CALCIUM SIGNALING

Full focus on calcium

Jan B. Parys[1]* and Andreas H. Guse[2]*

Intracellular calcium (Ca^{2+}) signals are of prime importance for cellular function and behavior and are underpinned by a plethora of Ca^{2+} channels, pumps, transporters, and binding proteins that are regulated in complex ways. A series of biennial meetings, the International Meetings of the European Calcium Society (ECS), focuses on a better understanding of these complex mechanisms in the framework of cellular and organismal (patho)physiology.

Realization of the vital importance of the calcium ion (Ca^{2+}) dates back a long time (back to Sydney Ringer in the 19th century, at least), but only in the past four decades, a large body of evidence became available, demonstrating that complex spatiotemporal Ca^{2+} signals regulate a plethora of intracellular processes through actions on various types of Ca^{2+}-binding proteins that serve as effectors (*1*). However, in apparent contrast to the accumulating knowledge about the various intracellular effects of Ca^{2+}, many mechanisms responsible for cellular Ca^{2+} homeostasis and signaling remained, until very recently, unidentified or are even now still elusive. The 15th International Meeting of the European Calcium Society (Hamburg, Germany, 9 to 13 September 2018; www.calciumsociety.eu) thus focused on a number of crucial aspects of intracellular Ca^{2+} signaling and its underlying mechanisms.

A recurrent topic was the emerging concept that each cell contains multiple Ca^{2+} stores [sarco/endoplasmic reticulum (SR/ER), mitochondria, and lysosomes in particular] and that each class of Ca^{2+} store contains several types of Ca^{2+} release channels. For a full understanding of the physiological, pathophysiological, and pathological relevance of intracellular Ca^{2+}, the respective roles of the various Ca^{2+} stores, their channels, and the stores' connection to the process of store-operated Ca^{2+} entry (SOCE) have to be understood and taken into consideration. In particular, data were presented about the structure, the regulation, and the occurrence in specific cellular microdomains of D-*myo*-inositol 1,4,5-trisphosphate (IP_3) receptor 1 (IP_3R1) to IP_3R3 and ryanodine receptor 1 (RYR1) to RYR3 located in the SR/ER and of TRPML1 and two-pore channels (TPCs) located in the lysosomes. It has also become increasingly clear in the past few years that the regulation of those various intracellular Ca^{2+} release channels occurs for a large part through associated proteins. An interesting example is the regulation of the IP_3R by neuronal calcium sensor 1 (NCS1), which itself is a multifunctional, Ca^{2+}-binding protein of the calmodulin superfamily. By binding to the IP_3R, NCS1 enhances IP_3-induced Ca^{2+} release; however, its interaction with the IP_3R is increased by the cytostatic drug paclitaxel, which binds to the hydrophobic cleft of NCS1 in its Ca^{2+}-bound state. The resulting Ca^{2+} oscillations that occur in neuronal cells are implicated in paclitaxel-induced peripheral neuropathy, an unwanted side effect of the chemotherapeutic treatment, through the activation of the protease calpain and the subsequent degradation of various important proteins, leading to neuronal damage (*2*). In addition, STAC3 has emerged as an essential protein in excitation-contraction coupling in skeletal muscle, interacting with the plasma membrane voltage-operated Ca^{2+} channel $Ca_V1.1$ and probably linking it to the RYR1 Ca^{2+} release channel (*3*). Last, data also indicated that the regulation of the IP_3R by antiapoptotic proteins is much more complicated than was initially thought. The finding of a previously uncharacterized Bcl-2–binding site in the IP_3-binding site of the IP_3R and the mutual competition that exists between both ligands indeed add additional possibilities for the fine tuning of cellular life-or-death signals depending on the intracellular context (*4*).

Although IP_3 was reported initially as the quintessential Ca^{2+}-releasing second messenger in cells (*1*), the adenine nucleotides nicotinic acid adenine dinucleotide phosphate (NAADP), cyclic ADP-ribose, and ADP-ribose constitute a superfamily of second messengers in Ca^{2+} signaling in their own right, as reported in a session specifically dedicated to adenine nucleotides. Interestingly, especially for NAADP, both the biosynthetic pathway and its exact target are still a matter of scientific debate. Evidence for NAADP acting on either RYR1 or TPC was presented during the meeting, suggesting various modes of action for the compound (*5*). New bifunctional probes, for example, ^{32}P-5-N_3-8-ethynyl-NAADP, were introduced as promising tools for the purification and identification of the NAADP receptor (*6*). Furthermore, a newly identified member of this growing superfamily, 2′deoxy-ADPR, was presented as an endogenous superagonist that acts on TRPM2, a Ca^{2+}-permeable, nonselective cation channel involved in the response to oxidative stress and inflammation (*7*).

Similar to the mode of action of NAADP, molecular details of the mechanisms underlying SOCE are still being unraveled. Although the basic mechanism that the emptying of ER Ca^{2+} stores triggers Ca^{2+} entry has been known for decades, the exact role of ORAI (and perhaps additional) channels and their regulators STIM1 and STIM2 are only beginning to become clear. STIM1 and STIM2 appear to act differentially, with STIM2 localized at plasma membrane (PM)–ER junctions, as the primary sensor for small local decreases in luminal Ca^{2+} concentration resulting from Ca^{2+} microdomains, for example, brief openings of RYR1 (*5*), but in addition also remodeling the C terminus of STIM1, thereby activating coupling of STIM1 and ORAI1 (*8*), as discussed in the "Berridge Lecture" of the meeting delivered by I. Ambudkar (NIH, USA). Furthermore, SOCE was reported to regulate the expression of key metabolic enzymes and major metabolite transporters by activation of nuclear factor of activated T cells (NFAT) and the mTOR nutrient-sensing pathway (*9*).

All of these mechanisms, acting together in a coordinated way, are responsible for the

[1]KU Leuven, Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine and Leuven Kanker Instituut, Campus Gasthuisberg O/N-1 B-802, Herestraat 49, BE-3000 Leuven, Belgium. [2]Calcium Signalling Group, Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany.
*Corresponding author. Email: jan.parys@kuleuven.be (J.B.P.); guse@uke.de (A.H.G.)

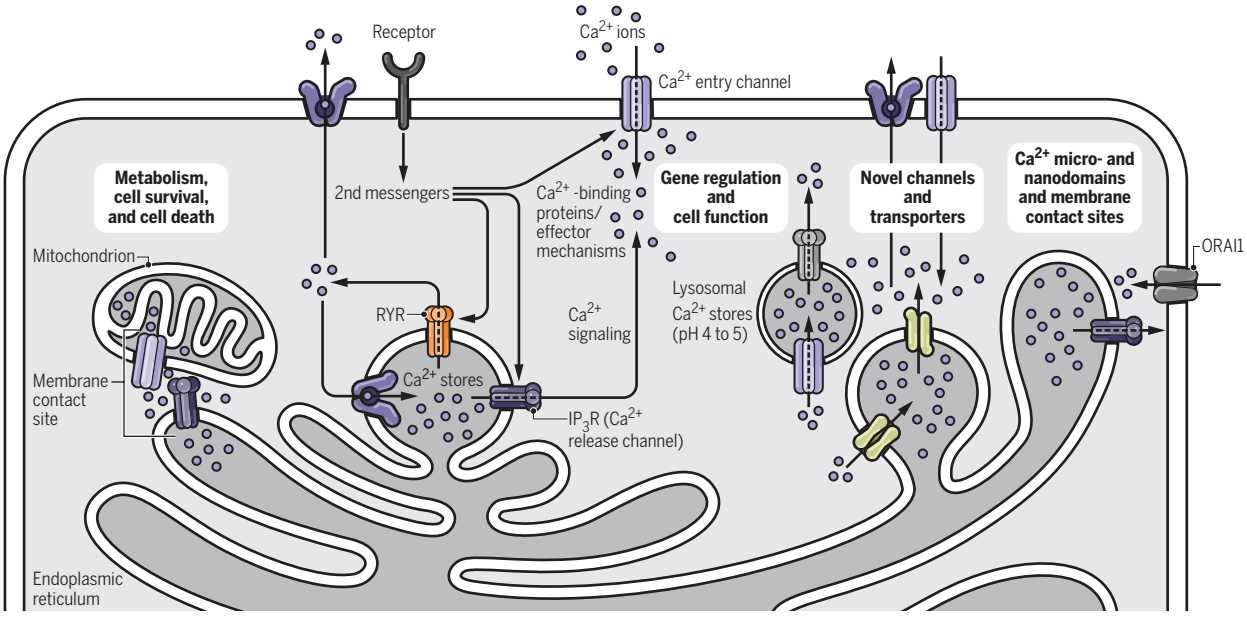

Fig. 1. Schematic representation of important ongoing topics in the field of Ca^{2+} signaling. A number of important topics will be discussed at the 16th International Meeting of the European Calcium Society (Cork, Ireland, 23 to 27 August 2020). These include the need for further study of the receptors, channels, transporters, binding proteins, and effector mechanisms involved in intracellular Ca^{2+} signaling; the effects of Ca^{2+} on metabolism, cell function, and cell death; roles for Ca^{2+} micro- and nanodomains and membrane contact sites; and roles for lysosomal Ca^{2+} stores. Additional topics to be discussed not depicted here include the evolution of Ca^{2+} signaling, roles for Ca^{2+} functions in neuronal physiology and pathology, and the use of new model systems, including organoids, to study Ca^{2+} signaling.

occurrence of precise and distinct Ca^{2+} signaling patterns that drive crucial cellular functions, including metabolism, cell motility, and vesicular trafficking. Such behaviors imply an important role for Ca^{2+} in the occurrence of pathological situations. In hepatocytes, for example, obesity leads to a decreased Ca^{2+}-pumping activity in the ER, an enhanced number of contact sites between ER and mitochondria, enhanced Ca^{2+} release through IP_3R1, and decreased SOCE, which leads to ER and mitochondrial dysfunctions. In neurodegenerative diseases, such as Alzheimer's disease and amyotrophic lateral sclerosis, changes in intracellular Ca^{2+} handling are reported and a role for Ca^{2+} in at least disease progression is presumed, although the exact relationships between Ca^{2+} and these pathologies still have to be fully elucidated. These few examples discussed at the meeting illustrate that, with respect to the relationship between intracellular Ca^{2+} and pathologies, we yet only see the proverbial tip of the iceberg and that the number of pathological cases in which abnormal Ca^{2+} handling is involved is bound to increase further (*10*). Obviously, a fuller understanding of the involvement of Ca^{2+} and Ca^{2+}-handling proteins in these processes will also provide us with new opportunities for therapeutic intervention.

The relationship between Ca^{2+} and neurodegenerative diseases and aging is the topic of the 8th ECS workshop (Coimbra, Portugal, 18 to 20 September 2019), but other urgent questions in the Ca^{2+} field will be tackled at our next meeting, the 16th International Meeting of the European Calcium Society (Cork, Ireland, 23 to 27 August 2020). These questions in particular concern the following problems (Fig. 1): How did Ca^{2+} signaling evolve throughout evolution? What is its role in plants and lower organisms? What is the exact role of Ca^{2+} in neuronal function? How does it affect brain function in pathological conditions? How do the various types of Ca^{2+} channels function, how do they affect each other, and what are their precise cellular roles? How do Ca^{2+} micro- and nanodomains function and what are the cellular advantages and disadvantages of such domains? What are the implications of organellar (and in particular lysosomal and mitochondrial) Ca^{2+} signals? What role does Ca^{2+} play in the regulation of metabolism, cell proliferation, and cell death? What are the implications for cancer and cancer therapy? How can organoids increase our knowledge about Ca^{2+} signaling? Answers to these questions will move the boundaries of our actual knowledge on Ca^{2+} further forward and lead to a more profound understanding of how Ca^{2+} exactly controls cellular behavior. This will enable acting on those Ca^{2+} signals, for example, in the framework of novel therapeutic approaches in pathological settings.

REFERENCES AND NOTES

1. M. J. Berridge, M. D. Bootman, H. L. Roderick, Calcium signalling: Dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* **4**, 517–529 (2003).
2. G. R. Boeckel, B. E. Ehrlich, NCS-1 is a regulator of calcium signaling in health and disease. *Biochim. Biophys. Acta Mol. Cell Res.* **1865**, 1660–1667 (2018).
3. B. E. Flucher, M. Campiglio, STAC proteins: The missing link in skeletal muscle EC coupling and new regulators of calcium channel function. *Biochim. Biophys. Acta Mol. Cell Res.* **1866**, 1101–1110 (2019).
4. H. Ivanova, L. E. Wagner II, A. Tanimura, E. Vandermarliere, T. Luyten, K. Welkenhuyzen, K. J. Alzayady, L. Wang, K. Hamada, K. Mikoshiba, H. De Smedt, L. Martens, D. I. Yule, J. B. Parys, G. Bultynck, Bcl-2 and IP_3 compete for the ligand-binding domain of IP_3Rs modulating Ca^{2+} signaling output. *Cell. Mol. Life Sci.*, 1–17 (2019).
5. B.-P. Diercks, R. Werner, P. Weidemüller, F. Czarniak, L. Hernandez, C. Lehmann, A. Rosche, A. Krüger, U. Kaufmann, M. Vaeth, A. V. Failla, B. Zobiak, F. I. Kandil, D. Schetelig, A. Ruthenbeck, C. Meier, D. Lodygin, A. Flügel, D. Ren, I. M. A. Wolf, S. Feske, A. H. Guse, ORAI1, STIM1/2, and RYR1 shape subsecond Ca^{2+} microdomains upon T cell activation. *Sci. Signal.* **11**, eaat0358 (2018).
6. G. S. Gunaratne, P. Su, J. S. Marchant, J. T. Slama, T. F. Walseth, 5-Azido-8-ethynyl-NAADP: A bifunctional, clickable photoaffinity probe for the identification of NAADP receptors. *Biochim. Biophys. Acta Mol. Cell Res.* **1866**, 1180–1188 (2019).

7. R. Fliegert, A. Bauche, A.-M. Wolf Pérez, J. M. Watt, M. D. Rozewitz, R. Winzer, M. Janus, F. Gu, A. Rosche, A. Harneit, M. Flato, C. Moreau, T. Kirchberger, V. Wolters, B. V. L. Potter, A. H. Guse, 2′-Deoxyadenosine 5′-diphosphoribose is an endogenous TRPM2 superagonist. *Nat. Chem. Biol.* **13**, 1036–1044 (2017).
8. K. P. Subedi, H. L. Ong, G.-Y. Son, X. Liu, I. S. Ambudkar, STIM2 induces activated conformation of STIM1 to control Orai1 function in ER-PM junctions. *Cell Rep.* **23**, 522–534 (2018).
9. M. Vaeth, M. Maus, S. Klein-Hessling, E. Freinkman, J. Yang, M. Eckstein, S. Cameron, S. E. Turvey, E. Serfling, F. Berberich-Siebelt, R. Possemato, S. Feske, Store-operated Ca^{2+} entry controls clonal expansion of T cells through metabolic reprogramming. *Immunity* **47**, 664–679.e6 (2017).
10. J. B. Parys, G. Bultynck, Calcium signaling in health, disease and therapy. *Biochim. Biophys. Acta Mol. Cell Res.* **1865**, 1657–1659 (2018).

Funding: The ECS conference "Calcium 2018" was supported by the Deutsche Forschungsgemeinschaft (grant no. GU360/18-1 to A.H.G. and V. Gerke, University of Münster, Germany), the NIH (grant no. 5065729 to G. Bird, NIEHS, Research Triangle Park, NC, USA), CoolLED Ltd. (Andover, UK), Biomol (Hamburg, Germany), SAGE Publications Inc. (Thousand Oaks, USA), Cell Calcium (Elsevier B.V.), and the European Calcium Society.
Competing interests: J.B.P. and A.H.G. are board members of the European Calcium Society.

10.1126/scisignal.aaz0961

Citation: J. B. Parys, A. H. Guse, Full focus on calcium. *Sci. Signal.* **12**, eaaz0961 (2019).