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The First Junior European Calcium Society Meeting: Calcium Research Across Scales, Kingdoms and Countries

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Abstract

The First Junior European Calcium Society Online Meeting, held October 20-21, 2020, aimed to promote junior researchers in the Ca^{2+} community. The meeting included four scientific sessions, covering Ca^{2+} research from molecular detail to whole organisms. Each session featured one invited speaker and three speakers selected based on submitted abstracts, with the overall aim of actively involving early-career researchers. Consequently, the meeting underlined the diversity of Ca^{2+} physiology, by showcasing research across scales and Kingdoms, as presented by a correspondingly diverse speaker panel across career stages and countries. In this meeting report, we introduce the visions of the Junior European Calcium Society board and summarize the meeting content.
Keywords: jECS, ECS, Ca\(^{2+}\) signaling, meeting report, junior researchers, online conference

The Junior European Calcium Society Board

The European Calcium Society (ECS) acts as a scientific interaction node for Ca\(^{2+}\) researchers, and prioritizes inclusion, support and promotion of early- and mid-career researchers. The ECS offers travel fellowships, poster prizes and oral presentation awards for junior members, and many of the awardees go on to contribute to the ensuing Special Issue published in *Biochimica et Biophysica Acta - Molecular Cell Research* (Decrock et al., 2017; Kim et al., 2017; Koenig et al., 2019; Stewart and Davis, 2019). As a consequence of these priorities, the Junior European Calcium Society (jECS) board was established in late 2018, following the 15\(^{th}\) International Meeting of the ECS in Hamburg, Germany.

In its original form, the jECS board was comprised of fifteen members from eight countries, ranging from PhD students to junior group leaders. An important agenda of the board was and is to create platforms for junior researchers in the Ca\(^{2+}\) field to collaborate, peer-mentor, share ideas and push boundaries. Accordingly, the jECS board opened an online space for the ECS community on Slack and a Twitter account (@EuroCalcium) (“jECS Twitter,” 2020). It became clear early on that the jECS would strive to not only provide a stage for young researchers to establish scientific networks and share their data, but also to share their experiences, to discuss challenges and to oppose the structural biases that affect many early career scientists, including gender issues.

The First jECS Meeting

To increase visibility of junior researchers, and to strengthen interactions between these, the jECS board planned the 1\(^{st}\) jECS Meeting as a one-day pre-meeting in connection with the 16\(^{th}\) ECS International Meeting in Cork, Ireland, August 2020. However, as SARS-CoV-2 rapidly spread around the world — grounding flights, closing universities and disabling conferences — plans for the meetings in Cork were tabled until 2022. Instead, the jECS board made the decision to host a virtual, stand-alone meeting on 20\(^{th}\)-21\(^{st}\) of October 2020.

A welcome consequence of this virtual meeting was the removal of both financial and geographic barriers to attendance (Sarabipour et al., 2020). The board could also expand its original format from an event for junior scientists, hosted by junior scientists, to accommodate a much broader
and larger audience. Consequently, the 1\textsuperscript{st} jECS Online Meeting had 190 registered participants from 28 countries across the world (Figure 1A). In accordance with the meeting vision, 71% of the registrants were junior researchers, while the remaining 29% included junior and senior group leaders, technical staff and medical professionals (Figure 1B).

**Figure 1: Meeting registrants across countries and career stages.**

A. Distribution of the 190 jECS meeting registrants across the world. Pins represent the location of speakers, by country. B. Distribution of registrants across career stages.
The meeting included a roundtable discussion on Equity and Diversity in Science, featuring Sarah Roberts-Thomson (Brisbane, Australia), Enikö Kallay (Vienna, Austria) and Geert Bultynck (Leuven, Belgium) as panelists. The discussion offered important debates about career positioning, minorities in science and cultural differences (Dukes, 2020). At the first jECS general assembly, the composition, structure and visions of the junior board were presented.

Sixteen scientific talks were given, divided in four sessions that broadly covered topics in $\text{Ca}^{2+}$ research across scales and Kingdoms, ranging from single channel studies to whole organisms (Figure 2). Each session featured one invited speaker and three short talks selected from abstracts, with the overall aim to have early-career researchers actively participating. An overview of the scientific content of the meeting is given below.

![Figure 2: Ca$^{2+}$ research across scales and Kingdoms.](image)

From left: Cryo-EM structure of TRPV5/Ca$^{2+}$CaM (Jenny van der Wijst), intravital brain imaging of mouse mitochondria (Maria Calvo-Rodriguez), Ca$^{2+}$ signal in a T cell (Feng Gu), Ca$^{2+}$ signal in A. thaliana leaf (Annalisa Bellandi). Cryo-EM, cryogenic electron microscopy; TRPV5, transient receptor potential vanilloid 5; CaM, calmodulin. Figure assets created with BioRender.com.
Session 1: Calcium Signaling in Disease

Investigations into dysregulated Ca\(^{2+}\) signaling in disease have provided insights into pathologies, novel therapeutic targets and mechanisms of action for existing therapies (Parys and Bultynck, 2018).

The versatility of the Ca\(^{2+}\) sensing receptor (CaSR) was highlighted by opening presenter Martin Schepelmann (Vienna, Austria). Originally studied in the context of calcitropic tissues and Ca\(^{2+}\) homeostasis regulation, the CaSR has emerged as a modulator of a number of other physiological and pathophysiological functions (Schepelmann et al., 2016). Inhibition of CaSR with inhaled negative modulators reduced airway hyperresponsiveness and inflammation (Yarova et al., 2015). In dextran sulphate sodium induced colitis, CaSR inhibition coincided with reduced intestinal inflammation while stimulation of the receptor exacerbated it (Elajnaf et al., 2019; Iamartino et al., 2020). Collectively, these results point to the CaSR as a novel therapeutic target for diseases such as asthma and colitis.

Jessica Moore (New Haven, USA) investigated the role of Ca\(^{2+}\) signaling in epidermal stem cell behaviour. Using a combination of live in vivo imaging, high-throughput image analysis and drug treatments, Moore observed synchronised Ca\(^{2+}\) fluctuations in neighbouring cells, which could be linked to coordinated cell cycle progression. These findings suggest intercellular Ca\(^{2+}\) communication via gap junctions is involved in balancing homeostatic stem cell differentiation and division, providing a framework to understanding associated pathologies.

Intercellular Ca\(^{2+}\) signaling was similarly in focus when explaining the pathology of infectious diarrhoea caused by rotavirus, as presented by Thomas Gebert (Houston, USA). Long-term time-lapse imaging studies revealed that rotavirus induces Ca\(^{2+}\) waves in remote, uninfected cells, which contributed to disease progression. Infected cells were found to secrete adenosine diphosphate (ADP), which activated purinergic receptor P2Y1 in neighbouring cells and stimulated Ca\(^{2+}\) waves. Inhibition of the P2Y1 receptor limited disease severity, positioning this process as a novel therapeutic target for the disease (Chang-Graham et al., 2020).

Mechanistic insights into cell death pathways, triggered by the Ca\(^{2+}\)-chelator BAPTA-AM in diffuse large B-cell lymphoma, were probed by Flore Sneyers (Leuven, Belgium). Sneyers asked why the
combination of BH3 mimetic B-cell lymphoma 2 (Bcl-2) antagonist venetoclax and BAPTA-AM provoked cell death synergistically, as venetoclax has been shown to function via a $\text{Ca}^{2+}$-independent pathway (Vervloessem et al., 2017). Functional and expression assays determined that treatment with BAPTA-AM, or its low $\text{Ca}^{2+}$-affinity variants, resulted in the loss of anti-apoptotic myeloid cell leukemia 1 (Mcl-1) protein, implying a $\text{Ca}^{2+}$-independent effect. The mechanisms by which BAPTA-AM variants lower Mcl-1 levels are currently investigated. However, these results serve as a reminder of the potential off-target effects of frequently used $\text{Ca}^{2+}$-chelators and indicators (Bootman et al., 2018; Smith et al., 2018).

**Session 2: Organellar Calcium Signaling**

$\text{Ca}^{2+}$ release from the endoplasmic reticulum (ER) and mitochondria is central for cellular signaling, underpinned by the wide spectrum of diseases involving dysregulation of organellar $\text{Ca}^{2+}$ handling (Raffaello et al., 2016).

The D-myo-inositol-1,4,5-trisphosphate (IP$_3$) receptor (IP$_3$R) $\text{Ca}^{2+}$ channels link extracellular signaling and ER $\text{Ca}^{2+}$ release (Rossi and Taylor, 2018). Ana Rossi (Cambridge, UK) addressed the fundamental question: How can IP$_3$Rs respond to an IP$_3$ stimulus independently of a preceding or succeeding stimulus to generate quantal $\text{Ca}^{2+}$ release? Using a synthetic IP$_3$R partial agonist, functional analyses and mathematical modelling, Rossi found that $\text{Ca}^{2+}$ signals evoked by sub-maximal concentrations of IP$_3$ arise from activation of a tiny number of IP$_3$Rs which rapidly inactivate. Most IP$_3$Rs thus retain the capacity to respond to additional stimulation, together allowing graded responses to IP$_3$.

Another trigger of organellar $\text{Ca}^{2+}$ release is nicotinic acid adenine dinucleotide phosphate (NAADP), which is the most potent $\text{Ca}^{2+}$ mobilizing agent produced in the first seconds after T cell activation (Guse and Diercks, 2018) (Figure 2). However, NAADP formation and signaling is far less understood than IP$_3$ generation and signaling. Feng Gu (Hamburg, Germany) characterized a family of newly identified NAADP forming enzymes. A double knockout T cell line of two of the isozymes was significantly compromised in $\text{Ca}^{2+}$ microdomain generation (Figure 2), suggesting that this family of enzymes is physiologically relevant.
Maria Calvo-Rodriguez (Boston, USA) used intravital multiphoton imaging to study mitochondrial Ca\textsuperscript{2+} levels in Alzheimer's disease (AD) (Calvo-Rodriguez et al., 2020) (Figure 2). Both in an AD mouse model and upon topical application of soluble amyloid beta onto the brain, Calvo-Rodriguez observed an increase in mitochondrial Ca\textsuperscript{2+} levels. This effect was abolished upon blocking the mitochondrial calcium uniporter (MCU). This work thus strengthens the hypothesis that Ca\textsuperscript{2+} dyshomeostasis contributes to the pathology of AD.

Mitochondrial dysregulation is also involved in changed Ca\textsuperscript{2+} homeostasis in cancer cells (Cardenas et al., 2020). This was the focus of Jillian Weissenrieder (Philadelphia, USA), who isolated cells from a pancreatic ductal adenocarcinoma (PDAC) mouse model where the MCU was knocked out. These cells did not produce tumours in an orthotopic model. However, upon re-expression of the MCU, the cells acquired cancer-associated phenotypes such as increased proliferation, motility and occasionally tumour formation. Thus, the MCU may be an important node for PDAC development and for potential treatment strategies.

Session 3: Calcium Signaling in Non-mammalian Systems

Model systems ranging from single-cell Dictyostelium over small organisms like Caenorhabditis elegans and Drosophila melanogaster to Arabidopsis thaliana are important to study conservation and mechanisms of Ca\textsuperscript{2+} signaling (Plattner and Verkhratsky, 2013).

Due to the short lifespan of D. melanogaster, the fly is a powerful model to study aging. Alba Delrio-Lorenzo (Valladolid, Spain) used this model to study sarcopenia - age-related loss of muscle-function. Delrio-Lorenzo and colleagues expressed the ER-targeted Ca\textsuperscript{2+} sensor GFP-aequorin protein (erGAP3) in flies to analyze the ER Ca\textsuperscript{2+} concentration in muscle cells and neurons during aging (Delrio-Lorenzo et al., 2020). Delrio-Lorenzo demonstrated a dramatic reduction in sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} concentration with age that correlated with loss of muscle, whereas there was no change in neuronal ER Ca\textsuperscript{2+} levels. Reduced SR Ca\textsuperscript{2+} levels could be explained by reduced protein levels of the sarco/endoplasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA) in the SR and potentially by leakiness of ryanodine receptor (RyR) Ca\textsuperscript{2+} channels.

The wounding of a major vein in an A. thaliana leaf generates a global Ca\textsuperscript{2+} wave that transmits from the wounding site to non-wounded systemic organs and tissues (Kudla et al., 2018) (Figure...
2). Using a novel experimental and computational method, Annalisa Bellandi (Norwich, UK) investigated how a local Ca\(^{2+}\) wave propagates across a leaf, from a wound that does not injure a major vein. Bellandi revealed details on the molecular components of cell-to-cell transmission and on the dynamics of local wound-induced Ca\(^{2+}\) responses. These responses appeared to have distinct phases involving different genetic components, and the Ca\(^{2+}\) wave depended on extracellular transmission and was supported by glutamate-like receptor channels.

Nishit Srivastava (Cambridge, UK and Paris, France) presented work on the role of mechanically activated Piezo ion channels for Dictyostelium cell migration (Srivastava et al., 2020; Wu et al., 2017). Dictyostelium can adopt either a bleb-driven or pseudopod-driven migration mechanism. With the invention of a “cell squasher” to apply uniaxial pressure (Srivastava et al., 2017), Srivastava found that bleb-driven motility was favoured under increased mechanical pressure. Srivastava also demonstrated that applied pressure is sensed through Piezo channel-mediated Ca\(^{2+}\) influx, ultimately resulting in bleb-driven motility.

Scarlett E. Delgado (Valparaiso, Chile) defied the time zone difference to talk about neuronal regeneration in C. elegans. Delgado used the MEC-4d C. elegans strain, which expresses a mutant form of the mechanosensitive Na\(^+\) channel MEC-4 that also permeates Ca\(^{2+}\) ions (Bianchi et al., 2004; Goodman et al., 2002). The expression of MEC-4d in C. elegans causes an imbalance in the intracellular Ca\(^{2+}\) concentration resulting in axonal death in touch receptor neurons (TRNs). Extraordinarily, C. elegans regenerates damaged TRNs during diapause in MEC-4d expressing animals (Caneo et al., 2019). Interestingly, using multiphoton microscopy, Delgado showed that increased Ca\(^{2+}\) levels were preserved in TRNs during diapause regeneration, opening the question of which mechanisms allow neurons to tolerate unphysiological Ca\(^{2+}\) levels.

Session 4: Structure and Molecular Mechanism of Calcium Binding Proteins

A wide range of Ca\(^{2+}\)-binding proteins modulate themselves or other targets upon Ca\(^{2+}\)-binding to convey Ca\(^{2+}\) signals (Berridge et al., 2003). A better understanding of these modulations has invaluable implications in health and disease.
Store-operated Ca\(^{2+}\) entry (SOCE) contributes to the control of Ca\(^{2+}\) homeostasis, cell proliferation and motility (Tajada and Villalobos, 2020; Villalobos et al., 2017). Lucía González Gutiérrez (Cáceres, Spain) revealed that several channel complexes involved in SOCE are remodeled in colorectal cancer cells. In particular, Gutiérrez found increased protein levels of transient receptor potential canonical 1 (TRPC1), Ca\(^{2+}\) release-activated Ca\(^{2+}\) channel protein 1 (Orai1) and stromal interaction molecule 1 (STIM1). Moreover, immunoprecipitation revealed increased dual interactions between TRPC1, Orai1, and STIM1, consequently enhancing SOCE. This enhancement was reversed by the ornithine decarboxylase inhibitor difluoromethylornithine (DFMO), which decreased the interaction between TRPC1 and STIM1. Reversing this Ca\(^{2+}\)-remodeling countered the enhanced proliferation and motility inherent to colorectal cancer cells.

Ca\(^{2+}\)-selective transient receptor potential vanilloid 5 (TRPV5) channels mediate the reabsorption of Ca\(^{2+}\) through the kidney epithelium. Channel closure is modulated by the Ca\(^{2+}\)-sensing protein calmodulin (CaM). Using single particle cryo-electron microscopy, Jenny van der Wijst (Nijmegen, Netherlands) and colleagues solved the structure of the TRPV5/Ca\(^{2+}\)CaM complex (Dang et al., 2019) (Figure 2). The structure revealed that the N- and C-terminal domains of Ca\(^{2+}\)CaM interact with individual C-terminal regions of TRPV5, with the CaM C-domain blocking the channel pore. Functional studies using Ca\(^{2+}\)-insensitive CaM variants are on their way to study the Ca\(^{2+}\)-dependence of CaM-mediated TRPV5 channel inactivation.

IP\(_3\)Rs require both IP\(_3\) and Ca\(^{2+}\) to activate and elicit Ca\(^{2+}\)-release from intracellular stores. While the IP\(_3\)-binding site is well-known, the identity of the Ca\(^{2+}\)-binding site remains unresolved. Inspired by the recent structure of IP\(_3\)R3 (Paknejad and Hite, 2018), Vikas Arige (Rochester, USA) investigated the involvement of residue E2002 in IP\(_3\)R1 Ca\(^{2+}\)-binding. Carbachol-stimulation of IP\(_3\)R1-expressing HEK293 cells revealed that E2002D channels attenuate Ca\(^{2+}\)-release compared to WT channels, whereas E2002Q channels did not respond to stimulation. Both variants gave rise to a reduced number of Ca\(^{2+}\)-puffs compared to WT. These results thus help to delineate the mechanistic basis for IP\(_3\)R regulation by Ca\(^{2+}\).

The meeting was closed by Gary Shaw (London, Canada) who highlighted the role of the Ca\(^{2+}\)-binding protein dysferlin in membrane repair. Cell rupture causes an influx of Ca\(^{2+}\), which triggers vesicle accumulation and fusion for membrane repair. Dysferlin aids wound closure through its C2
domains, which mediate membrane-tethering and protein trafficking and recruitment (Sula et al., 2014). Specifically, the C2A domain enables Ca\(^{2+}\)-mediated phospholipid-binding (Fuson et al., 2014). Using nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography, Shaw compared the Ca\(^{2+}\)-free and Ca\(^{2+}\)-bound dysferlin C2A domains. The comparison revealed a reduced flexibility of the Ca\(^{2+}\)-binding loops upon Ca\(^{2+}\)-binding, and provided mechanistic details into Ca\(^{2+}\)-dependent membrane repair by dysferlin (Wang et al., 2021).

**Concluding remarks and Perspectives**

Together, the two-day jECS online meeting emphasized the diversity of Ca\(^{2+}\) physiology, in high-quality presentations supported by an active audience. Annalisa Bellandi and Thomas Gebert were awarded the prizes for best presentations. The jECS board wishes to continue its conference activity, and while the prospects for 2021 are at present unclear, the board is considering to host another online conference. Moreover, the jECS still plans to organize a one-day pre-meeting for the 16\(^{th}\) ECS International Meeting in Cork, Ireland, in 2022.

To further promote junior researchers in the Ca\(^{2+}\) community, the jECS is engaging with the ECS webinars. The ECS webinar series was initiated by Martin Bootman (Milton Keynes, UK) and his team in May 2020, and recordings of previous talks are accessible on the jECS YouTube channel (“jECS YouTube Channel,” 2020). From 2021, every third webinar slot will be reserved for early-career Ca\(^{2+}\) researchers, coordinated by the jECS board, to give this group of researchers the valuable experience of presenting their data to the international Ca\(^{2+}\) community.

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References


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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Highlights:

- The junior European Calcium Society board organized their first online meeting.
- The board aims to promote junior researcher in the calcium community.
- The meeting facilitated scientific interactions with focus on diversity.
- Calcium research from molecular interactions to tissue-wide signaling was covered.