:30	**Lysosomal Ca^{2+} signalling** ***Chair: Sandip Patel***
10:40	O13: Teresa Alonso (University of Valladolid, Spain): "Endo-lysosomal Ca^{2+} signalling: a luminal view"
11:05	O14: José-Manuel Cancela and Lora Martucci (Paris-Saclay Institute of Neuroscience, France): "Endolysosomal two-pore channels (TPC) regulate social behaviour by controlling oxytocin secretion"
11:35	O15: Diego Medina (TIGEM at Pozzuoli, Italy): "TRP Mucolipins and TFEB"
12:00	O16: Lynn McKeown (University of Leeds, UK): "The role of NAADP-dependent calcium release on Rab46 activity in endothelial cells"
12:15	O17. Volodymyr Tsvilovskyy (University of Heidelburg, Germany): "OCaR1 confers exocytotic vesicles an autoregulatory competence to prevent Ca^{2+} release, exocytosis and pancreatic disorder"
12:30-13:45	Lunch
13:45-15:30	**Ca^{2+} signalling in nanodomains at membrane contact sites** **Sponsored by the journal *Contact*** ***Chair: Khaled Machaca***
13:45	O18: Manuela Zaccolo (University of Oxford, UK): "Regulation of cardiac calcium homeostasis by nano-domain cAMP signalling"

14:10	O19: Murali Prakriya (Northwestern University, USA): “Calcium regulation in the SOCE nanodomain: new insights into calcium-dependent inactivation of Orai1 channels”
14:35	O20: Patrick Hussey (Durham University, UK): “Interactions between the cytoskeleton and membranes: building an ER-PM contact site *in planta*.”
15:00	O21: Muhammad Yasir (University of Helsinki, Finland): “Three dimensional microscopy of endoplasmic reticulum I thyroid cells by serial block face SEM and TEM”
15:15	O22: Axel Tollance (University of Geneva, Switzerland): “Ca-independent role of Orai3 in the human muscle cell activation”

15:30-16:00 Flash Symposium
Chair: Amalia Dolga

***1. Vanessa Schwentner**, University Medical Centre Hamburg Eppendorf, Germany,*

Role of 3',5'-cyclic adenosine monophosphate during T cell activation.

***2. Rossini Michela**, Department of Biomedical Sciences, University of Padua, Padua, Italy*

A new reversible fluorescent probe to detect ER-mitochondria contact site dynamicity.

***3. Alejandro Marmolejo-Garza**, University of Groningen, The Netherlands*

Blockade of mitochondrial calcium uptake protects neurons against ferroptosis.

***4. Gaia Gherardi**, Department of Biomedical Sciences, University of Padua, Italy*

Mitochondrial calcium signaling regulates skeletal muscle homeostasis in adulthood and aging.

***5. Zhanat Koshenov**, Medical University of Graz, Austria*

Presenilin-1 controls pancreatic beta-cell metabolism by regulating mitochondrial Ca2+ sensitive NADH shuttle.

***6. Louisa Heßling**, Dept. of Biochemistry and Molecular Cell Biology, UKE, Hamburg, Germany*

Interaction of the NAADP binding protein HN1L/JPT2 with its target receptors in T cells.

***7. Jacek Kuznicki**, International Institute of Molecular and Cell Biology in Warsaw, Poland, The effect of stim2 knockout on zebrafish brain cells.*

16:00-16:30 Early Career Researchers meet the editors session

Muscra, Dúthalla and Ardmore meeting rooms, along the corridor from Devere Hall. NOTE change from Bearra to Dúthalla.

16:00-16:30 Early Career Researchers meet the editors session

Muscra, Bearra and Ardmore meeting rooms, along the corridor from Devere Hall

16:40-end	**Extended Poster Session 1 (Finger food included)** **odd numbers: 16:40-17:40 / even numbers: 17:40-18:40**

Tuesday 23rd August 2022

09:00-10:45	**Ca^{2+} signalling in Alzheimer's disease** ***Chair: Mark Rae***
09:00	O23: Ilya Bezprozvanny (University of Texas Southwestern, USA): "A gating mutation in ryanodine receptor type 2 rescues phenotypes of Alzheimer's disease mouse models by upregulating neuronal autophagy"
09:25	O25: Alexej Verkhrastsky (University of Manchester, UK): "Astroglial signalling in ageing and AD"
09:50	O26: Paloma Garcia-Casas (University of Padua, Italy): "ATAD3A, a novel interactor of Mfn2. Implications on Ca^{2+} signalling in the context of Alzheimer's disease"
10:05	O27: Gaiti Hasan (NCBS, TIFR, Bengaluru 560065, India): "Septin 7 knock-outs rescue SOCE-dependent gene expression and motor learning deficits arising from mouse cerebellar Purkinje neurons"
10:20-11:10	Extended Coffee break
11:10-12:15	**Ca^{2+} signalling and organoids** ***Chair: Carlos Villalobos***
11:10	O28: Joris Vriens (KU Leuven, Belgium): "Endometrial organoids as a model to study fetal - maternal crosstalk"
11:35	O29: Nathalie Vergnolle (INSERM Delegation Regionale Occitanie Pyrenees, France): "Organoid cultures for pharmacology studies" CANCELLED: ANNOUNCED DURING MEETING
12:00	O30: Amalia Dolga (University of Groningen, The Netherlands): "Alterations in mitochondrial metabolism and calcium pathways in familial Alzheimer's disease iPSC-derived neuronal cells and brain organoids"
12:15	Group Photo (weather permitting, in the amphitheatre immediately outside of the venue).
12:30-13:30	Lunch (a "packed lunch" to facilitate tourism)
13:30-end	**Tourism.** City bus tour of Cork

Wednesday 24th August 2022

08:30-10:15	**Ca^{2+} signalling in evolution** ***Chair: John Mackrill***
08:30	O31: Glen Wheeler (Marine Biological Association, Plymouth, UK): "Evolution of a novel class of voltage-gated calcium channels in marine diatoms"

08:55	O32: Silvia Moreno (University of Georgia, USA): “Divergent calcium signalling pathways of *Toxoplasma gondii*”
09:20	O33: Holly Shiels (University of Manchester, UK): “Calcium cycling in the avian heart - the 'missing link' in vertebrate EC coupling”
09:45	O34: Indu Nair (University College Cork, Ireland): “Characterization of a candidate inositol 1,4,5-trisphosphate receptor (IP_3R) in *Phytophthora infestans*- an oomycete pathogen causing Potato Late Blight”
10:15	O35: Rishav Mitra (NCBS, TIFR, Bengaluru 560065, India): “SOCE shapes developmental gene expression in dopaminergic neurons of the *Drosophila* flight circuit”
10:30-10:50	Coffee break
10:50-11:55	**Different dimensions in Ca^{2+} signalling in cancer** **Sponsored by the charity Breakthrough Cancer Research** ***Chair: Gregory Monteith***
10:50	O36: Natalia Prevarskaya (University of Lille, France): “Calcium oscillations in cancer cells”
11:15	O37: Julie Gehl (University of Copenhagen, Denmark): “How calcium can be used to treat cancer”
11:40	O38: Flore Sneyers (KU Leuven, Belgium): “BAPTA directly inhibits PFKFB3, thereby impeding mTORC1-driven Mcl-1 translation and killing Mcl-1-addicted cancer cells”
11:55	O39: Silke Chalmers (Aarhus University, Denmark & University of Queensland, Australia): “Uncovering the calcium conversation between breast cancer and the brain microenvironment”
12:10	O40: Danielle Love (CoolLED, Andover, UK): “LED light sources for Calcium Imaging”
12:15-14:15	**Extended poster session 2 (Finger food included)** **even numbers: 12:15-13:00 / odd numbers: 13:00-14:15**
14:15-16:00	**Neuronal calcium sensors from physiology to disease** ***Chair: Jose Naranjo***
14:15	O41: Karl-Wilhelm Koch (University of Oldenburg, Germany): “Control of photoreceptor function by NCS proteins”
14:40	O42: Daniele Dell'Orco (University of Verona, Italy): “Retinal dystrophies associated with point mutations in guanylate cyclase activating proteins”
15:05	O43: Valerio Marino "Ca^{2+} sensitivity of the prototypical Neuronal Calcium sensor recoverin is tuned by synergistic protein-protein and protein-membrane interactions."
15:20	O44: Florian Becker (University of Freiburg, Germany): “Conformational dynamics of calcineurin B homologous protein 3”

15:35	O45: Thomas Simmen (University of Alberta, Canada): "The Endoplasmic Reticulum (ER) Kinase PERK Mediates Metabolic Adaptation and Ca^{2+} Signaling Using the Oxidoreductase Ero1"
15:50-16:00	Brief break
16:00-17:00	ECS General Assembly
17:00-17:15	Brief break
17:15-18:15	**O46: Sir Michael Berridge Lecture** **Shmuel Muallem: "Ion Transporters and lipids at the ER/PM Junctions"**
19:15-12:00	Gala Dinner, Cork City Gaol.

Delegates to make their own way to the Gaol (unless they would like to walk there with some of the organizers). On arrival, there will be a prosecco reception and music. Dining will commence at approximately 20:00. Dinner will be followed by a night of music and dance, provided by Uilleann Ceoil (a traditional Irish band).

Thursday 25th August 2022

9:00-10:20	**Ca^{2+} signalling in plants** ***Chair: Doron Shkolnik***
09:00	O47: Ute Vothknecht (University of Bonne, Germany): "Calcium regulation of organellar function"
09:25	O48: Alex Costa (University of Milan, Italy): "*In vivo* calcium dynamics in plant cells: a holistic view"
09:50	O49: Philip Steiner (University Linz, Austria): "Pharmacological modulation of TPC1 regulates inter-organellar Ca^{2+} homeostasis in immune cells and plays an important role in allergic hypersensitivity"
10:05	O50. Andrea Crosino (University of Turin, Italy): "Investigating the role of clathrin-mediated endocytosis in Myc-factors perception in arbuscular mycorrhizas"

10:20-10:50	**jECS session** ***Chair: jECS Board members***
10:20	O51 (*see P42 for Abstract*): Mille Vissing (Zealand University Hospital and University of Copenhagen Denmark): "Investigation of Calcium Electroporation for Cancer in the Skin"
10:35	O52 (*see P25 for Abstract*): Jens Loncke (KU Leuven, Belgium): "Dysregulated MAM & Ca^{2+} homeostasis underlying Wolfram syndrome type 2-associated CISD2 deficiency"
10:50-11:15	Coffee break

11:15-12:15	**Late breaking session** ***Chair: Geert Bultynck***
11:15	O53: Irina Serysheva (The University of Texas Health Science Center at Houston, USA): "Structural dynamics of IP_3R underlying its gating and regulation"
11:40	O54: Sandip Patel (University College London, UK): "Segregated cation flux by TPC2 biases Ca^{2+} signalling through lysosomes"
12:05	O55: Axel Methner (University Medical Center Mainz, Germany): "TMBIM5 loss of function alters mitochondrial matrix ion homeostasis and causes a skeletal myopathy"
12:30-12:45	**Award ceremony and concluding remarks**
12:45-14:00	Farewell lunch

Abstracts for Oral Presentations

O1: BBA-Molecular Cell Research Opening Lecture

The Ca^{2+} regulated plasticity of vascular endothelial cells

Volker Gerke.

Center for Molecular Biology of Inflammation, University of Münster, Germany

Vascular endothelial cells can be considered gatekeepers of blood vessel homeostasis. They produce and release compounds regulating vascular tone, blood vessel growth and differentiation, plasma composition, coagulation and fibrinolysis, and also engage in interactions with blood cells thereby controlling hemostasis and acute inflammatory reactions. These activities have to be tightly controlled, typically by signalling pathways that originate at the plasma membrane and often involve cytosolic Ca^{2+} as a second messenger. Among other events, elevations in cytosolic Ca^{2+} trigger the exocytosis of organelles that store factors mediating interactions with leukocytes and platelets and they also induce processes facilitating the repair of mechanically (shear flow) induced plasma membrane lesions. This remarkable, Ca^{2+} controlled plasticity enables endothelial cells to cope with the many challenges faced as the cell sheet lining blood vessels. Our findings concerning Ca^{2+} regulated trafficking events that underlie endothelial responses to activation and injury will be discussed.

O2: PIEZO1 force sensor in physical exercise and cardiovascular health and disease

David J. Beech
School of Medicine, LIGHT Building 7.27, Clarendon Way, University of Leeds, Leeds, LS2 9JT, UK.

The two PIEZOs, PIEZO1 and PIEZO2, were first reported in 2010. These proteins form trimeric ion channels with little resemblance to other ion channels. A striking feature is their exquisite, robust and apparently specific sensitivity to activation by a range of mechanical forces. There is widespread agreement that they are bona fide direct sensors of force. We identified the importance in cardiovascular biology, first showing PIEZO1's activation by physiological force and its roles in embryonic vascular maturation and the sensing of fluid shear stress as generated by blood flow[1,2]. This and subsequent work firmly established PIEZO1's role in endothelial biology and showed its ability to integrate force with vascular architecture. By generating conditional genetic deletion in the adult mouse to avoid embryonic lethality we found that endothelial PIEZO1 is required for elevated blood pressure of whole body physical activity[3], necessary for capillary density in skeletal muscle[4] and critical in physical exercise performance[3,4]. Such functions require continuous activity of PIEZO1 channels and so it was perplexing how this could be possible when over-expression studies reveal powerful intrinsic inactivation gates in PIEZOs. However, we showed that native PIEZO1 channels of endothelial cells are non-inactivating. We discovered the mechanism by which the inactivation gate is disabled, unexpectedly through relationship of PIEZO1 to sphingomyelinase (SMPD3) and the membrane lipid ceramide[5]. We went on to show slow gating also in red blood cells (RBCs) with implications for understanding hereditary anaemia[6]. We showed that PIEZO1 is important for mechanical sensitivity of calcium-regulated protease activity (calpain and ADAM10), nitric oxide production via NOS3, cell interaction via NOTCH1, inflammation and fibrosis via p38, interleukin-6 and tenascin c and cell apoptosis via thrombospondin-2. Through medicinal chemistry studies of Yoda1 (a small-molecule agonist of PIEZO1) we found a Yoda1 antagonist (Dooku1) and new PIEZO1 agonists with improved reliability, efficacy, potency and physico-chemical properties. To understand the full-length mouse and human channels, their dynamics and responses to force, we began molecular dynamics simulations in model endothelial and RBC membranes. These models predict complex structural rearrangements and lipid interactions, some of which are now validated by laboratory techniques. In conclusion: PIEZO1 forms an exceptionally sensitive mechanical detector mechanism that responds rapidly to forces such as shear stress. It is important in endothelium, cardiovascular biology generally and in physical exercise responses.

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O3: Molecular Choreography of Piezo1

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A major unanswered question in biology is how mechanical forces are generated, detected, and transduced by cells to impact biochemical and genetic programs. Our work is aimed at uncovering the mechanical principles at play in cells and tissues using novel molecular, imaging, and bioengineering tools. Here we present insights gleaned from non-invasive approaches to measure and manipulate mechanotransduction in native cellular conditions. We find that the mechanically-activated ion channel Piezo1 transduces cell-generated traction forces to regulate a variety of biological processes. We show that cellular traction forces generate spatially-restricted Piezo1 Ca^{2+} flickers in the absence of externally-applied mechanical forces. However, Piezo1 channels are widely distributed on the cell surface and are mobile. Single particle tracking reveals a heterogeneity in the mobility behavior of individual channel puncta. We propose that Piezo1 Ca^{2+} flickers allow spatial segregation of mechanotransduction events and that mobility allows channel molecules to efficiently respond to mechanical stimuli.

O4: Examining the role of TMEM63s in mechanosensation

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OSCA/TMEM63 are a large family of mechanically activated calcium permeable ion channels. Since their discovery, *in vivo* mechanotransduction roles for the mammalian members of the family, Tmem63s, remain largely unknown. Here, we explore the contribution of TMEM63A in myelination. Many molecular factors that facilitate interaction between neurons and oligodendrocytes (OLs) drive myelination. Mechanical properties of OLs, as well as their extracellular environment and the axon, appear to play an essential role during OL development and myelination. How curvature/axon shaft morphology is sensed remains a mystery and the identity of potential OL mechanosensor(s) that would respond to axonal morphology is unknown. Single cell RNA seq data indicate that *Tmem63a* is enriched in Oligodendrocytes. Additionally, transient hypomyelination manifests in individuals with heterozygous TMEM63A variants. Our data suggests that these mutations in heterologously expressed TMEM63A cause loss of channel activity, suggesting that proper functioning of TMEM63A is required for myelination to progress normally in healthy individuals. Furthermore, to explore the role of TMEM63A in myelination, we are characterizing mechanosensitive currents and calcium signals in cultured OLs. *In vivo* myelin formation is being examined in the optic chiasm, spinal cord, and cortex at different developmental stages in mice, and behavior will be used to elucidate sensorimotor deficits in mice as a consequence of lack of myelination. Together, our work will begin to unravel how OLs sense mechanical forces or physical structures and convert information into calcium signaling events that allow for axon wrapping.

O5: TRPV4 channel activity in response to hypoxia is affected by cell density

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Transient receptor potential vanilloid 4 (TRPV4) is a polymodal Ca^{2+}-permeable channel involved in various hypoxia-sensitive pathophysiological phenomena. TRPV4 is activated by stretch and might thus be affected by change in cell rigidity. Using different cell densities in experimental conditions can indeed induce a modification of cell rigidity, and consequently, lead to modified TRPV4 activation. Here, we studied the effect of cell density on TRPV4 activity in response to hypoxia. Transiently TRPV4-transfected HEK293T cells were seeded at low ($1x10^4$ cells/cm²) or high ($3x10^5$ cellules/cm²) densities corresponding to non-confluent or confluent cells, respectively, on the day of experiments. Cells were then cultured under in vitro normoxia (21% O_2) or hypoxia (1% O_2) for 48h. First, TRPV4 activation was assessed in response to GSK1016790A (100 nM), a specific TRPV4 agonist. Channel activity was measured using patch-clamp, calcium imaging and Bioluminescence Resonance Energy Transfer (BRET) techniques. Indeed, TRPV4-mediated unitary currents were recorded in patch-clamp cell-attached mode and current amplitude, unitary conductance and open probability were analysed. Cytosolic calcium responses were measured by calcium imaging thanks to the ratiometric Fura2-LR-AM fluorescent probe. BRET was used to study calcium variation directly in the TRPV4 pore nanoenvironment with the expression of TRPV4 fused with a BRET probe containing a calcium sensor (Calflux) surrounded by nanoLuciferase (energy donor) and mNeonGreen (energy acceptor). Then, TRPV4 localisation to membrane was evaluated using confocal microscopy imaging and cell surface biotinylation. Another BRET construct allowed us to study channel internalization thanks to the expression of TRPV4 fused with nanoLuciferase and the bystander BRET probe mNeonGreen-CAAX (acceptor). Our results show that hypoxia exposure has a differential effect on TRPV4 activation depending on cell confluence. At low confluence level, TRPV4 response is increased in hypoxia, whereas at high confluence level, TRPV4 response is strongly inhibited. This diminution of TRPV4 activation can be explained by an internalization of the channel observed in hypoxia at high cell density. Thus, our study highlights the importance of specific culture condition, namely cell confluence, which can influence many cellular processes, especially regarding TRPV4 channel activity in response to hypoxia.

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O6: The role of the calcium-sensing receptor in the activation of the intestinal prostaglandin E2 pathway

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Introduction: The calcium-sensing receptor (CaSR) is a ubiquitously expressed G protein-coupled receptor. While its best characterized role is the regulation of calcium homeostasis, our recent studies suggested that the CaSR promotes colitis, which is a major risk factor for colorectal cancer. One of the main mediators of inflammation in the colon is the prostaglandin E2 (PGE2) pathway. Especially cyclooxygenase (COX)-2 is upregulated in colorectal carcinomas and inflammation. To understand how the CaSR affects the inflammation in the colon, we assessed the regulation of PGE2 pathway genes by the CaSR in vitro and in vivo.

Methods & Results: We measured gene expression levels in two colon cancer cell lines either stably overexpressing the CaSR fused to GFP (HT-29CaSR-GFP; Caco-2CaSR-GFP) or GFP alone (HT-29GFP; Caco-2GFP) after treatment with various CaSR ligands. The orthosteric ligand spermine and the positive allosteric CaSR modulator (PAM) NPS R-568 upregulated COX-2 expression both in HT-29CaSR-GFP cells (spermine ~8.0-fold, $p<0.001$; NPS R-568 ~6.4-fold, $p<0.01$) and Caco-2CaSR-GFP cells (spermine ~10.7-fold, NPS R-568 ~7.1-fold; both $p<0.001$). Ca2+ upregulated COX-2 expression significantly only in Caco2CaSR-GFP cells (~4.6-fold, $p<0.001$) compared with HT-29CaSR-GFP cells (~4.4-fold, $p>0.05$). In contrast, prostaglandin E synthase (PTGES) expression was upregulated by spermine (~2.9-fold, $p<0.01$) only in HT 29CaSR-GFP cells. Preincubating the cells with the negative allosteric CaSR modulator (NAM) NPS 2143 suppressed the effects of spermine and NPS R-568. Other genes involved in the PGE2 pathway were not responsive to the treatment and none of the studied genes were affected in HT-29GFP and Caco-2GFP cells lacking the CaSR.

In BALB/c mice with dextran sulfate sodium (DSS)-induced colitis, per os (p.o.) treatment with the clinically approved PAM cinacalcet significantly increased prostaglandin synthase expression in both the proximal (~4.0-fold, $p<0.0001$) and the distal colon (~2.6-fold, $p<0.001$), and the expression of the EP3 receptor in the proximal colon (~2.4-fold, $p<0.01$). However, contrary to our in vitro results, COX-2 expression was not affected. On the other hand, p.o. treatment with the NAM NPS 2143 increased the expression of the PGE2-degrading enzyme 15-PGDH (~1.9-fold, $p<0.01$) in the proximal colon. In healthy mice, neither modulator of the CaSR affected PGE2 pathway gene expression.

Conclusion: Activation of the CaSR induces several genes of the PGE2 pathway; interestingly, the affected genes of the pathway differ between the in vitro and in vivo models. Similarly to the lung, where NAMs are under investigation for treating asthma, they may thus be repurposed as a novel therapy against intestinal inflammation and colorectal cancer development.

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O7: The FDSS μCELL instrument for live calcium imaging in high-throughput

Geert Bultynck. KU Leuven, Belgium. (Sponsored by Hamamatsu)

O8: Ca^{2+} signals as modulators of ER-mitochondria contacts in health and disease

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The juxtaposition between different cellular organelles modulates a multitude of key signalling pathways, including Ca^{2+} homeostasis. In particular, Ca^{2+} exchange between endoplasmic reticulum (ER) and mitochondria is known to be favoured by their close connection. Nevertheless, whether specific Ca^{2+} signals modulate ER-mitochondria tethering has been much less investigated, partly due to the lack of probes allowing the measurement, with a sufficient spatial and temporal resolution, of dynamic changes in inter-organelle apposition. By a novel, multicolor chemo-genetic reporter, specifically tailored to detect organelle contacts in living cells, we found that the reduction of ER Ca^{2+} content triggers a rapid increase in ER-mitochondria connectivity. Current investigations to define mechanisms and possible pathological implications of this process will be discussed.

O9: Regulation by Calcium of neuronal metabolism. Role of MCU and Aralar-Malate-Aspartate Shuttle

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Neuronal activation increases cell workload to restore ion gradients altered by activation. Ca^{2+} is involved in matching increased workload with ATP production, but the mechanisms are still unknown. We present evidence that, in neurons using glucose as only fuel, glycolysis, pyruvate production, and neuronal respiration are stimulated upon activation in a Ca^{2+}-dependent way, independently of effects of Ca^{2+} as workload inducer. Mitochondrial calcium uniporter (MCU) does not play a relevant role in Ca^{2+} stimulated pyruvate production and oxygen consumption as both are unchanged in MCU-silenced neurons. However, Ca^{2+} stimulation is blunted in the absence of Aralar/AGC1/Slc25a12, the mitochondrial Ca^{2+}-binding aspartate-glutamate carrier, a component of the Malate-Aspartate Shuttle (MAS). Our findings suggest that Ca^{2+}-regulated Aralar-MAS activation upregulates glycolysis and pyruvate production, which fuels mitochondrial respiration, through regulation of the cytosolic NAD+/NADH ratio.

O10: Keeping zombies alive: the reticulum-to-mitochondria Ca^{2+} transfer in senescent cells

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Cellular senescence entails a permanent cell cycle arrest, characterized by apoptosis resistance, and a pro-inflammatory senescence-associated secretory phenotype (SASP). Physiologically, senescent cells promote tissue remodeling during development and after injury, but when accumulated over a certain threshold, as happens during aging or after cellular stress such as the one induced by chemo- or radiotherapy, contribute to the decline of the regenerative potential and function of tissues, causing several diseases such as type 2 diabetes, osteoarthritis and atherosclerosis. Moreover, the presence of senescent cells promotes tumorigenesis and cancer relapse by inducing de-differentiation, proliferation and metastasis. Thus, delaying senescent cell accumulation or reducing senescent cell burden presents a promising strategy to alleviate multiple diseases. Understanding the pathways that contribute to senescence is essential to reveal new therapeutic targets. Cellular senescence is accompanied by increased mitochondrial metabolism. However, how mitochondrial function is regulated and what role it plays in senescent cell homeostasis is poorly understood. Here we present preliminary evidence that shows an increased endoplasmic reticulum (ER)-mitochondria Ca2+ crosstalk that is fundamental to maintain senescent cell homeostasis and represents an attractive targetable pathway to selectively kill senescent cells. Constitutive IP3R-mediated Ca2+ transfer to the mitochondria is essential to maintain the mitochondrial function of senescent cells; its inhibition causes a bioenergetic crisis characterized by activation of SIRT1 which deacetylates p53 causing which trigger selective senescent cell death.

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CANCELLED: ANNOUNCEMENT MADE AT MEETING

O11: Anti-apoptotic Bcl-xL controls inositol 1,4,5 triphosphate (IP3) receptor mediated ER-mitochondria Ca^{2+} transfer

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Discovery of closely apposed membrane regions of ER and mitochondria, termed as ER-mito contacts, have opened new avenues in studying the organellar structure-function relationship and their implications on cell homeostasis. The ER-mito contacts locally convey Ca^{2+} signals that are essential for cell survival. The key player mediating release of Ca^{2+} from ER is the inositol 1,4,5 triphosphate receptor (IP3R). Voltage-dependent anion channel (VDAC) in the mitochondrial outer membrane enabling mitochondrial Ca^{2+} uptake, is another component. ER-mito Ca^{2+} transfer moderated by IP3R and VDAC is further finely regulated by emerging constituents of the contacts. A range of cancer cells have shown to evade cell death by modulating ER-mito Ca^{2+} transfer brought about by the B-cell lymphoma 2 family proteins. Of the anti-apoptotic members of Bcl2 family proteins, the role of ER resident Bcl2, as a modulator of ER Ca^{2+} homeostasis, has been well studied so far. Another anti-apoptotic Bcl2 family member, Bcl-xL, primarily found in outer mitocondrial membrane (OMM) has been reported to interact with IP3R and VDAC in individual studies. Previous studies have put forward Bcl-xL as a regulator of ER Ca^{2+} homeostasis with consequent effect on apoptotic protection. The interaction of Bcl-xL and IP3R has been demonstrated to enhance Ca^{2+} signals which further resulted in elevated mitochondrial bioenergetics. However, the mechanistic details of how Bcl-xL and IP3R are concerted to bring about changes in mitochondrial Ca^{2+} status is not fully known. In this study we found a link between Bcl-xL and functional ER-mito Ca^{2+} transfer. Using genetic targeting of Bcl-xL in mouse embryonic fibroblasts (MEFs) and fluorescence imaging of organelle targeted Ca^{2+} sensors we found that wild-type (WT) MEFs depict higher, faster and tighly coupled mitochondrial Ca^{2+} signal in response to IP3R-mediated cytoplasmic Ca^{2+} rise compared to Bcl-xLKO MEFs. Acute rescue with full-length Bcl-xL and stable rescue experiments with cytoplasm, ER and mitochondria localized BclxL revealed complementary results in case of full-length, ER and mitochondria specific BclxL reinforcing the importance of membrane targeted BclxL in ER-mito Ca^{2+} transfer. The difference in mitochondrial Ca^{2+} uptake was noticeable only in case of agonist stimulated local Ca^{2+} transfer and not under global Ca^{2+} challenge. In case of the latter, BCLxLKO cells exhibitted comparable/ better mitochondrial Ca^{2+} uptake with respect to WT MEFs. Taking these results into account, Bcl-xL was found to facilitate specifically local Ca^{2+} transfer from ER to mitochondria acting in concert with IP3R while not having similar effect on general cellular Ca^{2+} rise. This highlights a potential role of Bcl-xL in Ca^{2+} signal propagation in the context of ER-mito contacts. Considering the emergence of ER-mito contacts as Ca^{2+} hotspots affecting a wide range of diseases, insights into the mechanistic role of Bcl-xL impacting the structure-function of the Ca^{2+} nanodomain holds promise for exploring therapeutic strategies in future.

O12: Regulation of intracellular Ca^{2+} signaling: a novel function for the metabolic enzyme pyruvate kinase M2 (PKM2)

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Pyruvate kinase M2 (PKM2) is an alternatively spliced variant of the pyruvate kinase gene that is highly expressed in cancer cells. PKM2 controls the final, rate-limiting step of glycolysis, contributing to the cancer-specific Warburg effect (also known as aerobic glycolysis), and supporting anabolic processes during tumor formation. Recently, our group showed that PKM2 interacts in various tumor cell lines with inositol 1,4,5-trisphosphate receptors (IP_3R), a ubiquitously expressed endoplasmic reticulum (ER) Ca^{2+}-release channel. In hematopoietic T and B cells, we showed that PKM2 negatively modulates IP_3R activity (Lavik et al., 2022, Biochim. Biophys. Acta 1869:119206). Therefore, we aimed to further characterize the inhibitory effect of PKM2 on IP_3R activity and the implications of this modulatory effect on cell metabolism and tumor growth.

In this study, we generated HeLa cells in which PKM2 was knocked out (HeLa PKM2KO). Using the PKM2KO HeLa cells, we confirmed that PKM2 suppresses IP_3R-dependent Ca^{2+} signals without interfering with either ER Ca^{2+} store content or IP_3R expression levels. The inhibitory effect of PKM2 on the IP_3R was reflected by a lower mitochondrial Ca^{2+} uptake. Strikingly, quantification of ER-mitochondrial proximity using the SPLICS sensor indicated that PKM2 increases ER-mitochondrial contact sites. Furthermore, we demonstrated that PKM2 expression reduces mitochondrial membrane polarization and cell respiration.

It is known that PKM2 requires methylation at R445, R447 and R455 by the methyltransferase enzyme CARM1 in order to functionally interact with IP3R (Liu et al., 2017, Nature Cell Biol. 19:1358). To further unravel the role of PKM2 methylation in regulating the PKM2-IP3R interaction, we analyzed the functional consequences of the substitution of R445 and R477 to lysine (PKM2 R445/447K). We found that PKM2 R445/447K can still interact with IP_3R isoforms 1 and 3, though that it lacks the inhibitory effect of wild type PKM2 on IP_3R-dependent Ca^{2+} signals, when expressed in HeLa PKM2KO cells.

Together, our results show that PKM2 modulates cellular Ca^{2+} signaling via direct interaction with IP_3Rs. The modulation of Ca^{2+} signaling is a likely contributor to the survival and/or proliferation of cancer cells, as well as the poor prognosis seen in cancer patients with high levels of PKM2 expression. Our recent data reveal hitherto unknown aspects of PKM2 expression on mitochondrial function and organelle proximity.

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O13: Endo-lysosomal Ca^{2+} signalling: a luminal view

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Ca^{2+} is a universal second messenger that is compartmentalized in cytoplasmic organelles. Acidic organelles are Ca^{2+} stores capable of accumulating and releasing Ca^{2+} upon cell activation. Therefore, much effort has been devoted to develop genetically-encoded Ca^{2+} indicators as tools for measuring luminal Ca^{2+} in acidic compartments. However, this task is extremely challenging because most of the fluorescent Ca^{2+} indicators are pH-sensitive and the fluorescent signal is quenched at acidic pH. By contrast, bioluminescence-based Ca^{2+} sensors are potentially advantageous since they are relatively resistant to pH variations. Aequorin is a Ca^{2+} binding protein that emits blue light when reconstituted with its cofactor coelenterazine. Targeted aequorins are excellent Ca^{2+} indicators for monitoring subcellular Ca^{2+} dynamics. Here we targeted GFP-aequorin to the endo-lysosomal lumen and found that a significant fraction of the probe resides in a non-acidic compartment. We report that aequorin does not reconstitute at acidic pH and leveraged this to report calcium dynamics in this compartment. We show that luminal Ca^{2+} uptake is thapsigargin sensitive and that steady state Ca^{2+} levels are in the range of hundreds of micromolar, similar to the ER, but selectively reduced upon overexpression of an endocytic channel. We find the calcium mobilizing messenger IP3 evokes robust luminal responses in wild type cells but not in IP3 receptor knock-out cells. And that a fraction of endogenously tagged IP3 receptors colocalize with lysosomes. Stimulation with IP3-forming agonists evoke Ca^{2+} release in live intact cells.

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O14: Endolysosomal two-pore channels (TPC) regulate social behaviour by controlling oxytocin secretion

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Oxytocin (OT) is a prominent regulator of many aspects of mammalian social behaviour. OT is stored in large dense-cored vesicles (LDCVs) and is differentially released from different compartments of hypothalamic magnocellular neurones. Despite its importance, critical aspects of the Ca^{2+}-dependent regulatory mechanisms of its secretion remain to be identified. Here, we show that lysosomes are present in all neuronal compartments and surround dendritic neuropeptide secretory vesicles and demonstrate that the activation of endolysosomal two-pore channels (TPCs) provides the critical Ca^{2+} signals regulating OT secretion from and within the hypothalamus. We observed a dramatic reduction in plasma OT levels in TPC knockout mice, as well as an impaired somato-dendritic secretion of OT from the hypothalamus. Furthermore, we show that activation of type 1 metabotropic glutamate receptors (mGluR1) sustains somato-dendritic OT release by recruiting TPCs. We demonstrate that TPCs are mediator of OT secretion through a two-step mechanism involving the release and the modulation of neuropeptide vesicles availability (priming). Indeed, priming of LDCVs was increased by direct application of NAADP, the endogenous messenger activating TPCs or a selective TPC2 agonist, TPC2-A1-N or blocked by the NAADP/TPC antagonist Ned-19. Finally, we found that mice lacking TPCs exhibit impaired maternal and social behaviour that could be restored by direct OT administration. This study therefore shows an unexpected role for lysosomes and TPCs in critical specific steps of neuropeptide secretion, and in regulating social behaviour.

O15: TRP Mucolipins and TFEB

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Lysosomes are intracellular organelles deputed to the degradation of macromolecules and dysfunctional organelles. Deleterious mutations in lysosomal genes lead to the onset of monogenic diseases named lysosomal storage disorders (LSDs), characterized by the accumulation of undigested intracellular material, such as lipids and proteins, in the lysosomes. Recent evidence has shown that lysosomal biogenesis and autophagy are transcriptionally regulated by a gene network and by its master gene TFEB, a member of the Helix-Loop-Helix leucine zipper transcription factors that regulates the expression of lysosomal and autophagy genes (Sardiello et al., 2009, Settembre et al., 2011, Settembre et al., 2013). Our group contributes to demonstrating that TFEB activity responds to nutrients and is regulated by mTORC1-mediated phosphorylation, which occurs on the lysosomal surface (Settembre et al., 2012). More recently, we discovered that calcineurin, a Ca2+-modulated phosphatase, de-phosphorylates TFEB promoting its nuclear translocation. Also, we found that Calcineurin activity was modulated by lysosomal calcium through the lysosomal calcium channel mucolipin 1 (MCOLN1) (Medina et al., 2015). Together these results revealed, for the first time, the presence of lysosome-to-nucleus signaling mechanisms and changed the view of the lysosome from a “suicide bag” to a dynamic organelle that responds to environmental cues. The identification of global transcriptional regulation of lysosomal function was exploited by us first and then by other groups to boost lysosomal function in mouse models of human diseases. We found that the overexpression of TFEB on cellular models of LSDs promotes the clearance of pathologic lysosomal storage (Medina et al., 2011). Also, we found that TFEB induces clearance through the activation of a process called lysosomal exocytosis by inducing both the pre-docking of lysosomes close to the plasma membrane and the release of lysosomal calcium to promote lysosomal fusion (Medina et al., 2011). Viral-mediated TFEB gene transfer resulted in the clearance of accumulating substrates in cells and tissues from mouse models of several types of LSDs (Medina et al., 2011). This seminal approach has been replicated in varios disease models, including more common neurodegenerative disorders such as Parkinson’s, Alzheimer’s, and Huntington’s. Therefore, the possibility of modulating lysosomal function by acting in the TFEB network may lead to a novel therapeutic strategy with potential applicability to more than 50 LSDs. We are currently testing this possibility in our high content screening laboratory. Some of these studies resulted in the repurposing of drugs that regulates TFEB and reduce storage in LSDs (Moskot et al., 2014; Soldati et al., 2021; Capuozzo et al., 2022).

O16: The role of NAADP-dependent calcium release on Rab46 activity in endothelial cells

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Rab GTPases are master regulators of trafficking of intracellular vesicles. However, the mechanism by which context-dependent spatial and temporal regulation of Rab GTPase activity is controlled is poorly understood. Here we describe a mechanism by which localized calcium, released through an NAADP-dependent nanodomain, controls Rab46 (CRACR2A-L) activity.

Using high resolution quantitative imaging we show that, in endothelial cells, Rab46-mediated vesicular trafficking to the microtubule organising centre (MTOC) is stimulated by histamine, but not thrombin, and is independent of intracellular calcium. This acute trafficking pathway acts as a brake to vesicle degranulation, thereby preventing the 'all out' emergency response evoked by thrombin. Binding of calcium to the 2nd EF-hand is necessary for release of Rab46 from dynein at the MTOC. The nicotinic acid adenine dinucleotide phosphate (NAADP) antagonist Ned-19 and calcium channel inhibitor tetranadrine, inhibit the histamine, but not thrombin, evoked calcium response and induce clustering of Rab46 at the MTOC. In endothelial cells, siRNA mediated depletion of Rab46 has no effect on store-operated calcium signals but inhibits histamine-evoked calcium release.

Our data indicates that NAADP-mediated calcium release at the MTOC is necessary for Rab46 function. Moreover, Rab46 may play a role in the structural integrity of the NAADP-sensitive nanodomain thereby demonstrating a mechanism by which selective stimulants could spatially regulate the functionally coupling of NAADP synthesis to the target channels and organelles.

O17. OCaR1 confers exocytotic vesicles an autoregulatory competence to prevent Ca^{2+} release, exocytosis and pancreatic disorder

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Regulated exocytosis is triggered by increased Ca^{2+} levels within close proximity to secretory granules, which is prevented in resting cells. In acinar cells, exocytosis is triggered by Ca^{2+} release from intracellular organelles following hormonal stimulation with e.g. cholecystokinin (CCK). It is established that aberrant Ca^{2+} homeostasis is associated with pancreatic diseases, including pancreatitis. The contribution and importance of each of the intracellular Ca^{2+} stores in disease and key aspects of their regulation, in particular regulation of Ca^{2+} release from acidic organelles, is poorly understood.

Here we show that exocytosis of zymogen granules is regulated explicitly by Ca^{2+} release from the granules themselves. We identified OCaR1 as an Organellar Ca^{2+} Regulator protein that is localized in the membrane of lysosomes secretory granules in pancreatic acinar cells according to fluorescent microscopy as well as organellar proteomics. OCaR1 comprises up to 11 hydrophobic stretches, which likely represent 11 transmembrane helices. Freshly isolated acinar cells of OCaR1-/- mice have uncontrolled spontaneous nicotinic acid adenine dinucleotide phosphate (NAADP) dependent Ca^{2+} oscillations. OCaR1 determines Ca^{2+} release from NAADP-sensitive acidic compartments not only under basal condition but also after stimulation of the CCK receptors, as revealed by pharmacological inhibition of acidic Ca^{2+} store loading, NAADP antagonist and direct monitoring of Ca^{2+} arising from TPC2-containing granules using targeted Ca^{2+} sensors. Furthermore, OCaR1-/- acinar cell homeostasis is disturbed by spontaneously elevated plasma lipase and amylase and an increase in regulated exocytosis of zymogen granula. In mouse models of severe and chronic pancreatitis OCaR1 deletion exacerbates the disease indicated exaggerated release of digestive enzymes.

In summary, OCaR1 has a critical role in spontaneous and stimulated Ca^{2+} release and exocytosis. Moreover, OCaR1 deletion also exacerbated disease in murine models of severe and chronic pancreatitis. These findings established OCaR1 as a gatekeeper of Ca^{2+} release from NAADP-sensitive acidic granules, which confers secretory granules an autoregulatory competence to prevent uncontrolled exocytosis and pancreatic disorder.

O18: Regulation of cardiac calcium homeostasis by nano-domain cAMP signalling

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3’-5’-cyclic adenosine monophosphate (cAMP) is the key mediator of sympathetic regulation of calcium cycling in cardiac myocytes. Signalling by cAMP is organised in multiple distinct subcellular nanodomains regulated by cAMP-hydrolysing phosphodiesterases (PDEs) and this organisation is disrupted in heart disease. Although studies in cardiac myocytes have provided an understanding of the location and properties of a handful of cAMP nano-signalosomes, an overall view of the cellular landscape of cAMP nanodomains is missing. To fill this gap, we have applied an integrated (phospho)proteomics approach that takes advantage of the unique played by individual PDEs in the control of local cAMP to identify previously unrecognised cAMP nanodomains associated with β-adrenergic stimulation. We demonstrate that this is a valid approach to identify novel cAMP nano-signalosomes and to establish their specific function. This knowledge will provide a blueprint that can be exploited for the design of targeted therapeutics.

O19: Calcium regulation in the SOCE nanodomain: new insights into calcium-dependent inactivation of Orai1 channels

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Ca^{2+}-release activated Ca^{2+} (CRAC) channels mediate a process known as store-operated calcium entry (SOCE), which serves a wide variety of cellular functions ranging from gene expression, exocytosis, tissue development, and the immune response. CRAC channels are activated by the depletion of Ca^{2+} from the endoplasmic reticulum (ER), triggered physiologically through stimulation of a diverse set of metabotropic surface receptors. The best described CRAC channel is formed by the Orai1 protein and is gated by physical coupling of Orai1 with the ER Ca^{2+} sensor protein, STIM1. A key hallmark of CRAC channels is fast Ca^{2+}-dependent inactivation (CDI) which provides negative feedback inhibition to limit Ca^{2+} entry through CRAC channels and prevent Ca^{2+} overload at the Orai1-STIM1 nanodomain. However, the molecular basis of CDI remains obscure. In this talk, I will discuss our recent work in which we used a human gain-of-function Orai1 mutation that causes severe tubular myopathy to address the molecular mechanism of Orai1 CDI.

O20: Interactions between the cytoskeleton and membranes: building an ER-PM contact site *in planta*.

Patrick J Hussey, University of Durham, UK

In Eukaryotes, organelle interactions occur at specialised contact sites between organelle membranes. Interaction between different organelles is fundamental to a range of processes including exchange of small molecules such as calcium, lipid transfer, and autophagy. Our understanding of plant EPCS is still emerging, and the hypothesised roles of plant EPCS in processes such as calcium signalling or lipid exchange have yet to be characterised [1,2]. Contact sites are regulated by specialised tethering proteins, which bring organelle membranes into close proximity, and facilitates functional crosstalk between compartments. Whilst contact site proteins are well characterised in mammals and yeast, the regulators of plant contact site formation are only now beginning to emerge. The recently characterised NETWORKED proteins are the first dedicated family of plant-specific contact site proteins [3,4,5]. Research into the NET proteins and their interacting partners continues to uncover plant-specific mechanisms of organelle interaction and the importance of these organelle contacts to plant life. Here, we will present recent developments in our understanding of the mechanisms of plant organelle interactions and their functions.

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O21: Three dimensional electron microscopy of endoplasmic reticulum in thyroid cells by serial block face SEM and TEM

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The endoplasmic reticulum (ER) is the largest organelle, which extends throughout the cell. ER network is highly dynamic, undergoing continuous morphological rearrangements, extensions and movements. This multifaceted organization of the ER modulates a multitude of cellular processes ranging from sorting, modifying and transportation of newly synthesized proteins to regulate calcium dependent cell proliferation and migration. In this study/poster, for the first time, we present the 3Dstructural profile of ER in plastic embedded human normal thyroid cells using two powerful three dimensional electron microscopy (3D-EM) techniques, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). We observed a well-defined and extended ER throughout the cell, mostly tubular than sheets. As disruption in ER homeostasis triggers stress responses such as unfolded protein response (UPR) also known as an endoplasmic reticulum (ER) stress coping response, we investigated the expression of three key UPR-activated ER stress proteins PERK, IRE1α and ATF6 in normal thyroid and thyroid cancer ML-1 cells. The results showed an upregulation of PERK and IRE1 α proteins in thyroid cancer cells compared to normal thyroid cells. The same results were observed in papillary thyroid cancer patient tissues compared to the respective normal thyroid tissue. Taken together, we present the 3D-structure of ER of thyroid cells using SBF-SEM and TEM microscopy and that UPR activated ER-stress proteins expression is upregulated in thyroid cancer cells compared to normal thyroid cells. In future, our aim is to study how manipulation of intracellular calcium $(Ca^{2+})_i$, and induction of ER-stress manifests in ER morphology.

This project is financially supported by Academy of Finland, which is gratefully acknowledged.

O22: Ca-independent role of Orai3 in the human muscle cell activation

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Skeletal muscle repair relies on the activation of muscle stem cells, but the molecular pathways, especially the Ca^{2+} signalling required for muscle stem cell activation, are still unclear. Our work aims to study the activation of the quiescent muscle stem cells obtained *in vitro*, called reserve cells (RC). RC activation is defined as their ability to re-enter the cell cycle following stimulation.

We first tested the capacity of fetal calf serum (FCS) to induce the activation of the RC. Around 30% of the RC were positive for EdU (incorporated only in proliferating cells) after 24h of FCS stimulation, proving that FCS can trigger a subpopulation of quiescent RC to activate. We used the Ca^{2+} dye Fura-2 AM to measure the Ca^{2+} signals evoked by FCS stimulation and to establish whether the activation of the RC by FCS correlates with a specific Ca^{2+} response. Upon stimulation, we were able to differentiate several patterns of responses with a frequent development of Ca^{2+} oscillations, while around 5% of the cells did not display any Ca^{2+} elevation. Most of the Ca^{2+} responses were IP_3 dependent, and the Ca^{2+} oscillations were abrogated after removing extracellular Ca^{2+}. Inhibitors of the store-operated Ca^{2+} entry pathway (SOCE; a ubiquitous Ca^{2+} influx mechanism) only partially inhibited the Ca^{2+} oscillations. However, we observed that neither IP_3 production blockers nor Ca^{2+} influx blockers prevented the activation of RC. In addition, neither low Ca^{2+} medium nor BAPTA-AM (intracellular Ca^{2+} chelator) prevented the activation of RC. Hence, we concluded that RC activation is unexpectedly independent of Ca^{2+} signals. Nevertheless, by tracking each RC individually after FCS stimulation, we could determine that the cells displaying a Ca^{2+} response were migrating faster and farther than those not eliciting a Ca^{2+} response. In line, we showed that inhibitor of the IP_3 pathway and Ca^{2+} influx inhibitor strongly decreased the displacement of RC observed during the 48h after FCS stimulation.

We assessed the involvement of Orai channels in the RC activation and found that, while only Orai1 downregulation reduced the Ca^{2+} entry phase, Orai3 downregulation led to a 40% decrease in the RCs activation. In addition, Orai3 downregulation led to the formation of larger myotubes, suggesting an alteration of RC homeostasis. Thus, we have this paradoxical outcome that the Ca^{2+}-independent activation of RC required the presence of the Ca^{2+} selective channel Orai3. We are currently performing additional experiments to understand the implication of Orai3 in RC activation.

O23: A gating mutation in ryanodine receptor type 2 rescues phenotypes of Alzheimer's disease mouse models by upregulating neuronal autophagy

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It is well established that Ryanodine receptors (RyanR) are overactive in Alzheimer's disease (AD) and it has been suggested that inhibition of RyanR is potentially beneficial for AD treatment. However, the exact mechanism responsible for the beneficial effects of RyanR inhibition in AD models is not well understood. To resolve this question, in the present study we explored a potential connection between basal RyanR activity and autophagy in neurons. Autophagy plays an important role in clearing damaged organelles and long-lived protein aggregates, and autophagy dysregulation occurs in both AD patients and AD animal models. Autophagy is known to be regulated by intracellular calcium (Ca^{2+}) signals, and our results indicated that basal RyanR2 activity in hippocampal neurons inhibited autophagy through activation of calcineurin (CaN) and resulting inhibition of AMPK-ULK1 pathway. Thus, we hypothesized that increased basal RyanR2 activity in AD may lead to inhibition of neuronal autophagy and accumulation of β-amyloid. To test this hypothesis, we took advantage of the RyanR2-E4872Q knock-in mouse model (EQ) in which basal RyanR2 activity is reduced due to shortened channel open time. We discovered that crossing EQ mice with the APPKI and APPPS1 mouse models of AD rescued amyloid accumulation and LTP impairment in these mice. Our results revealed that reduced basal activity of RyanR2-EQ channels disinhibited the autophagic pathway and led to increased amyloid clearance in these models. These data may explain why pharmacological inhibition of RyanR results in beneficial effects in a variety of AD models and may also provide additional targets for therapeutic intervention in AD.

O24: Lipids and neurodegeneration: what your MAM can teach you

Estela Area-Gómez.

Columbia University, USA

Cancelled owing to exceptional circumstances

O25: Astroglial signalling in ageing and Alzheimer disease

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Ageing is associated with morphological and functional remodelling of astrocytes with a prevalence of morphological atrophy and loss of function, whci paves teh way to age-dependenr neurodegeneration. In particular ageing is associated with (i) decrease in astroglial synaptic coverage; (ii) deficits in glutamate and potassium clearance; (iii) reduced astroglial synthesis of synaptogenic factors such as cholesterol; (iv) decrease in aquaporin 4 channels in astroglial endfeet with subsequent decline in the glymphatic clearance; (v) decrease in astroglial metabolic support through the lactate shuttle; (vi) decreased adult neurogenesis resulting from diminished proliferative capacity of radial stem astrocytes; (vii) decline in the astroglial-vascular coupling and deficient blood-brain barrier and (viii) decrease in astroglial ability to mount reactive astrogliosis. Decrease in defencive capabilities of astroglia as well as degeneration and dystrophy of microglia are permissive for age-dependent neurodegenerative diseases. Neuroglial morphology and function can be influenced and improved by lifestyle interventions such as intellectual engagement, social interactions physical exercise, caloric restriction, and healthy diet. These modifications of lifestyle are paramount for cognitive longevity.

O26: ATAD3A, a novel interactor of Mfn2. Implications on Ca^{2+} signalling in the context of Alzheimer's disease

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Ca^{2+} signalling dysregulation is one of the key components in Alzheimer's disease (AD) physiopathology. Nowadays, it is well known that in familial AD (FAD) models, Ca^{2+} signalling is impaired even at the level of the mitochondria associated membranes (MAMs). Previous studies have demonstrated that mutated forms of Presenilin-2 (PS2) associated with FAD (FAD-PS2) strengthen ER-mitochondria tethering, potentiating the efficiency of ER-mitochondria Ca^{2+} transfer. This effect is mediated by the interaction of FAD-PS2 with Mitofusin-2 (Mfn2), a protein present at the level of the MAMs that prevents the excessive coupling between the two organelles. Therefore, FAD-PS2 acts as an antagonist of Mfn2 (Filadi, R et al. 2016).

To get insights in the molecular mechanism(s) through which FAD-PS2/Mfn2 modulate ER-mitochondria coupling, we investigated the PS2-modulated interaction of Mfn2 with other proteins. ATAD3A (ATPase family AAA-domain containing protein 3) has been shown to localize at MAMs, as well as take part in AD pathology through its oligomerization (Zhao, Y et al. 2022). ATAD3A interaction with Mfn2 is affected by FAD-PS2 but its effects on $Ca2^{+}$ signalling are unknown. Here, we have studied Ca^{2+} homeostasis, particularly at MAMs, upon downregulation of ATAD3A (ATAD3A-KD). ATAD3A-KD causes a reduction of the cytosolic, mitochondrial and ER Ca^{2+} concentration. Interestingly, although the concentration of Ca^{2+} in both organelles is reduced, the efficiency of Ca^{2+} transfer between them is increased, compared to controls. Moreover, the increased Ca^{2+} transfer between ER and mitochondria is not due to changes in MCU activity upon ATAD3A-KD. All these data suggests the involvement of ATAD3A in the modulation of the structural coupling between ER and mitochondria.

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O27: Septin 7 knock-outs rescue SOCE-dependent gene expression and motor learning deficits arising from mouse cerebellar Purkinje neurons

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Septins are filament forming cytoskeletal GTPases that assemble to form heteromeric complexes and regulate multiple cellular processes from yeast to humans. Septin 7 (SEPT7), a member of the Septin family, functions as a negative regulator of Orai, the store-operated Ca^{2+} entry channel, in *Drosophila* neurons and human neural progenitor cells. Flight deficits in *Drosophila* mutants of the inositol 1,4,5-trisphosphate receptor and of dSTIM, both essential components of intracellular Ca^{2+} signaling, can be rescued by *Septin 7* knockdown (1). Loss of STIM1 in mouse Purkinje neurons (PNs) leads to a change in their gene expression profile and attenuation of motor learning (2). Interestingly, PN specific knockout of *Sept7* restored expression of specific genes downregulated by *STIM1* gene knock-out. Moreover, motor deficits observed in PN-specific *STIM1* knockout mice were rescued by loss of one or both copies of the *Sept7* gene from PNs. Thus, reduction of SEPT7, a negative regulator of SOCE, can improve the function of Purkinje neurons (3). Cellular changes in STIM1+Sept7 double knockout human neuronal cells are currently under investigation and will be discussed. Our findings will help understand the therapeutic value of targeting Septin 7 in neurodegenerative conditions with reduced intracellular calcium signaling, such as Spino-cerebellar Ataxias, Parkinson's and Alzheimer's disease.

O28: Endometrial organoids as a model to study fetal-maternal crosstalk

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The embryo implantation process is a complex phenomenon characterized by the presence of an implantation-competent embryo, receptive maternal endometrium and the intricate crosstalk between both. Successful implantation is contingent on the optimal unity between these factors. Proteases have been described as blastocyst-secreted proteins involved in early implantation events. They stimulate intracellular calcium signaling pathways in endometrial epithelial cells, leading to optimal maternal receptivity and successful embryo implantation. However, the exact molecular players underlying protease-induced calcium signaling, and the subsequent downstream pathways remain elusive. By the use of human endometrial organoids, we were able to evaluate the effect of long-term stimulation by the serine protease trypsin in human endometrial organoids and investigated the molecular mechanisms at play. Calcium microfluorimetric experiments showed that long-term application of trypsin induced calcium waves in human organoids, dependent on phospholipase C, inositol 1,4,5-trisphosphate and store-operated calcium entry pathways. Moreover, we identified a new molecular key player as the molecular entity initiating protease-induced calcium responses and evaluated the *in vivo* consequences.

O29: Organoid cultures for pharmacology studies

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Organoid cultures have originally been developed as model systems to understand biological events, including development, tissue regeneration and oncogenesis. It is clear now that they have many other applications, including a strong potential for testing drug efficacy and potential toxicity. Miniaturization of organoid cultures now allows to perform many tests and to follow many readouts.

We have set up and miniaturized organoid cultures of human colon, using surgical resections or biopsies to isolate stem cells. Organoids from healthy individuals and from patients (Inflammatory Bowel Disease: IBD patients) were grown and demonstrated different phenotypes. The effects of drugs currently used in clinics and of newly developed targets were followed on the disease-associated phenotypes. Results demonstrated that such mini-gut cultures can be used to test drugs that favor the regrowth of functional epithelium. Other approaches have tested the effects of anti-cancer drugs on cancer organoid cultures. Calcium signaling in organoid cultures can be used as rapid readouts for pharmacology studies in human mini-organs.

Taken together, data are now available to suggest the use of organoid cultures not only for toxicology testing, but also for therapeutic testing, directly in diseased tissues. This could be an opening to personalized medicine.

CANCELLED: ANNOUNCED DURING MEETING

O30: Alterations in mitochondrial metabolism and calcium pathways in familial Alzheimer's disease iPSC-derived neuronal cells and brain organoids.

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The significant increase in life expectancy in the last 60 years urges an improvement in our treatments of age-associated diseases. Alzheimer's disease (AD) is currently considered the major cause of dementia. However, despite being one of the most studied neurodegenerative diseases, little therapeutic progress was achieved during the last decades. Familial Alzheimer's disease comprises of 5% of the cases and display an early onset, making it a valuable model for the study of the disease. One of the hallmarks of AD is its cell metabolism and calcium pathways disruption. In this research project we aimed to investigate the metabolic and calcium pathways altered in AD-derived brain cells. We successfully generated and characterized neuroprogenitor cells (NPCs) and brain organoids from familial AD, their respective isogenic controls, and healthy subjects-derived iPSCs. Our differentiation protocol makes use of a 15 days 3D embryoid body formation step, followed by FACS sorting of the NPC population ($CD271^-$, $CD44^-$, $CD184^+$, $CD24^+$, $CD15^{-/+}$) which renders a population of more than 90% NPC. The overall expression of metabolic pathways proteins was determined by proteomics. Proximity ligation assay was used to assess the ER-mitochondrial interaction. Metabolic activity was assessed by measurements of mitochondrial respiration, glycolytic rate, and metabolic substrate usage. We report an overall decrease in glycolytic proteins expression and substrate usage, and an altered fatty acid pathway and a decrease in OCR and ECAR parameters, detected by Seahorse Extracellular Flux analyzer. Furthermore, the mitochondrial calcium uptake machinery is impaired in iPSC-derived NPC cells. Our results indicate that metabolic alterations are already perceivable in AD-derived NPCs, which can be a target for early intervention.

O31: Evolution of a novel class of voltage-gated calcium channels in marine diatoms

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The four domain voltage-gated calcium channels (Ca_v) are central to many signalling processes in animals and are also important in unicellular eukaryotes, for example underlying the swimming responses of the green alga *Chlamydomonas*. Four domain Ca^{2+} and Na^+ channels are absent from prokaryotes, although a family of single domain voltage-gated Na^+ (BacNav) and Ca^{2+} channels have been identified in some bacteria. We recently identified that a group of eukaryote algae (the diatoms) also possess single domain voltage-gated channels (EukCatA - eukaryote cation channels) that resemble the BacNav family. Diatoms are unicellular silicified algae that play an important role in marine and freshwater ecosystems, representing one of the most abundant photosynthetic organisms on our planet. EukCatA channels expressed in heterologous systems are fast- activating Na^+/Ca^{2+} permeable channels, with similar characteristics to four domain Cav channels. Knockout of EukCatA2 in the diatom *Phaeodactylum tricornutum* demonstrates that EukCatA channels play a role in generating cytosolic Ca^{2+} elevations following depolarization and contribute to cellular signalling during gliding motility. The findings indicate that some eukaryote lineages possess alternative mechanisms for fast Na^+/Ca^{2+} signalling and provide insight into the evolution of the distinct types of voltage-gated ion channels.

O32: Divergent calcium signalling pathways of *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite that infects approximately one third of the world population. The infection may result in congenital disease and is clinically relevant in immunocompromised patients. *T. gondii* pathogenesis is directly linked to its lytic cycle, which consists of active invasion of host cells, replication inside a parasitophorous vacuole (PV) and egress resulting in lysis of the host cell. Egressed parasites seek another host cell to invade and reiterate the cycle. Ca^{2+} signals precede each one of the lytic cycle steps like invasion, egress, secretion of specific adhesins, extrusion of the apical conoid and motility, essential for invasion. Very few molecular players have been characterized until now. Ca^{2+} channels have been predicted to be present in apicomplexan parasites and they appear to be highly divergent. Plasma membrane Ca^{2+} influx has been characterized and is the result of the opening of more than one type of channel. We characterized a Transient Receptor-type channel (TgTRPPL-2) that can conduct Ca^{2+}, is modulated by cytosolic Ca^{2+} and is functional at the plasma membrane and the endoplasmic reticulum (ER). TgTRPPL-2 allows constitutive Ca^{2+} influx into the cytosol from the ER or the extracellular milieu. In addition, we characterized a divergent Two-Pore Channel, which localized to the apicoplast, a secondary endosymbiotic organelle. We observed a Ca^{2+} transfer activity from the ER to the apicoplast for which expression of TgTPC was essential. It is evident that *T. gondii* expresses divergent channels that represent important elements of the Ca^{2+} signaling toolkit and are essential for the optimal pathogenic cycle of the parasite.

O33: Calcium cycling in the avian heart - the 'missing link' in vertebrate EC coupling.

Holly Shiels.

University of Manchester, UK.

Bird cardiomyocytes are long, thin and lack t-tubules, similar to ectothermic non-avian reptiles. Paradoxically, birds can achieve greater contractile rates and developed pressures than mammals, whose wide cardiomyocytes contain a dense transverse (t)-tubular network allowing for uniform excitation-contraction coupling and strong contractile force. To address this apparent contradiction, this talk first functionally links recent electrophysiological studies on bird cardiomyocytes with decades of ultrastructure measurements. I then show new data demonstrating that the strong transsarcolemmal Ca^{2+} influx via the L-type Ca^{2+} current (I_{CaL}) and the high gain of Ca^{2+}-induced Ca^{2+} release (CICR) from the sarcoplasmic reticulum (SR), coupled with the internal SR Ca^{2+} release relay system, facilitates the strong fast contractions in the long thin bird cardiomyocytes, without the need for t-tubules. The talk will end with speculation on maintenance of the elongated myocyte morphology following the post-hatch transition from ectothermy to endothermy in birds in relation to cardiac load, myocyte ploidy level, and the cardiac regeneration potential of adult cardiomyocytes. Overall, the talk highlights how little we know about cellular Ca^{2+} dynamics in the bird heart and suggests how increased research efforts in this area would provide vital information for our quest to understand the role of myocyte architecture in the evolution of the vertebrate heart.

O34: Characterization of a candidate inositol 1,4,5-trisphosphate receptor (IP_3R) in *Phytophthora infestans*- an oomycete pathogen causing Potato Late Blight

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Potato late blight, caused by the oomycete pathogen *Phytophthora infestans*, is the most notorious plant disease known, that caused the Great Irish Famine and mass population decline in Ireland and much of Europe. *P. infestans* continue to wreak havoc on potato cultivations world-wide, currently causing about $10 billion per annum on crop loss and application of fungicides. Changes in EU legislation have banned the use of most fungicides and resistance developed by the pathogen makes it an immediate need to develop new approaches to tackle this disease. In this study, we aimed to enhance understanding of the *P.infestans* biology and to exploit its distinctive Ca^{2+} signalling mechanisms as a target for development of anti-oomycete fungicides. Cytoplasmic Ca^{2+} plays a critical role in controlling oomycete physiology. For example, zoospores are a motile stage of oomycetes, which contribute to the propagation of infection. Alteration of Ca^{2+} concentrations in the extracellular environment, or application of the voltage gated Ca^{2+} channel blockers, modifies the swimming patterns of zoospores, indicating roles for Ca^{2+} channels in the spread of this disease.

The second messenger inositol 1,4,5-trisphosphate (IP_3), is a molecule that couples extracellular stimuli to increases in cytoplasmic Ca^{2+}. Putative oomycete IP_3 receptor (ITPR) homologues were identified by searching conceptual translation of oomycete genomes with the protein sequence of human type 1 inositol 1,4,5-trisphosphate receptor (ITPR1), using the basic local alignment of sequences tool (BLAST) in the PubMed. All oomycete genomes investigated contain at least one ITPR homologue, including a single representative in *P.infestans*. We aimed to characterize this candidate ITPR homologue by biochemically isolating it from the membranes of *P. infestans* hyphae (cultured in liquid pea broth). The protein was extracted from the hyphae by grinding in liquid nitrogen followed by solubilization using the detergent Triton-X-100 in Tris HCl pH7.5 buffer containing EDTA and DTT. The solubilized protein from the hyphal mats were treated with IP_3-tagged biotin and subjected to affinity isolation using streptavidin coated magnetic beads. Positive controls were solubilized mouse brain tissue (rich in ITPR) and negative controls were solubilized hyphal samples treated with excess unlabelled IP_3 just prior to capture with IP_3-biotin. Among the IP_3-biotin isolated macromolecules, a 375kDa protein was identified by 7.5% reducing SDS-PAGE and Coomassie Blue R250 staining: this apparent molecular weight is consistent with that of the candidate *P. infestans* ITPR homologue. The identities of eluted proteins are currently being verified by in-gel trypsin digestion followed by LC-MS/MS and Western blotting using affinity-purified rabbit polyclonal antisera against synthetic peptides from *P. infestans* ITPR protein. These analyses will give insights into the candidate ITPR homologue in *P. infestans,* and interacting proteins associated with the receptor. Some of the distinctive properties of IP_3-mediated signalling in *P.infestans* indicate that it has the potential for development of new, oomycete-selective fungicides.

O35: SOCE shapes developmental gene expression in dopaminergic neurons of the *Drosophila* flight circuit

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Regulation of neuronal gene expression through activity-driven transcription of immediate-early genes has been well documented[1]. Neuronal Ca^{2+} signals, with slower altered dynamics, occur upon stimulation of metabotropic receptors followed by IP_3-mediated intracellular Ca^{2+} release and store-operated Ca^{2+} entry (SOCE). An understanding of how such IP_3/Ca^{2+} signals regulate gene expression is lacking in part because a Ca^{2+} responsive NFAT ortholog is absent in *Drosophila*. A series of transcriptomic screens in *Drosophila* neurons[2,3] helped identify gene families whose expression is regulated by the IP_3R and key components of SOCE. IP_3/Ca^{2+} signals and SOCE driven neuronal gene expression functions in different contexts ranging from the larval to pupal transition[2], flight circuit maturation[3] and others[4]. We have now investigated putative transcriptional mechanisms by which IP_3/Ca^{2+} signalling and SOCE regulate the expression of genes in pupal and adult neurons in the context of flight. In a recent study, we show that Set2, the Histone 3 lysine 36 methyltransferase forms part of a transcriptional feedback loop in a larval glutamatergic neuron subset to regulate the larval to pupal transition upon nutrient stress[5]. Here we investigate the role of Set2 in a subset of pupal and adult dopaminergic neurons required for maturation and function of the flight circuit. To bridge the gap between Ca^{2+} signals and expression of specific genes, including *Set2*, we used motif enrichment analysis across the upstream regions of genes targeted by SOCE and identified potential binding sites for a transcription factor (TF). We identify a critical developmental window wherein a novel signalling axis acts downstream of SOCE in a set of dopaminergic neurons. This pathway targets expression of specific voltage-gated ion-channel genes and thereby regulates neuronal excitability and affects flight. In summary, these findings identify a Ca^{2+} responsive TF in *Drosophila* neurons that amplifies changes in gene expression profiles through an activating histone modifier.

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O36: Calcium oscillations in cancer cells

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O37: How calcium can be used to treat cancer

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O38: BAPTA directly inhibits PFKFB3, thereby impeding mTORC1-driven Mcl-1 translation and killing Mcl-1-addicted cancer cells

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Intracellular calcium (Ca2+) signals control a plethora of physiological and pathophysiological processes. The main tool available and used worldwide, to chelate intracellular Ca2+ with rapid kinetics and high affinity is intracellular BAPTA (BAPTAi), which is usually introduced into cells as a membrane-permeant acetoxymethyl ester (BAPTA-AM). We previously demonstrated that loading cells with BAPTA (via incubation with BAPTA-AM) synergistically enhanced cell death induced by venetoclax, a selective Bcl-2 antagonist, in Bcl-2-dependent cancer cell models such as diffuse large B-cell lymphoma (DLBCL). These findings implied a novel interplay between intracellular Ca2+ signaling and anti-apoptotic Bcl-2 function. Here, we set out to identify the underlying mechanisms by which BAPTAi could enhance cell death. First, we observed that loading cells solely with BAPTAi induced apoptosis in lymphoma cell models that were highly sensitive to S63845, an Mcl-1 antagonist, but not in those resistant to S63845. BAPTAi loading provoked a complete and rapid decline in Mcl-1 protein levels by inhibiting mTOR-driven MCL-1 translation, a process that precedes apoptosis. Overexpression of a non-degradable Mcl-1 variant rescued BAPTAi-induced cell death. We further examined how BAPTAi diminished mTOR activity, which is known to be a nutrient sensor controlling protein synthesis. We found that loading cells with BAPTAi rapidly impaired glycolysis by inhibiting 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) activity, an up to now unappreciated effect of BAPTA in cells. All the aforementioned effects of BAPTAi were also elicited by loading cells with tetrafluoro-BAPTAi, a BAPTA analogue with low affinity for Ca2+. Moreover, EGTAi, a structurally different Ca2+ chelator with similar affinity for Ca2+ as BAPTAi, was less effective in downregulating Mcl-1-protein levels and provoking cell death. Thus, our work reveals PFKFB3 inhibition as an unconventional, Ca2+-independent mechanism by which BAPTAi and its structural analogues affect cellular metabolism and ultimately the survival of Mcl-1-dependent DLBCL cells. Our work has two important implications. First, direct inhibition of glycolysis, including through PFKFB3, emerged as a promising target in cancer treatment. Second, these findings show that cellular effects caused by BAPTAi are not related to Ca2+ signaling. Our data support the need for a reassessment of the role of Ca2+ in cell biological processes when findings were based on the use of BAPTAi.

O39: Uncovering the calcium conversation between breast cancer and the brain microenvironment

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The brain is unique among sites of breast cancer metastases for poor prognosis, limited treatment options and cellular landscape. The interaction between breast cancer and brain cells is thought to contribute to metastatic survival and outgrowth, however, these pathways are incompletely understood. Calcium signalling is deregulated in numerous pathologies, including cancers and their surrounding microenvironment. Recent work has proposed that communication of a calcium signal between breast cancer and the brain may contribute to metastatic survival. However, there is little evidence that such communication does occur in this metastatic setting, due in part to a scarcity of pre-clinical models. To address this, we developed a model suited to high-throughput investigations by combining breast cancer cells (MDA-MB-468), neural matrices consisting of astrocytes and neurons differentiated from human neural progenitor cells (ReNcell VM), and stable expression of calcium sensors GCaMP6m and jRCaMP1b, respectively. Investigations using this model determined that basal calcium activity did not appear to alter in breast cancer or neural cells as a result of co-culture. However, selective activation of calcium influx in breast cancer cells within co-culture resulted in increased calcium activity and significantly higher peak calcium levels in neural matrices ($p < 0.05$). Alterations in calcium activity were not observed in monoculture neural cells treated with the same activator. Furthermore, calcium activity in neural cells in co-culture occurred following a period of delay after agonist addition, suggesting the communication of a calcium signal from breast cancer to the brain. To further characterise this phenomenon, we developed single cell analysis tools to elucidate spatial information of all individual cells in co-culture. Separating responses in neural cells by proximity to breast cancer found a clear relationship between increased neural calcium activity and distance from an activated cancer cell. Collectively, this work provides the first live cell evidence of calcium communication between breast cancer and the brain microenvironment, a novel potential avenue for therapeutic targeting in this disease.

O40: LED light sources for Calcium Imaging

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O41: Control of photoreceptor function by NCS proteins

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Vertebrate photoreceptor cells are exquisite light detectors operating under very dim and bright illumination. These sensory cells achieve their exquisite ability to respond to single photons by a G protein-coupled receptor signaling cascade consisting of protein complexes that can form highly ordered supramolecular structures and control the homeostasis and mutual dependence of the secondary messengers cGMP and Ca^{2+}. Effective phototransduction requires feedback mechanisms, which enable photoreceptor cells to regain their responsiveness after light stimulation. The two most important feedback loops involve two subgroups of neuronal Ca^{2+}-sensor (NCS) proteins, named guanylate cyclase-activating proteins (GCAPs) and recoverins (Rec). Mammalian rod and cone photoreceptor cells express a small number of NCS protein isoforms, while zebrafish (*Danio rerio*) express six different GCAP paralogs in their retina, of which each member exhibits a unique expression and functional profile. The other prominent group of NCS proteins in the zebrafish retina comprises four zRec isoforms (zRec1a, zRec2a, zRec1b, zRec2b corresponding to genes rcv1a, rcv1b, rcv2a and rcv2b, respectively). GCAPs regulate guanylate cyclases in a Ca^{2+}-dependent manner, recoverin or its isoforms control the activity of opsin kinases. Our results indicate diverse recoverin and opsin kinase properties due to differential expression and interaction profiles. In summary, both NCS subclasses contribute to a complex signaling network in rod and cone cells, which is perfectly suited to match the requirements for sensitive cell responses and maintaining this responsiveness in the presence of different background light intensities.

O42: Retinal dystrophies associated with point mutations in guanylate cyclase-activating proteins

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The phototransduction cascade in vertebrates is finely regulated by subtle changes in intracellular Ca^{2+}, which follow the closure of cyclic nucleotide-gated channels. Guanylate cyclase-activating proteins (GCAPs) are neuronal calcium sensors that tune the light sensitivity of rods and cones by regulating membrane-bound guanylate cyclases (GCs) through a Ca^{2+}-mediated feedback mechanism, which makes enzyme activity dependent on intracellular [Ca^{2+}]. Up to three isoforms of GCAPs have been discovered in mammal photoreceptors, and six in teleost fish, thus raising the question of the physiological significance of this apparent redundancy. GCAPs show different affinity for Ca^{2+} and are able to replace Ca^{2+} with Mg^{2+}, thus switching from one signaling state to another. The best-known ubiquitous isoforms are GCAP1 and GCAP2, which are present both in rods and cones and whose biochemical and physiological characteristics in murine and bovine photoreceptors have been profoundly characterized. To date more than 20 mutations in the *Guca1a* gene coding for GCAP1 have been associated with human autosomal dominant cone or cone-rod dystrophies (CORD) and only one relatively rare missense mutation has been found in *Guca1b* coding for GCAP2. Recently, a missense point mutation in *Guca1c*, which encodes for GCAP3, a cone-specific isoform that is not expressed in murine and bovine retinas, has been associated with human retinitis pigmentosa (RP).

In recent years, a comprehensive biochemical and biophysical characterization of human GCAPs in their wild-type and disease-associated variants has been performed, including the role of protein myristoylation, dimerization and Mg^{2+}/Ca^{2+} exchange. Experimental investigations were combined with all-atom molecular dynamics simulations and numerical modelling of photoresponse kinetics. All results confirmed that the GCAP1-GC1 protein complex is the main regulator of the subtle interplay between cGMP and Ca^{2+} in human photoreceptors. GCAP2 does not play a prominent role in human phototransduction, unlike its bovine and murine orthologue, but may be involved in other biological processes yet to be elucidated. Similarly, GCAP3 shows peculiar biochemical properties and does not play an important role in regulating the phototransduction cascade.

Our data suggest that the knowledge assessed for bovine and murine GCAPs may not be directly applicable to human isoforms. In-depth knowledge of the structural and functional effects of point mutations in the GCAP1-GC1 complex may be extremely useful for the design of effective therapies for currently incurable inherited retinal degenerations.

O43: Ca^{2+} sensitivity of the prototypical Neuronal Calcium sensor recoverin is tuned by synergistic protein-protein and protein-membrane interactions

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The prototypical Ca^{2+}-sensor recoverin (Rec) participates in the Ca^{2+}-dependent feedback mechanism responsible for the shutoff of the phototransduction cascade by regulating in a Ca^{2+}-dependent fashion rhodopsin kinase (GRK1). In the dark, when intracellular [Ca^{2+}] in the photoreceptor outer segment is high, Rec prevents the functional interaction between GRK1 and the G protein-coupled receptor (GPCR) rhodopsin, by acquiring a relaxed (R) conformation in which the N-terminal covalently bound myristoyl group is anchored to the membrane, thus allowing the interaction with GRK1. When the intracellular [Ca^{2+}] drops to low nanomolar levels due to photon detection, Rec undergoes a paradigmatic Ca^{2+}-induced conformational change known as "myristoyl switch", acquires a tense (T) conformation where the fatty acid is sequestered in an hydrophobic crevice, and is thus able to freely diffuse in the cytosol, ultimately resulting in the relief of the inhibition of the phosphorylation of rhodopsin by GRK1. Notably, the apparent affinity of myristoylated Rec for Ca^{2+} (K_d^{app} ~17 µM) in vitro is almost two orders of magnitude too low to support the regulation of GRK1 in vivo, since Ca^{2+} levels in photoreceptor outer segments do not exceed 600 nM.

In this study we investigated the individual and synergistic role of the myristoyl group, the disc membrane and the GRK1 target in modulating the Ca^{2+} sensitivity of Rec by applying a combination of experimental and in silico techniques. Spectroscopic analyses highlighted that neither GRK1 nor the disc membrane alone are sufficient to trigger Rec conformational change within the physiological intracellular [Ca^{2+}] range, which can be achieved only in their simultaneous presence. Moreover, the concerted action of membrane and GRK1 allows the T to R transition to occur cooperatively through a conformational selection mechanism, which drives the structural transitions of Rec in the presence of multiple ligands [1].

Extensive, unbiased all-atom molecular dynamics (MD) simulations were employed to investigate the synergistic interaction of Rec with GRK1 and the disc membrane, which allowed us to monitor the spontaneous myristoyl-mediated association of Rec with the membrane both in the absence and in the presence of a peptide from GRK1 [2]. This process was found to be driven by persistent electrostatic protein membrane interactions between the polar heads of phosphatidylserine lipids and two arginine residues, namely R43 and R46, which recruit the N-terminal of Rec in proximity of the membrane, thus facilitating the correct orientation of the myristoyl moiety and its consequent anchoring on the membrane.

Finally, our results suggest that specific membrane composition and allosteric interactions are both necessary for the correct assembly and dynamics of functional Rec-GRK1 complex.

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O44: Conformational dynamics of calcineurin B homologous protein 3

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Calcineurin B homologous protein 3 (CHP3), also known as Tescalcin, is an EF-hand Ca^{2+}-binding protein involved in the regulation of cancerogenesis, stem cell differentiation, cardiac hypertrophy and neuronal development. Regulation of those processes is achieved via CHP3's interactions with sodium/proton exchangers (NHEs) (Fuchs et al. Sci. Rep 2018, Liang et al. Faseb J. 2020) and signalling proteins such as GSK3, subunit 4 of the COP9 signalosome and calcineurin A. CHP3 binds a single Ca^{2+} ion with micromolar affinity suitable for the transduction of intracellular Ca^{2+} signals. In addition to the Ca^{2+}-binding site in the EF-hand 3, CHP3 possesses an N-terminal myristoylation site. Ca^{2+} binding as well as the N-terminal myristoylation of CHP3 were demonstrated to be important for the stabilization of NHE1 at the plasms membrane, although the underlying molecular mechanism remains elusive. Here we will demonstrate with biochemical and biophysical methods that the conformational dynamics and ultimately the function of CHP3 are independently affected by Ca^{2+} binding and N-terminal myristoylation. Ca^{2+} binding induces an open conformation as indicated by the increased hydrophobicity and flexibility of this conformation, as compared to the closed, more rigid but also more stable Mg^{2+}-bound conformation. In the Ca^{2+}-bound conformation, CHP3 affinity to NHE1 is fivefold higher in comparison to the Mg^{2+}-bound state, and CHP3 stronger associates with lipid membranes. The N-terminal myristoylation increases the flexibility of the N-lobe of CHP3 and reduces the CHP3 affinity to NHE1 in a Ca^{2+}-independent manner. Interestingly, it has no effect on the association of the target-free CHP3 with lipid membranes. Taken together, these data exclude the Ca^{2+}-myristoyl switch proposed for CHP3. Instead, binding of a target peptide derived from NHE1 triggers the exposure of the myristic moiety in CHP3 independently of Ca^{2+} binding enhancing CHP3 association with lipid membranes. In conclusion, the interplay of Ca^{2+} binding, N-terminal myristoylation and target binding allows a context-specific regulation of CHP3's functions.

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O45: The Endoplasmic Reticulum (ER) Kinase PERK Mediates Metabolic Adaptation and Ca^{2+} Signaling Using the Oxidoreductase Ero1

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Unfolded proteins inside the endoplasmic reticulum (ER) tighten mitochondria-ER contacts (MERCs). PERK and Ire1 have been implicated in this process. However, how their upstream regulators adapt mitochondrial bioenergetics to a new equilibrium remains obscure. Reactive oxygen species (ROS) could potentially increase such mitochondria-associated membranes (MAMs). Accordingly, the ER chaperone calnexin controls MAMs through ROS derived from the ER oxidoreductase Ero1α and NADPH oxidase 4 (Nox4). We now show that Ero1α enters a protein complex during early ER stress. This complex requires the covalent interaction of the Ero1α C-terminal active site with cysteine 216 of PERK. The PERK-Ero1α complex controls mitochondrial dynamics, ROS levels and oxidation of MERC Ca^{2+} handling proteins. Using novel genetic tools, we determined that this function accelerates Ca^{2+} flux, increases mitochondrial ATP-producing efficiency and restores mitochondrial citrate levels. Therefore, ER stress adapts cellular bioenergetics based on PERK and Ero1α.

O46: The Sir Michael Berridge Lecture

Phosphatidylserine at the ER/PM junctions

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The membrane contact site ER/PM junctions are hub for numerous signaling pathways and are the site at which STIM1-Orai1 clustering and other channels are localized. Phosphatidylserine (PtdSer) mediates many physiological and pathological functions, however, the role of plasma membrane (PM) and ER PtdSer in junctional lipid composition, Ca^{2+} signaling and ion function is not understood. Junctional PtdSer is determined by the lipid transfer proteins ORP5 and ORP8 that exchange ER synthesized PtdSer for PM PI(4)P and by the extended synaptotagmin (E-Syts) 1-3. We will show how ORP5 and ORP8 function as a rheostat that reciprocally regulate the PtdSer/PI(4)P ratio to determine the activity of STIM1-Orai1 and the physiological receptor-evoked Ca^{2+} oscillations. We will also briefly discuss selective effect of the E-Syts on the HCO_3^- transporters, the Cl^- channel CFTR and $1Na^+/2HCO_3^-$ transporter NBCe1-B to show the importance of PtdSer is assembly of protein complexes at the junctions. The findings point to a novel role of PtdSer in Ca^{2+} signaling and the diversity of the ER/PM junctions.

O47: Calcium regulation of organellar function

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In plants, Ca^{2+} signaling in form of transient changes in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) has been shown to occur in response to many environmental and developmental stimuli such as light, touch, pathogenic elicitors, plant hormones, heat, cold, high salinity and drought. Changes in $[Ca^{2+}]_{cyt}$ are recognized by a toolkit of Ca^{2+} sensor proteins that convert? the signal into a cellular response via interaction with various target proteins, thus regulating cellular processes such as metabolic pathways, ion transport, protein transport, protein phosphorylation or gene expression.

Organellar biogenesis and function has to be carefully balanced in accordance with the developmental stage and metabolic requirements of the plant. To that end, organelles such as chloroplasts are tightly integrated into the signaling networks and regulatory circuits of the cell. Ca^{2+}-binding proteins, Ca^{2+}-dependent phosphorylation and Ca^{2+}-dependent enzyme activation/deactivation have been shown for chloroplasts.

It is also well known that stimuli-induced Ca^{2+} signals occur in chloroplasts. Studies performed with genetically encoded Ca^{2+} sensors targeted to the chloroplast stroma and the thylakoid lumen have revealed cytosol-dependent and autonomous plastidial Ca^{2+} fluxes in response to different environmental stimuli. While less well studied, it has furthermore become evident that this important regulatory pathway also exists in other plant organelles such as mitochondria, the nucleus and the endomembrane system.

O48: *In vivo* calcium dynamics in plant cells: a holistic view

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Permanently restricted to their site of germination, plants evolved mechanisms to react to ever- changing environmental conditions and harmful stresses. Local damage triggers inducible defence mechanisms that are often induced systemically in organs that are not damaged yet. Fast systemic responses are mediated by long-distance signalling that require the activity of Glutamate Receptor- Like channels (GLRs). GLRs are homologs of animal Ionotropic Glutamate Receptors (iGluRs) which are ligand-gated cation channels in the central nervous system. Even though iGluRs are gated through the binding with the L-Glutamate, the mechanism throughout GLRs is activated *in planta* is poorly understood. As an example, we still do not know if the GLRs binding of amino acids is necessary for their activity. Here we took the advantage of the recently obtained crystal structure of the *Arabidopsis thaliana At*GLR3.3 Ligand Binding Domain (LBD) to identify residues involved in the amino acid-binding. We, therefore, introduced single point mutations in the genomic sequence of the *At*GLR3.3 gene to prevent or abolish its amino acid-binding, and with the obtained constructs we complemented the *glr3.3* KO. By combining high-end imaging, genetics, we show that leaf injury, such as wound and burn, and root-applied hypo-osmotic stress induce the systemic apoplastic increase of L-Glutamate that activates GLR channels through their LBD. In addition, our work supports the evidence that in response to mechanical stress long-distance signalling is governed by a systemic change in the turgor state and that GLRs are downstream of it.

O49: Pharmacological modulation of TPC1 regulates inter-organellar Ca^{2+} homeostasis in immune cells and plays an important role in allergic hypersensitivity

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Mast cells and basophil granulocytes play an essential role in anaphylaxis and allergic reactions by releasing inflammatory mediators such as histamine and heparin. The cytoplasm of these innate immune cells contains a large number of granules comprising these messenger substances. We recently linked the endolysosomal two-pore channel TPC1 to systemic anaphylaxis *in vivo* and underlying mast cell function *ex vivo* [1]. TPC1-deficient mice developed enhanced systemic anaphylaxis, reflected by a drop in body temperature and slower recovery compared to wild-type animals. Genetic deletion or pharmacologic inhibition of TPC1 enhanced mast cell degranulation and histamine release owing to accelerated Ca^{2+} liberation. Signaling in mast cells is mainly regulated *via* the release of Ca^{2+} from the endoplasmic reticulum (ER) as well as from acidic compartments such as endolysosomes. Stimulation of TPC1 channel activity by one of its endogenous ligands namely nicotinic adenine dinucleotide phosphate (NAADP) or phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$) triggered the release of Ca^{2+} from the endolysosomes, thereby improving the effect of TPC1 on regulated mast cell degranulation. Apart from these important insights into TPCs physiology [2], there is a lack of ultrastructural knowledge that underlies these processes. We have therefore implemented 2D and 3D transmission electron microscopic (TEM) methods to investigate the ultrastructure of rat basophilic leukemia cells (RBL-1), treated with or without the plant alkaloid and TPC inhibitor tetrandrine. Our 2D TEM investigations with RBL-1 controls depicted that ER and endolysosomes formed inter-organellar contact sites. Moreover, 3D TEM tomography revealed the full extent of the large contact surfaces between the two organelles. In comparison, these contact surfaces significantly decreased in cells, treated with tetrandrine, further supporting the hypothesis that TPC function is essential for inter-organellar Ca^{2+} exchange. Here, we aim at a better understanding of the role of TPC channels in the regulation of the crosstalk between ER and endolysosomes at an ultrastructural level. Correlating our physiological and ultrastructural findings with analytical EM, as well as with further Ca^{2+} imaging, molecular biological and immunological experiments could help clarify whether TPC channels are indeed promising pharmacological targets for the treatment of allergic hypersensitivity.

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O50: Identification and functional characterization of a novel TRPM7 mutation associated with trigeminal neuralgia

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Trigeminal neuralgia (TN) is a unique pain disorder in which affected individuals experience intense paroxysmal pain in the territory of the trigeminal nerve. Although most cases of TN are sporadic, occurrence of familial TN suggests a genetic contribution to this disorder. Whole-exome sequencing analysis carried out in patients with TN and reporting positive family history of TN revealed multiple variants of ion channels including TRP channels.

Here we used Ca^{2+} and Na^{+} imaging and whole-cell patch clamp to assess a variant in the TRPM7 channel kinase, p.Ala931Thr, found in a man suffering from unilateral TN.

We found that A931T produced dramatic changes of TRPM7 channel properties. The current-voltage relationship of A931T was significantly different from WT TRPM7, with 20-fold higher inward currents than TRPM7 WT and unchanged outward currents. The inward current recorded in A931T had the following properties: i) it was carried by Na^{+} ions under physiological conditions; ii) it was completely inhibited by the TRPM7 antagonist NS8593, but was insensitive to the pore blocker Gd^{3+}; iii) it was observed when Na^{+} was replaced by Guanidinium, but it was completely suppressed by application of $NMDG^{+}$.

Our results support the notion that A931T, located in the S3 segment at the interface with the voltage-sensing transmembrane region S4, generates an omega current that carries Na^{+} influx in physiological conditions. A931T mutation may have consequences in the trigeminal axonal integrity since small persistent Na^{+} currents are known to trigger reverse Na^{+}/ Ca^{2+} exchange that can lead to increased intracellular Ca^{2+} and predispose axons to time-dependent injury.

O51: jECS Short Talk 1, to be selected at the jECS Satellite Meeting (20th August)

O52: jECS Short Talk 2, to be selected at the jECS Satellite Meeting (20th August)

O53: Structural dynamics of IP_3R underlying its gating and regulation

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Inositol-1,4,5-trisphosphate receptors (IP_3Rs) are activated by IP_3 and Ca^{2+} and their gating is regulated by various intracellular messengers that finely tune the channel activity that gives rise to many complex physiological processes from muscle contraction to the more enigmatic neuronal activities of the CNS such as learning and memory. In this work, we determined the cryo-EM structures of the IP_3R channel trapped in physiologically relevant states using key ligands that target channel gating. These near-atomic resolution structures provide unprecedented details governing binding of IP_3, Ca^{2+} and ATP in IP_3R1, revealing conformational changes that couple ligand-binding to channel opening. Using a deep learning approach and 3D conformational variability analysis, we extracted dynamic properties of the key protein domains. We found that IP_3 binding relies upon intrinsic flexibility of the cytoplasmic ARM2 domain. The atomic fluctuations identified in the constriction region at the gate suggest a modal gating mechanism of the channel opening. Our study highlights a key role of side chain dynamics of specific residues in regulation of IP_3R functions such as gating and ligand binding. Our structural findings and hypothesis were validated through mutagenesis and electrophysiology. From these studies, we correlate protein conformational changes that connect the ligand-binding regulatory sites to the channel gate. Our study provides a structural framework for understanding the allosteric mechanisms underlying ligand-mediated IP_3R activation and regulation.

O54: Segregated cation flux by TPC2 biases Ca^{2+} signalling through lysosomes

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Two-pore channels are endo-lysosomal cation channels with malleable selectivity filters that drive endocytic ion flux and membrane traffic. Here I discuss recent data showing that TPC2 differentially regulates its cation permeability when co-activated by its endogenous ligands, NAADP and PI(3,5)P_2. Whereas NAADP rendered the channel Ca^{2+}-permeable and PI(3,5)P_2 rendered the channel Na^{+}-selective, a combination of the two increased Ca^{2+} but not Na^{+} flux. Mechanistically, this was due to an increase in Ca^{2+} permeability independent of changes in ion selectivity. Functionally, cell permeable NAADP and PI(3,5)P_2 mimetics synergistically activated native TPC2 channels in live cells, globalizing cytosolic Ca^{2+} signals and regulating lysosomal pH and motility. This data shows that flux of different ions through the same pore can be independently controlled and identifies TPC2 as a likely coincidence detector that optimizes lysosomal Ca^{2+} signaling.

O55: TMBIM5 loss of function alters mitochondrial matrix ion homeostasis and causes a skeletal myopathy

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Ion fluxes across the inner mitochondrial membrane control mitochondrial volume, energy production, and apoptosis. TMBIM5, a highly conserved protein with homology to putative pH-dependent ion channels, is involved in the maintenance of mitochondrial cristae architecture, ATP production, and apoptosis. Here, we demonstrate that overexpressed TMBIM5 can mediate mitochondrial calcium uptake. Under steady-state conditions, loss of TMBIM5 results in increased potassium and reduced proton levels in the mitochondrial matrix caused by attenuated exchange of these ions. To identify the in vivo consequences of TMBIM5 dysfunction, we generated mice carrying a mutation in the channel pore. These mutant mice display increased embryonic or perinatal lethality and a skeletal myopathy which strongly correlates with tissue-specific disruption of cristae architecture, early opening of the mitochondrial permeability transition pore, reduced calcium uptake capability, and mitochondrial swelling. Our results demonstrate that TMBIM5 is an essential and important part of the mitochondrial ion transport system machinery with particular importance for embryonic development and muscle function.

Abstracts of Poster Presentations

P1: Computational re-estimation of the IP_3 diffusion coefficient

Roberto Ornelas-Guevara, Diana Gil, Valérie Voorsluijs and Geneviève Dupont

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Inositol 1,4,5-trisphosphate (IP_3) is a second messenger responsible for the release of intracellular Ca^{2+} ions from internal stores. This molecule has long been considered a global messenger with a diffusion coefficient ≈ 280 $\mu m^2/s$ in a water-like medium (Allbritton et al., 1992). This value was however re-estimated on the basis of the dynamics of local IP_3-evoked Ca^{2+} puffs triggered by IP_3 diffusing from a spot of photorelease (Dickinson et al., 2016). By combining these observations with a theoretical analysis of Ca^{2+} puff latencies, the authors calculated that IP_3 diffuses 30-fold slower than previously reported, implying that it should be considered as a local rather than a global messenger. Ineffective IP_3 binding to partially bound IP_3 receptor tetramers is thought to be responsible for the slowing down of IP_3 diffusion in intact cells (Taylor and Konieczny, 2016), although the reduction does not match quantitatively with current knowledge.

In this work we used stochastic modelling of IP_3R cluster dynamics (Voorsluijs et al., 2019) to re-examine this question. We performed sophisticated 2D and 3D spatiotemporal simulations in realistic cellular geometries, using COMSOL Multiphysics.

Simulations concluded that best agreement with the experimental observations of Dickinson et al (2016) is obtained with an IP_3 diffusion coefficient of about 100 $\mu m^2/s$, a value that is moreover practically unaffected by the presence of the ER.

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P2: Relationship between moderate ER Ca^{2+} depletion and induction of the unfolded protein response (UPR): experimental and modeling approach

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The Endoplasmic Reticulum (ER) is the primary site of folding and quality control of one third of cellular proteins and is the major intracellular Ca^{2+} store. Depletion of the luminal [Ca^{2+}] disrupts the correct folding environment leading to an alteration of ER homeostasis and accumulation of misfolded proteins inside the lumen. In order to restore ER proteostasis and normal cellular functions, cells have developed an adaptive mechanism consisting in 3 specific signalling pathways. This response is commonly referred to as the Unfolded Protein Response (UPR) and leads to an increase of the protein folding capacity of the ER and to homeostasis restoration. Although long-term and strong UPR activation is much studied, the consequences of small amplitude, more physiological, luminal Ca^{2+} depletions on the early activation of UPR has been largely unexplored. In this study, we investigate how moderate Ca^{2+} depletion impacts on the activation of the signalling pathways of the UPR. Ca^{2+} imaging experiments using genetically encoded Ca2+ indicators targeting the ER combined with Immunoblots, qPCR and imaging data allow us to reveal the early links between ER Ca^{2+} depletion and UPR activation. The concomitant development of a data-driven computational model allows us to decipher, formalize, and quantify these complex signaling pathways. Given that luminal Ca^{2+} depletion and alteration of correct ER proteostasis are involved in a variety of pathologies such as diabetes, neurodegenerative diseases or cancer, a better understanding of the reciprocal crosstalk between Ca^{2+} and UPR will provide insight into the mechanisms of progression of these diseases.

P3: Blockade of mitochondrial calcium uptake protects neurons against ferroptosis

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Introduction. Ferroptosis is an iron- and reactive oxygen species (ROS)-dependent form of regulated cell death, that has been implicated in Alzheimer's disease. Initiation of ferroptosis via cysteine/glutamate antiporter inhibition leads to mitochondrial fragmentation, mitochondrial calcium (Ca^{2+}) overload, increased mitochondrial ROS production, disruption of the mitochondrial membrane potential ($\Delta\Psi_m$) and cell death. Recent studies showed that mitochondrial dysfunction is a characteristic of ferroptosis, that makes preservation of mitochondrial function a potential therapeutic target in degenerative diseases. Mitochondrial calcium levels are controlled via the mitochondrial calcium uniporter (MCU), the main entry point of Ca^{2+} into the mitochondrial matrix. Therefore, we have hypothesized that inhibition of mitochondrial calcium uptake may confer protection against ferroptosis.

Objectives. To investigate the anti-ferroptotic potential of mitochondrial calcium uptake inhibition in conditions of increased oxidative stress.

Methods. In the present study we employ the HT22 murine hippocampal cell line, to model ferroptosis and oxytosis. To evaluate the potential protective capacity of targeting of the mitochondrial calcium uniporter against ferroptosis induced by erastin, RSL3 or glutamate, we pharmacologically targeted i) MCU with ruthenium red (RR), mitroxantrone (MX) and Ru265; and ii) MICU1, a regulator of the pore function of MCU, with MCU-i4. We measured hallmarks of ferroptosis, namely mitochondrial function, mitochondrial morphology, calcium uptake, mitochondrial ROS production and lipid peroxidation.

Results. Blocking the activity of MCU significantly reverted the calcium uptake, lipid peroxidation, and mitochondrial ROS that was initiated by erastin and RSL3 challenge, measured by flow cytometry. Co-treatment with RR, MX, Ru265 and MCU-i4 prevented erastin-, and RSL3-, and glutamate-induced cell death, as detected by bright-field microscopy, MTT assay and flow cytometry in a concentration-dependent manner. Additionally, ferroptosis impaired mitochondrial function decreasing the oxygen consumption rate.

Conclusions. An *in vitro* model of ferroptosis was employed to test the capacity of MCU inhibition to protect neurons against cell death. Taken together, our results demonstrate that MCU antagonism and mitochondrial calcium reduction is protective against ferroptosis. In conclusion, this study provides the foundation for further investigation into the therapeutic potential of MCU inhibition against ferroptosis or MICU1 deficiencies.

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P4: Orai1 Inhibitors as Potential Treatments for Pulmonary Arterial Hypertension

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Rationale: Pulmonary arterial hypertension (PAH) is characterized by progressive distal pulmonary artery (PA) obstruction, leading to right ventricular hypertrophy and failure. Exacerbated intracellular calcium (Ca^{2+}) signaling contributes to abnormalities in PA smooth muscle cells (PASMCs), including aberrant proliferation, apoptosis resistance, exacerbated migration, and arterial contractility. Store-operated Ca^{2+} entry (SOCE) is involved in Ca^{2+} homeostasis in PASMCs, but its properties in PAH are unclear.

Methods: Using a combination of Ca^{2+} imaging, molecular biology, in vitro, ex vivo, and in vivo approaches, we investigated the roles of SOCE channel Orai1 in PA remodeling in PAH, and determined the consequences of pharmacological Orai1 inhibition in vivo using experimental models of pulmonary hypertension (PH).

Results: Human PASMCs (hPASMCs) from patients with PAH (PAH-hPASMCs) showed upregulation of SOCE and Orai1 expression, in which ERK1/2, nuclear factor of activated T cells (NFAT), and nuclear factor-kappa B (NFκB) were contributing factors. Using siRNA and two Orai1 inhibitors, we found that Orai1 inhibition reduced SOCE, mitochondrial Ca2+ entry, aberrant proliferation, apoptosis resistance, migration, and excessive calcineurin activity in PAH-hPASMCs. Orai1 inhibitors reduced agonist-evoked constriction in human PAs. In experimental rat models of PH evoked by chronic-hypoxia or monocrotaline or Sugen/hypoxia, administration of Orai1 inhibitors (BTP2, JPIII, or 5J4) protected against PH.

Conclusions: In human PAH and experimental PH, Orai1 expression and activity are increased. Orai1 inhibition normalizes the PAH-hPASMC phenotype and attenuates PH in rat models, suggesting that Orai1 should be considered as a relevant therapeutic target for PAH.

P5: Communication is key: Investigating calcium crosstalk in the breast tumour microenvironment

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Breast cancer is a devastating disease that continues to take the lives of more than 650,000 women worldwide each year. Despite major advances in the molecular characterization of the disease, treatment options remain inadequate and therapy is often thwarted by resistance and metastasis. To develop effective, long-lasting therapies for breast cancer, we must continue to widen our tumour-centric approach to drug discovery and consider targeting interactions between cancer cells and the key, non-malignant cell types in the microenvironment.

Cancer-associated fibroblasts (CAFs) play a major role in steering breast cancer development and dissemination. These cells can arrange themselves to envelop and compartmentalize solid tumours, a phenomenon that has been linked to increased intratumoural pressure and a more aggressive tumour phenotype. Whilst calcium signals have independently been linked to both cellular contraction and cellular crowding, the role of calcium signaling in fibroblast-tumour interactions in this context has yet to be investigated.

By generating fibroblasts that express a fast, green-shifted genetically-encoded calcium indicator (GECI), we are able to explore whether fibroblast contractions are calcium signal-dependent and how this signal may help to coordinate CAFs as the dominant stromal cell type in breast cancer. Furthermore, by creating cancer cells that express red-shifted GECIs, we have been able to generate a multi-compartment co-spheroid model for the spatiotemporal evaluation of calcium signal crosstalk. By understanding and interrupting this cellular crosstalk, this research may open new avenues for therapeutic intervention in breast cancer and may help to define the next generation of breast cancer therapies.

P6: Mitochondrial calcium signaling regulates skeletal muscle homeostasis in adulthood and aging

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The second messenger Ca2+ regulates a broad repertoire of cellular processes. Upon physiological stimuli, skeletal muscle mitochondria rapidly and efficiently accumulate Ca2+ into their matrix via an electrogenic pathway, that relies on the driving force of a steep electrochemical gradient. A large [Ca2+]mt peak occurs dynamically in parallel to agonist-induced [Ca2+]cyt increases, thanks to the activity of the Mitochondrial Calcium Uniporter (MCU), the highly selective channel responsible for mitochondrial Ca2+ accumulation. MCU positively regulates myofiber size in physiological conditions, and counteracts pathological loss of skeletal muscle mass. We have previously demonstrated that skeletal muscle-specific MCU deletion inhibits mitochondrial Ca2+ uptake, impairs muscle force and exercise performance. Mitochondrial Ca2+ uptake is required for effective glucose oxidation, as demonstrated by the fact that, in MCU-/- myofibers, oxidative metabolism is impaired and glycolysis rate is increased. The decreased pyruvate dehydrogenase (PDH) activity is the main trigger of this metabolic rewiring. Nonetheless, mitochondrial activity is partially sustained by increased fatty acid (FA) oxidation. Recently, we have further investigated the MCU-PDH axis in skeletal muscle as potential pharmacological target during adulthood and aging.

P7: Identification and functional characterization of a novel TRPM7 mutation associated with trigeminal neuralgia

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Trigeminal neuralgia (TN) is a unique pain disorder in which affected individuals experience intense paroxysmal pain in the territory of the trigeminal nerve. Although most cases of TN are sporadic, occurrence of familial TN suggests a genetic contribution to this disorder. Whole-exome sequencing analysis carried out in patients with TN and reporting positive family history of TN revealed multiple variants of ion channels including TRP channels.

Here we used Ca^{2+} and Na^{+} imaging and whole-cell patch clamp to assess a variant in the TRPM7 channel kinase, p.Ala931Thr, found in a man suffering from unilateral TN.

We found that A931T produced dramatic changes of TRPM7 channel properties. The current-voltage relationship of A931T was significantly different from WT TRPM7, with 20-fold higher inward currents than TRPM7 WT and unchanged outward currents. The inward current recorded in A931T had the following properties: i) it was carried by Na^{+} ions under physiological conditions; ii) it was completely inhibited by the TRPM7 antagonist NS8593, but was insensitive to the pore blocker Gd^{3+}; iii) it was observed when Na^{+} was replaced by Guanidinium, but it was completely suppressed by application of $NMDG^{+}$.

Our results support the notion that A931T, located in the S3 segment at the interface with the voltage-sensing transmembrane region S4, generates an omega current that carries Na^{+} influx in physiological conditions. A931T mutation may have consequences in the trigeminal axonal integrity since small persistent Na^{+} currents are known to trigger reverse Na^{+}/ Ca^{2+} exchange that can lead to increased intracellular Ca^{2+} and predispose axons to time-dependent injury.

P8: Parallel regulation of IP_3 receptors by IP_3 and PIP_2

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While the majority of IP_3 receptors (IP_3Rs) in cells are mobile, localised Ca^{2+} puffs originate from a minor fraction of immobile IP_3Rs tethered to actin by KRas-induced actin-interacting protein (KRAP). These 'licensed' IP_3Rs are immobilised near contact sites between the endoplasmic reticulum and the plasma membrane (PM), where store-operated Ca^{2+} entry (SOCE) takes place. Uncovering the mechanisms that govern IP3R licensing is essential for understanding the spatial and temporal patterns of Ca^{2+} signalling. Phosphatidylinositol 4,5-bisphosphate (PIP_2) is a minor, but functionally diverse component of the PM which acts as the source of IP_3 in phospholipase C (PLC)- mediated Ca^{2+} signalling through IP_3Rs. Alongside its canonical role as IP3 precursor, PIP_2 is further involved in actin polymerisation beneath the PM and SOCE complex formation – processes which could relate PIP_2 to IP_3R licensing beyond IP_3 production. To address this possibility, we optimised and functionally characterised a rapamycin-inducible heterodimerisation tool for selective PIP_2 depletion at the PM. We show that selective PIP2 depletion at the PM reduces the frequency of Ca^{2+} puffs evoked by photolysis of caged IP_3 in HeLa cells without affecting puff amplitude or kinetics. As PIP_2 depletion may lead to reduction of basal IP_3 levels, we employ two complementary approaches to assess whether a loss of basal IP_3 is responsible for the reduced Ca^{2+} puff frequency. Reducing basal IP_3 levels by inhibiting PLC activity with U73122 or by overexpressing cytosolic IP_3 kinase C does not reduce the frequency of Ca^{2+} puffs evoked by photolysis of caged IP_3. We conclude that IP_3Rs are regulated by both PIP_2 and IP_3.

P9: A new reversible fluorescent probe to detect ER-mitochondria contact site dynamicity.

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Intracellular Ca2+ signalling has been extensively associated with domains of close proximity between ER and mitochondrial membranes (MAMs). MAMs have been proven to be crucial for the regulation of a series of fundamental cellular pathways among which cell death, autophagy, inflammasome activation and ER stress. Moreover, dysregulations at the level of these domains have been so far described in different pathologies, such as neurodegenerative and metabolic disorders and cancers. Thus, the need to study MAMs from both structural, i.e., ER-mitochondria contact sites, and functional points of view is urgent. To date, several efforts have been made to assess the visualization of organelle contacts, still failing to find a probe able to mark them in a clear and dynamic way in living cells [1]. At this purpose, we have developed and characterized a new reversible fluorescent probe to detect ER-mitochondria proximity, able to differentiate between very close contacts (short form: ≈10 nm) and wider ones (long form: ≈25 nm). The probe relies on splitFAST, a previously reported chemogenetic system to detect dynamic protein-protein interactions [2]. Stable HeLa clones have been created to express, in an inducible way, the short or the long form of the new probe. Importantly, the probe can be visualized as green or red thanks to the possibility to add different fluorogens for its fluorescence reconstitution. This feature could easily match with different types of applications and needs. We have proven the reversibility of our probe by measuring ER-mitochondria contacts during time, upon tunicamycin-induced ER stress. We detected a short term increase in organelle contacts, as already reported [3], followed, at later time, by a strong reduction of them. Moreover, the removal of the stressor from the cell media fully restored basal physiological organelle contact sites. Altogether, our results show the multiple potentialities of the novel probe and open new avenues in the dynamic investigation of organelle coupling.

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P10: Induction of cell death by a peptide corresponding to amino acids 2078-2098 of IP_3R1

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Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are channels located on the endoplasmic reticulum (ER) that after stimulation release Ca^{2+} ions. Cells have evolved complex mechanisms to control these Ca^{2+} signals, including by regulation of the IP_3R by interacting proteins. An experimental approach to explore the functional significance of IP_3R-interacting proteins is to identify their binding site on the IP_3R and use the corresponding sequence to synthesize a decoy peptide that can be delivered to intact cells. For instance, it was shown that the Lck tyrosine kinase binds to the type 1 IP_3R (IP_3R1) at a.a. 2078-2098. The synthetic peptide based on this sequence, referred as D5SD (Domain 5 Subdomain), functionally disturbs the Lck:IP_3R1 interaction and represses T cell receptor-mediated Ca^{2+} elevation (Harr et al., 2009, J. Biol. Chem. 284:31860).

Recently, we showed that D5SD can also act as a decoy peptide to displace from IP_3R the glycolytic enzyme pyruvate kinase M2 (PKM2), which is upregulated in cancer cells (Lavik et al., 2022, Biochim. Biophys. Acta 1869:119206). Here, we report that TAT-D5SD, a cell-permeable version of D5SD, induces cell death in a panel of hematological malignancies derived from T-cell and B-cell lineages, in which B-cells were substantially more sensitive to cell death caused by TAT-D5SD when compared to T-cell lymphomas and leukemia models. TAT-D5SD also displayed a significant cytotoxic effect on primary human chronic lymphocytic leukemia (CLL) and acute leukemia (AML) cancer cell lines. Using Jurkat T cells, we showed that TAT-D5SD elevates mitochondrial Ca^{2+} levels in a concentration-dependent manner. The mitochondrial Ca^{2+} uptake correlates with a decrease in mitochondrial membrane potential and with cell death. In the MEC1 B-CLL line, we showed that buffering intracellular Ca^{2+} using the cell-permeable form of BAPTA significantly reduces the cytotoxicity of TAT-D5SD.

To verify whether the disruption of the PKM2:IP_3R interaction by TAT-D5SD correlates with its cytotoxic effect, we compared Ca^{2+} signaling and cell death in HeLa WT, HeLa IP_3R 3KO (completely devoid of IP_3Rs), and HeLa PKM2KO cells (devoid of PKM2). TAT-D5SD similarly increased cytosolic Ca^{2+} levels in the HeLa WT and HeLa PKM2KO, while no elevations in cytosolic Ca^{2+} were observed in HeLa IP_3R 3KO cells. In accordance with these results, the TAT-D5SD peptide significantly increased the number of apoptotic HeLa WT and HeLa PKM2KO cells, while, the HeLa IP_3R 3KO cells were highly resistant to TAT-D5SD.

Together, these results indicate that TAT-D5SD induces cell death in a variety of hematological malignancies, including highly proliferative B-cells and acute myeloid cells. The cytotoxic effect of TAT-D5SD is dependent on IP_3R activity, but does not necessarily rely on PKM2 (or on Lck, which is not expressed in HeLa cells). The precise mechanism by which TAT-D5SD leads to IP_3R-dependent Ca^{2+} signals and cell death is yet to be fully established.

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P11: The Contribution of Annexin V to Mitochondrial Ca^{2+} Uptake

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Annexins are a family of 12 proteins playing an important role in apoptosis, membrane repair mechanism, and intracellular Ca^{2+} homeostasis. Among other annexins, one of the characterized functions of Annexin V (AnxV) is the ability to bind negatively charged phospholipids in Ca^{2+} dependent manner. Accordingly, upon cell membrane rupture, the Ca^{2+} level elevates in the cytosol which leads to translocation of AnxV to the ruptured area to fix the cell membrane. In addition to the cell membrane, several studies also demonstrated the translocation and localization of AnxV to mitochondria. However, the function of AnxV in the mitochondria is not clear yet.

Here, we investigate the contribution of AnxV to mitochondrial Ca^{2+} homeostasis by using various genetically encoded Ca^{2+} sensors targeted either in the cytosol, the intermembrane space (IMS), the mitochondrial matrix, or cristae lumen (CL). Our dynamic Ca^{2+} measurements revealed that CRISP/Cas9-mediated knockout (KO) of AnxV severely decreases mitochondrial Ca^{2+} elevations in IMS, the CL, and in the matrix upon stimulation of cells with an IP_3-generating agonist. Moreover, the cytosolic Ca^{2+} levels and mitochondrial membrane potential were not affected in AnxV-KO cells. Hence, mitochondrial-associated membranes (MAMs) remained the same in AnxV-KO cells indicating the direct involvement of AnxV in mitochondrial Ca^{2+} regulation without affecting the mitochondria-endoplasmic reticulum (ER) contact sites. Additionally, we demonstrate that AnxV is essential for mitochondrial Ca^{2+} uptake upon Ca^{2+} release from the ER but does not play a role in store-operated Ca^{2+} entry (SOCE). Our preliminary results indicate that AnxV regulates mitochondrial Ca^{2+} uptake via Voltage-Dependent Anion Channel 1 (VDAC1). Thus, our findings highlight the Ca^{2+} source-dependent involvement of AnxV in mitochondrial Ca^{2+} uptake. Currently, further studies are ongoing to identify the regulation of mitochondrial Ca^{2+} uptake via AnxV interaction of VDAC1.

P12: Three-dimensional model of sub-plasmalemmal Ca^{2+} microdomains evoked by the interplay between ORAI1 and $InsP_3$ receptors

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Ca^{2+} signaling plays an essential role in T cell activation, which is a key step to start an adaptive immune response. During the transition of a T cell from quiescent to fully activated, high-resolution Ca^{2+} imaging done by our group, revealed Ca^{2+} microdomains in the junctions between the plasma membrane (PM) and the endoplasmic reticulum (ER) in the absence of cell activation via TCR/CD3 (Diercks et al., 2018). These so called "non-TCR/CD3-dependent Ca^{2+} microdomains" show well-defined spatio-temporal dynamics and rely on d-*myo*-inositol 1,4,5-trisphosphate (IP_3) signaling and subsequent store operated Ca^{2+} entry (SOCE) via the ORAI/STIM system (Gil et al., 2021)**.** Similar, spatially and temporally reduced Ca^{2+} responses also appear as a signaling transition during the first ~15s following cell activation by TCR/CD3. We studied *a posteriori* their spatiotemporal Ca^{2+} dynamics in Gil et al. (2022).

We have used mathematical modelling to investigate the spatiotemporal characteristics of T cell Ca^{2+} microdomains and their molecular regulators. We developed a reaction-diffusion model using COMSOL Multiphysics to describe the evolution of cytosolic and ER Ca^{2+} concentrations in a three-dimensional ER-PM junction. Equations are based on a previously proposed realistic description of the junction, which is extended to take IP_3 receptors (IP_3R) into account that are located next to the junction (McIvor et al., 2018).

Furthermore, the existence of preformed clusters of ORAI1 and STIM2 slightly opens ORAI1 in conditions of a full ER and forms small Ca^{2+} microdomains. These ORAI1-based microdomains do not account on their own for the Ca^{2+} increase that corresponds to the experimental average. After a short activation of a few immobile IP_3Rs close to the junction, local Ca^{2+} depletion close to the IP_3R's pore activates further ORAI1 channels increasing Ca^{2+} in the junction allowing larger Ca^{2+} signals. The model predicts that Ca^{2+} microdomains with realistic amplitudes are formed when 2 to 5 IP_3Rs open simultaneously (Gil et al., 2021).

We conclude that the concerted activity of spontaneously opening of IP_3R's and of SOCE activation is necessary for non-TCR/CD3 dependent Ca^{2+} microdomains formation in T cells.

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P13: Three-dimensional model of sub-plasmalemmal Ca^{2+} microdomains evoked by T cell receptor/CD3 complex stimulation

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Ca^{2+} signaling plays an essential role in T cell activation, which is a key step to start an adaptive immune response. During the transition of a T cell from quiescent to fully activated, high-resolution Ca^{2+} imaging previously done by our group, revealed Ca^{2+} microdomains in the junctions between the plasma membrane (PM) and the endoplasmic reticulum (ER). These spatially and temporally restricted Ca^{2+} microdomains appear as a signaling transition during the first ~15s following cell activation by TCR/CD3. Subsequent NAADP signaling acts on ryanodine receptors type 1 (RYR1), leading to local store depletion and store operated Ca^{2+} entry (SOCE) via the ORAI/STIM system (Diercks et al., 2018). Similar Ca^{2+} microdomains occur before cell activation (non-TCR/CD3-dependent) but rely on D-*myo*-inositol 1,4,5-trisphosphate (IP_3) signaling. The spatiotemporal Ca^{2+} dynamics of IP_3-dependent Ca^{2+} microdomains was previously studied in Gil et al. (2021).

We have used mathematical modelling to investigate the spatiotemporal characteristics of T cell Ca^{2+} microdomains and their molecular regulators. We developed a reaction-diffusion model using COMSOL Multiphysics to describe the evolution of cytosolic and ER Ca^{2+} concentrations in a three-dimensional ER-PM junction. Equations are based on a previously proposed realistic description of the junction (McIvor et al., 2018), which is extended to take RYR1 into account located firstly inside and secondly around the junction.

Our previous model (Gil et al., 2021) predicted that non-TCR/CD3-dependent Ca^{2+} microdomains result from the concerted activity of IP_3 receptors and pre-formed ORAI1-STIM2 complexes. Simulations of pre-formed clusters of ORAI1, STIM2 and STIM1 reveal that ORAI1 slightly open in conditions of a minimal depleted ER, but that the resulting Ca^{2+} microdomains are too small to account for the Ca^{2+} increase that corresponds to the experimental average (Gil et al., 2022). Considering the increase in NAADP signaling, we concluded that ~7 RYRs, most probably located around the junction, can open simultaneously. The activation of SOCE due to the subsequent local ER Ca^{2+} depletion accounts for TCR/CD3 dependent Ca^{2+} microdomains formation in T cells.

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P14: Dimerization of Guanylate Cyclase Activating Protein 1 in wild-type and retinal dystrophy-associated conditions

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Guanylate Cyclase Activating Protein 1 (GCAP1) is a Ca^{2+}-sensor protein known to form dimers. GCAP1 is expressed in photoreceptors and regulates the enzymatic activity of retinal Guanylate Cyclase 1 (GC1) in a Ca^{2+}-dependent way, thereby helping to restore the dark-adapted homeostasis in photoreceptor cells. Over 20 missense mutations in the gene encoding GCAP1 have been linked to severe autosomal dominant forms of cone dystrophy (COD) and cone-rod dystrophy (CORD). A Glu to Val substitution in the high-affinity Ca^{2+} binding motif EF3 (E111V) associated with severe CORD has been previously characterized in vitro [1]. The present study provides insight into GCAP1 homo- and hetero-dimerization in patients carrying the E111V substitution by integrating biochemical and *in silico* methods.

The monomer-dimer equilibrium of both wild type (WT)-GCAP1 and E111V-GCAP1 and its dependence on Ca^{2+} and Mg^{2+} was evaluated by analytical size exclusion chromatography (aSEC). In addition, regulation of the catalytic activity of GC1 by WT and E111V GCAP1 was evaluated also in the presence of a peptide derived from retinal degeneration protein 3 (RD3), a strong inhibitor of GC1, to assess its potential effect as a therapeutic agent.

Rigid-body docking was used to identify the most probable poses defining homo- and hetero-dimers formation, while Molecular Dynamics (MD) simulations were carried out to assess the time-dependent conformational dynamics of both the WT and E111V-GCAP1 under Mg^{2+} and Ca^{2+}-saturating conditions, thus mimicking the GC1-activating and inhibiting state, respectively.

Both variants exhibited a monomer-dimer equilibrium with similar equilibrium constant in the presence of Mg^{2+} or Ca^{2+}. However, under Ca^{2+}-saturating conditions, as protein concentration decreased, the equilibrium clearly shifted towards a monomeric condition. On the contrary, the Mg^{2+}-bound form seems to partially disrupt the monomer-dimer equilibrium and results in a prevailing dimeric state of the protein.

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P15: Structural basis for activation and gating of IP_3 receptors

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Calcium (Ca^{2+}) is a universal and versatile cellular messenger used to regulate numerous cellular processes in response to external or internal stimuli. A pivotal component of the Ca^{2+} signaling toolbox in cells is the inositol 1,4,5-triphosphate (IP_3) receptors (IP_3Rs). IP_3Rs are intracellular Ca^{2+} channels, predominantly localized to the ER and activated by the binding of IP_3 generated in response to external stimulation of G-protein coupled receptors. Opening of the IP_3Rs results in the rapid release of Ca^{2+} from the ER into the cytoplasm triggering diverse signaling cascades to regulate physiological processes such as learning, fertilization, gene expression, and apoptosis. Dysfunctional IP_3Rs cause abnormal Ca^{2+} signaling and are associated with many diseases, including diabetes, cancer, and neurological disorders.

IP_3Rs are activated by IP_3 and Ca^{2+}, inhibited by Ca^{2+} at high concentrations, and potentiated by ATP. However, the underlying molecular mechanisms are unclear due to the lack of structures in the active conformation. We will present new cryo-electron microscopy (cryo-EM) structures of human type-3 IP_3R in multiple gating conformations; IP_3-ATP bound pre-active states with closed channels, IP_3-ATP-Ca^{2+} bound active state with an open channel, and IP_3-ATP-Ca^{2+} bound inactive state with a closed channel. The structures demonstrate how IP_3-induced conformational changes prime the receptor for activation by Ca^{2+}, how Ca^{2+} binding leads to channel opening, how ATP modulates the activity, and how the pore dilates, providing insights into the long-sought questions regarding the molecular mechanism of the receptor activation and gating. These structures will likely serve as foundations for future experiments addressing biophysical and functional questions related to IP_3Rs.

P16: Transcriptomics of intracellular calcium remodeling in rat hippocampal neurons aged *in vitro*

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Intracellular Ca^{2+} homeostasis plays an important role in control of neuronal activity including neurotransmitter release, synaptic plasticity and memory storage as well as neuron cell death. Aging is often associated to cognitive decline and it is the most important risk factor for neurodegenerative disorders. We have recently shown that long-term cultures of rat hippocampal neurons which resemble in many aspects aging neurons, undergo cell death after treatment with different neurotoxins involved in neurodegeneration that increase cytosolic [Ca^{2+}]. In addition, aged neurons display changes in intracellular Ca^{2+} homeostasis that may favour susceptibility to cell death in aging neurons. Accordingly, we have investigated calcium remodeling in aging neurons from a transcriptomic point of view. To this end, we used microarrays analysis in neonate rat hippocampal neurons cultured for 7 days in vitro (DIV) that resemble young neurons as well as long-term cultures (21 DIV), resembling aged neurons. Since the process of neuron isolation in long-term cultures is extremely difficult, we have carried out transcriptomic analysis from both mixed cultures (neurons and glia) and cultures devoid of neurons. Accordingly, we have four different experimental conditions: young mixed cultures, aged mixed cultures, young isolated glia and aged isolated glia. Then, since the number of neurons before and after "glial isolation" is known, it is possible to quantify differential expression throughout hypothesis contrast. Our results show that a few transcripts coding for plasma membrane calcium channels are overexpressed in aging neurons including Kainate receptor 4, the molecular players involved in store-operated Ca^{2+} entry Orai2 and Stim1, and the modulators SARAF and Septin4, as well as the members of the superfamily of TRP channels TRPM2 and 3. In addition, intracellular Ca^{2+} release channels IP3R 1 and 2 and ryanodine receptor 3 are also overexpressed in aging neurons whereas only the ryanodine receptor 2 is downregulated. In contrast, plasma membrane Ca^{2+} pumps PMCA1 and 3 are downregulated in aging neurons along with mitochondrial Ca^{2+} transport systems MCU, MICU2 and VDAC1,2 and 3. These results indicate that neuron aging is associated with a transcriptional remodeling of molecular players consistent with enhanced Ca^{2+} entry into the cytosol and decreased Ca^{2+} exit from the cytosol, which is according to the "calcium hypothesis of brain aging".

P17. Role of store operated Ca^{2+} entry in cholinergic activity in mouse primary bronchial smooth muscle

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Store operated Ca^{2+} entry (SOCE) is a major pathway in Ca^{2+} signaling which is activated upon depletion of Ca^{2+} from the SR [1]. Evidence suggests that SOCE is involved in contractions of airway smooth muscle (ASM), hence it is of interest as a target for treating bronchoconstriction [2]. Our aim was to examine the role of Ca^{2+} release activated Ca^{2+} (CRAC) channels in contraction and Ca^{2+} signaling in mouse primary bronchus using *in vitro* isometric tension recordings and Ca^{2+} imaging of single ASM cells isolated from GCaMP 8.1 mice. Cells were illuminated with a 488 nM laser and imaged using an iXon 897 EMCCD camera (Andor Technology, Belfast, UK) coupled to a CSU21 spinning disk confocal head (Yokogawa, Tokyo, Japan). In isometric tension recordings in bronchial rings, carbachol (CCh) caused concentration-dependent (0.1-10 μM) contractions that were well sustained over 10 min. GSK-7975A 10 μM, a CRAC blocker, reduced the responses to higher concentrations of CCh more effectively than lower concentrations and was more effective at inhibiting the sustained contractions (measured at 10 min) than the initial responses (measured at 2 min). The residual contractions in the presence of GSK-7975A were abolished on addition of nifedipine (1 μM). When nifedipine was applied alone, in contrast to GSK-7975A, it blocked the responses to lower concentrations of CCh more effectively than higher concentrations and did not distinguish between initial and sustained contractions. GSK-7975A abolished the residual contractions in the presence of nifedipine. Similar effects of both blockers were observed on Ca^{2+} signals in isolated ASMCs, where one blocker partially reduced responses to CCh (0.3 μM), while together the blockers abolished the responses.

To summarize, when either of the channels are blocked, the other channel can maintain the integrity of the SR and sustain Ca^{2+} signaling in response to cholinergic stimulation, therefore the tissue can continue contracting. Thus, our findings suggest that both L-type Ca^{2+} and CRAC channels are essential in maintaining cholinergic responses in ASM. This conclusion differs from that of Chen & Sanderson who suggested, on the basis of using high concentrations of GSK-7975A (50-100 μM), that CRAC channels could compensate when L-type Ca^{2+} channels were blocked in ASM, but not *vice versa* [2].

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P18: Loss of IP_3R fitness enables GNAQ/GNA11-mutated Uveal Melanoma survival by shutting down IP_3 signalling and preventing calcium overload-induced cell death

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Uveal melanoma (UM) is the most common primary intraocular malignancy in adults. In 50% of cases, liver metastases compromise patient's survival regardless of treatments. 90% of UMs are initiated by gain-of-function driver mutations in GNAQ/GNA11 encoding α subunits of the GPCR-associated G proteins while a small proportion are due to driver mutations in the Cysteinyl Leukotriene Receptor 2 (CYSLTR2) or the Phospholipase C beta (PLCB4). All these mutually exclusive oncogenic mutations have the same functional output, resulting in constitutive activation of the PLCβ enzyme which hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) in diacylglycerol (DAG) and inositol trisphosphate (IP_3). So far, UM research only addressed DAG-induced pro-oncogenic effects and identified its essential role in stimulating the MAPK pathway. As today, nothing is known on the role of the second arm of the deregulated PLC signalling, namely the IP_3/calcium (Ca^{2+}) pathway. Yet, Ca^{2+} controls several vital cell functions and any unregulated $[Ca^{2+}]i$ elevation is cytotoxic. Hence, UM cells probably have to remodel their Ca^{2+} homeostasome in order to cope with sustained IP_3 production and escape Ca^{2+} overload-induced cell death. Indeed, despite permanent IP_3 production, no major deregulation of the resting Ca^{2+} homeostasis was observed in GNAQ/GNA11-mutated UM cells. Our results further demonstrated that in UM cells, IP_3-induced ER Ca^{2+} depletion and the associated Ca^{2+} entry are prevented by the loss of responsive IP_3 Receptors (IP_3R) through multiple mechanisms. Altogether our data indicate that UM cells remodel their Ca^{2+} pathways to prevent ER stress and maintain their survival. It identified an UM cell vulnerability that opens up new perspectives for innovative uveal melanoma treatments.

P19: Recent updates into NAADP signaling – formation, binding protein(s) and target channel(s)

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most potent endogenous Ca^{2+} mobilizing second messenger known to date and is effective in the low nanomolar range in many cells. NAADP functions as a Ca^{2+} trigger in T cells by evoking an initial local Ca^{2+} release, termed Ca^{2+} microdomains, from intracellular stores. NAADP is rapidly formed within 10 seconds after T cell activation. We already demonstrated that the number of initial Ca^{2+} microdomains was significantly decreased in ryanodine receptor 1 knock-out (*Ryr1*$^{-/-}$) T cells [1] or when NAADP was antagonized by BZ194, a specific small molecule inhibitor [2]. The number of initial Ca^{2+} microdomains was not affected by functional double knock-out (KO) of TPC1/2, RYR3 or TRPM2 [1,2].

However, the formation of NAADP as well as its mode of action are still controversially discussed.

In T cells, we discovered a cytosolic soluble protein that binds NAADP, named hematological and neurological expressed 1-like protein (HN1L)/jupiter microtubule associated homolog 2 (JPT2) [3]. Gene silencing of HN1L/JTP2 in human Jurkat and primary rat T cells resulted in lower initial Ca^{2+} microdomains, a decreased signal onset velocity and amplitude of global Ca^{2+} signalling, similar to antagonism of NAADP-signaling by BZ194 or KO of RYR1. Furthermore, HN1L/JTP2 co-localizes with RYR in ER – PM junctions and was co-immunoprecipitated with RYR in membrane fractions.

CD38 can catalyze the formation of NAADP from NADP *in vitro* through base-exchange reaction. However, this reaction only proceeds at acidic pH and in the presence of excess nicotinic acid. In line, both subcellular and global Ca^{2+} signals were identical in WT and *Cd38*$^{-/-}$ murine primary T cells. Therefore, CD38 seems not to be the NAADP forming enzyme in T cells. However, we discovered the NADPH oxidase enzyme family (NOX/DUOX) able to form NAADP under physiological conditions with a pH optimum of approx. 7.5 *in vitro* [4]. T cells from functional DUOX1/DUOX2 KO mice showed no functional enzymatic activity and the number of initial Ca^{2+} microdomains as well as the global Ca^{2+} signals were significantly decreased compared to WT controls. Furthermore, blocking NAADP signaling by BZ194 in DUOX2 single KO rat T cells did not further decrease Ca^{2+} peak, plateau or signal onset velocity.

Taken together, we propose a new model for the formation and the signaling of NAADP. After T cell receptor stimulation NAADP is formed under physiological conditions by DUOX2 below the plasma membrane, binds to HN1L/JTP2 and evokes Ca^{2+} microdomains through the RYR1 as initial step in the adaptive immune response.

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P20: P2X4 and P2X7 are essential players in basal T cell activity and Ca^{2+} signaling milliseconds after T cell activation

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Initial T cell activation is triggered by the opening of Ca^{2+} channels like ryanodine receptor type 1 (RYR1) and ORAI1 leading to the formation of highly dynamic, spatiotemporally restricted Ca^{2+} signals milliseconds after T cell stimulation, called Ca^{2+} microdomains [1,2]. The ATP-gated cation channels P2X4 and P2X7, which were located at the plasma membrane, were shown to influence the free cytosolic Ca^{2+} concentration after T cell stimulation. Thus, an altered Ca^{2+} influx was shown several minutes after T cell activation upon inhibition of P2X channels [3]. We hypothesize that the two purinergic cation channels P2X4 and P2X7 are not only implicated in amplifying global Ca^{2+} signals but promote the formation of initial Ca^{2+} microdomains.

To analyze P2X4- and P2X7-dependent Ca^{2+} microdomains, high-resolution live-cell–imaging [4] was performed with different technical strategies, including knock-out mice, nanobodies and antagonists.

In the current study, we show that the two purinergic cation channels P2X4 and P2X7 are not only involved in the global Ca^{2+} signals, but promote the formation of initial Ca^{2+} microdomains after T cell stimulation [5], similar to NAADP targeting RYR1 or store-operated Ca^{2+} entry through ORAI1 [2]. Local Ca^{2+} signals were significantly decreased after stimulation in T cells from *P2x4*$^{-/-}$ and *P2x7*$^{-/-}$ mice or by chemically inhibiting P2X4 or blocking P2X7 by inhibitory nanobodies in WT T cells. In addition, we demonstrate that the mechanism of Ca^{2+} entry through P2X4 and P2X7 depends on ATP release from the cytosol via pannexin1. Using a pannexin1 inhibiting peptide called ^{10}panx1, Ca^{2+} microdomains were significantly reduced just seconds after T cell stimulation. Moreover, we show for the first time that basal ATP release by pannexin-1 in unstimulated cells activates P2X4 and promotes the formation of TCR/CD3-independent Ca^{2+} microdomains. Subsequently, T cell stimulation fosters ATP release and autocrine activation of both P2X4 and P2X7, amplifying initial Ca^{2+} microdomains already in the first second of T cell activation.

In summary, we suggest that basal T cell excitability depends on the purinergic axis of pannexin1/P2X4 triggering TCR/CD3-independent Ca^{2+} microdomain formation, which is fostered in frequency and amplitude after T cell activation by P2X4 together with P2X7.

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P21: Modulation of Ca^{2+} oscillations in murine airway smooth muscle cells by PGE.

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Prostaglandin E_2 (PGE_2) is an endogenously released lipid mediator that induces potent bronchodilation *via* activation of EP2 or EP4Rs (1). It is believed that PGE_2 mediates its effects through upregulation of cAMP and subsequent increases in protein kinase A (PKA). Studies have shown that the contractile state of airway smooth muscle (ASM) is correlated with the frequency of Ca^{2+} oscillations within SMCs (2). In the present study, confocal Ca^{2+} imaging was utilised to determine the effect of PGE_2 on spontaneous Ca^{2+} oscillations and carbachol (CCh)-evoked Ca^{2+} oscillations in freshly isolated murine ASM cells (ASMCs).

PGE_2 (300nM) significantly reduced the frequency of CCh (1μM)-evoked Ca^{2+} oscillations from 30 ± 4.0 to 5.5 ± 2.8 per min^{-1} and oscillation amplitude by 72% from 1.4 ± 0.43 to 0.35 ± 0.27 $\Delta F/F_0$. CCh-induced Ca^{2+} oscillations were also inhibited by the IP_3 receptor (IP_3R) and ryanodine receptor (RyR) antagonists, 2-APB (100μM) and tetracaine (100μM), respectively. 2-APB reduced CCh-evoked Ca^{2+} oscillation frequency by 95% from 20 ± 1.3 to 1 ± 1 per min^{-1} and mean oscillation amplitude by 92%, from 1.8 ± 0.41 to 0.14 ± 0.14 $\Delta F/F_0$. Tetracaine abolished CCh-induced Ca^{2+} oscillations in all cells tested (mean oscillation frequency and amplitude in control of 25 ± 2.7 per min^{-1} and 1.4 ± 0.44 $\Delta F/F_0$, respectively). Isolated ASMCs also developed spontaneous Ca^{2+} oscillations and these events were abolished by tetracaine, while 2-APB reduced the mean frequency and amplitude of these events by 85% (13 ± 1.3 to 2 ± 1.4 per min^{-1}) and 86% (0.93 ± 1.6 to 0.13 ± 0.09 $\Delta F/F_0$), respectively. However, unlike CCh-evoked Ca^{2+} oscillations, spontaneous Ca^{2+} oscillations were not affected by the application of PGE_2.

Taken together these data suggest that PGE_2 does not directly modulate Ca^{2+} release from intracellular stores and may mediate its effects on CCh-evoked calcium oscillations *via* uncoupling of muscarinic receptors from activation of phospholipase C.

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P22. Adhesion-dependent Ca^{2+} microdomains increase the sensitivity of T cells

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The activation of T cells is a hallmark of the adaptive immune response. Adhesion-dependent T cell stimulation may play a pivotal role, describing an adhesion-dependent change in T cell sensitivity that facilitates full T cell receptor (TCR)/CD3-mediated activation[1] . During an immune response, T cells migrate from the blood vessel walls into the inflamed tissue, crossing the endothelial cell layer and subsequently elements of the extracellular matrix (ECM). In this process, integrins facilitate binding to endothelial cells and ECM proteins. Using high-resolution live cell imaging, within tens of milliseconds after T cell activation, local Ca^{2+} microdomains represent the earliest Ca^{2+} signaling events2. Here, we hypothesized that Ca^{2+} microdomains observed without TCR/CD3 stimulation are elements of T cell sensitivity and occur in response to adhesion to ECM proteins. In a high-resolution live cell–imaging approach, we show that blocking with monoclonal antibodies directed toward integrin receptors that bind to collagen-IV/-VI, laminin-1 and the intercellular adhesion molecule (ICAM-1) results in a significant decrease of adhesion-dependent Ca^{2+} microdomains. Furthermore, adhesion of T cells to collagen-IV and laminin-1 resulted in a significant increase of Ca^{2+} microdomains. Moreover, these adhesion-dependent Ca^{2+} microdomains were significantly decreased by blocking the downstream integrin signaling pathway using focal adhesion kinase (FAK) inhibitor PF562,271, phospholipase C (PLC) inhibitor U73122 or by deletion of all three D-myo-inositol 1,4,5-trisphosphate receptor (IP_3R) subtypes. Blocking of FAK additionally resulted in reduced global Ca2+ signals or decreased NFAT translocation after TCR stimulation on adhesive poly-L-lysine coating. In summary, these data show that adhesion to the basement membrane proteins collagen-IV and laminin-1 results in an increased state of sensitivity of T cells, involving FAK, PLC, and IP_3Rs for the formation of Ca^{2+} microdomains.

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P23. Elucidating the role of TPC2 in Lysosomal Storage Disorders

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Lysosomal Storage Disorders (LSDs) are the main underlying cause of neurodegenerative dementias. Disturbed calcium (Ca^{2+}) ion homeostasis is a major cause of LSDs in both children and adults. In attempts to understand the progression of LSDs, defects in endo-lysosomal Ca^{2+} homeostasis in two families of Ca^{2+} channels, two-pore channels (TPC1-3) and transient receptor potential channel (TRPML1-2) have been widely studied. We aim to understand how these two channel families localized in lysosomes are involved in generating lysosomal (and cellular) Ca^{2+} ion signals and thereby influence Ca^{2+} homeostasis. We have begun elucidating the basic question to understand the link between how nicotinic acid dinucleotide phosphate (NAADP) mobilizes Ca^{2+} ions from these ion channels, with the initial focus on TPC2. For this, we have started looking at lysosomal-specific Ca2+ microdomains formed in Jurkat T cells with the aid of lysosomal specific agonist TPC2 channel namely TPC-A1-N (A1-N), to confirm how these differ from the ones generated by cell-permeable NAADP. Using our established protocol of high-resolution Ca^{2+} imaging and respective fluorescent indicators[1] , we identified that A1-N (60 µM) evoked the formation of Ca^{2+} microdomains within the first 10-20s post stimulation. We also aimed to understand the role of store-operated calcium entry (SOCE) and lysosomal de-acidification on A1-N evoked Ca^{2+} microdomain formation. Upon inhibition of SOCE by Synta66 (50 µM) and lysosomal de-acidification by bafilomycin A1 (12.5 nM), a significant reduction in A1-N evoked Ca^{2+} microdomain formation was observed. These results preliminarily indicate that A1-N mediated Ca^{2+} microdomain formation requires Ca^{2+}entry and intact lysosomes. This prompted us to further investigate the possible link between SOCE and TPC2. Furthermore, we are currently working on elucidating how A1-N evoked Ca^{2+} microdomain formation is influenced in the absence of ryanodine receptors, a novel identified NAADP-binding protein HN1L[2] and the NAADP-synthesizing DUOX enzymes[3]. This will help to understand the basic picture of the various players needed for efficient lysosomal Ca^{2+} ion release specifically from TPC2 in T-cells.

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P24: Presenilin-1 controls pancreatic beta-cell metabolism by regulating mitochondrial Ca^{2+} sensitive NADH shuttles

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Intracellular Ca^{2+} ions are known to regulate many metabolic processes in a cell. We have recently shown that presenilin-1 establishes an endoplasmic reticulum (ER) Ca^{2+} leak directed towards mitochondria in pancreatic beta cells (Klec et al. 2019, PMID: 30790505). This leak was found to be indispensable for glucose-stimulated insulin secretion in cultured beta cells and pancreatic islets (Klec et al. 2019, PMID: 31529929). The aim of current work was to elucidate the mechanism of presenilin-1 mediated regulation of pancreatic beta-cell metabolism. Silencing of presenilin-1 in pancreatic beta cells showed delayed and reduced cytosolic Ca^{2+} oscillations in response to glucose, but not pyruvate, pointing at possible importance of presenilin-1 for glycolysis. Additionally, presenilin-1 knockdown (KD) showed reduced pyruvate production upon glucose elevation, while lactate production was increased, indicating a metabolic switch. In support of the metabolic switch, we detected increased glutamine reliance of presenilin-1 KD cells in mass spectrometry analysis of cellular metabolites. Furthermore, cellular redox state was altered by presenilin-1 KD with reduced cytosolic NAD^+/NADH ratio, a possible reason for observed metabolic alterations. The latter finding is in line with our recently published work where we demonstrated that disrupted sub-cellular Ca^{2+} homeostasis can rewire cellular metabolism by modulating mitochondrial Ca^{2+} sensitive NADH shuttles (Koshenov et al. 2022, PMID: 35058562). Analysis of subcellular Ca^{2+} concentrations using genetically encoded Ca^{2+} sensors revealed reduced matrix and mitochondrial intermembrane space Ca^{2+}, while global cytosolic and ER Ca^{2+} levels were not altered by presenilin-1 KD. In detail, analysis of glycolytic intermediates by targeted mass-spectrometry showed an accumulation of metabolites preceding and including glyceraldehyde-3 phosphate (GAP) in presenilin-1 KD cells, pointing to the bottleneck at the reaction catalyzed by GAP dehydrogenase that requires NAD^+. These results led us to hypothesize that presenilin-1 mediated ER Ca^{2+} leak is important for mitochondrial intermembrane space residing Ca^{2+} sensitive NADH shuttles that recycle cytosolic NADH and provide NAD^+ for glycolysis. Overexpression of Ca^{2+} insensitive mutants of these shuttles rescued the effects of presenilin-1 KD. Thus, we have identified presenilin-1 as a regulator of pancreatic beta-cell metabolism, which acts by establishing an ER Ca^{2+} leak that controls mitochondrial Ca^{2+} sensitive NADH shuttles that recycle cytosolic NADH to maintain glycolysis and supply mitochondria with the substrate.

P25: Dysregulated MAM & Ca^{2+} homeostasis underlying Wolfram syndrome type 2-associated CISD2 deficiency

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CDGSH iron-sulfur domain 2 (CISD2) is a redox-active ER membrane protein that binds 2Fe-2S clusters. CISD2 impacts a broad range of cellular processes, including ROS and iron homeostasis, cellular longevity, autophagy, cell death and Ca^{2+} homeostasis. Loss-of-function mutations in *CISD2* are causative of Wolfram syndrome type 2 (WS2), a rare progressive disorder. Oppositely, high CISD2 levels are linked to poor prognosis in breast cancer, lung adenocarcinoma and several other types of cancer. The specific localization of CISD2 at the mitochondria-associated ER membranes (MAMs) is thought to be crucial for the role of CISD2 in cellular health and Ca^{2+} homeostasis. Indeed, highly focalized ER-mitochondrial Ca^{2+} transfer through the inositol 1,4,5-trisphosphate receptor (IP3R) occurring in the MAMs controls cell survival processes as well as cell death events.

Performing subcellular fractionation of HeLa cell lysates, we isolated pure MAM fractions and validated the presence of CISD2 in the MAMs. Moreover, we generated HeLa CISD2 KO cells via a Crispr/Cas9 based method. Using a MAM-specific fluorescent SPLICS probe (Cieri et al, 2017, Cell Death Diff), we found that HeLa CISD2 KO cells have reduced ER-mitochondrial contacts. Using co-immunoprecipitation assays, we demonstrated that CISD2 interacts with the IP3R. To determine a possible functional effect of CISD2 on IP3R-mediated Ca^{2+} release, we simultaneously imaged cytosolic Ca^{2+} and mitochondrial Ca^{2+} by using the cytosolic Ca^{2+}-sensitive fluorescent probe Fluo-4 and the mitochondrially-targeted genetically encoded Ca^{2+} sensor mitoCEPIA3. Ca2+ signals were evoked using extracellular agonists such as ATP. Interestingly, loss of CISD2 did not majorly affect agonist-induced, IP3R-mediated Ca^{2+} signals in the cytosol. However, the Ca^{2+} transfer from the ER to the mitochondria was significantly reduced in CISD2-deficient cells. Importantly, we excluded an altered mitochondrial membrane potential and reduced ER Ca^{2+} store content as causes for reduced Ca^{2+} transfer. Future work aims to assess the impact of disease-associated CISD2 mutants on ER-mitochondrial Ca^{2+} signaling and interaction with IP3Rs as well as to explore the impact of CISD2 loss on Ca^{2+} signaling in patient-relevant cortical neurons differentiated from induced-pluripotent stem cells.

For now, we conclude that CISD2 is a MAM-resident protein that is vital for MAM integrity and function. Cells lacking CISD2 show decreased ER-mitochondrial Ca^{2+} transfer. Possibly, CISD2 can positively regulate IP3R specifically in the MAMs, for example by taking part in a macrocomplex together with other IP3R modulators. Alternatively, the decreased ER-mitochondrial Ca^{2+} transfer can be independent of IP3R modulation and be solely attributable to decreased ER-mitochondrial contact.

P26: Neuronal mitochondrial responses require IP_3R and Parkin for viability and flight.

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Endoplasmic Reticulum (ER)-mitochondrial cross talk at Mitochondrial associated membranes (MAMs) is indispensable for neuronal health and physiology. It regulates mitochondrial quality and output, calcium homeostasis, and lipid metabolism. Such functions are dysregulated in neurodegenerative diseases like Parkinson's disease (PD), Alzheimer's diseases (AD) and Amyotrophic Lateral Sclerosis (ALS). We are interested in understanding interaction of IP_3R and Parkin protein in maintaining MAMs and cellular homeostasis for efficient neuronal function during ageing. The IP_3R is an ER- resident Ca^{2+} channel and an important component of intracellular Ca^{2+} signalling. It interacts with various proteins at MAMs and transfers Ca^{2+} to mitochondria through MAMs, required for mitochondrial function. Failure to do so usually leads to impaired Ca^{2+} homeostasis and mitochondrial dysfunction. If mitochondrial homeostasis is perturbed beyond repair, dysfunctional mitochondria are detected, removed, and replaced by PINK1 and Parkin proteins, mutations in which increase the chances of hereditary PD. Ageing imposes oxidative and metabolic stress on neuronal machinery, thereby compromising its function. Hence, ageing neurons require intricate balance of healthy mitochondrial function and removal of non-functional mitochondria for operating efficiently. In this study we tested if mutant IP_3Rs lead to dysfunctional mitochondria and thus impact PD -like progression in Parkin mutant *Drosophila*. Our preliminary observations demonstrate a strong genetic interaction between Parkin and IP_3R mutants. The cellular and molecular basis of this interaction is under investigation and will be discussed.

P27: Understanding polymodal activation of the lysosomal ion channel TPC2.

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TPC2 is a patho-physiologically relevant lysosomal ion channel that is activated directly by the phosphoinositide, $PI(3,5)P_2$ and indirectly by the Ca^{2+} mobilizing messenger NAADP acting through associated accessory proteins. It is highly unusual in toggling between a Na^+-selective and Ca^{2+}-permeable state in response to these cues – behaviour that can be mimicked by novel synthetic agonists. But the mechanisms underlying polymodal gating are unknown. Here, we used a combination of site-directed mutagenesis, gene editing, electrophysiology and Ca^{2+} imaging to probe the molecular determinants of TPC2 activation. We show that the $PI(3,5)P_2$ binding site is required for activation of TPC2 not only by $PI(3,5)P_2$ but also by NAADP. In contrast, activation of TPC2 by the small molecule NAADP mimetic, TPC2-A1-N, is $PI(3,5)P_2$-independent. We further show that activation of endogenous TPC2 by TPC2-A1-N is unaffected by blocking $PI(3,5)P_2$ synthesis or knockout of the recently described NAADP-binding proteins, JPT2 and LSM12. Our data reveal a central role for $PI(3,5)P_2$ in mediating local and remote activation of TPC2 by endogenous cues, despite radically different effects on ion permeation, but that it can be bypassed by a synthetic agonist. Convergent and divergent routes to channel activation and ion selectivity switching emerge.

P28. Calcium oscillations regulate lipid flux *in vivo* during zebrafish liver development

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During starvation, vertebrates utilize the liver as a lipid storage organ. For instance, patients suffering from anorexia nervosa[1], overnight fasted mice[2], and starved zebrafish have been reported to develop fatty liver. Hepatic steatosis, in this case, creates and energy reservoir that allows survival during long-term caloric deprivation. Nutrient availability clears steatosis and returns the liver to baseline lipid deposition[3]. In contrast to hepatic steatosis caused by excessive calorie intake (i.e. Non-alcoholic fatty liver disease), the mechanisms of starvation induced hepatic steatosis are barely studied at all.

Here we take advantage of the transparency of the zebrafish larvae to develop a setup that allows *in vivo* imaging of lipid droplets and intracellular calcium dynamics in the zebrafish liver. We show a negative correlation between calcium oscillations and lipid accumulation in the zebrafish liver. Specifically, starvation induced hepatic steatosis and inhibited calcium waves. Further, feeding recommences calcium waves in the liver, correlating with a decrease in the amount of lipid droplets in the organ. To functionally test the relationship between calcium signaling and steatosis, we buffered calcium signaling using a genetically encoded calcium scavenger. This accelerated the process of steatosis development, suggesting calcium flux to be a regulator of lipid accumulation.

Withing the cell, calcium stored in endoplasmic reticulum (ER) is considered to be responsible for calcium transients[4]. However, other organelles such as endo-lysosomes contains calcium at similar concentration as ER[5,6] and has recently gained much attention as a regulator of intracellular calcium level. Thus, we evaluated the potential role of endo-lysosomal calcium to regulate intracellular calcium oscillations *in vivo* in zebrafish liver. Our preliminary data suggests that activation of a lysosomal cation channel, TPC2 increases calcium transients. Interestingly, this decreases steatosis during starved state without introduction of food. This suggests that mobilization of endo-lysosomal calcium stores could induce calcium oscillations in the liver cells, which further would induce clearance of lipid droplets.

Currently, we are developing tools for sub-cellular imaging of calcium release from endo-lysosomal compartment *in vivo*, and are interested to investigate the link between TPC2 activity and lipophagy under physiological conditions. Additionally, it would be of interest to test TPC2 activation as a method for reducing hepatic steatosis in pathological conditions, such as in alcoholic and non-alcoholic liver disease.

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P29: Role of 3',5'-cyclic adenosine monophosphate during T cell activation

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3',5'-cyclic adenosine monophosphate (cAMP), a second messenger, functions as a key modulator of the immune response[1] . For a long time, cAMP has been thought to be solely a negative regulator in T cells, with several reports showing major inhibitory effects of sustained increases of cAMP levels on T cell activation1 . However, already in 2009 Conche et al. were able to demonstrate that a transient cAMP increase upon TCR/CD3 ligation contributes to T cell activation and precedes the Ca^{2+} response upon interaction of a T cell with an antigen presenting cell. These findings suggest that the kinetics of cAMP signaling plays also a major role in initial T cell activation rather than just negatively regulating T cells in the long run[2]. Furthermore, preliminary data of the existence of local cAMP pools in other cell types suggest that the differential effects of cAMP signaling on T cell activation might be due to compartmentalization not only on a temporal level, but on a spatial level as well, leading to the discussion of a possible role of cAMP microdomains in T cell activation1 . So far, this phenomenon could not be observed in live cell imaging due to technological limitations. However, we were able to overcome this obstacle by performing high-resolution live cell imaging in combination with novel genetically-encoded cAMP-sensors called cAMPFIRE (cAMP Fluorescence Imaging Reporters based on Epac)[3]. These highly sensitive FRET-sensors are suitable for subcellular imaging and can therefore be used to visualize cAMP signaling after T cell stimulation. Using electroporation, we transfected Jurkat T cells and performed live cell imaging. To verify the sensor and optimize the settings, we stimulated the cells with a membrane-permeable cAMP-derivate called pro-cAMP, as well as forskolin, which activates the adenylyl cyclase, and IBMX, an inhibitor of phosphodiesterase. To investigate the effect of T cell receptor stimulation on cAMP signaling, we stimulated the cAMPFIRE-transfected Jurkat cells with soluble α-CD3 antibodies as well as antibody coated-beads to investigate the effect of T cell receptor stimulation on cAMP signaling. Analysis of local cAMP signals was performed by a newly developed custom-made deconvolution and detection script. Data obtained with this innovative method will be discussed at the conference.

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P30: Interplay of calcium and cGMP in the photoreceptor guanylate cyclase complex.

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Photoreceptor guanylate cyclases (GCs) are controlled by guanylate cyclase-activating proteins (GCAPs) that belong to the class of neuronal calcium-sensor proteins. They regulate the calcium-dependent synthesis of cGMP catalyzed by GCs in rod and cone cells, which is a critical step in photoreceptor light adaptation in the retina. Mutations in human GC-E and in particular in GCAP1 can cause an imbalance in calcium-cGMP homeostasis leading to retinal dystrophies. In humans, three different isoform of GCAPs (1, 2 and 3) are known, which differ with respect to their calcium-sensing and regulatory properties. The third isoform GCAP3 is however not well characterized for its regulatory properties or target specificity. We aim to unravel the molecular mechanism of photoreceptor GC-E switching from the inactive to the active state under control of GCAPs. We tested the hypothesis, whether a rotation model of activation involving an α–helix rotation in GC-E is the critical switch that operates under control of GCAPs. We simulated experimentally this α-helix rotation by integration of alanine residues close to the transmembrane region by site directed mutagenesis and functional studies conducted by GC-Assays. We compared the enzymatic catalytic parameters of wildtype and the retinal disease-related mutant V902L of GC-E, which is a constitutively active mutant. We extended the analysis to photoreceptor GC-F and the regulatory features mediated by GCAP isoforms, particularly GCAP3 and investigated their different calcium concentration profiles. Surprisingly, our data do not support the α-helix rotation model in GC-E. This finding distinguishes sensory GCs from hormone-receptor GCs and points to their unique calcium-dependent control by GCAPs. Furthermore, the point mutation in position V902L in GC-E leads to the GC active transition state, which is in wildtype GC-E stabilized by GCAPs. Our findings are also relevant for understanding the molecular basis of retinal diseases that are caused by a distortion of calcium-sensing processes.

Reference: Shahu, M.K.; Schuhmann, F.; Scholten, A.; Solov'yov, I.A.; Koch, K.-W. The Transition of Photoreceptor Guanylate Cyclase Type 1 to the Active State. Int. J. Mol. Sci.2022,23,4030. https://doi.org/ 10.3390/ijms23074030

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P31: The cyclic nucleotide cADPR does not activate TRPM2

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Cyclic ADPR (cADPR) is a NAD metabolite that releases Ca^{2+} from intracellular stores by activation of ryanodine receptors. cADPR also evokes Ca^{2+} entry over the plasma membrane which could either reflect activation of capacitative Ca^{2+} entry or direct activation of a Ca^{2+} channel. TRPM2 is a non-selective, Ca^{2+}-permeable cation channel. A C-terminal domain homologous to the ADPR pyrophosphatase NUDT9led to the identification of ADPR as activating ligand. Early reports indicated that cADPR may also activate TRPM2, either alone or in synergy with ADPR, with one report indicating that this occurs only at 37°C [1,2]. Later these reports were questioned by the observation that commercial cADPR contains ADPR and that removal of ADPR abrogates the effect of cADPR [3,4]. Recently, the identification of an additional nucleotide binding site in the N-terminal MHR1/2 domain of TRPM2 that binds ADPR in a horseshoe-like conformation, as well as the cADPR antagonist 8-Br-cADPR [5], and a publication that shows activation of TRPM2 by binding of cADPR to the NUDT9H domain [6] raised the question of the role of cADPR in TRPM2 activation again and led us to revisit the topic

By HPLC analysis, we confirmed that commercial preparations of cADPR can contain significant amounts of ADPR. We tested the purest preparation of cADPR available to us (<2% ADPR) in whole cell patch clamp experiments using HEK293 cells with stable overexpression of human TRPM2, but observed no activation of the channel, regardless of temperature, Ca^{2+} buffering, or extracellular cation composition [7]. We also found no synergistic effects when we co-infused cADPR with subthreshold ADPR under conditions of low Ca^{2+} buffering. To assess the role of the MHR1/2 and the NUDT9H domain, channel variants with mutations in either binding site were tested for activation by ADPR and the superagonist 2′-deoxy-ADPR. Changes in either domain abrogated activation of TRPM2 by both agonists. To address the binding of cyclic nucleotides to the MHR1/2 domain we expressed the isolated MHR1/2 domain from zebra fish TRPM2 in bacteria and measured the binding by ITC. Neither cADPR nor 8-Br-cADPR bind to the isolated MHR1/2 domain while ADPR and 8-Br-ADPR bind with high affinity under the same conditions [7].

While both nucleotide binding sites are required for activation of TRPM2 by ADPR and 2′-deoxy-ADPR, we found no evidence for activation of the channel by cADPR or binding of the cyclic nucleotides to the MHR1/2 domain.

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P32: Now Oral Number O43

P33: The effect of *stim2* knockout on zebrafish brain cells

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Zebrafish is an established model organism to study human neurological diseases [1-3]. Its major advantages are >70% genetic similarity to humans, the easiness of genetic manipulation, the availability of various transgenic zebrafish lines, and transparent body and fast external development that allow for *in vivo* observations at the cellular and organism level. All this makes zebrafish well suited for studying the interaction between neurons and microglia [4]. Microglia are brain resident immune cells engaged in a wide spectrum of regulatory processes in the brain parenchyma [5]. Many microglia functions rely on Ca^{2+} signaling; for instance their activity was reduced upon inhibition of store operated calcium entry (SOCE) [6]. The aim of this work was to better understand the role of Stim2 protein in the interplay between different brain cells analyzed *in vivo* in 5 days post fertilization (dpf) larvae. Using CRISPR/Cas9 we generated *stim2a* [7], *stim2b* [8] and double *stim2a;stim2b* zebrafish knockout lines and studied them using a variety of techniques. We found that zebrafish devoid of both Stim2 proteins have elevated number of microglia, disturbance in neuronal Ca^{2+} oscillations observed *in vivo* by lightsheet microscopy, and higher susceptibility to seizures. The transcriptomic analysis was performed using RNAseq and scRNA-seq to identify genes underlying this phenotype. Expression of several genes including *annexin3a* has been elevated several fold in the brain of these fish. Annexin A3 has been postulated to be a marker of activated microglia, but its function in these cells is unknown. Our data indicated that overexpressed ANNEXIN A3 modulates SOCE activity in HeLa and HEK cells [9]. Decreased expression of *il1b* and *p53* was detected by qPCR, and acridine staining increased in the brain of *stim2a;stim2b* suggesting enhanced cell death. The observed changes might be due to the effects of microglia activity on the neuronal compartment. The interaction between mCherry microglial positive cells and GCaMP positive neurons in the brain will be established by analysis *in vivo* using lightsheet microscopy.

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P34: Acidification of endothelial Weibel-Palade bodies is mediated by the vacuolar-type H^+-ATPase

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Weibel-Palade bodies (WPB) are unique secretory granules of endothelial cells that store the procoagulant von-Willebrand factor (VWF) in a highly compacted form. Upon exocytosis the densely packed VWF unfurls into long strands that expose binding sites for circulating platelets and thereby initiate the formation of a platelet plug at sites of blood vessel injury. Dense packing of VWF requires the establishment of an acidic pH in the lumen of maturing WPB but the mechanism responsible for this acidification has not yet been fully established. We show here that subunits of the vacuolar-type H^+-ATPase are present on mature WPB and that interference with the proton pump activity of the ATPase employing inhibitors of different chemical nature blocks a reduction in the relative internal pH of WPB. Furthermore, depletion of the V-ATPase subunit V0d1 from primary endothelial cells prevents WPB pH reduction and the establishment of an elongated morphology of WPB that is dictated by the densely packed VWF tubules. Thus, the vacuolar-type H^+-ATPase present on WPB is required for proper acidification and maturation of the organelle.

P35: Lack of MICU-dependent gatekeeping of the mitochondrial Ca^{2+} uniporter of *Trypanosoma cruzi* at low extramitochondrial Ca^{2+} concentrations

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The mitochondrial Ca^{2+} uptake, which is important to regulate bioenergetics, cell death and cytoplasmic Ca^{2+} signaling, is mediated via the calcium uniporter complex (MCUC). In animal cells the MCUC is regulated by the mitochondrial calcium uptake 1 and 2 dimer (MICU1-MICU2), which acts as gatekeeper preventing mitochondrial Ca^{2+} overload at low cytosolic Ca^{2+} levels. In contrast to animal cells, knock out of either MICU1 or MICU2 in *Trypanosoma cruzi*, the etiologic agent of Chagas disease, did not affect Ca^{2+} uptake at low Ca^{2+} concentrations and it was though that in the absence of one MICU the other would replace its role. However, previous attempts to knockout both genes were unsuccessful. Here, we designed a strategy to generate *TcMICU1*/*TcMICU2* double knockout cell lines using CRISPR-Cas9 genome editing. Ablation of both genes was confirmed by PCR and Southern blot analyses. The absence of both proteins significantly decreased the mitochondrial Ca^{2+} uptake at different extramitochondrial Ca^{2+} concentrations ($[Ca^{2+}]_{ext}$), without dissipation of the mitochondrial membrane potential, and increased the $[Ca^{2+}]_{ext}$ set point needed for Ca^{2+} uptake, as we have seen with *TcMICU1*-KO and *TcMICU2*-KO cells. Mg^{2+} was found to be a negative regulator of MCUC-mediated mitochondrial Ca^{2+} uptake at low $[Ca^{2+}]_{ext}$. Occlusion of the MCUC pore by Mg^{2+} could partially explain the lack of mitochondrial Ca^{2+} uptake at low $[Ca^{2+}]_{ext}$ in *TcMICU1*/*TcMICU2*-KO cells. In addition, *TcMICU1*/*TcMICU2*-KO epimastigotes had a lower growth rate, while infective trypomastigotes have a reduced capacity to invade host cells and to replicate within them as amastigotes.

P36: HINT1 deficiency impairs actin cytoskeleton and calcium signaling

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Loss of functional histidine triad nucleotide binding protein 1 (HINT1) causes a rare form of inherited peripheral neuropathy with neuromyotonia (NMAN). Patients suffer from motor greater than sensory neuropathy with an age on onset within the first decade. HINT1 is a ubiquitously expressed phosphoramidase and SUMOylase. It acts as a transcriptional inhibitor of pro-oncogenic transcription factor and as an adaptor protein of the endocannabinoid signaling pathway in the central nervous system (CNS). Yet, its role in the peripheral nerves is uncharacterized. We created HeLa cell lines deficient for *HINT1* using CRISPR/Cas9 genome editing technology and studied their transcriptome profile. Gene ontology and pathway analysis indicated integrin signaling and actin cytoskeleton as affected pathways. Additionally, we identified intracellular signaling as a recurrent term within the most significantly affected pathways. Therefore, we characterized, the calcium signaling response to extracellular stimuli using genetically encoded calcium indicators (GECOs). We identified a weaker response in the *HINT1* KO cells likely caused by reduced calcium loading into the endoplasmic reticulum. Our findings identify and characterized two affected pathways as a results of loss of HINT1 suggesting new disease mechanisms.

P37: The butterfly effect: long-term impact of abnormal calcium signaling at fertilization on offspring health

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Fertilization triggers a chain of events that transform the mature egg into a totipotent one-cell embryo. These events are driven in mammalian eggs by repetitive increases in cytosolic calcium (Ca2+), or Ca2+ oscillations. Ca2+ oscillation patterns in fertilized eggs can be modified in vitro by changing the ionic composition of culture media, such as in assisted reproduction, or by conditions affecting mitochondrial function, such as obesity and inflammation. In mice, in vitro manipulation of Ca2+ oscillations after fertilization has a long-term impact on offspring growth. It is unknown if this outcome is a result of the combined effects of in vitro manipulation and altered Ca2+ signaling. Here we tested the hypothesis that, even without manipulation in vitro, abnormal Ca2+ signaling after fertilization impacts offspring growth. Plasma membrane Ca2+ ATPase 1 (PMCA1) is a pump that extrudes Ca2+ from the cytosol following Ca2+ release events and is highly expressed in mouse eggs. To disrupt Ca2+ signaling in vivo, we generated conditional KO (cKO) females that lack PMCA1 only in the eggs. As anticipated, in vitro fertilized cKO eggs had increased Ca2+ exposure relative to controls (~65% more) due to persistent prolonged Ca2+ transients. To assess their impact on offspring growth, we mated cKO females to WT males to generate pups that had high Ca2+ exposure at fertilization. Because these offspring were heterozygous, control pups that had normal Ca2+ at fertilization were generated by mating WT females to heterozygous males and selecting the heterozygous pups. Pups were weighed weekly to trace their growth trajectory. Offspring had comparable weights at birth. However, at 12 weeks-of-age, experimental females were 6% larger than controls while experimental males were 9% larger than controls and had altered glucose metabolism and body composition. Our results reveal that abnormal Ca2+ signaling after fertilization in vivo has a long-term impact on offspring health. These findings are relevant for environmental or health conditions affecting the egg's ability to handle Ca2+, such as obesity, inflammation, mutations in Ca2+-handling genes and use of assisted reproduction technologies.

P38. STIM1 and STIM1L in skeletal muscle: distinct isoforms for distinct functions?

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Muscle fibers are excitable cells whose main function is contraction. To achieve this process, they rely mainly on their own calcium stores in the sarcoplasmic reticulum (SR) that are released during excitation-contraction coupling. The majority of the calcium released is then repumped into the SR through the sarcoplasmic/endoplasmic calcium ATPase (SERCA) to terminate muscle contraction. However, a small amount is extruded and a mechanism to compensate this loss has been proposed : the store-operated calcium entry (SOCE).

SOCE is a phenomenon triggered by SR Ca^{2+} store depletion that involves an interaction mainly between two proteins: stromal interaction molecule 1 (STIM1), a Ca^{2+} sensor of the SR that binds to and activates Orai1, a plasma membrane Ca^{2+} channel. We previously reported that in skeletal muscle two isoforms of STIM1 are highly expressed: STIM1 and a longer splice variant, called STIM1L. Although it has been clearly established that SOCE plays an important role in myogenesis, our understanding of the physiological role of these two STIM1 isoforms in the formation, maturation and maintenance of the internal calcium circuitry in myotubes remains to be clarified. Hence, the aim of the present study was to determine the specific functions of STIM1 and STIM1L during the different steps of myogenesis.

To do so, we infected human primary myoblasts with a lentivirus encoding a doxycycline-inducible miRNA expression to downregulate both STIM1/1L isoforms (STIM1all) or only STIM1L. This knock-down was triggered at early (day 0) or later (day 6) step of *in vitro* myogenesis and experiments were performed at day 4 and day 11, respectively targeting the differentiation and the maturation processes. We showed that STIM1L downregulation severely affected the internal organization of the myotubes, with a strong impact on the sarcomere (the contractile unit of skeletal muscles) formation and maintenance. In addition, electrical stimulations-induced Ca^{2+} transients revealed that STIM1all and STIM1L down-regulation impacts maintenance of Ca^{2+} transients only at early stage of maturation. Interestingly, the cytosolic Ca^{2+} clearance capacity was reduced upon STIM1all and STIM1L down-regulation, both at early and late stages of myotube maturation. Using siRNA against the different extrusion systems (PMCA1/4 and NCX3), we showed that PMCA1 is the main extrusion protein in myotubes. We are now investigating whether STIM1 indeed modulates that activity of PMCA1.

Overall, our data show that STIM1L is required for the proper internal architecture organization during myogenesis while STIM1 seems to be more importantly involved as a regulator of Ca^{2+} homeostasis, especially at early stage of differentiation.

P39: Interaction of the NAADP binding protein HN1L/JPT2 with its target receptors in T cells

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During the adaptive immune response, activation of T cells significantly depends on changes in free cytosolic calcium (Ca^{2+}) concentrations. Early Ca^{2+} signals upon T cell activation are mostly regulated by Nicotinic acid adenine dinucleotide phosphate (NAADP), the most potent Ca^{2+} mobilizing second messenger. NAADP is formed within seconds upon T cell stimulation[1] . However, the target channels of NAADP are controversially discussed: while in some cell types, NAADP acts on two-pore channel 1 and 2 (TPC1 and TPC2) [2] , in T cells NAADP was shown to activate the ryanodine receptor type 1 (RYR1), located in the membrane of the endoplasmic reticulum (ER)[3] . Surprisingly, when using photoaffinity labeling (PAL) to identify binding partners of NAADP, neither RYR1 nor TPCs were labeled, but small cytosolic proteins[4] . Thus, a unifying hypothesis was formulated that one or more NAADP binding proteins (NAADP BPs) activate different Ca^{2+} channels localized at different organelles – depending on cell type and signaling pathway involved[5] . Recently, two labs independently identified hematological and neurological expressed 1–like protein (HN1L) / jupiter microtubule associated homolog 2 (JPT2) as NAADP binding protein [6,7]. The essential role of HN1L/JPT2 during T cell activation was proven by showing decreased local and global Ca^{2+} signals in human Jurkat and primary rat T cells with gene knock-out of Hn1L/Jpt2. Also, co-localization of HN1L/JPT2 with RYR1 in T cells upon their T cell receptor stimulation was shown in super resolution imaging[6] . Here, we want to understand the exact mechanisms connecting HN1L/JPT2 and RYR1 to NAADP signaling. Data obtained regarding NAADP binding to HN1L/JPT2 and posttranslational modifications of HN1L/JPT2 will be discussed at the conference.

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P40: Alternative splicing of STIM is an important regulator of neuronal Ca^{2+} signaling in physiology

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Store-operated Ca^{2+}-entry (SOCE) regulates basal and receptor-triggered Ca^{2+} signaling with STIM proteins sensing the endoplasmic reticulum (ER) Ca^{2+} content and triggering Ca^{2+} entry by gating Orai channels in the plasma membrane. Both *STIM1* and *STIM2* genes have similar exon numbers with differences in the length of exons 1 and 11, translating into mature proteins with a diverging distal cytosolic region. Although crucial for immune cells, the role of STIM proteins in neuronal Ca^{2+} homeostasis is less understood. Here, we characterize two novel variants, namely STIM1B and STIM2.3, which are predominantly expressed in the nervous system. In both variants, splice insertion of a shorter alternative exon between exons 11 and 12 results in truncated proteins lacking the microtubule associated EB binding sites, serine/proline rich region and the polybasic domain (PBD). While overexpression of STIM1B reduced SOCE in a partially residue-dependent manner, the gain-of-function phenotype of STIM2.3 was reproduced by the larger C terminal deletion. Deletion of the PBD in STIM2 diminished SOCE which was rescued by the simultaneous mutation of both EB binding motifs. Thus, full-length STIM2 requires the PBD for proper unfolding, detachment from MT and subsequent Orai activation. Despite a STIM2.3 mediated increase in basal Ca^{2+} levels, clustering under basal conditions, as seen with STIM2, was abolished. Moreover, Tg-induced clustering and cluster size distributions of both variants, STIM1B and STIM2.3, was significantly reduced in the absence of endogenous STIM. The neuronal variant STIM1B preferentially localizes to neurites and presynaptic regions in contrast to the more somatic localization of STIM1. Additionally, repetitive high frequency stimulation of autaptic hippocampal neurons overexpressing STIM1B leads to a strong enhancement of synaptic transmission due to increased vesicle replenishment that is absent in STIM1 expressing cells. This effect is mediated by a four amino acid motif within STIMB's additional domain and requires the interaction with Orai channels, suggesting a requirement for SOCE in high demand situations[1]. The physiological role of the shorter STIM2 variant, STIM2.3, is currently being investigated. However, it has been shown, that mutation of the EB binding motif in STIM2 leads to a reduced number of mushroom spines regulating synaptic plasticity[2]. Our findings demonstrate that alternative splicing is potent means to adapt Ca^{2+} signaling to specific cellular needs such as neurons.

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P41: Loss of IP_3 receptors in human induced pluripotent stem cells alters metabolism but not differentiation potential

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Human cells contain 3 genes (*ITPR1*, *ITPR2* and *ITPR3*) that encode for inositol 1,4,5-trisphosphate receptors (IP3Rs), thereby yielding 3 isoforms : IP3R1, IP3R2 and IP3R3, respectively. These IP_3Rs are partially redundant as knockout of all 3 subtypes is lethal in rodents while single gene knockouts produce tissue-specific pathology. Mutations in *ITPR1* cause ataxia. We recently reported *ITPR3* mutations in patients with Charcot-Marie-Tooth neuropathy, suggesting a role in the peripheral nervous system. Here, we set out to create a model for elucidating cell type-specific functions of the human IP3 receptors. Our first goal was to determine their role in stem cell survival and pluripotency. Therefore, we generated *ITPR1*, *ITPR2*, *ITPR3* and *ITPR1/2/3* triple knockout (TKO) cell lines of human induced pluripotent stem cells (iPSC).

CRISPR/Cas9 gene editing was used to knockout the *ITPR* genes. TKO iPSC lines were generated by knocking out the individual genes in successive manner. Generated knockout cell lines were analyzed by Sanger sequencing, Western blotting and quantitative reverse transcription PCR (qPCR) to ensure successful editing. The resulting knockout cell lines were then analyzed by gene expression panel targeted to stem cell-specific genes, immunocytochemistry and trilineage assay to examine the pluripotency of the cell lines. We also confirmed the successful editing of the generated TKO cells with functional Ca^{2+} imaging and examined the metabolite profile of the generated knockout cell lines with targeted metabolomics.

The desired gene editing events were successfully implemented. The qPCR analysis indicated the loss of *ITPR2* and *ITPR3* expression, whereas some *ITPR1* expression was retained in edited cell lines. Nevertheless, immunoblotting confirmed that the expression of all 3 IP3R isoform proteins was absent. Importantly, Ca^{2+} imaging revealed that the TKO iPSC, though having adequately loaded ER Ca2+ stores, did not respond to IP_3-generating agonists. Despite the loss-of-function, gene expression panel identified comparable levels of pluripotency markers between edited and control cell lines. The expression of pluripotency marker NANOG was confirmed with immunocytochemistry, Trilineage assay by directed differentiation revealed that all iPSC lines could differentiate into all three germ layers, despite distinct alterations in citric acid cycle metabolites in the TKO iPSC, identified by targeted metabolite profiling.

To conclude, this work is the first report of complete functional loss of IP3Rs in human iPSC. Our results suggest that IP3Rs control stem cell metabolism but are not required for maintenance of pluripotency.

P42. Investigation of Calcium Electroporation for Cancer in the Skin

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Background: Mutations may impair cancer cell-calcium homeostasis and cellular stress with calcium-ions can induce cell death. Calcium electroporation is a novel, local cancer treatment using intratumorally injected calcium chloride and manually applied pulsed electric fields. The pulses create transient pores in bi-lipid membranes allowing an increased flux of calcium-ions. Normal cells may restore homeostasis and recover whilst cancer cells die, making the treatment a safe, efficient and selective tool for targeted local treatment of cancer with limited side effects. Reports of long-term local disease control and systemic responses following treatment in initial small-cohort trials imply a positive effect on cancer immunity caused by calcium induced cell death. We report two clinical studies investigating the histopathological effect of calcium electroporation (CaEP-B) and clinical response (CaEP-R).

Materials and methods: Both studies are non-randomized phase II trials including patients with cutaneous malignancy of any histology. Patients are followed up to 12 months. The CaEP-B study will include 24 patients treated once and retreated after one month, depending on number of included tumours, with sequential biopsies taken at baseline and after treatment. The primary endpoint is the change in proportion of tumour-infiltrating lymphocytes two days after treatment. The samples will be analysed for immune markers as well as necrosis, changes in vasculature and inflammation. Circulating tumour DNA from sequential blood samples will be analysed in a subgroup of patients. Secondary endpoints include response, PD-L1 expression and importance of radiation. The CaEP-R study aims to investigate response to calcium electroporation in 30 patients. Patients are treated once and the primary endpoint is overall response rate at two months. The trial is a collaboration between three cancer centres. In one patient subset, MRI is used to verify treatment area. In another subset, qualitative interviews have been performed to uncover patient experience.

Results: Trials are ongoing and a total of 30 patients have been included with a variety of cancer subtypes. Few side effects have been observed and healthy tissues have been spared. Nine patients have been interviewed and the qualitative data of this subgroup is being analysed. MRI has been used to verify treatment areas in three patients. Preliminary results from the first eight patients in CaEP-R show an overall response rate of 42% (CI 23-63 %) across all tumours after two months (n = 24). In CaEP-B, one patient showed improved response to immunotherapy in untreated tumour after treatment.

Conclusion: This is the most comprehensive project to date investigating the effect of calcium electroporation on malignant tumours and their microenvironment. The results of this trial may illuminate mechanisms underlying this promising new treatment and reveal synergistic effects with the immune system.

P43: A simple methodology to study the effects of plant nutrient homeostasis on Ca^{2+} signaling

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Increasing crop yields by using eco-friendly practices is of high priority in tackling problems regarding food security and malnutrition worldwide. The scientific community aims to understand plant nutrients homeostasis by deciphering the nutrient sensing and signaling mechanisms of plants. Several lines of evidence about the involvement of Ca2+ as the signal of impaired nutrient availability have been reported. Moreover, an interesting aspect is to test how the nutrient status can affect Ca2+ signaling in response to environmental challenges. To do so, we aimed at developing a reliable approach to combining plant hydroponic cultures with in vivo Ca2+ imaging. Whereas hydroponics offers precise control of the media composition and easy access to every plant tissue for phenotypical and molecular analysis, modern genetically encoded Ca2+ indicators (GECIs) allow to carry out whole-plant imaging at low magnification.

Here we report the development of a pipeline to perform Ca2+ imaging analysis in adult Arabidopsis plants grown in hydroponic conditions. To standardize and automatize the image analyses we report the development of a customized data analysis pipeline, a python based napari plugin, that will be shared with the community and the design of a 3D-printed custom-made chamber for a quick exchange of the nutrient solution as well as osmotic or salt stress treatments.

To test the effects of nutrient availability on the generation and propagation of long-distance Ca2+ waves we are performing leaf wounding experiments in wild type and mutants Arabidopsis plants, grown in media with different compositions.

P44: Are STIM1L KO mice fatigued?

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Store Operated Calcium Entry (SOCE) is activated to refill the endoplasmic/sarcoplasmic reticulum (ER/SR) with calcium and relies on the activity of the calcium sensors from the STIM family (STIM1, 2) and the plasma membrane Orai calcium channel family (Orai1, 2, 3). Since skeletal muscle contraction mobilizes calcium from the SR, the mechanisms for SR calcium refilling are essential to maintain optimal contraction capacity. Calcium extrusion occurs during the prolonged contraction; thus, SOCE could be decisive in helping to maintain sustained contractions.

Our laboratory discovered that skeletal muscles highly express alternative splicing of STIM1, called STIM1L. Because the expression of STIM1L allows the SOCE activation to take place after a few seconds (faster than STIM1), we hypothesized that skeletal muscles need the muscle-specific expression of STIM1L to maintain prolonged contractions. To test this hypothesis, we generated constitutive STIM1L KO mice without affecting the expression of the ubiquitous STIM1 isoform.

The study used both males and females according to ARRIVE guidelines. STIM1L KO mice develop normally and do not exhibit a disabling phenotype. A spontaneous activity test using running wheels showed that 18-week-old STIM1L KO mice ran less than the control mice, both males, and females. This phenotype, which may reflect muscle fatigue, is not observed in younger mice. Assessment of muscle contractions ex vivo is currently carried out to confirm the muscular origin of the fatigue observed in STIM1L KO mice.

Our results indicate that the calcium sensor STIM1L specifically expressed in skeletal muscles could have a fundamental role in resistance to muscle fatigue.

P45: Leukocyte adhesion is governed by endolysosomal two pore channel 2 (TPC2)

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In response to pro-inflammatory challenges including pathogenic attack and tissue damage, the endothelial cell surface is rearranged to present leukocyte-engaging cell surface receptors. Cell surface presentation of the leukocyte receptor P-selectin together with the stabilizing co-factor CD63 is needed for leukocyte tethering and rolling and is mediated via demand-driven exocytosis from endosomal compartments. Focusing on endolysosomal Ca^{2+} circuits possibly regulating post-endolysosomal transport of CD63, we report that the recruitment of neutrophils to the activated vessel walls is strongly reduced in mice with specific gene deletion of the endolysosomal non-selective cation channel TPC2, as revealed by intravital microscopy. Investigating human primary endothelial cells with impaired TPC2 functionality through both RNA interference-mediated TPC2 gene silencing and pharmacological TPC2 inhibition, we found that TPC2 was needed to ensure CD63 transfer from late endosomes/lysosomes via Weibel Palade bodies to the plasma membrane to retain P-selectin on the endothelial cell surface. A significantly reduced P-selectin-dependent adhesion of human neutrophils to histamine-activated human primary endothelial cells with pharmacological TPC2 block under flow conditions confirmed the physiological relevance of TPC2 functionality for leukocyte interaction with the endothelium. Our findings establish the endolysosome-localized TPC2 Ca^{2+} channel as a key element in the maintenance of proper endothelial functions and a potential pharmacological target in the control of inflammatory leukocyte recruitment.

P46: Development of an adrenocortical cell model of calcium signaling modulation to decipher the molecular mechanisms responsible for primary aldosteronism

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Introduction: Primary aldosteronism (PA) is the most frequent form of secondary hypertension. The identification of germline or somatic mutations in different genes coding for ion channels and defines PA as a channelopathy. These mutations promote activation of calcium signaling, the main trigger for aldosterone biosynthesis.

Objective: The objective of our work was to elucidate, using chemogenetic tools, the molecular mechanisms underlying the development of PA by modulating sodium entry into the cells, mimicking some of known mutations identified in PA.

Method: We have developed an adrenocortical H295R_S2 cell line stably expressing a chimeric ion channel receptor formed by the extracellular ligand-binding domain of the α7 nicotinic acetylcholine receptor fused to the ion pore domain of the serotonin receptor 5HT3 α and named α7-5HT3. Mutations have been introduced in the ligand binding domain to allow only synthetic drugs to activate this channel receptor. Activation of α7-5HT3 by a specific drug, PSEM-817 leads to sodium entry into the cells. This cell line was characterized in terms of intracellular calcium concentrations, cell proliferation, aldosterone production, steroidogenic expression and electrophysiological properties.

Results: Treatment of α7-5HT3 expressing cells with increasing concentration of PSEM-817 (from 10^{-9} to 10^{-5} M) induced a significant increase in intracellular calcium concentrations, similarly to potassium (12 mM) or angiotensin II (10^{-8} M). This stimulation of calcium signaling did not affect cell proliferation, but was responsible for an increase in *CYP11B2* expression and aldosterone production after 24h of treatment. However, while increased intracellular calcium concentrations were observed starting from 10^{-8} M of PSEM-817, *CYP11B2* expression and aldosterone production were only affected starting from 10^{-7} M, suggesting a dose dependent effect. Finally, whereas cells were hyperpolarized in absence of stimulation (around -60 mV), PSEM-817 induced a strong depolarization, cells rising to a membrane potential around -10mV.

Conclusion: This cell line, in which we can modulate the intracellular calcium concentration "on demand", is a useful tool for a better understanding of the alterations of intracellular ion balance and calcium signaling in the pathophysiology of PA.

P47: Store-Operated Ca^{2+}-Entry in Uterine Endometrial Cancer

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Worldwide, uterine endometrial carcinoma is the sixth most common form of cancer in women. Early diagnosis resulting from patient-reported postmenopausal bleeding leads to excellent 5-year survival rates. However, for women with advanced or recurring EC, prognosis is relatively poor [1]. Consequently, there is a need for new therapeutic strategies to tackle these forms of this disease.

The "Ca^{2+}-toolkit" is characteristic of each cell-type and alters during proliferation, differentiation, oncogenesis and death [2]. Using The Cancer Genome Atlas accessed via the UALCAN resource [3], we examined differences in the expression of 196 genes encoding Ca^{2+}-toolkit proteins between EC tumours (n = 546) and normal adjacent tissues (n = 35). All genes encoding components of the store-operated Ca^{2+}-entry (SOCE) pathway showed significant differences in transcription. Transcription of *ORAI 1-3* genes was increased in EC tumours relative to normal tissue, whereas *STIM1* and *STIM2* were significantly decreased. Of these changes, only increased expression of *ORAI2* was associated with alterations in patient outcomes: by Kaplan-Meier analyses, those with the highest 50% of *ORAI2* levels showed significantly poorer survival ($p = 0.005$).

In order to investigate the roles of Orai channels in EC biology, we examined the effects of small molecule antagonists (GSK7975A, Synta66 and 2-aminoethoxydiphenyl borate (2-APB)) on SOCE [4] in the human EC cell-line RL95-2. Changes in cytoplasmic Ca^{2+} levels were monitored by fluorescent videomicroscopy using the Ca^{2+} fluorophore, fura-2. In nominally Ca^{2+}-free HEPES-buffered saline, RL95-2 cells were incubated with 1 mM thapsigargin, to deplete the endoplasmic reticulum of Ca^{2+}. Extracellular Ca^{2+} (2 mM) was then added back to the medium, to quantify SOCE. Pre-incubation with any of the antagonists significantly decreased the rate and/or magnitude of SOCE relative to vehicle pretreated with vehicle (DMSO) alone.

The ability of cells to form colonies is a hallmark of cancer cells. To examine the role of SOCE in this process, RL95-2 cells were seeded at low-density (3×10^3 cells/well in 12-well plates) and cultured for two weeks in the presence of different concentrations of GSK7975A, Synta66 or 2-APB. All three antagonists significantly inhibited clonogenesis of RL95-2 EC cells, with half-maximal inhibitory concentrations at low micromolar levels.

Overall, these findings indicate that SOCE plays a role in colony formation of RL95-2 cells and that in EC, high *ORAI2* expression is associated with poor patient survival. This highlights the potential of targeting *ORAI2* in the development of new chemotherapeutic strategies for the treatment of EC.

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P48: Intracellular calcium signaling is significantly dysregulated in iPSC-derived neural progenitor cells containing the M139V presenilin 1 mutation.

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Alzheimer's disease (AD) is an untreatable neurodegenerative disorder, the underlying cause(s) of which remain unknown. Recent research by us and others supports the so-called 'calcium hypothesis of AD', which posits that the disruption/dysregulation of neuronal intracellular calcium regulation may be one of the initiators of AD pathology. To date however, most of these studies have been conducted using animal models of AD. As such, it remains unclear if these findings of neuronal calcium homeostatic dysregulation are replicated in human brain tissue carrying specific AD-linked mutations, due to the paucity of living human brain tissue available for research purposes.

The current study sought to determine if previous work in our lab, demonstrating that neuronal calcium homeostasis was dysregulated disrupted in primary hippocampal neurons from a transgenic mouse model of AD (3xTgAD mouse) (relative to wildtype neurons)1, could be replicated using human induced pluripotent stem cell (iPSC) derived neural progenitor cells (NPCs) containing a familial AD mutation of the presenilin 1 (PS1) protein (M139V), and a healthy donor control (Cntrl).

NPCs were prepared from commercially available human iPSCs containing the M139V PS1 mutation, and from healthy control iPSCs, by dual inhibition of TGF-β/BMP-dependent SMAD signaling. Neural stem lineage was confirmed by SOX2 and βIII tubulin immunostaining. 5-9 day post-culture cells were loaded with the calcium-sensitive dye Calbryte 520 AM (4μM; 1h) and placed in a chamber superperfused with

HBSS (2ml/min). Intracellular calcium ([Ca2+]i) responses to extracellular 50mM K+ and the Ca2+ ionophore ionomycin (10μM) were recorded using WinFluor software. Data are expressed as mean±S.E.M. N values are displayed as n = x cells, x experiments. All results presented were analysed using a two-tailed Welch's t test.

We initially observed that basal [Ca2+]i levels in the M139V NPCs (n = 64, 5) were significantly elevated relative to Cntrl (n = 71, 9) (14.4±1.2 arbitrary units vs 7.1±0.4 arbitrary units; $p<0.0001$). Furthermore, we found that the ΔF/F relative percentage change of K+-evoked [Ca2+]i signals was significantly greater in M139V NPCs compared to Cntrl 732.1%±77.6 vs 108%±8.3, $p<0.0001$). Similarly, the ΔF/F of ionomycin-evoked [Ca2+]i signals was also significantly greater in M139V NPCs compared to Cntrl (431.5%±42.0 vs 184.4%±10.6, $p<0.0001$).

Our results indicate that, in accordance with our previous work using primary cultured murine hippocampal neurons, both basal and evoked [Ca2+]i levels in presenilin mutant cells were significantly increased relative to controls. The profound differences in calcium responses observed in NPC populations prior to maturation supports the suggestion that AD pathogenesis commences before more overt, neurohistopathological changes.

[1] Kaar, A, 2019 , 'Investigating neuronal calcium homeostasis in murine models of Alzheimer's disease.' PhD Thesis, University College Cork.

P49: Use of genetically-encoded calcium indicators to measure intracellular calcium signalling dynamics between cell-in-cell structures

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Cell-in-cell (CIC) structures, where one cell is completely enclosed within another, have long been observed under both physiological and pathological conditions (1, 2, 3). CIC structures can result from a variety of processes such as cell cannibalism and emperipolesis (2, 3). One such pathological state where CIC structures are observed includes breast cancer, particularly in fluid exudates taken from clinical samples from patients with more advanced and more aggressive cancers (3, 4, 5). Calcium (Ca2+) signalling plays a crucial role in many cellular processes and alterations in Ca2+ signalling are well-established as playing a role in diseases such as breast cancer (6, 7, 8). How Ca2+ may be involved in the formation of CIC structures and how cells may interact through Ca2+ signalling remains to be established. During these experiments, cells were maintained in suspension under conditions that promote the formation of CIC structures. Cells were genetically modified to express genetically-encoded calcium indicators (GECI) GCaMP and jRCaMP. The use of GECI allowed for the observation of these CIC structures over many hours and for the observation of changes in intracellular Ca2+ after the formation of CIC formation. Cells were stimulated using the Ca2+ mobilising agonist ATP and changes in intracellular Ca2+ were observed using confocal microscopy. Post stimulation with ATP, a differential Ca2+ response was seen between the inner and outer cell of the CIC structure. These results indicate that the Ca2+ dynamics of CIC structures are complex and differ between inner and outer cells of these structures and Ca2+ signalling may play a role in the development and maintenance of CIC structures.

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P50: Role of calcium entries in the physiopathology of glioblastoma stem cells

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Calcium regulates various cellular processes in physiological and physiopathological conditions. Calcium signaling has been involved in the regulation of cancer stem cells that are the cells responsible for tumor initiation and recurrence. Many of the calcium toolkit proteins (pumps, exchangers, voltage-dependent or non-dependent channels and intracellular proteins) are overexpressed or display functional changes in various cancer stem cells. SOC (store operated calcium channels) and ROC (receptor operated calcium channels) mediate most of calcium entries in non-excitable cells and modulate the intracellular calcium concentration, which can regulate cancers stem cells activities as cell proliferation, differentiation, migration, invasion or chemoresistance.

As previous studies of transcriptome analysis show that human glioblastoma stem cells (GSC) are enriched in calcium signaling genes, this work focuses on variation of calcium influx in GSC and its involvement in the regulation of GSC activity. SOC and ROC are functional in GSC derived from patients with mesenchymal (the most aggressive glioblastoma group) and non mesenchymal glioblastoma. The pharmacological inhibition of SOC induces the decrease of proliferation, impairs self-renewal, and reduces expression of the stem cell marker SOX2 in GSC. To investigate the impact of SOCE signaling in GSC migration, tridimensional environments have been used to mimic the different substrates that GSC will find in the brain. Biocompatible polyacrylonitrile-derived nanofibrous scaffolds have been developed in Institut Européen des Membranes with two nanofibers organizations (preferentially aligned and planar isotropic orientation) and two stiffnesses (3 kPa and 166 kPa). These scaffolds have been used to analyze the impact of stiffness and organization on calcium influx in GSC as well as proliferation and migration of these cancer stem cells.

To complete knowledge on the role of calcium signaling in GSC, ROCE (ROC entry) were also studied, especially TRPC6 and TRPC3. Both channels are expressed in GSC and TRPC3 has a lower expression level in non mesenchymal GSC compared to mesenchymal GSC. ROCE were analyzed as well as these involvements in GSC proprieties.

Our data indicate that calcium signaling plays a crucial role in GSC physiopathology and could be an interesting therapeutical target to regulate GSC.

P51: S100A8/A9 regulated by miRNA-132: a key player in Ca^{2+} -regulated cytokine mobilization and secretion during neutrophil-mediated inflammation

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Neutrophils are important members of the immune system and participate in the release of cytokines. This process must be highly orchestrated, to constrain inflammatory responses to pathogens and injury, and avoid overactivation and development of diseases. However, this elegant regulation is still only partially known. Increasing evidence over the years show that Ca^{2+} is actively involved in cytokine secretion but our knowledge underlining for such a mechanism need to be filled. To this end, our objective is to study the Ca^{2+}-dependent mechanisms underlying cytokine secretion in neutrophils and also at the earlier stage, cytokine production. For that, the differentiated myeloid cell line HL-60 (dHL-60) was used as cellular model, since neutrophils are terminally differentiated cells and unable to be genetically modified. By stimulating dHL-60 with the pro-inflammatory stimulus fMLF, we found that several cytokines and notably IL-8 gene expression were up-regulated upon stimulation as well as secretion in a time-dependent manner. Incubation of cells in the absence of extracellular Ca^{2+} ($[Ca^{2+}]_e$) or after treatment with the intracellular Ca^{2+} ($[Ca^{2+}]i$) chelator BAPTA, led to a strong inhibition of IL-8 secretion and intracellular production. The effect of $[Ca^{2+}]_i$ and $[Ca^{2+}]_e$ entry were dependent on store-operated Ca^{2+} entry mechanism since the cytokine secretion were inhibited by 2-APB. Next, we investigated the role of S100A8/A9 since these Ca^{2+}-binding proteins have been previously involved in other key neutrophil functions (e.g. NADPH oxidase activation). To determine if S100A8/A9 is also involved in these processes, knockdown studies were performed in dHL-60 cells. Our results showed that both secretion and intracellular production of IL-8 were dysregulated in these cells, indicating the unneglectable role of S100A8/A9. Then, the post-transcriptional regulation mechanism of S100A8/A9 was investigated. Using TargetScan prediction, we found that microRNA-132 was upregulated upon fMLF stimulation and thus, it constitutes an upstream regulator of S100A8/A9. Moreover, stable overexpression of miR-132-5p in dHL-60 cells led to a strong inhibition of S100A8/A9 expression. Here, for the first time, we proved that intracellular S100A8/A9, negatively regulated by miR-132-5p, is a very important player in cytokine secretion and production process during neutrophil-like cells mediated inflammation. Further investigations are required to fully understanding the relationship between miRNA-S100A8/A9- Ca^{2+}-cytokines with the potential hope to reveal new targets to control inflammatory disorders.

P52. Direct monitoring of endo-lysosomal Ca^{2+} signalling with a novel bioluminescent indicator

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Ca^{2+} is a universal second messenger that is compartmentalized in cytoplasmic organelles. Acidic organelles are Ca^{2+} stores capable of accumulating and releasing Ca^{2+} upon cell activation. Therefore, much effort has been devoted to develop genetically-encoded Ca^{2+} indicators as tools for measuring luminal Ca^{2+} in acidic compartments. However, this task is extremely challenging because most of the fluorescent Ca^{2+} indicators are pH-sensitive and the fluorescent signal is quenched at acidic pH. By contrast, bioluminescence-based Ca^{2+} sensors are potentially advantageous since they are relatively resistant to pH variations. Aequorin is a Ca^{2+} binding protein that emits blue light when reconstituted with its cofactor coelenterazine. Targeted aequorins are excellent Ca^{2+} indicators for monitoring subcellular Ca^{2+} dynamics. Here we targeted GFP-aequorin to the endo-lysosomal lumen and found that a significant fraction of the probe resides in a non-acidic compartment. We report that aequorin does not reconstitute at acidic pH and leveraged this to report calcium dynamics in this compartment. We show that luminal Ca^{2+} uptake is thapsigargin sensitive and that steady state Ca^{2+} levels are in the range of hundreds of micromolar, similar to the ER, but selectively reduced upon overexpression of an endocytic channel. We find the calcium mobilizing messenger IP3 evokes robust luminal responses in wild type cells but not in IP3 receptor knock-out cells. And that a fraction of endogenously tagged IP3 receptors colocalize with lysosomes. Stimulation with IP3-forming agonists evoke Ca^{2+} release in live intact cells.

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