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Editorial

Calcium Waves in Warszawa

Signaling between cells is an essential property of multicellular organisms. Since it was recognized that external signals—first messengers—have to be translated inside cells into specific responses mediated by second messengers, it became evident that next to cyclic AMP, the classical second messenger, Ca^{2+} plays a central role in converting extracellular signals into a multitude of intracellular responses in time and space. Even if the history of Ca^{2+} as a second messenger actually started more than 100 years ago with the discovery of Ringer that calcium is essential for the contraction of the heart muscle [1], it took decades until the First International Conference on Calcium-Binding Proteins was organized by Witold Drabikowski and Ernesto Carafoli back in 1973 in Jablonna, Poland [2]. Though the knowledge of Ca^{2+} as second messenger at that time was still rather limited a basic principle of calcium-binding proteins as mediator of the Ca^{2+} signal was already presented by Bob Kretsinger at that first calcium meeting [3]: the module of the EF-hand which later proved to be so important for the functional understanding of calcium-binding proteins.

When the European Calcium Society (ECS) decided to held in September 2010 its 11th Symposium on Calcium-Binding Proteins in Warsaw, Poland, Jacek Kuznicki, the chairman of the meeting and former student of Drabikowski, decided to start the symposium with lectures in memoriam of the late Drabikowski, presented by his colleagues or former students. Ernesto Carafoli (University of Padua, Italy) gave a detailed account on the seminal influence of Drabikowski in helping to develop the field of calcium-binding proteins and their importance for Ca^{2+}-signaling. A detailed review on the special structural properties of EF-hand containing calcium-binding proteins was given by Zenon Grabarek (BBRI, Watertown, USA) followed by Marek Michalak (University of Alberta, Canada) who reported on the importance of calcium buffering chaperones of the endoplasmic reticulum which are responsible for the folding and quality control of newly synthesized glycoproteins. David MacLennan (University of Toronto, Canada) closed this memorial session with his in-depth view of the importance of the ryanodine receptor, the Ca^{2+} release channel of the sarcoplasmic reticulum, for various muscle diseases, caused by mutations of the release channel or of calsequestrin, the calcium buffering protein of the reticular systems. Two mechanistic models were proposed to explain how dysregulation of Ca^{2+} homeostasis could lead to muscle diseases.

Seven different sessions were organized spanning a wide range of different topics of the calcium field including imaging techniques and structural studies, calcium transport and signaling, the role of calcium in gene expression, alternative splicing, differentiation, neuronal function and disease. These sessions included formal lectures and oral presentations selected from submitted abstracts. In addition, two poster sessions were organized for the more than 100 posters.

1. Calcium Toolkits

As life science in general, also the calcium society profited enormously from the breathtaking technical development of different tools such as PCR, fluorescent dyes, imaging techniques or NMR to name a few. Olga Garaschuk (University of Tuebingen, Germany) used an *in vivo* two-photon imaging technique to provide evidence that in microglia transient Ca^{2+} signals act as rapid, highly sensitive and very localized sensors of neuronal damage. Using the same technique enabled Spencer L. Smith (University College London, UK) to simultaneously monitor the firing rates of almost 50% of neurons during visual stimulation in an imaged region of the primary visual cortex of the mouse. This technique permitted for the first time to obtain visual receptive field maps with high spatial and temporal resolution. An interesting improvement of the application of the energy transfer technique has been developed by Takeharu Nagai (Hokkaido University, Japan) and his group using a bioluminescent protein to transfer its resonance energy to a fluorescent protein with high quantum yield to enhance luminescence intensity. This allows single-cell imaging without external light illumination which permitted to develop an autoluminescent Ca^{2+} indicator to visualize Ca^{2+} dynamics at the single-cell level with a temporal resolution of 1 Hz. In a poster the same group also presented the development of ultra-sensitive Ca^{2+} indicators using the FRET-based technique which permit the detection of Ca^{2+} in the range between 15 and 150 nM combined with large signal changes which enables detecting subtle Ca^{2+} transients. An interesting contribution was presented by Paola Pizzo (University of Padua, Italy) who studied Ca^{2+} transients in the trans-compartment of the Golgi by using a FRET-based Ca^{2+} probe specifically targeted to the Golgi. She could show that Ca^{2+}-handling in the trans-Golgi is insensitive to IP_3 and accumulates Ca^{2+} solely through SPCA1, the calcium pump of the secretory pathway which is homologous to the SERCA pump. Knocking down SPCA1 not only altered the Ca^{2+} content of the Golgi resulting in major changes of protein trafficking within the secretory pathway, but also influenced the morphology of the Golgi apparatus.

A long time unanswered question was how is the mechanism of store operated calcium entry (SOCE). Since the discovery of Orai as (part of) the calcium entry channel in the plasma membrane and STIM as the Ca^{2+} sensor of the endoplasmic reticulum the interaction between these two proteins became a highly active field of research. Mitsu Ikura (University of Toronto, Canada) by using NMR identified key structural details of STIM which contribute to the properties of its Ca^{2+} sensory function, and which permit the transmission of the luminal Ca^{2+} signal into the activation of SOCE. Ikura also dwelled on some structural properties of the IP_3 receptor, the Ca^{2+}-release channel of the endoplasmic reticulum. The overall structure of the IP_3 receptor consists of 3 different regions: the N-terminal ligand-binding

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region, a central regulatory region and the C-terminal channel region. Ikura's group studied the conformational properties of the ligand-binding N-terminal region by using different biophysical methods including NMR and small angle X-ray scattering (SAXS). The presented data strongly suggest ligand induced conformational changes in the N-terminal region of the receptor resulting in a more compact structure due to the binding of IP_3 whereas the presence of Ca^{2+} drives the structure to a more extended conformation.

A number of studies were devoted to the largest class of EF-hand containing proteins, the S100 calcium-binding proteins. Olga Moroz (University of York, UK) reported on the structural properties of S100A12 in its apo- and metal bound forms, since S100A12 like some other S100 proteins can bind zinc and copper next to calcium. The finding that zinc binding enhances the affinity for calcium is especially important for target binding. Of special interest was the interaction of some of the S100 proteins with the RAGE receptor (RAGE = receptor for advanced glycation endproducts), involved in inflammatory processes. Guenter Fritz (University of Freiburg, Germany) together with the group of Walter Chazin (Vanderbilt University, USA) investigated the interaction between RAGE and S100B and S100A12 (but also S100A8/A9) by X-ray crystallography and NMR. X-ray crystallography provided evidence that Ca^{2+}-loaded S100B assembles into large multimers resulting in a decreased dissociation from RAGE which may lead into a sustained activation of the receptor signal cascade with possible pathological consequences. They also determined the structure of the ligand-binding domain VC1 of RAGE, and mapped the VC1 ligand-binding surface onto the structure by NMR titration measurements of S100B to generate a model for the VC1-S100B complex. An interesting observation was reported by Wiktor Prus and Anna Filipek (Nencki Institute, Warsaw, Poland) who demonstrated that the nuclear translocation of Sgt1, a chaperone-like protein, induced by heat shock was dependent on the interaction with S100A6 (calcyclin).

Calmodulin plays a central role in regulating and transmitting the calcium signal which is especially important in cell proliferation and cell cycle progression. David Sacks (Harvard Medical School, Boston, USA) reported on the interaction of calmodulin and of IQGAP1, a calmodulin-binding protein due to its "IQ" motif, with members of the ErbB family of receptors like EGFR or HER2/Neu. These receptors play a central role in cell growth and development, aberrant receptor expression is often observed in the development of cancer tissues. HER2 is overexpressed in 30% of human breast tumors. Sacks demonstrated that binding of IQGAP1 to the HER2 receptor stimulates the HER2-dependent tissue proliferation which can be prevented by calmodulin. Similarly, on 2 posters Antonio Villalobo (University of Madrid, Spain) and his group presented their results on the influence of calmodulin on the ligand-dependent activation of EGFR and regulation of its expression using conditional calmodulin-knockout DT40 cells. A novel calmodulin-binding motif was presented by Mitcheell Maestre-Martinez (MPI for Biophysical Chemistry, Goettingen, Germany) who studied the interaction between calmodulin and Munc13 which is involved in the regulation of synaptic neurotransmitter release. The structure of the complex of calmodulin interacting with the binding domain of Munc13 solved by NMR revealed a novel calmodulin-binding motif involving both, the N-and C-terminal domains of calmodulin in an extended, dumbbell-like shape.

2. Calcium Transport

Ludwig Neyses (University of Manchester, UK) gave an impressive account on some new roles of the calmodulin-dependent calcium pump of plasma membranes (PMCA). Four different genes generating numerous spliced isoforms have been reported in mammals. Neyses pointed out that PMCA1 is strongly associated with blood pressure and hypertension, common causes of cardiovascular disease. Spliced isoforms PMCA1b and 4b, both expressed in cardiac tissues, contain a PDZ domain at the C-terminus. Interestingly, PMCA4b interacts via its PDZ-domain with the cardiac neuronal form of the NO synthase and regulates its activity. PMCA4b is part of a multiprotein key signaling complex of the heart regulating beta-adrenergic signal transmission which, if fails, can lead to sudden cardiac death. PMCA spliced variants are not only developmentally regulated, but also topically targeted as demonstrated by Katalin Paszty (Semmelweis University, Budapest, Hungary). It was shown that PMCA2w/b, the prominent isoform of the lactating mammary gland, was concentrated in the apical membrane of MDCK cells, whereas PMCA2z/b was predominantly baso-lateral. It was further shown that localization of PMCA2w/b to the apical membrane needed an intact cytoskeleton and the presence of the apical scaffold protein NHERF-2 to anchor PMCA2w/b to the apical membrane via its PDZ domain.

The receptor for the Ca^{2+}-mobilizing messenger NAADP was long time unknown. Recently the group of Antony Galione (University of Oxford, UK) identified a two-pore channel (TPC) as the NAADP receptor, a new family of Ca^{2+} release channels. Interestingly, TPC2 identified as the NAADP-dependent calcium release channel seem to be exclusively located in the endolysosomal system, not in the endoplasmic reticulum, an unexpected finding that lysosomes may play a key role in cellular Ca^{2+} homeostasis and Ca^{2+} signaling. TPCs contain 2 repeats of a six transmembrane segment in contrast to the voltage-gated Na^+ and Ca^{2+} channels which contain 4 of such segments. TPC2, reconstituted into lipid bilayers, was shown to form NAADP-gated cation conductances which could be blocked by NED-19, a NAADP antagonist.

3. Ca^{2+} Signaling

Jim Putney (NIEHS, Research Triangle Park, North Carolina, USA) who first proposed the concept of the capacitative or store-operated calcium entry (SOCE) in 1986 [4] gave a cutting edge review on the regulation and function of SOCE. It was known for a long time that if Ca^{2+} was released from the ER by IP_3 through the IP_3 receptor calcium release channel the ER had to be refilled with Ca^{2+} by a calcium entry mechanism as proposed by Putney. Only recently the molecular basis for this mechanism was unraveled by the discovery of STIM, the calcium sensor of the ER, and Orai which at least is part of the calcium entry channel at the plasma membrane. Upon store depletion STIM rearranges to sites close to the plasma membrane thereby interacting with and activating SOCE channels of the Orai family. The current underlying SOCE is referred to as Ca^{2+} release-activated Ca^{2+} current or I_{CRAC}; Orai was identified as the pore-forming subunit of the CRAC channel, highly selective for calcium. In mammalian organisms 2 STIM isoforms exist, STIM1 and STIM2, with very different Ca^{2+} sensing characteristics, STIM1 could be described as the "decoder" of Ca^{2+} oscillations, a suggestion which is supported by oscillating movements of STIM1 toward the plasma membrane following in synchrony Ca^{2+} oscillations. Three isoforms of Orai have been described, Orai1, 2, 3. Orai molecules are predicted to have 4 transmembrane regions with the N- and C-terminus located on the cytosolic site of the plasma membrane. A detailed analysis of STIM1-Orai1 interaction was analyzed in osteoclasts indicating that the presence of Orai1 is essential for osteoclastogenesis. Martin Bootman (Babraham Institute, Cambridge, UK) reviewed the role of Ca^{2+} signaling in atrial myocytes which is much less studied in comparison to ventricular cells. Both Ca^{2+}-release channels, the IP_3- and the ryanodine receptors, have been identified in atrial myocytes, they co-localize within the subsarcolemmal space of the cells. Of the 3 RyR isoforms RyR2 is the dominant Ca^{2+}-release channel required for excitation–contraction coupling (ECC) in the heart. On the other hand, the functional importance of IP_3 receptors in the heart remained controversial. However, recent reports underlined the importance of IP_3-dependent Ca^{2+}-release for ECC in atrial myocytes which express up to 10 fold higher levels of IP_3R2 in atrial compared to ventricular cells. It was

also shown that IP_3-induced Ca^{2+} release (IICR) is important for the generation of arrhythmias, specifically chronic atrial fibrillation which may lead to ischemia and cardiac failure. It is also important to note that IICR can be stimulated by endothelin-1 via its $G\alpha_q$-coupled receptor to induce hypertrophic signaling in cardiac myocytes.

Ca^{2+} signaling is an essential mechanism for neuronal and glial function. Alexei Verkhratsky (University of Manchester, UK) and Vladimir Parpura (University of Alabama, Birmingham, USA) reviewed various aspects of the underlying mechanisms important for astro-glial cells. Vekhratsky concentrated on the role of ionotropic receptors like the sodium–calcium exchanger, the ionotropic glutamate receptor, the glutamate transporter, or the sodium, potassium pump which enable glia cells to respond to signals long time assumed to act only on neurons. These receptors are activated by a synaptic release of neurotransmitters, and thus mediate rapid neuronal–glial communications. Parpura reviewed the different Ca^{2+} sources important for the exocytotic release of glutamate from astrocytes. Inositol-1,4,5-trisphosphate- and ryanodine receptors are responsible for the release of Ca^{2+} from the reticular store which is necessary and sufficient to release glutamate exocytotically. Like in other cells the Ca^{2+} depleted ER is refilled using a store operated calcium entry mechanism. Cytosolic calcium entry can also be provided by voltage-gated calcium channels. Parpura pointed out that astrocytes could modulate the kinetics of the glutamate transmitter release by using different Ca^{2+} delivery pathways.

4. Calcium and gene expression, alternative splicing and differentiation

In this session the most recent results of calcium-dependent gene expression and the influence of calcium on alternative splicing and differentiation have been reviewed. Jose Naranjo (University of Madrid, Spain) who discovered the calcium-dependent transcriptional repressor DREAM reported his interesting finding that the nuclear localization of DREAM is dependent on SUMOylation. His Lab identified several SUMOylation sites which, if mutated, demonstrate a reduced nuclear localization. Another interesting result was the finding that DREAM controls a number of transcription factors like MEF2C, important for heart development, or Npas4, important for the development of inhibitory synapses in the brain.

Next to the regulation of transcription the regulation of splicing is another important step in the regulation of protein synthesis. Douglas Black (HHMI, UCLA, Los Angeles, USA) gave an impressive account of the involvement of Ca^{2+} signaling in the regulation of alternative splicing, a topic first time discussed at a calcium meeting. Alternative splicing is a mechanism to generate a number of different protein isoforms from a single gene in a developmentally and tissue-specific manner, a mechanism especially important for neuronal development. Black discussed the properties of an important regulator of alternative splicing, the Fox protein, related to the *feminizing on X* protein of *Caenorhabditis elegans*. Three mammalian homologues are known, each containing a highly conserved RNA recognition motif (RRM) which recognizes the intronic splicing regulatory element UGCAUG, an element which is important in controlling the splicing of many alternative exons. All 3 paralogs of Fox are expressed in neurons, Fox-1 and -2 also in muscles. Fox-1 and -2 are involved in the regulation of alternative splicing of the voltage-gated calcium channel $Ca_V1.2$ by repressing the splicing of exon 9A and activating exon 33 of the channel protein. The inclusion or exclusion of those exons specifically affect the electrophysiological properties of the channel, indicating that the Fox proteins play an important role in tuning the channel properties during neuronal development. Another interesting example in which alternative splicing affects the properties of a protein concerned the glutamate receptor NMDA which plays an important role in modulating synaptic functions in the brain. Black demonstrated that splicing of exon 21 of the NMDA receptor which controls the trafficking of the NMDA receptor to the plasma membrane is regulated by the calmodulin-dependent kinase CaMKIV through 2 intronic CaMKIV-responsive RNA elements (CaRRE1 and 2) in response to neuronal depolarization. Interestingly, one of these CaRREs was also responsible for the regulation of alternative splicing of one exon of isoform 1a of PMCA by CaMKIV.

In recent years it became evident that Ca^{2+} signaling is an important pathway for neuronal development. Catherine Leclerc (University of Toulouse, France) described her latest results on the influence of Ca^{2+} signaling on early neurogenesis during embryonal development of *Xenopus laevis*. Leclerc provided evidence that an increase of intracellular Ca^{2+} concentration was necessary and sufficient to induce neural development out of the ectoderm. For this developmental change an increased expression of voltage-gated, dihydropyridine sensitive calcium channels could be observed which were responsible for the increased Ca^{2+} influx, and which are possibly activated by the fibroblast growth factor FGF. Leclerc also underlined the importance of the Ca^{2+} induced expression of the arginine N-methyltransferase xPRMT1b which plays a pivotal role during neural development. This enzyme induces the expression of a number of early neural genes, among them the zinc-finger Zic3 which is an important transcription factor during early embryonal development.

5. Calcium and disease

In this last session of the meeting several presentations reported on the influence of Ca^{2+} signaling on various diseases. The session was opened by the lecture given by Simonetta Camandola (NIH, Bethesda, USA) from the group of Mark Mattson who reported on the role of calcium on neuronal plasticity and its influence on neurodegenerative diseases such as Alzheimer (AD). Currently 5 million Americans and about 30 million individuals worldwide suffer from AD with the estimation that by the year 2050 this number will be doubled. Evidence is accumulating that alterations in synaptic calcium handling are early and pivotal events during development of the disease process with the consequence that chronical disturbance of Ca^{2+} homeostasis controlled by the endoplasmic reticulum (ER) may lead to dysfunction and degeneration of neurons. Presenilin-1 (PS1) of which mutations lead to the inheritable, early onset AD is an integral membrane protein of the ER. These mutations of PS1 enhance Ca^{2+} release through IP_3- and ryanodine-sensitive channels thereby exaggerating Ca^{2+} signaling in synaptic terminals which may lead to the dysfunction and degeneration of neurons. Carlos Enrich (University of Barcelona, Spain) gave an interesting overview how annexinA6 may induce cholesterol imbalance. AnnexinA6 due to its phospholipid-binding property can sequester cholesterol into late endosomes, thereby reducing the levels of cholesterol in the Golgi. This annexinA6-dependent redistribution of cholesterol may reduce phospholipase A2 activity with consequences for the activity of the Golgi apparatus. In addition, annexinA6 may also function as a scaffold protein for a number of signaling proteins such as p120GAP leading to tumor suppressor activity due to the downregulation of Ras activity.

Two poster prizes were given to young researchers:

1) Magali Savignac (University of Toulouse, France) for the poster entitled: "ER stress response is up-regulated in Darier keratinocytes and impairs trafficking of E-cadherin, a key component of adherens junctions"
2) Ilse Vandecaetsbeek (University of Leuven, Belgium) for the poster entitled: "The unique carboxy-terminus of SERCA2b is responsible for the higher apparent Ca^{2+}-affinity of the pump"

In addition, a Drabikowski award was given for the best short oral presentation to Joanna Jung (University of Alberta, Edmonton, Canada), the title of her presentation was: "The role of endoplasmic reticulum quality control system in the biology of the major peripheral myelin glycoproteins".

All 3 award winners summarized their work in this special issue of the 11th European Symposium on Calcium.

6. 2nd Sir Michael Berridge Lecture

The Sir Michael Berrigde lectureship was inaugurated in 2008 at the 10th Symposium of the ECS Society in Leuven, Belgium, to celebrate the 25th anniversary of the seminal paper of Berridge and his colleagues published in Nature [5] to demonstrate the IP_3-dependent calcium release from a nonmitochondrial intracellular store which later turned out to be the endoplasmic reticulum. The first lecture was delivered by Chakashi Toyoshima in Leuven who solved the structural principles of the activation cycle of the Ca^{2+} pump of the sarcoplasmic reticulum [6]. Katsuhiko Mikoshiba (RIKEN Brain Research Institute, Hirosawa, Japan) who discovered the IP_3 receptor gave the second Berridge lecture to close the symposium in Warsaw. He summarized the breath-taking amount of data he and his Lab collected over the years on the structure-function relationship of the IP_3 receptor and its role in cell function and disease, only a few of them can be mentioned here. Originally, the IP_3 receptor was discovered by Mikoshiba as a P400 protein since mice deficient of a protein with a molecular mass of 400 kDa showed degenerated Purkinje cells with poor dendrites. The absence of Ca^{2+} spikes in those mice and the discovery of high affinity binding of IP_3 to P400 convinced Mikoshiba that P400 was actually the IP_3 receptor which demonstrated IP_3-dependent Ca^{2+}-releasing activity after incorporation into liposomes. Inositol 1,4,5-triphosphate turned out to be the only molecule among the different inositol phosphates and phospholipids to target a Ca^{2+} channel. In mammalian organisms 3 different isoforms exist whereas only one isoform has been found in Starfish, *C. elegans* or *Drosophila*. It was shown that the N-terminal part of the receptor contains the ligand-binding sites for IP_3 and IRBIT, the IP_3R-binding protein released with IP_3, the C-terminal part contains the channel domains. Next to the ligand-binding domain the N-terminal region also contains a suppressor domain which plays a critical role in the coupling between ligand binding and channel gating. Three-dimensional structural studies provided evidence that the receptor exists in a tetrameric form which can be in an open or closed state, regulated by Ca^{2+}. IRBIT interacts with the IP_3 receptor within the IP_3-binding core. It is released upon IP_3 binding, i.e. IRBIT is a modulator for IP_3-induced Ca^{2+} release. Since IRBIT also binds to the Na^+/HCO_3 cotransporter which is important for the regulation of the intracellular pH, this suggests the possibility that IRBIT acts as a signaling molecule downstream of the IP_3R. Furthermore, the IP_3 receptor is important for fertilization and development, during which it is essential for the dorso-ventral axis formation. It was interesting to see that IP_3R1-/- mice which are impaired in their brain development, or IP_3R2-/- mice showed no defect in cardiac development, but the double knockouts IP_3R1,2-/- mice died *in utero* and showed defects in the myocardium indicating an essential role of the IP_3 receptor in cardiogenesis, partly through the regulation of calcineurin/NFAT signaling. Since a variety of proteins like ankyrin, the phosphatases PP1 and PP2A, ERp44 or chromogranin involved in different signaling pathways bind to IP_3 receptors Mikoshiba concluded that the IP_3 receptor works like a scaffolding protein to form a signaling center as a calcio-signalosome.

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