http://www.ulb.ac.be/assoc/ecs

3rd ECS Workshop
Calcium In and Out

Seix(France)
June14th-17th, 2011

Organizing Committee:
Catherine Leclerc, Lucette Pelletier,
Christian Mazars, Jacques Haiech, Roland Pochet
& Marc Moreau

And supported by

GDR 2688 and GDRE

Welcoming address

The organizing committee is pleased to welcome the third workshop of ECS in Seix, this small Pyrénean village perfectly dedicated to science. This is the second ECS workshop in Seix. Indeed, no way to escape and to visit museums, or going out for shopping or theatre or cinemas. You can just enjoy the wildness of surrounding mountains, may be rafting and devote yourself to Science and calcium. The comfort is far away from the usual 4 stars hotels, but the cooking is exceptional. The brain has no escape way, than to be focused on the interesting talks mixing plant and animal calcium signalling that will be given during these four days. In this rustic frame, we hope that you will have good discussions and may be establish new cooperation in a friendly atmosphere.

The organizing committee

Don’t forget !

The 12th meeting of ECS will be held in Toulouse (France) the 2nd week of September 2012

Programme

Tuesday 14th June 2011

12h00 arrivals

12h30 – 14h00 Lunch

16h00 -16h30 **Opening**

Ca^{2+} Pumps
Chair Roland Pochet

16h30-17h30 ***Plenary Lecture***	*Régis Bobe (France)*	SERCA family and their involvement in intracellular calcium signalling.
17h30-18h00	*Y Boursiac (France)*	Implication of the vacuole in calcium signalling unravelled by *aca4/aca11*, a double mutant disrupted in vacuolar calcium pumps in Arabidopsis thaliana
18h00-18h30	*Bela Papp (France)*	SERCA proteins, endoplasmic reticulum calcium uptake and cancer cell differentiation

Short communications for selected posters

19h30 Welcome party & 20h00 Diner

Wednesday 15th June 2011

Channels I : VDCC
Chair Lucette Pelletier

9h00-10h00 ***Plenary Lecture***	*Jörg Striessnig (Austria)*	Neuronal L-type calcium channels: exciting in health and human disease
10h00-10h30	*Steve Dubel (France)*	Beyond the Pore of the Voltage Dependent Calcium Channels : a Spotlight on the Auxiliary Subunits

10h30-11h00 Coffee break

11h00-11h30	*Daphné Atlas (Israel)*	Ca^{2+} bound at the pore of the calcium channel during depolarization triggers fast fusion of vesicles tethered to the channel via synaptotagmin, syntaxin 1A, and SNAP-25

Short communications for selected posters

12h30 – 14h00 Lunch

Channels II: CRAC/ARC/TRP/Mechanosensitive channels
Chair: Catherine Leclerc

14h30-15h30 ***Plenary Lecture duo***	*Olivier Mignen (France)* *&* *Thierry Capiod (France)*	ARC channels: When ORAI and STIM proteins decide to be independent…from store depletion. Relative importance of external and luminal calcium on cell proliferation.
15h30-16h00	*Jean Marie Frachisse (France)*	How to connect mechanosensitive channels and Ca^{2+} signalling in plants?

16h00 -16h30 Coffee break

16h30-17h00	*Mohamed Trebak (USA)*	Orai channels in vascular disease
17h00-17h30	*Klaus Groschner (Austria)*	Molecular organization and function of TRPC signaling microdomains in cardiac cells

17h30-18h00
Pause

Ca^{2+} micro-domains & Imaging
Chair Marc Moreau

18h00-18h30	*Jan Parys (Belgium)*	Regulation of IP_3-induced Ca^{2+} release at the ER-mitochondria microdomain: a role for Bcl-2-family members.
18h30-19h00	*Maud Frieden (Switzerland)*	Differential requirement of TRPCs channels and STIM1/Orai1 molecules in Ca^{2+} signaling and endothelial cell tube formation.

20h00 Diner

Thuesday 16th June 2011

Signal deciphering & CaBP
Chair Christian Mazars

9h00-10h00 ***Plenary Lecture***	*Steve Moss (England)*	How annexins shape and respond to Ca^{2+} signals
10h00-10h30	*Annette Draeger (Switzerland)*	Plasma membrane repair and cellular damage control

10h30-11h00 Coffee break

11h00-11h30	*Kenji Hashimoto (Germany)*	Calcineurin B-like proteins and their interacting protein kinases in decoding calcium signals in plants.
11h30-12h00	*Lorella Navazio (Italy)*	Calcium-mediated communications between rhizobia and plants in the establishment of nitrogen-fixing symbiosis

12h30 – 14h00 Lunch

Afternoon free
(rafting for courageous volunteers)

18h00-20h00
ECS Board meeting for ECS conference 2012

20h00 Diner

21h30 - 22h30
Discussion on standardisation & harmonisation of Ca^{2+} imaging protocols

Friday 17th June 2011

Modelling
Chair Jacques Haiech

8h30-9h30 ***Plenary Lecture duo***	*Martin Bootman (UK)* *&* *Rüdiger Thul (UK)*	*No title for the moment* A minimal 3-dimensional model of an atrial myocyte with a realistic distribution of calcium release sites
9h30-10h00	*Christian Brière (France)*	Biological wikis: network tools for collaborative development and sharing of biological data and information

10h00-10h30 Coffee break

10h30-11h00	*Lu Li (UK)*	*No title for the moment*
11h30-12h00	***Concluding remarks***	

12h00 – 14h00 Lunch

14h00 shuttle bus to Toulouse
Arrival at 16h00

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Ca^{2+} pumps

Plenary lecture

SERCA family and their involvement in intracellular calcium signalling.

Jose J. Lopez[1] , Lahouaria Hadrib[2], Alain Stepanian[1], Roger Hajjar[2], Stephane N. Hatem[3], Larissa Lipskaïa[2], Jocelyne Enouf & **Regis Bobe[1]**

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Calcium homeostasis is a ubiquitous second messenger involved in a multitude of cellular functions, including secretion, motility, neuronal activity, cell proliferation, differentiation or apoptosis in every human/eukaryotic cell. Among the players involved in Ca^{2+} homeostasis are Sarco/Endoplasmic Reticulum Calcium transport ATPases (SERCAs) that actively accumulate Ca^{2+} in the endoplasmic reticulum(ER). The SERCA family is composed of three distinct genes that give rise to multiple isoforms through alternative splicing in the C-Terminal part of the corresponding proteins. To date, about 12 isoforms have been described for human and some specificity has been observed between species. SERCAs form the only calcium uptake mechanism in the ER; therefore the nature of SERCA system in the different cell types and its function constitute a key mechanism to regulate calcium homeostasis in the endoplasmic reticulum. Increase in SERCA expression or activity can induce Ca^{2+} overload into the ER, while treatment using thapsigargin, a high affinity selective pan-SERCA inhibitor will empty the store in Ca^{2+}. Interestingly both can lead to ER stress and apoptosis indicating that ER stores need to be tightly regulated. The SERCA activity participates to the amplitude, the duration and frequency of calcium transients and oscillations not only inside the ER compartments but also into the cytosol. We recently showed that expression of SERCA2a in human smooth muscle cells totally modifies the pattern of calcium signaling; decreasing the calcium capacitive entry and inhibiting NFAT activation and translocation to the nucleus in response to serum and thrombin stimulations.

These data indicate that SERCAs modulate the calcium mobilisation from the ER as well as the calcium influx and therefore strongly control the whole intracellular Ca^{2+} signaling leading to differential effects from ER stress to activation of transcription factors.

Implication of the vacuole in calcium signalling unravelled by *aca4/aca11*, a double mutant disrupted in vacuolar calcium pumps in Arabidopsis thaliana.

Yann BOURSIAC[1,3], Shawn ROMANOWSKY[1], Woo Sik CHUNG[2] and Jeffrey F. HARPER[1]

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Calcium (Ca^{2+}) signals regulate many aspects of plant development, including a programmed cell death pathway that protects plants from pathogens (Hypersensitive Response, HR). Cytosolic Ca^{2+}-signals result from a combined action of Ca^{2+}-influx through channels, and Ca^{2+} efflux through pumps and cotransporters. Plants utilize calmodulin activated Ca^{2+} pumps (ACA, Autoinhibited Ca^{2+}-ATPase) at the plasma membrane (PM), endoplasmic reticulum (ER) and vacuole. The current study addresses in particular the role of the vacuolar calcium pumps, ACA4 and ACA11. A double knockout mutation of those pumps in Arabidopsis results in a high frequency of HR-like lesions. The appearance of macrolesions could be suppressed by growing plants with increased levels (> 15 mM) of various anions, providing a method for conditional suppression. By removing plants from a conditional suppression, lesion initials were found to originate primarily in leaf mesophyll cells, as detected by aniline blue staining. Initiation and spread of lesions could also be suppressed by disrupting the production or accumulation of salicylic acid (SA), as shown by combining *aca4/11* mutations with a *sid2* mutation or expression of a SA degradation enzyme (NahG). This indicates that the loss of the vacuolar calcium pumps by itself does not cause a catastrophic defect in ion homeostasis, but rather potentiates the activation of a SA-dependent PCD pathway. Together these results provide evidence linking the activity of the vacuolar Ca^{2+} pumps to the control of a SA-dependent programmed cell death pathway in plants. Additional functions are also indicated - such as modulating calcium signals that regulate transpiration. Since animal cells do not have a large central vacuole, this study highlights a plant specific mechanism for using calcium pumps to regulate calcium homeostasis.

SERCA proteins, endoplasmic reticulum calcium uptake and cancer cell differentiation

Bela Papp

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Calcium accumulated in the lumen of the endoplasmic reticulum is essential for several important intracellular processes such as IP3-induced calcium signaling or the control of capacitative calcium influx, and is also required for calcium-dependent intraluminal protein chaperoning. Sarco/Endoplasmic Reticulum Calcium transport ATPases (SERCA enzymes) constitute a "nodal point" in calcium signalling, because calcium is accumulated in the endoplasmic reticulum, and is thus made available for these processes, exclusively by SERCA-driven calcium transport.

In many cell types the ubiquitous SERCA2b isoform is co-expressed with SERCA3 enzymes. Interestingly, whereas the expression levels of SERCA2 are relatively stable, SERCA3 expression displays significant changes during cell differentiation. Retinoic acid-induced differentiation of acute promyelocytic leukaemia cells, as well as short-chain fatty acid-induced differentiation of colon and gastric carcinoma cells is accompanied by the selective induction of SERCA3 expression. On the other hand, although abundant in T and B lymphocytes, SERCA3 expression is decreased during the activation of T lymphoid cells, as well as during Epstein-Barr virus-induced immortalization of B cells. In addition, work performed *in situ* on human colon tumour samples shows that the expression levels of SERCA3 protein are inversely correlated with the degree of malignancy of the lesions, whereas normal epithelium expresses SERCA3 abundantly.

The biochemical characteristics of SERCA2 and SERCA3 differ significantly, and SERCA isoenzymes may be associated with functionally distinct endoplasmic reticulum sub-compartments. Therefore, the selective modulation of the expression of SERCA2 and SERCA3 within a cell constitutes a new mechanism that allows for the fine-tuning of the biochemical characteristics of calcium uptake into the endoplasmic reticulum, depending on the cell type, its function, degree of differentiation and state of activation. Cells in which SERCA expression levels change may serve as interesting new experimental models for the better understanding of the specialization of the endoplasmic reticulum, and of the involvement of this organelle in differentiation and tumorigenesis.

Moreover, in selected cell types in which SERCA levels are modulated during differentiation, plasma membrane calcium pump expression levels are also modulated. When taken together, these data suggest that the entire cellular calcium transport machinery may be remodeled during cell differentiation, and that this phenomenon may be deficient in cancer.

Channels I : VDCC

Plenary lecture

Neuronal L-type calcium channels: exciting in health and human disease

Jörg Striessnig

Department of Pharmacology and Toxicology, Institute of Pharmacy; Center for Molecular Biosciences, University of Innsbruck, Austria.

Voltage-gated L-type calcium channels in heart and vascular smooth muscle are a well-established drug target for organic calcium channel blockers since decades and are valuable therapeutics to treat hypertension and myocardial ischemia. However, these channels are also expressed in endocrine cells, sensory cells, and neurons. In most cells the Cav1.2 and Cav1.3 L-type channel subtypes coexist but, due to their different biophysical properties, serve distinct functional roles. Using unique animal models we have shown that Cav1.3 serves as sinoatrial node pacemaker channel, triggers excitation-secretion coupling in cochlear hair cells, and regulates neuronal excitability. Cav1.3 therefore is not only required for normal cardiac automaticity and hearing, but also for emotional and drug taking behaviors. We have recently discovered a novel regulatory mechanism within the Cav1.3 C-terminus which is a strong determinant of the calcium- and voltage-dependent gating of these channels. Since this regulatory domain is absent in short Cav1.3 splice variants, alternative splicing can produce Cav1.3 channels with distinct gating properties. We have recently discovered a novel human disease (SANDD) resulting from a loss-of-function mutation in the pore-forming Cav1.3 α1-subunit gene (CACNA1D). Affected individuals suffer from congenital deafness and sinoatrial node dysfunction, emphasizing the important role of these channels for cardiac pacemaking and neurotransmitter release in sensory cells also in humans. Cav1.3 channels mediate calcium transients in dopaminergic substantia nigra pacemaker neurons which may underlie their high susceptibility to neurodegeneration in Parkinson's disease. Selective Cav1.3 channel blockers are therefore pursued as novel neuroprotective agents for Parkinson's disease therapy. Support: Austrian Science Fund (P20670, W11)

Beyond the Pore of the Voltage Dependent Calcium Channels: a Spotlight on the Auxiliary Subunits

Stefan J. Dubel

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Research on voltage dependent calcium channels over the last two decades has been focused on both the biochemical isolation, genetic cloning and expression of the alpha1 subunits of both high voltage-activated (HVA) and low voltage-activated (LVA) calcium channels from a wide number of organisms. The pore forming subunit is a favorite pharmacological target for modifying the electrophysiological properties due to its central role in conducting calcium ions into a cell and its use as a good target for therapeutics. As well, several genetic maladies including migraine, epilepsy, and deafness were identified and the corresponding mutations directly impact on the behavior of these channels making them key players in so-called “channelopathies”. Alongside the HVA channels stands a variety of accessory/auxiliary/regulatory subunits some of which have become critical to define an archetypal macromolecular HVA calcium channel complex. These subunits (alpha2/delta, betas and gammas) both have a clear functional impact on the biophysical properties and trafficking of HVA channels. The role of these subunits in regulating LVA channel function was also investigated but whether LVA channels comprise any auxiliary subunit is still unclear. Finally, the alpha2/delta subunit has emerged as a central target for the treatment of epilepsy and pain making by the use of the drug Gabapenin. My presentation will focus on the genetics, biochemistry, regulation and trafficking of these auxiliary subunits and I will discuss how these subunits impact on calcium channel function.

Plenary lecture

Ca^{2+} bound at the pore of the calcium channel during depolarization triggers fast fusion of vesicles tethered to the channel via synaptotagmin, syntaxin 1A, and SNAP-25

Daphne Atlas

Daphne Atlas, Dept. of Biological Chemistry Institute of Life Sciences The Hebrew University of Jerusalem, Jerusalem, 91904 Israel

Voltage gated calcium channels (VGCC) are involved in a myriad of cellular Ca^{2+} signaling processes including exocytosis, a Ca^{2+} dependent release of hormones and neurotransmitters. Much progress has been made in the understanding the mode of action of VGCC in exocytosis, a process distinguished by two sequential yet independent Ca^{2+} binding reactions, which are triggered during membrane depolarization.

The first reaction is Ca^{2+} binding to the selectivity filter, the EEEE motif of the open pore of the channel. Secondly, subsequent to a brief and intense Ca^{2+} inflow, Ca^{2+} bind to synaptotagmin, a vesicular protein that reorganizes the vesicle and primes it for secretion.

Inquiries into the functional and physical interactions of the channel with synaptic proteins has demonstrated that exocytosis is triggered during the initial Ca^{2+} binding at the open channel, prior to Ca^{2+} entry into the cell. This model was proven experimentally when secretion was triggered by impermeable La^{3+} that substituted for Ca^{2+} in chromaffin and pancreatic beta cells (*Lerner et al., 2006; Trus et al., 2007*), and a Ca^{2+} impermeable L-type calcium channel that substituted for normal channel, triggered secretion without conduction Ca^{2+}, in bovine chromaffin cells (*Hagalili et al., 2008; Marom et al., 2010; Atlas, 2010*)

Accordingly, a cycle of secretion begins by the occupancy of the open-pore during an incoming stimulus. This stimulates the release of a releasable pool of Ca^{2+} primed vesicles tethered to the channel. At the same time, the transient increase in $[Ca^{2+}]_i$ primes a fresh set of non-releasable pool of vesicles by binding to synaptotagmin. These vesicles will be ready for release by the next incoming stimulus.

We propose a model in which conformational change induced during depolarization and Ca^{2+} binding at the EEEE motif is the primary event that triggers secretion. In this model synaptotagmin acts as a vesicle docking protein and the channel serves as the molecular On/Off signaling switch, where the predominance of a conformational change of a Ca^{2+}-bound channel provides for the fast secretory process.

Trus et al., Biochemistry 2007 Dec 18;46(50):14461-7
Hagalili et al., 2008 Biochemistry 2008 Dec 30;47(52):13822-30
Marom et al., J Biol Chem. 2010 Mar 5;285(10):6996-7005
Atlas, Cell Signal. 2010 Nov;22(11):1597-603.

Channels II:
CRAC/ARC/TRP/Mechanosensitive channels

Plenary Lecture

ARC channels: When ORAI and STIM proteins decide to be independent…from store depletion.

Olivier Mignen[1]

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Calcium signals induced in response to agonists that act through the activation of phospholipase C involve both an $InsP_3$-mediated release of Ca^{2+} from intracellular stores, and the activation of an enhanced entry of extracellular Ca^{2+}. Until fairly recently, attention has focused on the store-operated mechanism of Ca^{2+} entry store-operated channels (SOC) responsible for this entry. The archetypal version of SOC is the Ca^{2+}-release activated Ca^{2+} channels (CRAC channels). However, studies on of the entry of Ca^{2+} under low levels of stimulation led to the discovery of a novel mode of entry whose activation was entirely independent of store depletion. The channels responsible for this entry are activated by low concentrations of arachidonic acid and were described as ARC channels (***A*rachidonate-*R*egulated *C*a^{2+} channels)**. Characterization of the properties of ARC channels has demonstrated that, ARC and CRAC channels possess certain unique biophysical and pharmacological features that show that these are entirely distinct, but co-existing, entities and play a unique and non-overlapping role in Ca^{2+} signaling.

During the course of the past 6 years, the entire field of calcium signaling has been fundamentally transformed – initially, by the identification of the Stromal Interacting Molecule (STIM) proteins by the identification of the Orai proteins. The current model of store operated calcium entry emphasizes the role of STIM1 located in the endoplasmic reticulum membrane as the sensor of the status of the intracellular Ca^{2+} stores. Based on this, the idea rapidly developed that the action of STIM1 on Ca^{2+} entry was specifically, and exclusively, associated with the store-operated mode of entry. However ARC channel activity is also regulated by STIM1. This regulation exclusively involves the pool of STIM1 that constitutively resides in the PM, and is entirely independent of store depletion. These shared ability to be regulated by STIM1, together with the observation that ARC and CRAC channels share some basic biophysical properties, led to consider that these two conductances might be molecularly related. Recent key studies identified members of the Orai proteins as a critical component of the SOC channels and demonstrated that Orai1 is an essential subunit of the CRAC channel pore. Our results revealed that, like the store-operated CRAC channels, the molecular composition of the ARC channels also involves members of the Orai proteins. However, whilst the CRAC channel pore appears to be comprised of only Orai1, both Orai1 and Orai3 contribute to the ARC channel pore.

Plenary Lecture

Relative importance of external and luminal calcium on cell proliferation.

Thierry Capiod[1] and Anne-Sophie Borowiec[2].

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Both increases in the basal cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$) and $[Ca^{2+}]_{cyt}$ transients play major roles in cell cycle progression, cell proliferation and division. Calcium transients are observed at various stages of cell cycle and more specifically during late G_1 phase, before and during mitosis. These calcium transients are mainly due to calcium release and reuptake by the endoplasmic reticulum (ER) and are observed over periods of hours in oocytes and mammalian cells. Calcium entry sustains the ER Ca^{2+} load and thereby helps to maintain these calcium transients for such a long period. Calcium influx also controls cell growth and proliferation in several cell types. About 40 years ago, several scientists have established that external calcium controls cell division and proliferation. Since then, the involvement of calcium channels in normal and pathological proliferation has been investigated but there are two important points which can be emphasized in these early studies. First, they showed that low concentrations of external calcium, well below physiological levels, can trigger cell proliferation. Cells proliferate maximally in the presence of as little as 50 to 100µM free calcium. Second, transformed cells have a much lower sensitivity to external calcium and proliferate even in the presence of 20µM external calcium. Therefore, the role of calcium channels and calcium influx in cell proliferation can be questioned. Various calcium channels are involved in this process and the tight relation between the expression and activity of cyclins and calcium channels also suggests that calcium entry may be needed only at particular stages of the cell cycle. Consistent with this idea, the expression of L-type and T-type calcium channels and SOCE amplitude fluctuate along the cell cycle. But, as calcium influx regulates several other transduction pathways, the presence of a specific connection to trigger activation of proliferation and cell division in mammalian cells will be discussed.

How to connect mechanosensitive channels and Ca^{2+} signalling in plants?

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Mechanical perception is at the basis of plant interaction with biotic and abiotic agents. Plethora of example involving bacteria or virus cell invasion or plant motion induced by wind shows as early event a variation in cytosolic Ca^{2+}. Although Ca^{2+} signalling events have been extensively studied, the mechanical event per se and its early coupling with Ca^{2+} has been poorly investigated.

All living organism contain in their membrane pore forming proteins activated by membrane stretch. These channels called MechanoSensitive (MS) channel are very efficient transducer of mechanical force into transmembrane electrochemical gradient variation [1]. Their activities are involved in general function in animal such as hearing, touching and in bacteria such as osmoprotection.

In plant many MS channels have been described in term of electrophysiological characteristics. Among them some are Ca^{2+} selective. It is the case of the SA calcium channel [2] identified at the plasma membrane of *Vicia faba* guard cells. This channel, activated by membrane tension controlled by cytoskeleton integrity, is involved in stomatal open/closure, allowing gas exchange between plant and atmosphere. Unfortunately this channel has not been identified at the molecular level. MCA1 that partially complement the *mid1* yeast mutant lacking a component of stretch-activated Ca^{2+} channel, was proposed by Nakagawa et al [3] to be a plant mechanosensory channel. Expressing MCA1 in CHO cells induced a novel Ca^{2+} permeability elicited by stretch. Although the knockout plant showed a reduced ability of its roots to penetrate a hard substrate, the precise role of MCA remains enigmatic. Another gene candidate family is represented by ten MscS-Like (MSL) genes in *Arabidopsis* [4] homolog of bacterial MscS. Among these ten candidates MSL9 and MSL10 have received most attention. The proteins expressed in an overlapping but not identical pattern are found mainly in the plasma membrane of root cells. Electrophysiological analysis of protoplasts derived from the root cortex showed that the mechanoresponsive channel activity is due to the anion conductance.

Plant channels candidate introduced above will be discussed. Based one their calcium or anion permeability and on their electrophysiological analogy with mammal mechanosensitive channels, a scheme linking mechanosensitive channel with Ca^{2+} signalling will be proposed.

[1] Sachs, Physiology 2010, 25:50; [2] Zhang et al., Plant Physiol. 2007, 143:1140; [3] Nakagawa et al., 2007, PNAS 104:3639; [4] Haswell et al., 2008, Curr. Biol., 18:730

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Orai channels in vascular disease

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Calcium is a universal second messenger that controls many cell functions including proliferation and migration which are hallmarks of proliferative disease such as vascular occlusive diseases and cancer.

STIM and Orai proteins have recently emerged as the molecular correlates for the highly calcium selective conductances mediated by store-operated calcium entry (SOCE) and store-independent Arachidonate-regulated calcium (ARC) channels. Store-operated calcium entry (SOCE) is controlled by the extent of filling of the internal calcium stores, mainly the endoplasmic reticulum (ER), while ARC channels are activated downstream of arachidonate production by mechanisms that remain unclear.

Vascular smooth muscle cell (VSMC) proliferation and migration is a hallmark of vascular occlusive disease such as atherosclerosis and restenosis. We previously showed that proliferative migratory VSMCs (called "synthetic") display up-regulated STIM1, Orai1 and Orai3 proteins. Here we will describe recent findings from our group of STIM and Orai isoform-specific requirement for mediating store-dependent and store-independent calcium entry pathways in VSMCs. We will also discuss the requirement of STIM and Orai proteins in VSMC proliferation and migration *in vivo* using animal models of vascular occlusive diseases and will discuss the implications of these findings in the pathophysiology of vascular diseases.

Molecular organization and function of TRPC signaling microdomains in cardiac cells

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TRPC channels are potential determinants of excitability, Ca^{2+} homeostasis and gene expression in cardiac muscle. TRPC3-mediated Ca^{2+} entry has been proposed as a crucial upstream signal for the control of nuclear factor of activated T-cells (NFAT)-dependent control of transcriptional programs. The linkage between TRPC channel activity and NFAT translocation is still incompletely understood. TRPC conductances may govern calcineurin activity and thereby NFAT nuclear translocation by supplying Ca^{2+} either directly through the TRPC pore into a regulatory microdomain or indirectly via promotion of voltage-dependent Ca^{2+} entry. We recently identified a single point mutation in the TRPC3 selectivity filter (E630Q), which disrupts Ca^{2+} permeability but preserves monovalent permeation. This Ca^{2+} permeation-deficient mutant was used to test for a role of TRPC3 channels in terms of a direct Ca^{2+} source for calcineurin and NFAT activation. Elimination of Ca^{2+} permeation through TRPC3 channels uncoupled channel activation from NFAT translocation in HEK293 cells as well as in murine HL-1 atrial myocytes. Nonetheless, the E630Q mutation fully retains the ability to convert phospholipase C-linked stimuli into L-type ($Ca_V1.2$) channel-mediated Ca^{2+} entry in HL-1 cells, thereby generating a dihydropyridine-sensitive Ca^{2+} signal that is apparently isolated from the NFAT pathway.

Prevention of PKC-dependent modulation of TRPC3 by either inhibition of cellular kinase activity or mutation of a critical phosphorylation site in TRPC3 (T573A; moonwalker), which disrupts targeting of calcineurin into the channel complex, converts cardiac TRPC3-mediated Ca^{2+} signaling into a transcriptionally silent mode. Thus, we provide evidence for control of cardiac gene transcription by a mechanism involving TRPC3-mediated local Ca^{2+} entry and dynamic, phosphorylation-dependent targeting of the Ca^{2+} effector calcineurin into the signaling microdomain. Moreover, we demonstrate a dichotomy of TRPC-mediated Ca^{2+} signaling in the heart constituting two distinct pathways that are differentially linked to gene transcription. Our results identify TRPC3 as a pivotal signaling gateway in Ca^{2+}-dependent control of cardiac gene expression.

Ca^{2+} micro-domains and imaging

Regulation of IP_3-induced Ca^{2+} release at the ER-mitochondria microdomain: a role for Bcl-2-family members.

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The IP_3 receptor (IP_3R) is a ubiquitously expressed intracellular Ca^{2+} channel, responsible for the majority of the Ca^{2+} signals regulating cellular processes. IP_3Rs are mainly located on the endoplasmic reticulum (ER), mediating Ca^{2+} release to the cytosol upon activation by IP_3. Mitochondria can be found in close proximity to the ER and even physically couple to it. As a result, part of the released Ca^{2+} is delivered to a microdomain located between ER and mitochondria in which the local Ca^{2+} concentration can increase to a much higher level than in the cytosol. From this microdomain, Ca^{2+} released from the ER can therefore enter the mitochondria despite the relatively low affinity of the Ca^{2+}-uptake mechanisms, and regulate Ca^{2+}-dependent processes, like ATP production. The Ca^{2+} concentration in the matrix will depend on the IP_3R activity and the efficiency of the coupling between ER and mitochondria.
An impaired flux of Ca^{2+} ions to the mitochondrial matrix will decrease ATP production, leading to AMP-activated protein kinase activation and subsequent autophagy. In contrast, an excessive flux of Ca^{2+} ions will lead to the opening of the permeabilization transition pore, matrix swelling and rupture of the outer mitochondrial membrane, eventually leading to apoptosis. Proteins regulating the IP_3R-mediated Ca^{2+} flux to the mitochondria have therefore either pro-apoptotic (caspase 3, cytochrome C, PML) or anti-apoptotic (Bcl-2, Bcl-Xl, protein kinase B) properties.
Here, we will especially focus on the regulation of the IP_3R by anti-apoptotic Bcl-2-family members. Bcl-2 itself has a complex action: it acts both on the ER and on the mitochondria, and modulates Ca^{2+} homeostasis at various levels. Our results indicate that Bcl-2 can inhibit IP_3-induced Ca^{2+} release. This occurs by the binding of Bcl-2 through its BH4 domain to a.a. 1389-1408 in the centre of the regulatory domain of the IP_3R. Interestingly, the anti-apoptotic protein Bcl-Xl, despite its functional and structural homology with Bcl-2, does not interact with the IP_3R through its BH4 domain and therefore does not inhibit IP_3-induced Ca^{2+} release. The difference in action between Bcl-2 and Bcl-Xl seems to be related to a single residue difference (lysine versus aspartate) in their respective BH4 domains. Understanding the exact mechanism of IP_3R regulation by Bcl-2-family members can be exploited to stimulate Ca^{2+}-mediated apoptosis for therapeutic purposes e.g. in cancer. The regulatory role therein of other proteins interacting with Bcl-2 and IP_3R must however also be taken into consideration.

Differential requirement of TRPCs channels and STIM1/Orai1 molecules in Ca^{2+} signaling and endothelial cell tube formation.

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In non-excitable cells like endothelial cells, Ca^{2+} entry occurs through the well-described store-operated Ca^{2+} entry (SOCE) pathway, involving the proteins STIM1 and Orai1, but also through channels gated by second messengers. These channels belong to the large family of transient receptor potential (TRP), and in particular to the TRPCs. Depending on the cell type, TRPCs are also engaged in SOCE, while the precise nature of molecules activated by physiological concentration of agonist remains often controversial.

The aim of this study was to determine the molecules involved in Ca^{2+} entry elicited by store depletion using the SERCA blocker thapsigargin (TG), and those implicated in histamine-induced Ca^{2+} entry, in an endothelial cell line derived from human umbilical vein, the EA.hy926. Furthermore, we assessed the molecules involved in cell proliferation and in spontaneous tube formation (*in vitro* angiogenesis assay). The invalidation of STIM1 or Orai1 reduced by about 55% TG-induced Ca^{2+} entry. Surprisingly, the remaining part of the entry was abolished after phospholipase C (PLC) or protein kinase Ceta (PKCeta) inhibition. siRNA against TRPC3 decreased Ca^{2+} entry and the double TRPC3/STIM1 invalidation reduced Ca^{2+} entry by more than 85%. Hence, in EA.hy926 cells, TG stimulation engaged not only STIM1 and Orai1, but also TRPC3 by an unusual signaling pathway involving PLC and PKCeta.

Beside TRPC3, the EA.hy926 cells expressed TRPC1, TRPC4, TRPC5 and TRPC6. The invalidation of each of these channels, except TRPC6 significantly reduced Ca^{2+} entry elicited by 1 µM histamine. Surprisingly, even though this level of stimulation did not lead to a measurable Ca^{2+} store depletion, siRNA against STIM1 and Orai1 impacted on the cytosolic Ca^{2+} elevation. Cell proliferation was altered by Orai1, TRPC3 and TRPC5 invalidation while the other molecules (STIM1, TRPC1, TRPC4, TRPC6) did not appear to be involved in this process. When putted on a basement membrane matrix (Matrigel), the EA.hy926 cells formed spontaneously, within 10-14 hours, tubular structures that reflect *in vitro* angiogenesis. siRNA against STIM1 and Orai1 did not decrease, and even increased tube formation. On the contrary, the invalidation of TRPC3, TRPC4 and TRPC5 greatly impaired tubulogenesis. The reduction of tube formation was associated with the lack of spontaneous Ca^{2+} oscillations observed 1 hour after plating the cells on the Matrigel. These data showed that several TRPCs are required for cell proliferation and tubulogenesis, while STIM1 does not seem to be implicated in these processes.

Signal deciphering and CaBP

Plenary lecture

How annexins shape and respond to Ca^{2+} signals

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The annexin family constitutes an ancient group of highly conserved proteins found in most species from bacteria to modern vertebrates, with 12 members expressed in Homo sapiens. The defining biochemical properties of annexins are their ability to bind Ca^{2+}, and to bind to negatively charged phospholipids in the presence of low μM Ca^{2+}. Although there are exceptions to this generalisation, the prevailing view is that most annexins are free cytosolic proteins in resting cells, which become peripherally associated with intracellular membranes upon elevation of intracellular Ca^{2+}.

Since many of the functions of individual annexins are directly regulated by Ca^{2+} these proteins are widely accepted as being archetypal Ca^{2+} effectors, but there is also evidence that some members of the family have roles in shaping Ca^{2+} signals. This is particularly true for annexins 5 and 6, the two members of the family that will be the focus of my presentation. Annexin 5 was for many years considered to be a candidate Ca^{2+} channel, a theory predicated on two key observations. One of these is that, in common with certain other annexins, it can selectively and specifically mediate Ca^{2+} transfer across a lipid bilayer. The second is that annexin 5 has within it's structure a central pore that mutagenesis studies revealed to be the likely route of Ca^{2+} conductance.

Our own in vivo investigations, in which we used targeted gene disruption to knock-out annexin 5 in chick DT40 cells revealed changes in Ca^{2+}-signaling and in susceptibility to Ca^{2+}-dependent apoptosis. We also investigated annexin 6 using gene knock-out in mice, and again observed changes in Ca^{2+}-signaling in cardiomyocytes and fibroblasts. In the latter cells we showed this to be linked to anomalies in mitochondrial morphogenesis and function. Targeted disruption of other annexin genes did not lead to changes in Ca^{2+} signaling, showing that within the family annexins 5 and 6 may be specifically linked to regulating cellular Ca^{2+} signals and susceptibility to apoptosis.

Plasma membrane repair and cellular damage control

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Subject to mechanical, chemical or immunological damage, the life of a cell is a hazardous one. In view of the many dangers, the efficient repair of plasmalemmal lesions is critical for cellular survival.

Ca^{2+} -influx through the ruptured site is considered the danger signal which sets the repair machinery in motion. Injuries can be patched by the delivery of internal membranes via exocytosis. Depending on the cell type or on the nature of the injury, the damaged site is quarantined and taken up into the cell's interior by endocytosis or shed in the form of microparticles.

Ubiquitously expressed, the annexin protein family function as intracellular Ca^{2+} sensors. Most cells contain multiple annexins, which interact with distinct plasma membrane regions promoting membrane segregation and membrane fusion. Recent results have implicated a role for the annexins in plasma membrane repair. An injury-induced Ca^{2+} elevation triggers the translocation of annexins from the cytoplasm to the plasma membrane, where they reseal the injury. According to their individual Ca^{2+} profile, highly Ca^{2+} sensitive annexins will translocate preferentially and act as a first line of defense, whereas annexins with a lower Ca^{2+} sensitivity will be used if the injury persists and/or early attempts at resealing fail. Thus, multiple annexins within one cell allow a graded response to membrane injury, discerning between a limited and a sustained lesion.

Calcineurin B-like proteins and their interacting protein kinases in decoding calcium signals in plants.

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Plants possess diversely evolved Ca^{2+} binding proteins that form complex networks. A small family of Ca^{2+} binding protein in plants exhibits structural similarity with Calcineurin B and has therefore been designated as Calcineurin B-like proteins (CBLs). No homologs of the phosphatase Calcineurin A have been identified in plant genomes. Instead, CBLs specifically interact with a family of CBL-interacting serine/threonine protein kinases (CIPKs) and regulate their kinase activities through relief of autoinhibition. Therefore, CBL-CIPK complexes could be considered as bimolecular Ca^{2+} sensors that translate Ca^{2+} signals into phosphorylation of downstream target proteins.

In the model plant Arabidopsis, 10 CBL-type calcium sensor proteins form an interaction network with 26 CIPKs. Preferential complex formation of individual CBLs with defined subsets of CIPKs appears to be one of the mechanisms generating the temporal and spatial specificity of calcium signals in plant cells.

Reverse genetics approaches have unraveled the function of several members of both protein families in physiological processes like salt tolerance and nutrient uptake. Moreover, our genetics and cell biological analyses indicate that alternative complex formation of CIPK-type kinases with different CBLs enables the simultaneous regulation of ion transport processes at different compartments of the plant cell. On this conference I will summarize the recently gained knowledge about the physiological function of CBL-CIPK complexes and the mechanistic principles of this signaling network and will discuss how CBL-CIPK complexes contribute to deciphering of Ca^{2+} signals in plants.

Calcium-mediated communications between rhizobia and plants in the establishment of nitrogen-fixing symbiosis

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The rhizobium-legume symbiosis is a complex multi-step process that involves the coordinate expression of specific genes in both plants and nitrogen-fixing bacteria, collectively called rhizobia. This mutualistic association endows legumes with an independence from nitrogen fertilizers and sustains crop productivity. In the presence of appropriate rhizobial species, legume hosts form unique structures – the root nodules – in which nitrogen fixation occurs. Nodulation requires extensive plant-microbe communications, involving a range of signalling molecules of both plant and microbial origin. Calcium signalling is known to play a crucial role in the symbiotic signalling pathway activated in the plant host by the microbial lipochitooligosaccharide Nod factor. We have investigated the potential involvement of Ca^{2+} in the perception of plant symbiotic signals by rhizobia. Transgenic cultures of *Mesorhizobium loti* and *Rhizobium leguminosarum* bv. *viciae* expressing the bioluminescent Ca^{2+} reporter aequorin were generated and used in Ca^{2+} measurement assays after challenge with host plant root exudates and molecules therein contained. Specific plant flavonoids were found to activate in *R. leguminosarum* a signalling cascade involving a transient elevation in intracellular Ca^{2+} concentration, which precedes and primes the activation of nodulation (*nod*) genes. This novel, early event in the symbiotic route was found to be positioned upstream of the activity of the transcriptional regulator NodD, suggesting that the signalling pathway induced by flavonoids in the bacterial microsymbiont may be more complex than has hitherto been thought. Recent work in our laboratory has also uncovered the participation into the plant-microbe molecular dialogue of oligogalacturonides, pectic fragments of the plant cell wall that activate cytosolic Ca^{2+} changes and hydrogen peroxide accumulation in rhizobia. Our data shed light on a previously uninvestigated facet - bacterial Ca^{2+} signalling - of the two-way partner signal exchange and provide in the microsymbiont a neat counterpart to the well-established crucial role played by Ca^{2+} in plants during the early stages of rhizobium-legume interaction.

Modelling

Plenary lecture

Calcium signalling during excitation-contraction coupling in atrial myocytes

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Electrical depolarisation of ventricular myocytes produces homogenous, global increases in calcium to trigger contraction during each heart beat. Such global calcium transients arise due to the simultaneous recruitment of many calcium spark sites throughout each cell. An essential component of ventricular myocytes is their 'transverse tubule system' (T-tubules), which conveys the action potential deep into the cells to ensure that calcium sparks are simultaneously triggered throughout the volume of a myocyte.

Atrial myocytes share many structural and functional features with their ventricular counterparts, but also have some significant differences. In particular, the atrial myocytes in many species do not express T-tubules, and consequently their calcium signals are spatially distinct (*1*). Atrial myocyte calcium transients originate in sub-sarcolemmal locations, and give rise to a sharply-defined ring of elevated calcium around their periphery. Positive inotropic agents induce globalisation of action potential-evoked calcium signals in atrial myocytes, and correspondingly a significant increase in the contractility of the cells, by promoting the centripetal, inward propagation of the calcium transients so that calcium can reach the contractile filaments (*2*).

From the description given above, it could appear that atrial myocytes are simply ventricular myocytes without T-tubules. However, comparison of calcium signalling in atrial myocytes and chemically de-tubulated ventricular myocytes indicates that atrial myocytes have unique calcium signalling kinetics that are not mimicked by simply removing the T-tubules from ventricular myocytes (*3*).

We have developed a mathematical model of atrial myocyte calcium signalling based on the realistic distribution of calcium spark sites (ryanodine receptor clusters). Our model recapitulates the sub-sarcolemmal initiation of calcium signals in atrial myocytes, and their subsequent centripetal propagation as calcium waves. Furthermore, the model allows predictive analysis concerning the movement of calcium within/between z-disks, the potentially pro-arrythmic effects of altering the refractory period of calcium spark sites or introducing randomness in calcium release thresholds.

1. M. D. Bootman, D. R. Higazi, S. Coombes, H. L. Roderick, *J Cell Sci* **119**, 3915 (2006).
2. L. Mackenzie, H. L. Roderick, M. J. Berridge, S. J. Conway, M. D. Bootman, *J Cell Sci* **117**, 6327 (2004).
3. I. Smyrnias *et al.*, *Cell Calcium* **47**, 210 (2010).

A minimal 3-dimensional model of an atrial myocyte with a realistic distribution of calcium release sites

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We here develop a 3-dimensional model of an atrial myocyte whose geometry is directly inferred from experimental data. The cell is described by a continuous cylinder, while calcium liberation from the sarcoplasmic reticulum (SR) to the cytosol is restricted to discrete release sites within spatially separate z-planes. Transport of calcium within the cell is modelled by diffusion, with SR re-filling modelled with a linear pump. This is described mathematically with a linear second order partial differential equation (PDE). Calcium liberation within the z-planes is realised via a calcium-induced-calcium release mechanism. We take this to be a threshold process that releases calcium from the SR at a constant rate for a fixed duration comparable to the lifetime of a calcium spark. These calcium release events are regarded as source terms in the linear PDE, triggered when cytosolic calcium exceeds threshold, which mimics calcium excitability. By solving the dynamics between release events (exploiting the linearity of the PDE) we formulate a minimal model solely in terms of behaviour at the release sites. This minimal description contains all the original biology and geometry of the full model, while being computationally inexpensive. The main overhead lies in the summation of release unit activity that contributes to the spatio-temporal evolution of the calcium concentration throughout the whole cell. In contrast to ventricular myocytes, atrial myocytes do not possess a T-tubule system and hence the issue of calcium wave propagation to the cell interior is much more important for subsequent cell contraction. Our computational model allows us to exhaustively probe the dependence of wave properties (speed, shape, path through the cell), on stimulus protocols, release strength, pump-rates, and values of the effective diffusion coefficient. Importantly we find that a diffusive gap, i.e. the absence of release sites, in the sub-sarcolemmal space (consistent with experimental findings) can lead to propagation failure of centripetal waves if the initial stimulus is too weak. Thus the model is useful for exploring the functional consequences (e.g. the degree of myocyte contraction) of hormonal or electrical stimulation.

Biological wikis: network tools for collaborative development and sharing of biological data and information

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Biological databases are essential tools for most biologists. But building and maintenance is is a key issue for many databases developed by a small group of people, due to the increasing amount of information to collect. An interesting way to tackle this problem is to develop collaborative databases that are curated and maintained by a large number of researchers working in the field. Wiki systems, which are directly inspired by the Wikipedia project, offer a variety of advantage for the collaborative management of biological data and information. A wiki is a website that allows creation and editing of any number of interlinked web pages by any number of contributors (end users or experts) with or without access control. A wiki is essentially a database for creating, browsing, and searching through information. Biological wikis are indexes of biological data that emerge from focused and rapidly developing communities. However when developing a wiki website several important issues need to be addressed: the reliability and quality of user information, the format of information incorporated in the database (text, images, diagrams,…), information feedback into "authoritative" databases. A number of biological websites are based on the concept of wiki (see for instance http://en.wikipedia.org/wiki/List_of_biological_wikis or http://www.bioinformatics.org/wiki/BioWiki). An interesting example is the "gene wiki" project (http://en.wikipedia.org/wiki/Gene_Wiki) whose purpose is to facilitate transferring information on human genes to Wikipedia articles. Other functional currently-active examples of biological wikis are Wikigenes (http://www.wikigenes.org), which gathers information about a large number of genes, or Wikipathways (http://www.wikipathways.org), which is "dedicated to the curation of biological pathways by and for the scientific community".

These concepts will be used to develop an encyclopedia dedicated to sharing information about the "Calcium signalling toolkit". Using the wiki concept to develop this encyclopaedia will facilitate the feeding and update of the database by a panel of calcium signalling experts. *In fine*, existence of such encyclopaedia, maintained actively and dynamically by researchers working in the field of calcium signalling, will be an invaluable novel tool for conducting new experiments and to develop a system biology approach of calcium networks.

Calcium input frequency, duration and amplitude differentially modulate the relative activation of calcineurin and CaMKII

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N-methyl-d-aspartate (NMDA) receptor dependent long-term potentiation (LTP) and long-term depression (LTD) are two prominent forms of synaptic plasticity, both of which are triggered via post-synaptic calcium elevation and the consequent activation of calmodulin. Calmodulin activates calcium/calmodulin-dependent protein kinase II (CaMKII), inducing LTP; calcium also activates protein phosphatase 2B (PP2B, also called calcineurin), triggering LTD. In this talk, I will first present a detailed allosteric model for calmodulin activation by calcium. The simulation results from this model are consistent with published experimental data and showed that the steady increase of calcium concentration can differentiate the activations of CaMKII and PP2B. In order to further study how calcium selectively stimulates two opposing events in a time range where calcium concentration cannot be stabilised, we extended this allostoric model and studied different calcium spike frequencies, amplitudes, and durations. We show that the activation of calmodulin is dependent on the calcium spike frequency. When the total amount of calcium ions remains the same, high frequency pulses stimulate more calmodulin and increase the duration of its activation. Besides, high frequency of calcium input, despite activating both PP2B and CaMKII, shifts the balance of relative activation from PP2B to CaMKII. In addition, the change in total amount of calcium ions input, by means of changing the spike number and spike amplitude, adjusts the sensitivity of the system towards calcium spike frequencies. At the same frequency, the more calcium ion is input, the higher proportion of CaMKII is activated. Thus, a smaller amount of calcium ions input requires a higher frequency firing to induce the potent intracellular calcium elevation in order to activate CaMKII; while a larger amount of calcium ions requires less frequent firing. To summarise, the sensitivity of synaptic weights to calcium depends on the frequency, amplitude and duration of signals.

Posters abstracts

Golgi Apparatus sub-compartments Calcium homeostasis: effect of Familial Alzheimer's disease linked Presenilin 2 mutations

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Calcium (Ca^{2+}) is one of the major intracellular messengers that impacts nearly every aspect of cell life. In particular, it plays essential roles in neuronal development, synaptic transmission and plasticity, as well as in the regulation of metabolic pathways and cell fate decisions.

A perturbed Ca^{2+} homeostasis has been demonstrated in Alzheimer's disease (AD) (1), one of the most devastating neurological disorder of the elderly. The majority of AD cases is sporadic but a small fraction is inherited in a dominant pattern (Familian AD, FAD). Of the three genes involved in the pathogenesis of FAD, two encode for the ubiquitously expressed homologous proteins presenilin (PS) 1 and 2 and FAD mutations in these latters have been variably correlated to alterations of Ca^{2+} signaling.

We have recently shown that FAD mutations in PS2 alter the cellular Ca^{2+} handling, reducing the Ca^{2+} content of the two major intracellular Ca^{2+} stores, the endoplasmic reticulum (ER) and the Golgi apparatus (2). These results, obtained using specifically targeted aequorin Ca^{2+} sensors and performing experiments at the cell population level, were further deepened by investigating the mechanism by which PS2 mutants exert their effect and a reduced Sarco-Endoplasmic Ca^{2+} ATPase (SERCA) activity was demonstrated in cells expressing the FAD mutant (3).

The aim of my work is to study in details the effect of FAD-linked PS2 mutants on Ca^{2+} homeostasis in different sub-compartments of the Golgi apparatus, at the single cell level. The Golgi apparatus is indeed very heterogeneous in terms of Ca^{2+} handling and substantial differences can be found between the cis- and the trans-part of the organelle (4). Using a FRET-based Ca^{2+} probe specifically targeted to the trans-Golgi, already available in the lab (4), I have investigated the effect of the PS2 FAD mutant T122R on the Ca^{2+} content of this sub-compartment. In particular, since Ca^{2+} uptake mechanisms in the overall Golgi complex rely on both the well-known SERCA and the less characterized secretory-pathway Ca^{2+} ATPases 1 (SPCA1) and this latter seems to be the only present in the trans-compartment (4), I wanted to check whether PS2-T122R mutant is also able to affect the activity of this pump. In addition, using a specific cis-Golgi targeting sequence (5), a new fluorescent Ca^{2+}-probe was generated and used to study how Ca^{2+} handling by this Golgi sub-compartment is regulated and whether is altered by FAD-PS2 expression.

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References

1. Zampese E, et al., *Current Trends in Neurology*, 2009.
2. Zatti G, et al., *Cell Calcium* 2006.
3. Brunello et al., *Journal of cellular and molecular medicine*, 2009
4. Lissandron V, et al., *PNAS* 2010
5. Zerfaoui M, et al., *Glycobiology*, 2002

Effects of Presenilin mutants, linked to Familial Alzheimer's Disease, on ER-mitochondria interactions and Ca^{2+} cross-talk.

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Alzheimer's Disease (AD) is the most frequent form of dementia and a small percentage of cases is inherited and is referred to as Familial AD (FAD). These cases are due to autosomal dominant mutations on three genes coding for Amyloid Precursor Protein, Presenilin-1 (PS1) and Presenilin-2 (PS2), keys component of the γ-secretase complex.

Calcium (Ca^{2+}) is a key intracellular second messenger involved in the regulation of many metabolic pathways and the observation that cellular Ca^{2+} dynamics are altered in early stages of AD suggests the possibility of an involvement of Ca^{2+} alteration in its pathogenesis. On this line, numerous studies reported a role for FAD-linked PS mutations in cellular Ca^{2+} alterations, although different and contradictory data were obtained. In our lab it was previously shown that several FAD PS2, but not PS1, mutants reduce endoplasmic reticulum (ER) and Golgi apparatus Ca^{2+} content mainly by interfering with the activity of the SERCA Ca^{2+} pump (Zatti G, et al. 2004; Brunello L, et al. 2009).

Here, by the use of different genetically encoded Ca^{2+} probes, we show that over-expression of the same FAD-PS2 mutants and, to a lower extent, of PS2 wt, lead to an increased mitochondrial Ca^{2+} uptake, compared to controls induced by a similar intracellular Ca^{2+} release. Confocal microscopy images have demonstrated that this effect is due to an increased interaction between mitochondria and ER. Moreover, endogenous PS1 and PS2 knockdown, by specific siRNAs, revealed that only PS2 is involved in the apposition between the two organelles and so could influence Ca^{2+} uptake by mitochondria. By expressing the PS2 mutant D366A, loss-of function for γ-secretase activity, similar results were obtained, indicating that PS2 enzymatic activity seems to be not involved on this its new cell function. Finally, in order to clarify the mechanism(s) by which PS2 mediates ER-mitochondria tethering, possible PS2 molecular partners are currently investigated.

Mitochondrial Ca^{2+} uptake is involved in numerous metabolic pathways and, if excessive or altered, can lead to apoptosis. Altogether these results offer new insights in clarifying the PS2 role on cellular Ca^{2+} homeostasis, suggesting new possible approaches for the investigation of its FAD mutant effects linked to the pathogenesis of FAD.

Store-Operated Calcium Entry in Neurons

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Calcium sensors STIM1 and STIM2 and calcium channel forming protein ORAI1 are the proteins involved in the process of Store-Operated Calcium Entry (SOCE). STIM proteins interaction with ORAI1 is a crucial element of calcium homeostasis in non-excitable cells. However, the molecular mechanism of SOCE in neurons remains unclear. Here we show that cultured rat cortical neurons exhibit SOCE and that STIM1 and STIM2 can activate ORAI1 channels and play distinct roles in this process.

We noticed that the complexes of STIM1/ORAI1 and STIM2/ORAI1 are formed in non-transfected and transfected neurons and that their numbers increase when calcium levels in the endoplasmic reticulum are depleted. To understand the mechanism of STIM proteins translocation from the ER to/near PM, we studied the effects of simultaneous expression of ORAI1 and YFP-STIM1 or ORAI1 and YFP-STIM2 on intracellular calcium level during SOCE using a Ca^{2+} imaging method and Fura-2. After ER store depletion intracellular calcium level was enhanced in neurons expressed with YFP-STIM1/ORAI1, but not with YFP-STIM2/ORAI1, which correlated well with the number of complexes formed. The use of two SOCE inhibitors, ML-9 and 2-APB, additionally corroborated the differential activity of STIM1 and STIM2. What more, an increase in constitutive Ca^{2+} entry was observed in neurons with YFP-STIM1/ORAI1 and YFP-STIM2/ORAI1 expression but not with YFP-STIM expression alone. However, 2-APB-mediated constitutive Ca^{2+} entry was enhanced only with YFP-STIM2/ORAI1 transfected neurons.

Our data indicate that in neurons both STIM proteins are involved in calcium homeostasis. However, STIM1 is a major player in activating SOCE to restore calcium level in ER, while STIM2 regulates the resting calcium level in ER and Ca^{2+} leakage with the additional involvement of STIM1. Our work is one significant step towards an understanding of the mechanism of Store-Operated Calcium Entry into neurons and will help explain the impairment of calcium homeostasis observed in neurodegenerative diseases.

Protective role of calmodulin in the development of acute pancreatitis

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Acute pancreatitis is generally initiated by premature trypsinogen activation in pancreatic acinar cells mediated by excessive intracellular calcium release from internal stores. One of the major causes of acute pancreatitis is excessive alcohol intake; however the molecular mechanism of this severe inflammatory disease is not completely understood. We have shown that in two-photon permeabilized mouse pancreatic acinar cells even a relatively low ethanol concentration as well as its non-oxidative metabolite palmitoleic acid ethyl ester (POAEE) elicit calcium release from intracellular stores and also induce intracellular trypsinogen activation. Adding the calcium sensor calmodulin (at a normal intracellular concentration) to the permeabilized cells markedly reduced ethanol-induced calcium release and trypsinogen activation. Both ethanol-elicited and POAEE-induced calcium liberation and trypsin activity were significantly reduced in acinar cells from mice in which type 2 inositol trisphosphate receptors had been knocked out. Double knock out of inositol trisphosphate receptors of both types 2 and 3 further reduced ethanol-induced or POAEE-induced calcium release and trypsinogen activation to very low levels. Thus the inositol trisphosphate receptor calcium release channels, that are responsible for normal pancreatic stimulus-secretion coupling, also play a major role in the toxic action of ethanol and its metabolites. Calmodulin provides a protective mechanism, regulating the sensitivity of the calcium release process.

References:

Gerasimenko J.V et al (2011) Calmodulin protects against alcohol-induced pancreatic trypsinogen activation elicited via Ca^{2+} release through IP_3 receptors. PNAS, 108(14):5873-8

Gerasimenko J.V. et al (2009) Pancreatic protease activation by alcohol metabolite depends on Ca^{2+} release via acid store IP_3 receptors. PNAS, 106(26):10758-63.

Calcium regulation of apoptosis in pancreatic acinar cells

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We have studied calcium regulation of induction of apoptosis in pancreas. In pancreatic acinar cells, the earliest events were found to be cytosolic calcium elevations due to release of calcium from intracellular stores[1]. As a result of that calcium levels also increased in mitochondria aiding mitochondrial depolarisation and mPTP. High mitochondrial calcium at the time of oxidant stress was found to be the crucial factor in the cell fate[2]. When mitochondrial calcium was low, then apoptosis did not occur regardless of other stores' content. We also studied Bcl-2 family members, well known regulators of apoptosis involved in regulation of intracellular calcium homeostasis[3]. Most interesting was a potential link between Bcl-2 family proteins and a calcium induced calcium release (CICR) from the intracellular stores. Inhibition of antiapoptotic proteins induced calcium release from the ER that lead to the formation of calcium plateau while inhibition of either IP_3Rs or RyRs reduced but did not abolish calcium release. Furthermore, we have shown that loss of Bcl-2 protein significantly affected calcium extrusion as well as apoptosis/necrosis ratio in pancreatic acinar cells. In conclusion we suggest that Bcl-2 regulates vast majority of main components of calcium signalling, serving its crucial role in regulation of cell death.

1. Baumgartner HK, Gerasimenko JV, Thorne C, Ferdek P, Pozzan T, Tepikin AV, Petersen OH, Sutton R, Watson AJ, Gerasimenko OV. (2009) Journal of Biological Chemistry 284(31): 20796-803.
2. Gerasimenko O, Gerasimenko J. (2010) Methods in Molecular Biology 591: 201-10.
3. Gerasimenko J, Ferdek P, Fischer L, Gukovskaya AS, Pandol SJ. (2010) Pflügers Archiv European Journal of Physiology 460(5): 891-900.

Ca_v1 channels are specifically implicated in calcium signalling and cytokine production by Th2 lymphocytes, a T-cell subset responsible for allergic asthma.

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T helper (Th)2 lymphocytes are effector $CD4^+$ T cells that produce interleukin (IL-)4, IL-5 and IL-13. They are implicated in the elimination of parasites but they may cause allergic diseases in predisposed subjects. T-cell receptor (TCR)-driven intracellular Ca^{2+} rise is required for cytokine production. Classically, TCR stimulation induces IP3 formation, depletion of intracellular Ca^{2+} stores and activation of store-operated Ca^{2+} (ORAI) channels. The implication of other Ca^{2+} channels as voltage-dependent Ca_v1 channels in non-excitable lymphocytes is a matter of debate. Ca_v1 channels are formed by the $\alpha1$ subunit (coded by four genes from $Ca_v1.1$ to $Ca_v1.4$) and auxiliary, including β subunits. We showed that Th2 cells differentiated in vitro selectively expressed $Ca_v1.2$ and $Ca_v1.3$, which could account for the differential regulation of $[Ca]_i$ between Th2 and other T-cell subsets.

In order to study the role of Ca_v1 channels in Ca^{2+} signalling of Th2 cells, 1) we have cloned the $Ca_v1.2$ $\alpha1$ subunits expressed by Th2-cells. Comparison with database showed that they correspond to neuronal isoforms, and that they contain the putative calcium sensors characterizing excitable neurons. We also found that Th2 express all the auxiliary subunits (β and α-2δ) required to form a functional channel. 2) We transfected Th2 with Ca_v1 antisense specific oligonucleotides (Ca_v1AS) and tested the effect on TCR-dependent Ca^{2+} response by microspectrofluorimetry and TIRFM. We showed that Ca_v1AS selectively decreased the expression of Ca_v1 (50 to 70% of inhibition) without any effect on the expression of ORAI or of major components of Ca^{2+} signalling in T-cells. Ca_v1AS profoundly decreased TCR-driven $[Ca]_i$ increase but had no effect on thapsigargin-induced $[Ca]_i$ increase (implicating ORAI channels). The effect of Ca_v1AS on TCR-induced Ca^{2+} increase results in impaired nuclear translocation of the Ca^{2+}-regulated transcription factor NFAT, which plays a major role in *il4* gene transcription, and defective cytokine production by Th2 cells. Ca_v1AS had no effect on TCR-driven Ca^{2+} response and the production of interferon-γ by another T-cell subset. 3) We showed that TCR stimulation favours the redistribution of the Ca_v1 channels at the cell membrane by staining with anti-Ca_v1 monoclonal antibodies, and increases Ca_v1 coding gene transcription.

These results show that Ca_v1 channels play a prominent role in Ca^{2+} signalling specifically in Th2 cells. We now focus on how these channels are regulated in Th2 cells, the membrane of which does not depolarize upon activation.

Calcium compartmentation and more specifically nuclear calcium controls programmed cell death induced by dihydrosphingosine (d18:0) in a ROS independent way in tobacco BY-2 cells

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We have recently shown that sphingoid long-chain bases (LCBs) are able to trigger nuclear calcium responses in tobacco BY-2 cells constitutively expressing the calcium probe aequorin in the nucleus (Xiong et *al.* 2008, Cell Calcium 43, 29-37). We further investigated the signalling pathway of these lipids on the tobacco cells by assessing the effect of the first member of the sphingolipid biosynthesis pathway, dihydrosphingosine or d18:0 (DHS) on BY-2 cells. We show here that this compound immediately induces dose-dependent cytosolic and nuclear calcium increases. In addition, DHS leads to a burst of Reactive Oxygen species (ROS). Upon 30 min of treatment, cell death symptoms appear and display programmed cell death (PCD) hallmarks such as cell shrinkage, chromatin condensation and caspase-like activities. Lanthanum chloride, a general blocker of calcium entry, suppresses the cellular calcium variations, the burst of ROS and the PCD induced by DHS. We thus evaluated the importance of ROS and of each calcium compartment in the signalling pathway leading to cell death. We report here that DL-2-amino-5-phosphopentanoic acid (AP5) and [(+)-dizocilpine] (MK801), two inhibitors of animal and plant ionotropic glutamate receptors, suppress DHS-induced cell death symptoms by selectively inhibiting the variations of nuclear calcium concentrations. We also show that suppression of ROS with the NADPH oxidase inhibitor diphenyleneiodonium chloride (**DPI**) does not impact on cell death. Altogether our work highlights the crucial role of nuclear calcium signature in controlling DHS-induced cell death in tobacco cells in a ROS-independent manner.

Calcium: an emerging key player for renal organogenesis

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From the formation of a simple kidney in amphibian larvae, the pronephros, to the formation of the more complex mammalian kidney, the metanephros, calcium is present throughout numerous steps of tubulogenesis and nephron induction. Several calcium-binding proteins such as regucalcin/SMP-30 and calbindin-D28k are commonly used to label pronephric tubules and metanephric ureteral epithelium, respectively. However, the involvement of calcium and calcium signaling at various stages of renal organogenesis was not clearly delineated. In recent years, several studies have pinpointed an unsuspected role of calcium in determination of the pronephric territory and for conversion of metanephric mesenchyme into nephrons. Influx of calcium and calcium transients have been recorded in the pool of renal progenitors to allow tubule formation, highlighting the occurrence of calcium-dependent signaling events during early kidney development. Characterization of nuclear calcium signaling is emerging. Implication of the non-canonical calcium/NFAT Wnt signaling pathway as an essential mechanism to promote nephrogenesis has recently been demonstrated. We examine the current knowledge of the impact of calcium ions during embryonic development of the kidney. It focuses on Ca^{2+} binding proteins and Ca^{2+} sensors that are involved in renal organogenesis.