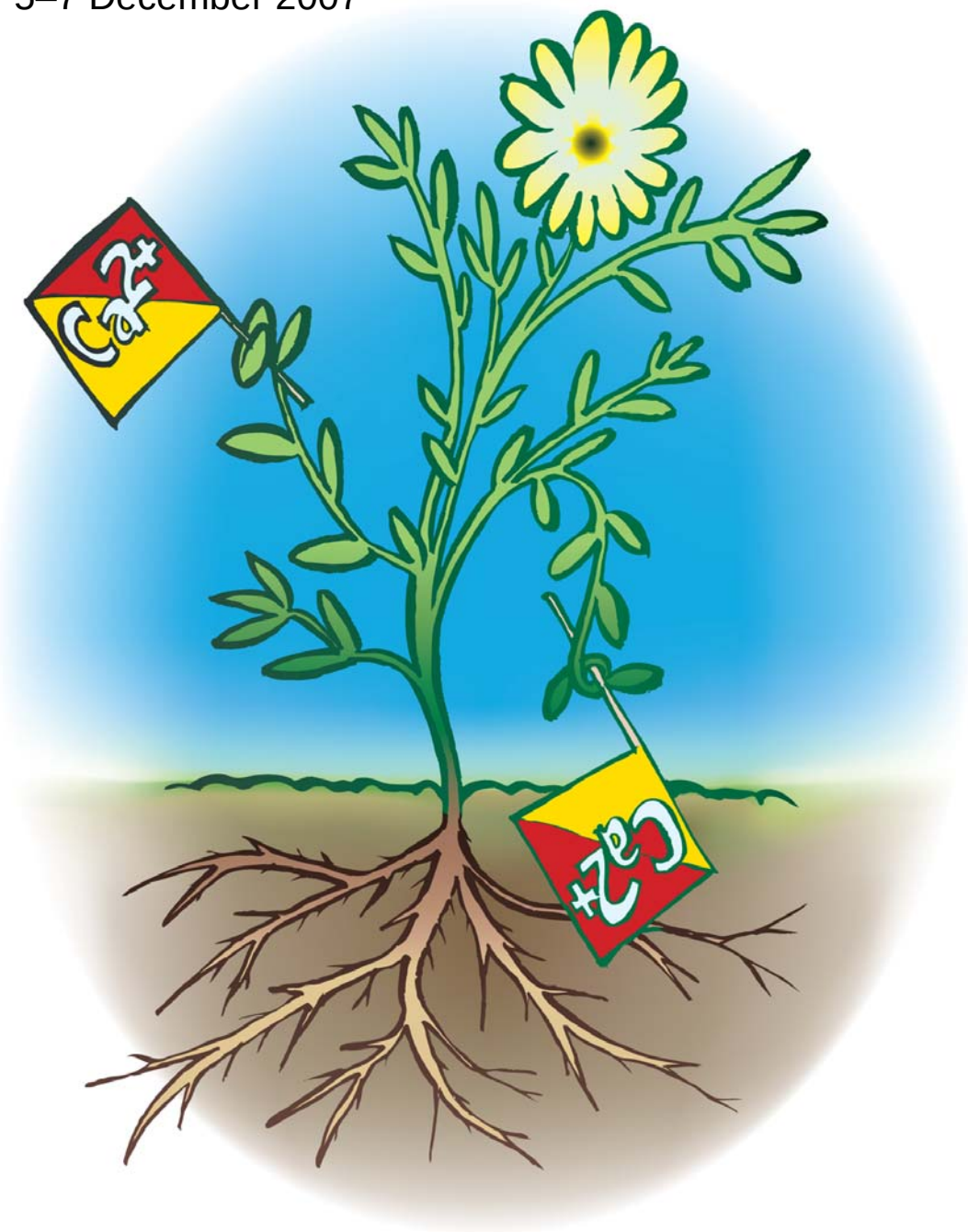
18th New Phytologist Symposium

Calcium-based signalling systems in plants

Royal Dublin Society, Dublin, Ireland
5–7 December 2007



Programme, abstracts and participants

New Phytologist

Programme, abstracts & participants

18th New Phytologist Symposium

Calcium-based signalling systems in plants

Royal Dublin Society, Dublin, Ireland
5–7 December 2007

Acknowledgements

The 18th New Phytologist Symposium is funded by the New Phytologist Trust

New Phytologist Trust

The New Phytologist Trust is a non-profit-making organization dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.

Programme, abstracts and participant list compiled by Helen Pinfield-Wells. Calcium illustration by Sam Day, www.samday.com

Table of contents

Wednesday 5 December	
8:00–8:45	**Registration**
9:00–9:05	**Welcome – Alistair Hetherington**
Session 1 *Chair: Carl Ng, UCD, Ireland*	
9:05–9:45	**1.1. Calcium channels and calcium signalling** Dale Sanders FRS, University of York, UK
9:45–10:25	**1.2. The control of stress gene expression and tolerance by calcium** Marc Knight, University of Durham, UK
10:25–11:05	Coffee/tea
11:05–11:45	**1.3. Differential changes in free calcium in the cytosol vs. nucleus in response to physical and chemical stimuli** Raoul Ranjeva, CNRS Toulouse, France
11:45–12:25	**1.4. The role of sphingosine-1-phosphate in guard cell signalling** Alistair Hetherington, University of Bristol, UK
12:25–12:45	**1.5. Short lecture A: Ca^{2+} and calmodulin involvement in photoperiodic signalling** John Love, University of Exeter, UK
12:45–14:00	Lunch
Session 2 *Chair: Marc Knight, University of Durham, UK*	
14:00–14:40	**2.1. Signalling in symbiosis** Giles Oldroyd, JIC, Norwich, UK
14:40–15:20	**2.2. Calcium/calmodulin-regulated transcription activators: Integrating developmental cues and stress responses** Hillel Fromm, Tel Aviv University, Israel
15:20–15:40	**2.3. Short lecture B: Arabidopsis vacuolar Ca^{2+}/H^{+} exchangers CAX1 and CAX3 pay a role in abiotic stress tolerance.** Jon Pittman, University of Manchester, UK
15:40–16:30	Coffee/tea
Plenary 1 *Chair: Alistair Hetherington, University of Bristol, UK*	
16:30–17:30	**Calcium signalling mechanisms** Sir Michael Berridge FRS, Brabraham Institute, University of Cambridge, UK
17:30–19:30	Reception and posters

Thursday 6 December	
Session 3 *Chair: Raoul Ranjeva, CNRS Toulouse, France*	
9:00–9:05	**Announcements – Alistair Hetherington**
9:05–9:45	**3.1. Ca^{2+}, ROS and pH – tipping the balance for root hair growth** Simon Gilroy, University of Wisconsin, USA
9:45–10:25	**3.2. Systems analysis of circadian calcium signals in Arabidopsis** Alex Webb, University of Cambridge, UK
10:25–11:05	**Coffee**
11:05–11:45	**3.3. Calcium signalling mechanisms in plants and algae: Evolutionary implications from physiological and genomic studies** Colin Brownlee, Marine Biological Association, Plymouth, UK
11:45-12:25	**3.4. Guard cell ion channel signalling, CO_2, abscisic acid and a model for calcium specificity** Julian Schroeder, University of California San Diego, USA
12:25–12:45	**3.5. Short lecture C: Mutations in AtCML9, a calmodulin-like protein from *A. thaliana*, alter plant responses to abiotic stress and abscisic acid** Jean-Philippe Galaud, Castanet-Tolosan, France
12.45–14:00	Lunch
14:00–14:45	**Discussion** **Future priorities and directions in calcium-based signalling research in plants** Janet Braam, Rice University, Houston, USA
14:45–16:30	Posters and coffee
Plenary 2 *Chair: Marc Knight, University of Durham, UK*	
16:30–17:30	**Pollen tubes, vesicles and CPK; what is the actual function of Ca^{2+} oscillations?** Tony Trewavas FRS, University of Edinburgh, UK
19:30	Conference Dinner, Minerva Suite, Royal Dublin Society

Friday 7 December	
Session 4 *Chair: Dale Sanders, University of York, UK*	
9:00–9:05	**Announcements – Alistair Hetherington**
9:05–9:45	**4.1. Calcium-induced activation of programmed cell death** Paul McCabe, UCD, Ireland
9:45–10:25	**4.2. A calcium sensor / protein kinase network for decoding calcium signals in plants** Jörg Kudla, University of Münster, Germany
10:25–11:05	**Coffee**
11:05–11:45	**4.3. Substrates of calcium-dependent protein kinases** Alice Harmon, University of Florida, USA
11:45-12:25	**4.4. Calcium/calmodulin signalling is critical for reprogramming plant growth and response to biotic and abiotic cues** Joe Poovaiah, Washington State University, USA
12:25–12:45	**Short lecture D: Is Nod factor induced calcium spiking in legume root hairs really required for nodule induction?** Giulia Morieri, JIC, Norwich, UK
12.45–13:00	**Poster prize presentation and conclusions – Alistair Hetherington**
13:00–14:00	Lunch

Speaker Abstracts

Session 1
Chair: Carl Ng, UCD, Ireland

1.1. Calcium channels and calcium signalling

SANDERS DALE, MAATHUIS FRANS, HARRIS RICHARD, PEITER EDGAR
Department of Biology, University of York, York YO10 5YW, UK

A multitude of electrophysiological studies on plant membranes have revealed the existence of many classes of calcium permeable channel that can be identified through hallmark characteristics: conductance, ionic selectivity, gating, and pharmacology. However, the identity of the channels that underlie calcium currents has remained obscure, impeding deeper understanding of calcium signalling. The Slow Vacuolar channel is a calcium-activated calcium-permeable channel that dominates the electrophysiological properties of the tonoplast. Electrophysiological and transcriptomic analyses, as well as measurements of cytosolic free calcium, indicate that TPC1 mediates specific responses to hydrogen peroxide. This is supported by the finding that hydrogen peroxide-induced elevations in cytosolic free calcium are attenuated in a *tpc1* knockout mutant. In contrast, stomatal responses to hydrogen peroxide were not found to be dependent on TPC1, indicating a diversity of hydrogen peroxide-related signalling networks. The possibility that TPC1 plays a more general role in mobilisation of cations other than calcium has been investigated rigorously: negative results were forthcoming. In an effort to identify further calcium permeation pathways, we have studied functions of three related genes, all of which encode membrane proteins that are upregulated by calcium starvation. Properties of the mutants indicate a complex function in divalent cation homeostasis.

1.2. The control of stress gene expression and tolerance by calcium

HELEN J. RUSHTON[1], RICHARD G. CAPPER[1], JOY M. BOYCE[1], HEATHER KNIGHT[2] & MARC R. KNIGHT[2]

[1]*Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB.*
[2]*School of Biological Sciences, Durham University, South Road, Durham DH1 3LE.*

Our lab is interested in understanding the molecular mechanisms of calcium regulation of gene expression. In the course of this work we have considered the issues of necessity, sufficiency and specificity of calcium. Our earliest studies involved correlations of *in planta* calcium measurements with stress gene expression, followed by tests of sufficiency and necessity using pharmacological agonists/antagonists of calcium. These approaches, along with data from us and others on stress tolerance itself, demonstrate a very widespread role for calcium. Calcium regulates the expression of genes responding to, and is required for tolerance of, a very wide range of abiotic and biotic stresses. This raises the question of how specificity might be encoded in calcium signalling? Correlation of specific calcium signatures with specific primary stimuli suggested the possibility of stress-specific information being encoded by calcium. In order to move our work on gene expression forwards and to probe the issue of specificity we undertook a reverse genetic approach. We examined the effect of loss and gain of function of calmodulin (CaM), calcium-dependent protein kinases (CDPK) and calcium pumps (ECAs/ACAs), and obtained largely no useful data. Apart from the practical difficulty this presents, it also illustrates the redundancy of such components of calcium signalling, making their study difficult. Forward genetic screens also produced very few mutants, all of whom had very weak calcium phenotypes, indicating again, perhaps, the robustness of the calcium signalling system in plants to chemical/genetic intervention. Most recently we have been examining the regulation of the whole transcriptome by calcium in an attempt to obtain information on specificity and also to circumvent the problems of the genetic approach. This approach has revealed specific transcription factor/DNA motifs which are the likely targets of calcium signalling pathways *in planta*. We aim to build upon this data by identifying the mechanisms by which these components are regulated by calcium. This will hopefully allow us to construct calcium signalling networks regulating gene expression in plants.

1.3. Differential changes in free calcium in the cytosol vs. nucleus in response to physical and chemical stimuli

RAOUL RANJEVA & CHRISTIAN MAZARS

Signaux et Messages Cellulaires chez les Végétaux, UMR5546 -CNRS Université P. Sabatier, Chemin de Borde-Rouge, BP 42617 Auzeville f-31326 Castanet-Tolosan Cédex.

(A collective work done in collaboration with the groups of A. PUGIN, Dijon and A. MITHOEFER MPI, Jena)

Calcium-dependent processes are distributed between different cellular organelles where they are regulated by changes in free calcium concentrations in these compartments. Here, we consider the particular case of the nucleus which is surrounded by a double membrane punctuated with pores that are large enough to allow the reversible diffusion of calcium from the cytosol to the nucleoplasm. We present and critically discuss data showing that depending upon each situation, free calcium concentration changes may be coincident, delayed or even completely disconnected in the cytosol and the nucleus. We show further that isolated nuclei are impermeable to exogenously added free calcium but are able to convert physical and/or chemical stimuli into intensity-dependent and specific elevations in nucleoplasmic calcium. We discuss data taken from the literature showing that the nucleus contains structures which, in addition to the nuclear envelope, may constitute a source of mobilizable calcium for the nucleus. We propose that the nuclear compartment contains all the biochemical equipment that allows the generation/regulation of calcium signals and suggest that the nucleus may generate its own calcium signals independently of the cytosol.

1.4. Sphingosine-1-phosphate-based signalling in guard cells

ALISTAIR HETHERINGTON
School of Biological Sciences, University of Bristol, Bristol, UK

In previous work we showed that treating guard cells with sphingosine-1-phosphate (S1P) resulted in an increase in guard cell cytosolic free calcium concentration and a reduction in stomatal aperture.
To investigate this further we identified, characterized and manipulated genes potentially capable of metabolizing S1P. Sphingosine kinase1 (SPHK1) encodes an enzyme capable of phosphorylating sphingosine and other sphingoid long chain bases and is expressed in guard cells. The stomata of sphk1-kd lines were less sensitive, while the stomata of spk1-oe lines were more sensitive than WT to ABA. Reducing expression of either a putative S1P phosphatase (SPPASE) or the DPL1 gene, which encodes an enzyme with S1P lyase activity, individually had no effect on guard cell ABA-signalling, however stomatal responses to ABA in SPPASE RNAi DPL1 RNAi plants were compromised. We also found evidence that expression of SPHK1 and SPPASE were co-ordinately regulated. I shall discuss how this might constitute a possible mechanism for the homeostatic control of S1P levels and as such contribute to robustness in S1P-based signalling pathways.

1.5. Short lecture A: Ca^{2+} and calmodulin involvement in photoperiodic signalling

JOHN LOVE, ANDREW MURPHY AND GEORGE LITTLEJOHN
School of Biosciences, Geoffrey Pope Building, University of Exeter EX4 4QD

Photoperiodism is a key regulator of plant development. Our laboratory aims to apply any means available to investigate the functional integration of cell signalling and molecular pathways involved in photoperiodic flowering. We have previously shown that phase changes in diurnal and circadian $[Ca^{2+}]_{cyt}$ oscillations may be associated with photoperiod sensing in *Arabidopsis thaliana*. Currently, we are using aequorin- and cameleon-based *in vivo* Ca^{2+} imaging and qRT-PCR analysis of the expression of key flowering genes to investigate the role of cytoplasmic and chloroplastic Ca^{2+} signals in photoperiodism. In addition, we have empirical and bioinformatic evidence suggesting that calmodulin is involved in modulating essential components of the flowering pathway. Our results and hypotheses for the involvement of Ca^{2+}-dependent signalling systems will be presented.

Session 2
Chair: Marc Knight, University of Durham, UK

2.1. Signalling in symbiosis

GILES OLDROYD
Department of Disease and Stress Biology, John Innes Centre, Norwich, UK

Legumes form symbiotic interactions with both nitrogen fixing rhizobial bacteria and mycorrhizal fungi. Both of these micro-organisms release diffusible signals that are recognised by the legume: Nod factors from rhizobia and as yet uncharacterised Myc factors. There is conservation in the plant signal transduction pathway responsible for the recognition of both Nod factor and Myc factor. Repetitive oscillations in cytosolic calcium in root hair cells are one of the earliest plant responses to Nod factor and Myc factor, however the structure of the calcium response differs for these two signals. These calcium responses are embedded in the conserved symbiosis signalling pathway with a calcium/calmodulin dependent kinase (CCaMK) being apparently responsible for decoding both Myc factor and Nod factor induced calcium oscillations. Gain of function mutations in CCaMK activate nodulation without the need for Nod factor or rhizobial elicitation. This gain of function requires the activity of *NSP1* and *NSP2,* both of which encode putative transcriptional regulators in the GRAS family, as well as *ERN,* a newly defined ERF transcription factor. *NSP1, NSP2* and *ERN* have specific functions in Nod factor signalling and represent a nodulation specific branch downstream of CCaMK. We propose that Nod factor induced calcium oscillations that occur in both the cytosol and the nucleus regulate the DMI3 kinase through a combination of calcium and calmodulin binding. DMI3 activates downstream components, including the transcriptional regulators NSP1, NSP2 and ERN. CCaMK must be able to discriminate between the Nod factor and Myc factor induced calcium oscillations and we presume that equivalent mycorrhizal specific components are induced under the appropriate conditions.

2.2. Calcium/calmodulin-regulated transcription activators: Intergrating developmental queue and stress responses

HILLEL FROMM[1], ROY NAVE[1], RONI ALONI[1], ALIZA FINKLER[1], JOY BOYCE[2], MARC R. KNIGHT[3], AND YAEL GALON[1].

[1]Department of Plant Sciences, Tel Aviv University, 69978 Tel Aviv, Israel; [2]Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK; [3]School of Biological and Biomedical Sciences, Durham University, South Road, Durham, DH1 3LE, UK.

E-mail for correspondence: hillelf@post.tau.ac.il

In spite of the importance of Ca^{2+} signalling in plants, the mechanisms linking Ca^{2+} signalling to the transcription machinery are poorly understood. We seek to characterize the *cis*-regulatory elements, *trans*-acting factors (TFs) and other cellular factors that mediate Ca^{2+}-regulated gene expression. By linking artificial cytosolic Ca^{2+} transients with rapid transcriptome changes we have previously reported that abscisic-acid responsive elements (*ABREs*) may function as Ca^{2+}-responsive *cis* elements[1,2]. In addition, we have been studying a family of Ca^{2+}/calmodulin-binding TFs (designated CAMTAs[3]). These add to other plant TFs that bind Ca^{2+}/calmodulin, including specific members of the WRKY and Myb TF families. Phenotypic and transcriptome analyses of single and double knockout mutants of *Arabidopsis CAMTA*s revealed their importance in both biotic and abiotic stress responses. *CAMTA* promoter:*GUS* fusions in transgenic plants revealed that the expression profile of one *CAMTA* resembles that of auxin-responsive genes. However, the spatial expression profile of this *CAMTA* gene differentially changes in response to different stresses. Considering that CAMTAs regulate the expression of *AVP1,* a gene encoding a vacuolar pyrophosphatase[4] that controls cellular pH and regulates auxin transport[5], we suggest that *CAMTAs* respond to auxin signals and integrate stress signals into their expression pattern and function, possibly through *ABRE*-related *cis*-regulatory elements.

[*]Research supported by (a) the Israel Science Foundation (grant No. 201/05), and (b) BBSRC (UK) grant No. P20471.

[1]Kaplan B. et al. (2006) Plant Cell 18: 2733-2748

[2]Finkler A. et al. (2007) Plant Signalling and Behavior 2: 17–19

[3]Finkler A. et al. (2007) FEBS Letters 581: 3893–3898

[4]Mitsuda N. et al. (2003) Plant Cell Physiology 44: 975-981

[5]Li J. et al. (2005) Science 310: 121-125.

2.3. Short lecture B: *Arabidopsis* vacuolar Ca^{2+}/H^{+} exchangers CAX1 and CAX3 play a role in abiotic stress tolerance

JON K. PITTMAN[1], CLARE EDMOND[1], SIMON ACTON[2], KATHRYN NORTH[2], JIAN ZHAO[3], KENDAL D. HIRSCHI[3] & MARTIN R. MCAINSH[2]

[1]Faculty of Life Sciences, University of Manchester, Manchester, M13 9PT, UK; [2]Department of Biological Sciences, Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK; [3]USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX 77030, USA

Abiotic stress can significantly perturb plant growth, thus plants have specific adaptive responses which are activated following stress signal perception and signal transduction. A common response to abiotic stress is the generation of cytosolic Ca^{2+} elevations with specific dynamics which are thought to elicit a specific response. It is unclear what components are involved in generating these Ca^{2+} signatures. *Arabidopsis* vacuolar Ca^{2+}/H^{+} exchangers encoded by CAX genes mediate high capacity Ca^{2+} transport into the vacuole. Analysis of *cax1* and *cax3* knockout mutants suggest that these transporters are involved in specific abiotic stress responses including low temperature, salinity and oxidative stress. Furthermore, CAX1 and CAX3 are both regulated in response to abiotic stress. *CAX1* and *CAX3* are transcriptionally up-regulated following stress treatments. In addition, stress-dependent phosphorylation was observed for both proteins; however, the stress-dependent regulation profile differs between CAX1 and CAX3 with respect to specific stresses. Inhibition of vacuolar Ca^{2+}/H^{+} exchange activity affects the generation of stress-induced Ca^{2+} signatures. Alterations in the dynamics of cold- and H_2O_2-induced cytosolic Ca^{2+} signatures were observed in the *cax1* knockout. These results suggest that CAX1 and CAX3 may be central components in controlling cytosolic Ca^{2+} dynamics under specific stress conditions.

Plenary 1
Chair: Alistair Hetherington, University of Bristol, UK

Calcium signalling mechanisms

MICHAEL J. BERRIDGE
Laboratory of Molecular Signalling, The Babraham Institute, Babraham, Cambridge CB2 4AT, UK.

Calcium (Ca^{2+}) is a highly versatile intracellular signal capable of regulating many different processes. To achieve this versatility, cells have access to a very extensive Ca^{2+} signalling toolkit from which each cell type expresses a unique set of components to create Ca^{2+} signalling systems with widely different spatial and temporal properties. Spatial properties are particularly relevant for fast responses where components of the ON reactions and their downstream effectors are closely associated. This spatial contiguity is less apparent for the slower responses such as gene transcription, fertilization and cell proliferation where Ca^{2+} signals tend to operate more globally and where temporal properties of signalling become increasingly important with signalling represented as repetitive Ca^{2+} transients and waves. Such Ca^{2+} signalling systems are not fixed in stone, but are constantly being remodelled to adapt to changing circumstances. Ca^{2+} itself plays a critical role in an internal assessment mechanism that remodels its own signalling pathway. A number of important disease states (hypertension, congestive heart failure, manic depressive illness, Alzheimer's disease) may result from abnormal remodelling of Ca^{2+} signalling systems.

Session 3
Chair: Raoul Ranjeva, CNRS Toulouse, France

3.1. Ca^{2+}, ROS and pH - tipping the balance for root hair growth

GABRIELE B. MONSHAUSEN[1,2], TATIANA N. BIBIKOVA[2], MARC A.MESSERLI[3], CHEN SHI[2], & SIMON GILROY[1,2]
[1]Department of Botany, University of Wisconsin, Madison WI 53706, USA; [2]Department of Biology, Pennsylvania State University, University Park, PA 16802, USA; [3]BioCurrents Research Center, Marine Biological Laboratory, Woods Hole, MA 02543, USA

Root hairs show highly localized cell expansion focused to their growing tips that is regulated by a host of cytoplasmic factors such as Ca^{2+}, the cytoskeleton, reactive oxygen species (ROS) and monomeric G-proteins. We have been able to show that root hairs exhibit pulsatile growth that is associated with oscillatory increases in extracellular pH and ROS, driven by an oscillating Ca^{2+} gradient focused on the expanding tip. Consistent with a role for these changes in growth control, artificially manipulating wall pH and ROS leads to altered growth. Ca^{2+}-dependent production of extracellular ROS is disrupted in the *rhd2-1* mutant, which lacks a functional version of the NADPH oxidase ATRBOH C. Roots hairs in this mutant burst at the transition to tip growth implying a lesion that compromises the ability of the cell to locally resist the expansive forces of turgor. However, this phenotype can be rescued by elevating the growth medium pH to ≥6.0. These rescued root hairs show normal growth, including generation of the tip focused Ca^{2+}-gradient. Such observations suggest a model where root hair elongation is coupled to spatially distinct Ca^{2+}-dependent regulation of extracellular pH and ROS production and that these systems interact to regulate tip growth.

3.2. Systems analysis of circadian calcium signals in Arabidopsis

ALEX A.R. WEBB
Department of Plant Sciences, Downing Street, Cambridge CB2 3EA
Alex.webb@plantsci.cam.ac.uk

Cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) oscillates with a period of 24 hours in Arabidopsis due to regulation by the circadian clock. We are dissecting the mechanisms by which these oscillations of $[Ca^{2+}]_{cyt}$ are generated and decoded with the goal of determining the function of the circadian Ca^{2+} signalling network. Investigating a signalling pathway that operates in the time scale of days rather than minutes necessitates an alternative experimental approach to the intracellular techniques typically used to analyse Ca^{2+} signalling networks. We have adopted a systems approach that couples non-invasive imaging of aequorin and luciferase luminescence, reverse genetics, transcriptomics, bioinformatics, mathematical modelling and automated physiological assays. Our work has demonstrated that circadian oscillations of $[Ca^{2+}]_{cyt}$ are generated by a circadian oscillator which is genetically separable from the oscillator regulating other circadian outputs such as gene expression, leaf and stomatal movements. We find that circadian oscillations of $[Ca^{2+}]_{cyt}$ are driven by oscillations in the cellular concentration of cyclic ADP ribose (cADPR). cADPR forms part of a cytosolic arm of the circadian clock that also regulates the transcription of several genes encoding circadian oscillator components. This novel role for a cytosolic signalling molecule as a circadian clock component may explain our observation that the circadian clock gates the low temperature Ca^{2+} signalling network. New data describing a mathematical model of the dual regulation of $[Ca^{2+}]_{cyt}$ by the circadian clock and light and progress in identifying the molecular targets for circadian oscillations of $[Ca^{2+}]_{cyt}$ will be described.

3.3. Calcium signalling mechanisms in plants and algae: Evolutionary implications from physiological and genomic studies.

COLIN BROWNLEE[1], ALISON TAYLOR[1,2], GARRY FARNHAM[1], NIALL RAUH[1,3] GLEN WHEELER[4] , JOHN BOTHWELL[1]AND FREDERIC VERRET[1]

1 Marine Biological Association, Plymouth, UK, 2 University of North Carolina, Wilmington, USA, 3 University of Bristol, UK, 4 Plymouth Marine Laboratory, UK

The eukaryotic algae comprise a phyletically divergent group of organisms. Those on the green lineage had ancient origins quite distinct from the heterkonts (brown algae, diatoms), haptophytes (coccolithophores) and dinoflagellates which together account for the bulk of marine eukaryotic biomass. We have been studying the plasma membrane properties of representatives of these algae with a view to improving knowledge on the evolution of cell signalling and ionic homeostasis. Certain members of the brown algae have provided excellent cell physiological models for dissecting out the components of Ca^{2+} signals that underlie cell volume control. The Ca^{2+} signalling machinery of the highly polarised *Fucus* zygote shows a high degree of spatial organization involving fluxes at both plasma membrane and endomembrane locations. The molecular counterparts to the channel activity underlying these responses remain to be characterised though the availability of the full genome sequence of the brown alga *Ectocarpus* now provides a platform on which to base these studies. Representative species of other algal groups, including the diatoms, and coccolithophores exhibit very fast animal-like sodium/calcium-based action potentials that have not previously been shown in photosynthetic organisms. We have identified three distinct classes of 'animal-type' Na/Ca channels in the genomes of several evolutionarily divergent algae, including the green alga *Chlamydomonas* but these are distinctly absent from higher plants. The evolutionary and functional significance of these findings will be discussed.

3.4. Guard cell Ca^{2+} and ion channel signalling: CO_2, abscisic acid and a model for calcium specificity.

JULIAN I. SCHROEDER, YONG-FEI WANG, MARIA ISRAELSSON, ROBERT S. SIEGEL, NORIYUKI NISHIMURA, MAIK BÖHMER, IZUMI MORI, TRIIN VAHISALU[2], HANNES KOLLIST[2], YOSHIYUKI MURATA[1], JAAKKO KANGASJARVI[2], YINGZHEN YANG

Cell and Developmental Biology Section, Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093-0116, USA.

[1]Dept. of Agriculture Okayama University, Okayama 700, Japan.

[2] Dept of Biol and Environ Sciences, University of Helsinki, Finland.

Guard cells have been developed as a model system for dissecting ion channel functions and early signal transduction mechanisms in plant cells. Although many studies have shown a central role for intracellular Ca^{2+} in guard cell ion channel regulation, genetic loss-of-function mutants in Ca^{2+} sensors that impair abscisic acid regulation of these ion channels have been lacking. In addition, a Ca^{2+}-independent branch functions in the abscisic acid (ABA) response. We have recently identified two calcium-dependent protein kinases (CDPKs) that function in abscisic acid and intracellular Ca^{2+} regulation of guard cell anion, and Ca^{2+}-permeable channels and stomatal closing (I. Mori et al., *PLoS Biol.*). Furthermore, signal transduction analyses will be presented, that point to a model for how plant cells can achieve specificity in calcium signalling through “priming” and “de-priming” of intracellular Ca^{2+} sensitivity (J. Young et al., *PNAS*). New evidence from independent analyses will be presented that correlates with this “Ca^{2+} sensitivity priming” hypothesis. In addition, evidence for a parallel signalling branch in the ABA signalling network will also be presented. A central target of ABA and intracellular Ca^{2+} signalling is the activation of S-type anion channels in guard cells. However, plasma membrane Cl^-/anion channel-encoding genes have not yet been characterized in plants and genetic evidence for the functions S-type anion channels is still lacking. Mapping of a guard cell response mutant has led to identification of a new gene encoding a plasma membrane protein that is essential for mediating ABA- and Ca^{2+}-activated anion channel activity. Genetic guard cell signal transduction analyses will be presented that test the signalling functions of these anion channels.

Our research on guard cell hyperpolarization-activated Ca^{2+}-permeable channels has led to a model for oxidative stress regulation of Ca^{2+} channels (Pei et al., 2000 *Nature*; J.M. Kwak et al., *EMBO J.*; Mori et al., *PLoS Biol.*). Data will be presented from rat basophilic leukemia cells and Jurkat T cells analyzing a role for oxidative regulation of store-operated Ca^{2+} channels.

3.5. Short lecture C: Mutations in AtCML9, a calmodulin-like protein from *A. thaliana*, alter plant responses to abiotic stress and abscisic acid

FABIENNE MAGNAN, BENOIT RANTY, MARTINE CHARPENTEAU, JEAN-PHILIPPE GALAUD & DIDIER ALDON

UMR 5546 CNRS-Université Toulouse, Pôle de Biotechnologie végétale, 24 chemin de Borde-Rouge, BP42617, 31326 Castanet-Tolosan, France.

Many stimuli such as hormones and abiotic stress factors elicit changes in intracellular calcium levels that serve to convey information and activate appropriate responses. The Ca^{2+} signals are perceived by different Ca^{2+} receptors, and calmodulin (CaM) is one of the best characterized Ca^{2+} sensors in eukaryotes. Calmodulin-like (CML) proteins sharing sequence similarity with the ubiquitous and highly conserved CaM exists in plants but their roles at physiological and molecular levels are largely unknown. We report here data for *Arabidopsis thaliana* CML9 (AtCML9) that exhibits 46% amino acid sequence identity with CaM. *AtCML9* gene expression is finely regulated during plant development and in response to abiotic stress and ABA treatment. Using *cml9* knockout mutants, we present evidence that AtCML9 plays essential roles in modulating responses to abiotic stress and ABA. Seed germination and seedling growth for the mutant lines are hypersensitive to ABA and salt stress. Mutations of *AtCML9* gene also confers enhanced tolerance to water deficit and alters the expression of several stress regulated genes. These data indicate that AtCML9 is involved in abiotic stress tolerance through its effects on ABA-mediated pathways.

Plenary 2
Chair: Marc Knight, University of Durham, UK

Pollen tubes, vesicles and CPK; what is the actual function of Ca^{2+} oscillations?
TONY TREWAVAS
University of Edinburgh, UK

Since the early 80?s a succession of excellent people in my laboratory have established that a variety of behavioural signals, red and blue light, gravity, touch cold, elicitors, circadian variations modify cytosolic, and in some cases nuclear, calcium using dye and aequorin measurement technology. In early work we also established by artificial elevations of $[Ca^{2+}]_i$ that Ca^{2+} changes were sufficient on their own to induce growth, or turgor controlled, behavioural changes.

In this lecture I intend to concentrate on research in the pollen tube. Again excellent researchers in my laboratory established that incompatibility factors that institute out-breeding initiated immediate changes in $[Ca^{2+}]_i$and further we learned how to steer pollen tubes round corners by spatially manipulating tip-high $[Ca^{2+}]_i$.
Finally this was followed by the clearest demonstration yet that cyclic AMP had a signalling role in plant cells by imaging cyclic AMP distributions in growing and direction-changing pollen tubes. I want in this lecture to describe work much of which remains unpublished because time ran out on us and I retired just over three years ago.

This research by Masaaki Watahiki commenced in 1997 to sort out CDPKs in pollen tubes and largely ceased by 2002 as required by the funding of other grant programmes. The hope had been to better identify downstream substrates of calcium dependent phosphorylation but I returned the final funding obtained for this purpose from lack of suitable post doctoral candidates. Some of what was found was confirmed by another laboratory which published in 2006. But there are sufficient differences that merit description here. The two CDPK?s identified in tobacco pollen tubes are found in part attached to the sperm cell outer membrane and to the cell wall-containing vesicle membrane and are thus useful markers for these cell sites.
Characteristics of the binding regions were looked at in detail in one CDPK.

Finally I want to suggest why the tip-high $[Ca^{2+}]_i$of so many different pollen tubes (we have examined six altogether) oscillates as does the growth rate. It is possible that Ca^{2+} oscillations construct an oscillating gate via critical but oscillating phase transitions in gel structure at the tip that permits a discrete but limited number of vesicles into the cellular region for tip fusion. Since the gate, via $[Ca^{2+}]_i$ oscillation, is controlled by negative feedback, a mechanism for sensitively controlling growth rates becomes available.

Session 4
Chair: Dale Sanders, University of York, UK

4.1. Calcium-induced activation of programmed cell death

PAUL F. McCABE, MAGDALENA ZIMON, ELIZABETH M. MOLONY
University College Dublin, Ireland

Programmed cell death (PCD) is a fundamental cellular process that is involved in plant defence, development and response to stress. Using an Arabidopsis cell suspension culture our group has developed an *in vitro* model system to study aspects of the regulation of plant programmed cell death. The system allows us to activate cell death synchronously in a cell culture and to then accurately monitor rates of cell viability, PCD and necrosis within a cell population. We primarily use cell morphology and viability stains to identify cells undergoing PCD but we have confirmed cell death by assaying cytochrome c release from the mitochondria and PCD associated gene regulation. Because we can monitor individual cells the system is very sensitive and can report on either large, or subtle, changes in PCD activation levels. Using this model system we have previously investigated the roles of cell signalling, senescence and mitochondria in the regulation of plant PCD. More recently we have been studying the effect of *Fusarium* mycotoxins, sphingolipids and calcium influxes on programmed cell death rates in plant cells. I will describe the Arabidopsis model system in detail and I will discuss our recent findings that a calcium influx can induce PCD in Arabidopsis cells. I will also show evidence that blocking a calcium influx can inhibit the initiation, but not the progression, of PCD in plant cells.

4.2. A calcium sensor / protein kinase network for decoding calcium signals in plants

JÖRG KUDLA
Universität Münster, Institut für Botanik und Botanischer Garten, Schlossplatz 4, 48149 Münster, Germany

Intracellular release of calcium ions belongs to the earliest events in signal perception. Calcium-binding proteins are involved in sensing and relaying these signals to downstream signalling and adaptation responses. Calcineurin B-like proteins (CBLs) represent a plant-specific group of calcium sensor proteins that are closely related to Calcineurin B and Neuronal Calcium Sensors (NCS). CBLs exclusively interact with a group of serine-threonine kinases designated as CBL-interacting protein kinases (CIPKs). In Arabidopsis, 10 CBL-type calcium sensor proteins form an interaction network with 25 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 and cell biological approaches have begun to unravel the functional principles of this signalling network and to unravel the function of some members of both protein families. I will present results of our characterization of cbl and cipk loss-of-function mutants and of our investigation of the sub-cellular localization of all CBLs from Arabidopsis. These studies suggest that CBL/CIPK complexes function predominantly at cellular membranes and can decode Ca^{2+} signals in different compartments. In this context, dual lipid modification by myristoylation and palmitoylation appears to play an important role in determining the membrane targeting of CBL/CIPK complexes. Our reverse genetics analyses indicate that alternative complex formation of CIPK-type kinases with different CBLs enables the simultaneous regulation of ion transport processes in different compartments of the plant cell. In this way CBL/CIPK complexes contribute to regulating the extrusion of Na^+ ions in root tissues and in addition regulate the sequestration of Na^+ in the vacuole in green tissues.

4.3. Substrates of calcium-dependent protein kinases

JEFFREY F. HARPER[1], JOHN C. CUSHMAN[1], AND ALICE C. HARMON[2]
[1]Department of Biochemistry, University of Nevada, Reno, Nevada, 89557; [2]Department of Botany and Program in Plant Molecular and Cellular Biology, University of Florida, Gainesville, FL, 32611-8526

Calcium-dependent protein kinases (CDPKs) transduce micromolar calcium signals via binding of Ca^{2+} to their C-terminal calmodulin-like domains. To identify targets of CDPK signalling, we have used a variety of approaches. In soybean, the substrate serine acetyltransferase2;1 was discovered by interaction cloning. This enzyme, which catalyzes the first step in cysteine synthesis, is phosphorylated in vivo in response to oxidative stress. Phosphorylation of SAT2;1 prevents feedback inhibition by cysteine, and thus may play a role in protection against reactive oxygen species. Screening of a yeast-two hybrid library with Arabidopsis CPKs 4 and 11 yielded several substrates including Di19-1, a drought stress-induced protein. Di19-1 is phosphorylated within its nuclear localization signal. Testing of synthetic peptide libraries containing 650 peptides based on known Arabidopsis phosphoproteins, yielded 110 substrates. Phosphorylation of at least 20 of these sites in vivo has been confirmed. This work not only identified new CDPK substrates, but also demonstrated both overlap and specificity for substrates of CDPK family members.

4.4. Calcium/calmodulin signalling is critical for reprogramming plant growth and response to biotic and abiotic cues

JOE B.W. POOVAIAH, TIANBAO YANG, & LIQUN DU
Center for Integrated Biotechnology and Dept. of Horticulture, Washington State University, Pullman, WA 99164-6414, USA

Calcium/calmodulin-mediated signalling plays a crucial role in plant growth and development as well as plant response to environmental stimuli. How these stimuli induce downstream actions and how one can manipulate these events to alter plant response is an area of major interest. Recently our laboratory has made progress in understanding the functional significance of several intriguing calcium/calmodulin-regulated proteins. This presentation will focus on a calcium/calmodulin-regulated metabolic enzyme (DWF1); a chimeric calcium/calmodulin-dependent protein kinase (CCaMK); and a family of calcium/calmodulin-regulated transcription factors (AtSRs or CAMTAs). These proteins play critical roles in plant growth (Du and Poovaiah, Nature, 437:741-745, 2005), plant-microbe interactions (Patil et al., PNAS, 92: 4897-4901, 1995; Gleason et al., Nature, 441:1149-1152, 2006) and plant response to multiple environmental signals (Yang and Poovaiah, JBC, 277: 45049-45058, 2002; Du et al., Nature, under revision). The identification and manipulation of calcium-binding and calmodulin-binding sites in these proteins provide new ways to rebuild the proteins and engineer plants to obtain desired traits. Supported by grants from the National Science Foundation, U.S. Department of Agriculture and the Agricultural Experiment Station, Washington State University.

4.5. Is Nod-factor-induced calcium spiking in legume root hairs really required for nodule induction?

GIULIA MORIERI[1], SONJA KOSUTA[2], HIROKI MIWA[1], KRZYSZTOF SZCZYGLOWSKI[2], GILES E.D. OLDROYD[1], J. ALLAN DOWNIE[1]

[1] John Innes Centre, Norwich, UK; [2] Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, Ontario, Canada

In rhizobia-legume symbioses bacterial infection results in the formation of plant derived nitrogen-fixing root nodules. The establishment of this symbiosis requires the plant to perceive a bacterially derived molecule called Nod factor which induces many events associated with nodulation, including periodic calcium oscillations in root hair cell. This calcium spiking is considered to be a central component of the signal transduction pathway that leads to the nodule formation. However, two recent *L. japonicus* mutants, *nup85* and *nup133*, do form a few nodules yet fail to induce calcium spiking. In this work, we show another example of a mutant which undergoes nodule morphogenesis without the activation of calcium spiking. Most mutations of a transmembrane receptor kinase, *SymRK*, completely block all nodulation associated events. However, the weak allele *symrk-14,* forms nodules, but lacks calcium spiking. These observations raise the question: is Nod-factor-induced calcium spiking in legume root hairs really required for nodule induction?

Poster Abstracts

Listed alphabetically by first author, presenting author is underlined

1. Ca^{2+} and Mg^{2+} cooperate in the signalling pathway of blue light-induced chloroplast movements

ANIELSKA-MAZUR, ANNA & GABRYŚ, HALINA
Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland.

Directional chloroplast movements are controlled by blue light in higher land plants. Regulation of calcium homeostasis appears to be crucial for chloroplast responses. However, the role of calcium in their mechanism and secondary messenger(s) involved in the light signal transduction remain to be identified. We compared effects of wortmannin, the inhibitor of phosphoinositide-3-kinase and of the calmodulin inhibitor, trifluoperazine, on chloroplast responses in transgenic *N. tabacum.* Mature leaves of the transgenic tobacco expressed plastin-GFP which enabled parallel investigation of the actin cytoskeleton. Light-induced chloroplast responses were normal in the transgenic plants. 10 μM wortmannin caused full inhibition of the weak light-induced accumulation response and 100 μM wortmannin was necessary to block the avoidance response induced by strong light. Similar effects were previously reported for *Lemna trisulca* but at the wortmannin concentration lower by two orders of magnitude. Ca^{2+} and Mg^{2+} partly reactivated the accumulation and fully reactivated the avoidance response. Similarly, very strong inhibitory effects of trifluoperazine were reversed by calcium and magnesium, with Mg^{2+} twice as effective as Ca^{2+}. The actin cytoskeleton was strongly disrupted by trifluoperazine. This disruption was also reversible by Ca^{2+} or Mg^{2+}. Our results point to a possible cooperation of both ions in the signalling pathway of blue light-induced tobacco chloroplast movements.

2. Dual lipid modification and differential targeting determine the localization of CBL/CIPK complexes

BATISTIC, OLIVER & KUDLA, JÖRG
Universität Münster, Institut für Botanik und Botanischer Garten, Schlossplatz 4, 48149 Münster, Germany

Intracellular release of calcium ions belongs to the earliest events in signal perception by plant cells. Calcineurin B-like proteins (CBLs) represent a novel group of calcium sensor proteins likely to function in deciphering such calcium signals. CBLs exclusively interact with a group of serine-threonine kinases designated as CBL-interacting protein kinases (CIPKs). In Arabidopsis, 10 CBL-type calcium sensor proteins form an interaction network with 25 CIPKs. Differential targeting of distinct CBL/CIPK complexes to membranes of diverse calcium stores can contribute to achieve specificity within the calcium signalling network.

Here we present our localization analysis of all ten CBL proteins from Arabidopsis that indicates differential cellular localization of these calcium sensors as mechanisms contributing to spatial specificity in calcium signalling. Moreover, we report that alternative complex formation of the kinase CIPK1 with either CBL1 or CBL2 results in distinct complex location at the plasma membrane or the tonoplast. Furthermore, we will show that CBL1 is targeted to the plasma membrane via its lipid-modified N-terminus and that the lipid-modification of the calcium sensor determines the cellular localization and trafficking of preassembled CBL/CIPK complexes.

3. Ca^{2+} signals co-ordinate zygotic polarization and cell cycle progression in the brown alga, *Fucus serratus*

BOTHWELL, JOHN H.F.[1], KISIELEWSKA, JOLANTA[2], GENNER, MARTIN J.[1], MCAINSH, MARTIN R.[3], BROWNLEE, COLIN[1]

1. Marine Biological Association of the UK, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK 2. University of Newcastle-upon-Tyne, Institute of Cell and Molecular Biosciences, Medical School, Framlington Place, Newcastle NE2 4HH, UK 3. Department of Biological Sciences, Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

Zygotes of the fucoid brown algae are excellent models in which to ask fundamental questions about zygotic symmetry breaking. Although the acquisition of polarity is tightly co-ordinated with the timing and orientation of the first asymmetric division - with zygotes having to pass through a G1/S-phase checkpoint before the polarization axis can be fixed - the mechanisms behind the interdependence of polarization and cell cycle progression remain unclear. In this study, we combine in vivo Ca^{2+} imaging, single cell monitoring of S-phase progression and high-throughput Ca^{2+} buffer loading experiments to determine whether
Ca^{2+} signals co-ordinate polarization and cell cycle progression in the
Fucus serratus zygote. Consistent with earlier studies on this organism, and in contrast to animal models, we observe no fast Ca^{+} wave following fertilization. Instead, single-cell 2-photon microscopy suggests that distinct slow, localized Ca^{2+} requirements exist for polarized actin nucleation, S-phase progression and subsequent rhizoid growth. Inhibitor experiments show that these processes can, surprisingly, be uncoupled, with cell cycle progression through S-phase being independent of concomitant actin polarization. This absence of a morphogenesis checkpoint, together with the observed Ca^{2+}-dependence of S-phase and polarization, shows that the regulation of zygotic division in the brown algae differs from that in other eukaryotic model systems, such as yeast and Drosophila.

4. The mutation of two Ca^{2+} pumps ACA4 and ACA11 provides evidence for communication between the tonoplast and plasma membrane in the regulation of Ca^{2+} signals leading to a programmed cell death

BOURSIAC YANN, ROMANOWSKY SHAWN & HARPER JEFFERY
Biochemistry department, MS200, University of Nevada Reno, 1664 North Virginia Street, Reno, NV 89523, USA

Arabidopsis has 10 calmodulin-activated Ca^{2+} pumps (ACAs, Autoinhibited Ca^{2+} P-type ATPases), with isoforms localized to the plasma membrane (PM), ER or tonoplast. These efflux pumps function to move Ca^{2+} to specific locations and/or regulate Ca^{2+} dynamics. Evidence indicates that 2 tonoplast pumps, ACA4 and ACA11, function to regulate several features of plant development, including the rate of transpiration, flowering transition, and a programmed cell death (PCD) pathway. Two independent sets of *aca4/11* double mutants show leaves with a lesion mimic phenotype similar to a hyper-sensitive response. This phenotype can be suppressed by blocking increased levels of salicylic acid, implicating the vacuolar pumps in regulating a Ca^{2+} signalling pathway as opposed to a nutritional function. Interestingly, lesions can also be suppressed by elevated nitrogen (20mM), with new lesions originating in mesophyl cells within 48 hours after transfer of plants to low nitrogen. The lesion phenotype is exacerbated in a quadruple mutant between *aca4/11* and mutations in two PM NADPH oxidases genes*, rbohD* and *rbohF.* This supports a working hypothesis that RBOH D and F can function at the PM to suppress a PCD pathway that is triggered by Ca^{2+} signals modified by the tonoplast Ca^{2+} pumps.

5. Mathematical modelling of calcium homeostasis in the nucleus of plant cell

BRIÈRE C., XIONG T.C., GRAT S., JAUNEAU A., RANJEVA R., MAZARS C.
UMR 5546, CNRS/Université de Toulouse Surfaces Cellulaires et Signalisation chez les Végétaux, Pôle de Biotechnologie Végétale, 24 Chemin de Borde Rouge, BP 42617 Auzeville, 31326 Castanet-Tolosan, France

The calcium ion (Ca^{2+}) is firmly established as a ubiquitous intracellular second messenger mediating responses to a variety of biotic/abiotic signals in plants. Here, we show that nuclei isolated from cell suspension cultures of BY-2 tobacco, harbouring the bioluminescent Ca^{2+} probe aequorin in the nucleoplasm, convert changes in temperature or mechanical stress into changes in free Ca^{2+} concentrations in the nucleoplasm. The resulting elevations of $[Ca^{2+}]_{nuc}$ proceed in a pH –dependent manner. Our experimental data suggest that isolated plant nuclei must be considered as a closed system where free calcium concentrations would be regulated by reversible movements between the nucleoplasm and nuclear stores. By combining mathematical modeling and pharmacological approaches, we investigated how calcium homeostasis might be regulated in the nucleus. Results of the numerical simulations predict that the buffering capacity of the nucleoplasm and the activities of putative calcium transporters/exchangers located on the inner nuclear membrane might be highly coordinated. Collectively, our data strengthen the fact that nucleus is partially autonomous in term of calcium signalling and may control downstream nuclear Ca^{2+}-dependent events.

6. Bacterial extracellular polysaccharides are polyanionic, bind calcium in the apoplast and prevent detection of defence elicitors or MAMPs.

COOPER, RICHARD M. [1], MORRISSEY, KATE L.[1], ASLAM, SHAZIA N [1], JACKSON, ROBERT W. [1], KNIGHT, MARC R. [2] , CHINCHILLA, DELPHINE [3], BOLLER, THOMAS [3], ERBS, GITTE [4], TANDRUP JENSEN, TINA [4], NEWMAN, MARI-ANNE [4]

[1]Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY; [2]School of Biological and Biomedical Sciences, Durham University, South Road, Durham, DH1 3LE; [3]Botanisches Institut der Universitat, Hebelstrasse 1, CH 4056, Basel, Switzerland; [4]Faculty of Life Sciences, Dept of Plant Biology, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark.

Plant pathogens form an intimate association with host cells when their presence is revealed by various defence elicitors or MAMPs (microbial associated molecular patterns) such as bacterial flagellin, LPS, elongation factor (EF-Tu), fungal glucan or chitin oligomers. Calcium influx to the cytosol from the apoplastic pool is a prerequisite for consequent induction of most defence responses to microbial pathogens. Sequestration of this pool would be a logical strategy from pathogens but it has not so far been considered. Many studies have implicated extracellular polysaccharides (EPS) secreted by bacterial pathogens in pathogenicity and fitness, usually in a protective role. However, the role of these very long molecules may be more crucial to infection. Polyanionic EPSs, such as alginate (*Pseudomonas syringae*), xanthan (*Xanthomonas campestris* pv*. campestris*), amylovoran (*Erwinia amylovora*) and EPS1 from mutualistic *Sinorhzobium meliloti* bind calcium. We hypothesized that, after invasion of host intercellular spaces, sequestration by EPS of the Ca^{2+} ions prevents calcium flux to the cytosol and defence activation. We have shown with aequorin-transformed *Arabidopsis* that pure EPSs can suppress calcium flux associated with MAMP recognition. After EPS treatment of *Arabidopsis* cells, we could still measure specific binding activity of radiolabelled flg22 on those cells, suggesting that EPSs do not block in a non-specific manner binding of elicitor to the cell surface. An EPS knockout mutant of *X. campestris* triggers more rapid and greater calcium influx than its respective wild type. Defence gene expression (monitored by real time PCR) and callose deposition are elicited by MAMPs and by EPS-defective mutants, but are also suppressed by EPS supplied in pure forms or produced from wild type cells. In contrast most EPSs were not able to trigger defence genes, oxidative burst and apoplast alkalinisation. Interestingly, calcium-saturated xanthan but not free xanthan triggered defence genes PR1 and PDF 1.2 suggesting a potential role as elicitor of the saturated form and a suppression of MAMP activity only by the free form. The widespread production of these ion-binding acidic polymers by diverse bacterial pathogens suggests that EPSs play a key role in establishing compatibility by suppressing MAMP recognition.

7. Extracellular ATP activation of plasma membrane Ca^{2+} channels requires NADPH oxidase; a plant purine-signalling system

DEMIDCHIK, V.[1], SHANG, Z.L.[1], SHIN, R.[2], THOMPSON, E.[1], RUBIO, L.[1], GLOVER, B.J.[1], SCHACHTMAN, D.P.[2], SHABALA, S. N.[3], DAVIES, J.M[1].

1. Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3 EA, UK. 2. Danforth Plant Science Center, St. Louis, MO 63132, USA. 3. School of Agricultural Science, University of Tasmania, Private Bag 54, Hobart, Tasmania 7001, Australia.

Extracellular ATP (eATP) is implicated in the regulation of cell viability, growth and stress responses. Although there are no apparent equivalents of animal plasma membrane purinoceptors, eATP has been shown to elevate cytosolic free calcium in *Arabidopsis* organs and regulate calcium-dependent transcription. It has been postulated that eATP signalling originates in the cell wall. However, here eATP was found to stimulate net calcium influx at the *Arabidopsis* root epidermis and elevate cytosolic free calcium in protoplasts from these cells. Patch clamping of the protoplasts revealed eATP activation of the hyperpolarisation-activated calcium conductance (HACC) and a 20pS calcium-permeable channel that could mediate transient calcium influx. Root epidermal ROS accumulation in response to eATP was lower in the *rhd2/AtrbohC* NADPH oxidase mutant. eATP-induced transcription of a MAP kinase involved in (oxidative) stress signalling was impaired in *rhd2/AtrbohC* roots. The eATP-activated HACC was absent in *rhd2/AtrbohC* and reducing conditions prevented activation in wild type, indicative of eATP activation of HACC *via* NADPH oxidase. Overall, the results support the plasma membrane as the site of eATP perception and delineate a novel signalling pathway.

8. cGMP acts upstream of the calcium transient during hydrogen peroxide-promoted stomatal closure in *Arabidopsis* guard cells

DUBOVSKAYA, LYUDMILA V.[1], KOLESNEVA, EKATERINA V.[1], MCAINSH, MARTIN R.[2], HETHERINGTON, ALISTAIR M.[3], VOLOTOVSKI, IGOR D.[1]

[1]Institute of Biophysics and Cell Engineering NAS of Belarus, 220027 Minsk, Academicheskaya, 27, Belarus; [2]Department of Biology, Lancaster University, Lancaster LA1 4YQ, UK; [3]School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK.

H_2O_2 was shown to induce the closure of pre-opened stomata in *Arabidopsis* plants. The guanylyl cyclase inhibitor LY 83583 entirely suppressed the H_2O_2-induced effect in wild type plants and treatment with 8-bromo-cGMP, a cell-permeable analog of cGMP, reversed the LY inhibitory influence, suggesting that cGMP is required for H_2O_2-induced stomatal closure. Furthermore, H_2O_2 was revealed to induce a rapid significant increase in cGMP concentration being detectable within 30 sec, reaching a maximum within 1 min and decreasing to the prestimulation level in 5 min. Thus, the effect of H_2O_2 correlates well with cGMP dynamics. To provide the evidence suggesting that H_2O_2-activated cGMP signalling pathway is calcium dependent we investigated the interaction between cGMP and Ca^{2+}. Using transgenic Arabidopsis seedlings expressing apoaequorin in the cytosol, we showed that pre-treatment of seedlings with LY suppressed entirely the H_2O_2-induced $[Ca^{2+}]_{cyt}$-transient registered after a lag-phase of 40 sec peaked in 1 min while 8-bromo-cGMP reversed the LY inhibitory effect. In addition, the increase in [cGMP] in response to H_2O_2 appeared to be faster than $[Ca^{2+}]_{cyt}$ increase, suggesting that cGMP acts upstream of the calcium transient.

9. Identification of putative calcium sensor elements in the *Arabidopsis thaliana* circadian clock

HUBBARD, KATHERINE E.[1], GARDNER, MICHAEL J.[1], BRAAM, JANET [2] AND WEBB, ALEX A.R. [1]

[1]*Department of Plant Sciences, University of Cambridge, Cambridge, CB2 3EA, UK*

[2]*Department of Biochemistry and Cell Biology, Rice University, Houston, TX77251-1892, USA*

A wide variety of plant physiological processes are circadian-regulated. Studies of the model plant *Arabidopsis thaliana* have demonstrated the existence of a molecular clock based on a transcriptional feedback loop, with a free running period of 24 hours. There are circadian oscillations of cytosolic free calcium concentration ($[Ca^{2+}]_{cyt}$), but signalling processes both upstream and downstream of these $[Ca^{2+}]_{cyt}$ rhythms are unclear at present. We have conducted a reverse genetic screen of Ca^{2+} signal transduction mutants to identify components involved in rhythmic processes such as circadian rhythms of leaf movement and photoperiodic flowering time. This has identified at least one putative Ca^{2+} sensor that affects both circadian rhythms and photoperiodic responses. The position of this Ca^{2+} sensor in the circadian signalling network is being determined. Determining the function of Ca^{2+} sensors in the control of rhythmic processes will help determine roles for Ca^{2+} in cytosolic signalling pathways related to the circadian clock.

10. Calcium homeostasis in Arabidopsis V-ATPase mutants

KREBS, MELANIE, LIU, TZU-YIN & SCHUMACHER, KARIN
ZMBP-Plant Physiology, Universität Tübingen,
Auf der Morgenstelle 1, 72076 Tübingen, Germany

In plant cells, multiple organelles serve as intracellular Ca^{2+}-stores and their differential use may contribute to the spatial and temporal characteristics of $[Ca^{2+}]_{cyt}$-signatures. Our analysis of *det3*, a mutant with reduced activity of the V-ATPase, a proton pump that drives Ca^{2+}/H^{+}-antiport in the endomembrane system, has provided initial evidence that different intracellular Ca^{2+}-stores might be involved in creating stimulus-specific Ca^{2+}-signatures in guard cells. We have since identified organelle-specific V-ATPase isoforms and were able to show that the severe growth phenotype of *det3* can be attributed to defects in secretory and endocytic trafficking. Accordingly, a double mutant that lacks both tonoplast-localized V-ATPase isoforms is viable and lacks the severe growth retardation but shows symptoms of Ca^{2+}-deficiency comparable to *cax1cax3*-mutants. We will present our recent results concerning calcium homeostasis, functional interplay of V-ATPase, V-PPase and members of the CAX-family of Ca^{2+}/H^{+}-antiporters as well as potential regulators of V-ATPase activity.

11. Calcium channel and peroxidase activity of maize annexins

LAOHAVISIT, A., MORTIMER, J. C., DEMIDCHIK, V., NICHOLS, C., COXON, K. M., MIEDEMA, H., MACPHERSON, N., WEBB, A. A. R., BROWNLEE C., BATTEY, N. H., DAVIES, J.M.

Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3 EA, United Kingdom.

Annexins are a multi-functional family of soluble proteins. Characteristically, annexins undergo Ca^{2+}-dependent binding to negatively charged phospholipids and in some cases Ca^{2+}-independent binding at low pH. Annexins can be cytosolic, membrane-associated or membrane-integral. Mammalian annexins are involved in Ca^{2+}-based signal transduction and some form voltage-regulated Ca^{2+}-permeable channels. Here, ability of maize annexins to modulate plant cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) and form Ca^{2+}-permeable channels has been tested. Addition of an annexin-enriched preparation to root epidermal protoplasts isolated from *Arabidopsis* constitutively expressing apoaequorin caused dose-dependent transient and long term increases in $[Ca^{2+}]_{cyt}$ that were sensitive to the cation channel blocker Gd^{3+}. This preparation also caused formation of hyperpolarisation-activated Ca^{2+}-permeable channels in artificial planar lipid bilayers. The main contaminant in the preparation was identified as a lipid-binding 23 kDa protein harbouring a C2 domain. Maize annexins purified to homogeneity retained the ability to form hyperpolarisation-activated Ca^{2+}-permeable channels in bilayers. Additionally, soluble annexins exhibited peroxidase activity with a K_m for peroxide of 14 µM. Calcium-stimulated annexin binding to liposomes enhanced peroxidase activity. Overall the results suggest that annexins may act as a control point in Ca^{2+} transport and control of reactive oxygen species.

12. Day length signalling and crosstalk between cytoplasmic and chloroplastic calcium oscillations in *Arabidopsis thaliana*.

LITTLEJOHN, GEORGE & LOVE, JOHN
Division of Plant and Microbial Sciences, School of Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter. EX4 4QD

Much is known of the genetics of photoperiodic flowering in *Arabidopsis*, but cellular signalling processes have not received as much attention. Ca^{2+} is involved in phytochrome signalling and posttranslational modulation of CONSTANS (CO), a transcription factor which induces the expression of *FLOWERING LOCUS T* (*FT*) and is central to the molecular model of flowering in *Arabidopsis.* FT in turn, orchestrates the transition in meristem identity from vegetative to reproductive. Circadian oscillations in cytoplasmic free calcium concentration ($[Ca^{2+}]_{cyt}$) have been described which may encode information influencing the transition to flowering. In addition, an observed peak in chloroplastic $[Ca^{2+}]$ ($[Ca^{2+}]_{chlo}$) seen soon after dark, may signal nightfall and influence relevant genetic components.

We aim to define the role of photoperiodic oscillations in cytoplasmic and dark-induced bursts in chloroplastic $[Ca^{2+}]$ in determining the transition to, and timing of, flowering. In order to do this, we are taking a transgenic approach to manipulating subcellular $[Ca^{2+}]$ in plants grown in various photoperiods. $[Ca^{2+}]$ will be monitored via expression analysis of key genes, luminescence of cytoplasmic and chloroplast-targeted aequorin and FRET seen in a new cameleon calcium sensor, YC6.6 (ECFP-CKKp-Venus) targeted to the cytoplasm or chloroplast.

13. Mutations in AtCML9, a calmodulin-like protein from *A. thaliana*, alter plant responses to abiotic stress and abscisic acid

MAGNAN, FABIENNE, RANTY, BENOIT, CHARPENTEAU, MARTINE, GALAUD, JEAN-PHILIPPE & ALDON, DIDIER

UMR 5546 CNRS-Université Toulouse, Pôle de Biotechnologie végétale, 24 chemin de Borde-Rouge, BP42617, 31326 Castanet-Tolosan, France.

Many stimuli such as hormones and abiotic stress factors elicit changes in intracellular calcium levels that serve to convey information and activate appropriate responses. The Ca2+ signals are perceived by different Ca2+ receptors, and calmodulin (CaM) is one of the best characterized Ca2+ sensors in eukaryotes. Calmodulin-like (CML) proteins sharing sequence similarity with the ubiquitous and highly conserved CaM exists in plants but their roles at physiological and molecular levels are largely unknown. We report here data for *Arabidopsis thaliana* CML9 (AtCML9) that exhibits 46% amino acid sequence identity with CaM. *AtCML9* gene expression is finely regulated during plant development and in response to abiotic stress and ABA treatment. Using *cml9* knockout mutants, we present evidence that AtCML9 plays essential roles in modulating responses to abiotic stress and ABA. Seed germination and seedling growth for the mutant lines are hypersensitive to ABA and salt stress. Mutations of *AtCML9* gene also confers enhanced tolerance to water deficit and alters the expression of several stress regulated genes. These data indicate that AtCML9 is involved in abiotic stress tolerance through its effects on ABA-mediated pathways.

14. Calcium signalling in the regulation of gliding motility and direction reversal in a benthic diatom

MCLACHLAN, D.H.[1,2], TAYLOR, A.R.[1], UNDERWOOD, G.J.C.[2], GEIDER, R.[2] & BROWNLEE, C.[1]
[1]*Marine Biological Association, Citadel Hill, Plymouth, PL1 2PB, U.K.*
[2]*University of Essex, Colchester, CO4 3SQ, U.K.*

Motility in response to light allows organisms to maximise photosynthetic efficiency while avoiding deleteriously high irradiance. Many types of motile microalgae have been observed to accumulate in areas with moderate fluence-rates and disperse from areas of high fluence. Two distinct motile responses to light were defined in the pennate diatom *Navicula perminuta*. Confocal scanning laser microscopy and fluorescent indicators were used to image intracellular Ca^{2+} dynamics during the response to high intensity blue light. Calcium transients were involved in stimulus-induced reversal of cell direction. The calcium signal was to the cell apex, occurred 4 s after the cell was exposed to the light stimulus and corresponded with reversal of cell direction. Inhibitor experiments suggested that the calcium required for this response originated from intracellular stores whereas calcium-dependent motility required influx from the external medium. The results suggest that regulation of the cytoskeletal organisation that underlies directional secretion and motility is under the control of calcium signalling.

15. Integration of *Arabidopsis* root growth through Ca^{2+}, pH and ROS

MONSHAUSEN, GABRIELE B., MILLER, NATHAN D., SPALDING, EDGAR P. & GILROY, SIMON

Department of Botany, University of Wisconsin-Madison, Birge Hall, 430 Lincoln Dr, Madison, WI 53706, USA

Roots must sense and respond to a variety of stimuli such as the direction of gravity, availability of nutrients and water and mechanical signals from objects they touch and integrate these to an appropriate growth response. We are interested in defining the regulatory processes that underlie such integration of stimuli and how this system translates to tropic growth control and development of root system architecture. Using confocal fluorescence microscopy, we have found that vertical root growth is associated with highly complex and dynamic oscillatory changes in cytosolic Ca^{2+}, surface pH and ROS centered over the entire elongation zone. Exposure to mechanical stresses, e.g. when the growing root encounters a barrier or when it is touch stimulated, rapidly triggers Ca^{2+}-dependent surface alkalinization and extracellular ROS production in these same regions. These observations suggest a possible link between the mechanical forces inherent in growth and changes in wall properties that mediate local growth control. Current work aims at defining the molecular mechanism behind Ca^{2+}-dependent apoplastic alkalinization and ROS production and its relationship to dynamic growth control of the root as a whole. This work is supported by NSF.

16. Investigating the role of calcium in plant programmed cell death

MOLONY, ELIZABETH M., & McCABE, PAUL F.
School of Biological and Environmental Science, Science Education and Research Centre (West), University College Dublin, Belfield, Dublin 4, Ireland.

Programmed cell death (PCD) is an indispensable facet of plant stress and defence responses and several developmental processes. PCD is associated with several hallmark features, specifically a condensed morphology and nuclear DNA degradation. We are currently investigating the signalling mechanism that triggers PCD in *Arabidopsis thaliana* and have identified a pivotal role for calcium.

Using *Arabidopsis* suspension cultures we induced a calcium influx with the ionophore A23187. This resulted in the death of cells displaying the specific corpse morphology associated with PCD. Next we blocked calcium influx into the cell. A heat treatment of 55°C was used as a death-inducing stimulus. The calcium permeable channel blocker, lanthanum chloride, was added to cultures before, during and after, heat shock. When lanthanum was added before and up to 4 minutes following initiation of heat treatment, PCD morphology was inhibited whereas 8 minutes into the shock or thereafter, morphology rates were unaffected.

Another marker for PCD is DNA cleavage by endonucleases. Fragment End Labeling (FragEL™) is an enzymatic detection kit which identifies nuclei with DNA fragmentation. FragEL data shows that lanthanum inhibits DNA cleavage following a PCD activation treatment. These data suggest that calcium regulates PCD, and blocking a calcium influx inhibits the onset of PCD following a lethal heat treatment.

17. Is Nod-factor-induced calcium spiking in legume root hairs really required for nodule induction?

MORIERI, GIULIA[1], KOSUTA, SONJA[2], MIWA, HIROKI[1], SZCZYGLOWSKI, KRZYSZTOF[2], OLDROYD, GILES E.D.[1], DOWNIE, J. ALLAN[1]

[1] John Innes Centre, Norwich, UK; [2] Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, Ontario, Canada

In rhizobia-legume symbioses bacterial infection results in the formation of plant derived nitrogen-fixing root nodules. The establishment of this symbiosis requires the plant to perceive a bacterially derived molecule called Nod factor which induces many events associated with nodulation, including periodic calcium oscillations in root hair cell. This calcium spiking is considered to be a central component of the signal transduction pathway that leads to the nodule formation. However, two recent *L. japonicus* mutants, *nup85* and *nup133*, do form a few nodules yet fail to induce calcium spiking. In this work, we show another example of a mutant which undergoes nodule morphogenesis without the activation of calcium spiking. Most mutations of a transmembrane receptor kinase, *SymRK*, completely block all nodulation associated events. However, the weak allele *symrk-14,* forms nodules, but lacks calcium spiking. These observations raise the question: is Nod-factor-induced calcium spiking in legume root hairs really required for nodule induction?

18. GFP-Calspermin: A novel high affinity CaM antagonist

MURPHY, ANDREW & LOVE, JOHN
School of Biosciences, Geoffrey Pope Building, University of Exeter. EX4 4QD

We cloned, expressed and purified a chimeric fusion between a soluble green fluorescent protein (smGPF) and the calmodulin binding protein, calspermin. We have shown that the fusion protein, (smGN), has a K_i in the calmodulin-dependent cyclic nucleotide phosphodiesterase activity assay of 1.966 nM, *i.e.* 3,800 times smaller than that of the commonly used calmodulin inhibitor W7. Using surface plasmon resonance we determined The $k_a = 1.24 \bullet 10^6$ M^{-1} s^{-1}, the $k_d = 5.49 \bullet 10^{-3}$ M^-1 s^{-1} and the $K_D = 4.39 \bullet 10^{-9}$ M. We found that the GFP moeity was important for successfully binding calspermin to the surface of the flow cell at a sufficiently high concentration for SPR, and that this procedure may be used for SPR analysis of other acidic polypeptides, (pI ≤4). To determine whether smGN might also bind to other calmodulin-like proteins in a heterologous system, we purified proteins from a plant total cell extract or a plant total protein extract by affinity chromatography against smGN. The purified proteins were identified as calmodulins by SDS-PAGE and LC-MS-MS, indicating a high level of specificity. We conclude that the high and affinity and specific binding between smGN and calmodulin make it an easily localised, recombinant alternative to chemical calmodulin inhibitors.

19. Involvement of Calmodulin in Expression of Circadian Clock and Flowering Time Related Genes in *Arabidopsis thaliana*.

MURPHY, ANDREW & LOVE, JOHN
School of Biosciences, Geoffrey Pope Building, University of Exeter. EX4 4QD

In flowering plants, including *Arabidopsis,* changes in day length serve as important seasonal cues which are perceived and interpreted within the photoperiodic pathway.
We have tested the hypothesis that calmodulin (CaM) is involved in transduction of the floral signal within the photoperiodic pathway.

A. thaliana seedlings at the ten leaf stage were treated daily for 14 days with the CaM antagonist W7 (N-(6-Aminohexyl)-5-chloro-1-naphthalene sulphonamide-HCL). Seedlings were then harvested every 4 h for a period of 28 h.
RT-qPCR analysis of the expression of, *TIMING OF CAB EXPRESSION 1* (*TOC1*), *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*), revealed changes in phase and level of expression in CaM antagonist treated plants compared to controls.
These changes are most noticeable under short day conditions and provide evidence of the involvement of CaM in floral signature propagation.

20. *Arabidopsis* vacuolar Ca^{2+}/H^{+} exchangers CAX1 and CAX3 play a role in abiotic stress tolerance

PITTMAN, JON K. [1], EDMOND, CLARE [1], ACTON, SIMON [2], NORTH, KATHRYN [2], ZHAO, JIAN [3], HIRSCHI, KENDAL D. [3] & MCAINSH, MARTIN R. [2]

[1]Faculty of Life Sciences, University of Manchester, Manchester, M13 9PT, UK; [2]Department of Biological Sciences, Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK; [3]USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX 77030, USA

Abiotic stress can significantly perturb plant growth, thus plants have specific adaptive responses which are activated following stress signal perception and signal transduction. A common response to abiotic stress is the generation of cytosolic Ca^{2+} elevations with specific dynamics which are thought to elicit a specific response. It is unclear what components are involved in generating these Ca^{2+} signatures. *Arabidopsis* vacuolar Ca^{2+}/H^{+} exchangers encoded by CAX genes mediate high capacity Ca^{2+} transport into the vacuole. Analysis of *cax1* and *cax3* knockout mutants suggest that these transporters are involved in specific abiotic stress responses including low temperature, salinity and oxidative stress. Furthermore, CAX1 and CAX3 are both regulated in response to abiotic stress. *CAX1* and *CAX3* are transcriptionally up-regulated following stress treatments. In addition, stress-dependent phosphorylation was observed for both proteins; however, the stress-dependent regulation profile differs between CAX1 and CAX3 with respect to specific stresses. Inhibition of vacuolar Ca^{2+}/H^{+} exchange activity affects the generation of stress-induced Ca^{2+} signatures. Alterations in the dynamics of cold- and H_2O_2-induced cytosolic Ca^{2+} signatures were observed in the *cax1* knockout. These results suggest that CAX1 and CAX3 may be central components in controlling cytosolic Ca^{2+} dynamics under specific stress conditions.

21. Interaction between plant phospholipase D and actin is regulated by calcium ions

PLESKOT, ROMAN [1,2], LINEK, JAN[1], POTOCKÝ, MARTIN[1,2], KŘEČEK, PAVEL[2], KRINKE, ONDŘEJ [1], ŽÁRSKÝ, VIKTOR[2,3], VALENTOVÁ, OLGA[1], NOVOTNÁ, ZUZANA [1] & MARTINEC, JAN[2]

[1]*Institute of Biochemistry and Microbiology, Institute of Chemistry Technology, Technická 5, 166 28 Prague 6, Czech Republic*

[2]*Institute of Experimental Botany, Academy of Science of the Czech Republic, Rozvojová 263, 165 02 Prague 6, Czech Republic*

[3]*Department of Plant Physiology, Faculty of Science, Charles University in Prague, Viničná 5, 128 44 Prague 2, Czech Republic*

Phospholipase D (EC 3.1.4.4, PLD) hydrolyses membrane lipids to yield phosphatidic acid (PA) and free-head group. This enzyme plays important role in many processes of plant cell. It is involved in vesicular trafficking, cytoskeleton rearrangement, remodeling and degradation of membranes, cell proliferation and signal transduction. However, mechanisms of activity regulation at molecular level are less understood and obviously uncompleted. Calcium ions, phosphoinositides, heterotrimeric G-proteins and local changes in physical state of membrane are important regulatory factors. Experiments *in vitro* with recombinant PLD [1] indicate that interaction of PLD with actin cytoskeleton is another regulatory mechanism. Monomeric actin (G-actin) inhibits PLD activity and polymeric form of actin (F-actin) has an opposite effect. In our work we investigate interaction between phosphatidylinositol-4,5-bisphosphate (PIP_2)–dependent PLD from tobacco (*Nicotiana tabacum* L.) and actin. In this report we show that interaction is significantly enhanced in presence of calcium ions. Tobacco PLD binds to the actin via conserved actin binding region (ABR) [2], and this interaction is isoform specific. Upon sequence analysis of eukaryotic ABR we speculate that actin-specific binding is probably mediated by a few aminoacids rather than conformational change of the whole region.

References:

[1] Kusner D. J., Barton J. A., Qin C. ,Wang X. & Iyer S. S. (2003). *Arch. Biochem. Biophys*., **412**: 231-241.

[2] Lee S. , Park J. B., Kim J. H., Kim Y., Shin K. J., Lee S. J., Ha S. H., Suh P. G. & Ryu S. H. (2001). *J. Biol. Chem*., **276**: 28252-28260.

Acknowledgement:

This work has been supported by the grant of the Czech Ministry of Education, Youth and Sports number LC 06034.

22. Microtubules are a target for self-incompatibility signalling in Papaver pollen

POULTER, NATALIE & FRANKLIN-TONG, NONI
University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

Perception and integration of signals into responses is of crucial importance to cells. Both the actin and microtubule cytoskeleton are known to play a role in mediating diverse stimulus-responses. Self incompatibility (SI) is an important mechanism to prevent self-fertilization. SI in *Papaver rhoeas* triggers a Ca^{2+}-dependent signalling network to trigger programmed cell death (PCD), providing a neat way to inhibit and destroy incompatible pollen. We previously established that SI stimulates F-actin depolymerization and that altering actin dynamics can push pollen tubes into PCD. Very little is known about the role of microtubules in pollen tubes. Here we investigated whether the pollen tube microtubule cytoskeleton is a target for the SI signals. We show that SI triggers very rapid apparent depolymerization of cortical microtubules. SI-induced microfilament and microtubule alterations are quite different. Moreover, actin depolymerization can trigger microtubule depolymerization, but not *vice versa*. Surprisingly, sustained alterations in microtubule dynamics did not trigger PCD, though taxol reduced SI-induced caspase-like activity, suggesting that tubulin depolymerization is required for SI-induced PCD to progress. Together, our data provide good evidence that SI signals target the microtubule cytoskeleton and for signal integration, implicating one-way “cross-talk” between the microfilament and microtubule cytoskeleton.

23. Identification of regulators and targets of circadian calcium signalling

ROBERTSON, FIONA C., HOTTA, CARLOS T., GARDNER, MICHAEL J., HUBBARD, KATHARINE E., BAEK, SOENG L., DODD, ANTONY N., WEBB, ALEX AR.
Department of Plant Sciences, University of Cambridge, Downing, Cambridge, CB2 3EA, UK

The plant circadian clock is an endogenous timekeeper that regulates a wide variety of physiological processes. Circadian oscillations in the concentration of cytosolic free calcium ($[Ca^{2+}]_{cyt}$) occur in plants. We are elucidating the mechanisms involved in their generation and identifying the targets for circadian Ca^{2+} signalling. Recent data from our lab has shown that the oscillations of $[Ca^{2+}]_{cyt}$ can be separated from other circadian outputs using inhibitors of cyclic ADP ribose action or the *timing of chlorophyll A binding protein 1*-1 (*toc1*-1) mutation. We are using these conditions to identify transcripts that are co-regulated with $[Ca^{2+}]_{cyt}$ to identify regulators and targets of circadian oscillations of $[Ca^{2+}]_{cyt}$ In parallel, we are continuing to reconstruct the genetic network regulating circadian oscillations of $[Ca^{2+}]_{cyt}$ using a reverse genetic approach.

24. *Arabidopsis* annexin 1 cation channel formation and requirement for ROS-stimulated Ca^{2+} influx

SHANG, Z.L.[1], LAOHAVISIT, A.[1], MACPHERSON, N.[1], RUBIO, L.[1], CUIN, T. A.[2], MORTIMER, J.C.[1], COXON, K. M.[1], WEBB, A. A. R. [1], BROWNLEE C.[3], BATTEY, N. H.[4], MILLER, A.J.[5], SHABALA, S. N.[2], DAVIES, J.M[1].

1. Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3 EA, United Kingdom. 2. School of Agricultural Science, University of Tasmania, Private Bag 54, Hobart, Tasmania 7001, Australia. 3. Marine Biological Association, Citadel Hill, Plymouth PL1 2PB, United Kingdom. 4. Plant Science Laboratories, University of Reading, Whiteknights, Reading RG6 6AS, United Kingdom. 5. Rothamsted Research, West Common, Harpenden AL5 2JQ, United Kingdom.

In *Arabidopsis* root epidermis, extracellular hydroxyl radicals activate plasma membrane Ca^{2+}- and K^+-permeable channels to mediate Ca^{2+} influx and K^+ efflux respectively (*J. Cell Sci.* 116, 81; *Nature* 422, 422). The encoding genes are unknown. Here, we tested whether an annexin (ANN) forms these channel conductances. Annexins are small, soluble proteins capable of binding and inserting into membranes. Some animal annexins can form voltage-gated cation channels. *Arabidopsis* ANN1 was found to lie at the periphery of root epidermal cells. Patch clamping root epidermal protoplasts of the *Atann1* mutant revealed loss of both the hydroxyl radical-activated Ca^{2+} influx and K^+ efflux conductances. The *gork* mutant (lacking the epidermal K^+ outward rectifier channel) showed a normal response. AtANN1 purified to homogeneity (from a yeast expression system) formed cation-permeable channels in planar lipid bilayers. The *Atann1* mutant had shorter roots than wt and when expressing aequorin differed from wt in its root $[Ca^{2+}]_{cyt}$ response to ROS. These data, combined with extracellular flux determinations, support the hypothesis that AtANN1 forms the hydroxyl radical-activated Ca^{2+} influx and K^+ efflux pathways.

25. Calcium spiking in *Medicago truncatula* root cells during rhizobial infection

SIEBERER BJÖRN J., CHABAUD MIREILLE, FOURNIER JOËLLE, TIMMERS ANTONIUS C. & BARKER DAVID G.
Laboratory of Plant-Microbe Interactions, INRA-CNRS, 31326 Castanet-Tolosan, France

Calcium spiking is one of the earliest responses of legume root hairs to rhizobial signalling molecules known as Nod-factors and appears to be an essential component of a signal transduction pathway leading to the expression of symbiosis-specific early nodulin genes. Our objective is to investigate whether cytosolic calcium signalling also plays a role during rhizobial infection, a complex process which involves the elaboration of a specialized intracellular invagination called the infection thread. For this, we have made use of two cytoplasmic cameleon calcium reporters (YC 2.1 or YC 3.60), which have been introduced into *Medicago truncatula* roots using *Agrobacterium rhizogenes*-mediated transformation. Experiments using transgenic composite plants have shown that, following *Sinorhizobium meliloti* inoculation, calcium spiking resembling that elicited by Nod factors can be observed in the majority of growing root hairs. We are now examining in detail the subsequent stages of root hair curling, infection thread initiation and infection thread growth down the root hair. We also plan to use nuclear-tagged cameleons to specifically study the role of nuclear-localized Ca^{2+} spiking and to extend our observations to later stages of infection in the root cortex.

26. Rapid transcriptome changes induced by thiourea reveal the role of calcium and calmodulin related proteins in multistress tolerance of *Brassica juncea*

SRIVASTAVA, A.K., RAMASWAMY, N.K., D'SOUZA, S.F.
Nuclear Agriculture and Biotechnology Division (Fiply), Bhabha Atomic Research centre, Mumbai 400085, India

Our field trial experiments could identify 'Thiourea' as an effective bioregulatory molecule, which could impart multistress tolerance in *Brassica juncea.* In understanding the molecular mechanism of stress tolerance, we found that thiourea exhibits differential gene expression under salinity stress. This prompted us to have the complete transcriptome profile in the presence of thiourea. Microarray analysis has shown the presence of more than thirty genes either up or down regulated under salinity stress. However in the presence of thiourea the expression of these genes are regulated at the level of distilled water control. The expression profiles of selected genes are also validated by Real-time PCR under similar treatment and multistress conditions. The data obtained suggest the involvement of calmodulin related protein in the functional regulation by thiourea. Further, the pretreatment of seeds with EGTA makes thiourea ineffective in regulating the target genes of calmodulin related proteins like calcium transporter under the stress, which confirms the above contention. Thiourea mediated change in the cytosolic calcium was also monitored by using the calcium specific fluorescent dye. All these results supports the view that change in the gene expression by thiourea, as an effector biomolecule accounts for the increase in the multistress tolerance seen under field conditions.

27. Spatial control of tip growth by RHD2/NADPH oxidase during root hair development

TAKEDA, SEIJI [1], GAPPER, CATHERINE [1], KAYA, HIDETAKA [2], KUCHITSU, KAZUYUKI [2] & DOLAN, LIAM [1]

[1]*Cell and Developmental Biology, John Innes Centre, Norwich NR4 7UH, UK*

[2]*Department of Genome and Drug Research Center, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan*

Root hairs are long tubular structures that initiate from root epidermal cells and elongate by tip growth. They function in the uptake of nutrients from the soil and in the interaction with other organisms in the soil. We have shown that accumulation of reactive oxygen species (ROS) at the hair tip is crucial for tip growth, and that *ROOT HAIR DEFECTIVE 2* (*RHD2*) encodes an NADPH oxidase that catalyses this local ROS production in *Arabidopsis thaliana*. Here we report progress towards understanding the role of RHD2 in the control of localised cell growth. We show that RHD2 protein accumulates at the site of ROS production at the growing hair tip, indicating that the localization of RHD2 at the tip is important for tip growth. The RHD2 protein carries putative phosphorylation residues and two calcium-binging EF-hand motifs at its amino-terminal, indicating that the RHD2 is activated by phosphorylation and calcium. We have carried out experiments that indicate that ROS production of RHD2 is activated by phosphorylation and calcium synergistically through these protein domains. We will present a model mechanism for the spatial control of cell growth by RHD2.

28. Perception of *Piriformospora indica* elicitor by *Arabidopsis thaliana*: The calcium response by roots

VADASSERY, JYOTHILAKSHMI & OELMÜLLER, RALF
Friedrich-Schiller-Universität Jena, Institut für Allgemeine Botanik und Pflanzenphysiologie, Dornburger Str. 159, 07743 Jena, Germany

Piriformospora indica, an endophytic fungus of sebacinaceae family colonizes the roots of wide variety of plants, but most importantly of *Arabidopsis thaliana* that does not form symbiotic association with mycorrhizal fungi. It can be grown in axenic cultures also and hence this system is ideal to study plant fungus symbiotic interactions. Signal molecules released by fungus, which are involved in this dialogue, have not been identified yet and our project aims for this. We were able to isolate a crude cell wall extract from *P.indica* that promotes growth in *Arabidopsis.* To investigate the recognition of the fungal factor by plant cell, we measured the cytosolic calcium level in the transgenic aequorin plants. The crude cell wall extract showed a root- specific calcium spiking in *Arabidopsis.* To find if there is subsequent activation of defence responses we measured the level of hydrogen peroxide. But the elicitor induced no defence specific H_2O_2 burst suggesting that *P.indica* factor does not induce a typical defence response. This root specific calcium increase is now being used as a marker to identify the elicitor and also to screen for mutants, which do not show such a response. This will help in identifying the genes involved in early recognition between the symbionts.

29. Involvement of a putative Glutamate-receptor in cryptogein-induced plant defense responses

VATSA, PARUL [1], BOURQUE, STEPHANE [1], RANJEVA, RAOUL [2] & PUGIN, ALAIN [1]
[1] *UMR INRA 1088 - CNRS 5184 - Université de Bourgogne, Plante – Microbe - Environnement,*
17 Rue Sully, BP 86510, 21065 Dijon Cédex
[2] *Signaux et Messages Cellulaires chez les Végétaux, Centre National de la Recherche Scientifique, Université Paul Sabatier, Castanet-Tolosan, France*

Cryptogein, a 98 amino acid protein secreted by the oomycete *Phytophthora cryptogea*, triggers Systemic Acquired Resistance (SAR) and a programmed cell death/hypersensitive response (HR) on tobacco plants. The mode of action of this elicitor has been studied using tobacco cell suspensions. After recognition by high affinity binding sites located on the plasma membrane, cryptogein induces a large calcium influx necessary for the induction of many events including protein phosphorylation, MAPK activation, anion efflux, plasma membrane depolarisation, NADPH oxidase activation leading to active oxygen species (AOS) production and cytosol acidification, nitric oxide (NO) production and free calcium elevations in the cytosol and the nucleus. Relationships between free calcium, AOS and NO have now been well established (Lecourieux *et al.* 2006, *New Phytologist* 171: 249-269, Garcia-Brugger *et al* .2006, MPMI,19:711-724).
In this work we demonstrate and characterize the involvement of a putative plant homolog of neuronal ionotropic glutamate receptors (iGRLs) in calcium signalling in response to cryptogein. Using transformed tobacco cell suspensions expressing aequorin in the cytosol or in the nucleus our preliminary results have shown that glutamate induces a strong and transient $[Ca^{2+}]_{cyt}$ elevation without $[Ca^{2+}]_{nuc}$ changes. Glutamate-induced $[Ca^{2+}]_{cyt}$ elevation was a result of calcium influx from the extracellular medium and was inhibited by different glutamate receptor (GLR) inhibitors. This data and the desensitization of calcium elevation monitored after successive applications of glutamate suggest the presence of functional calcium channels of GLRs-type in tobacco. Nevertheless, glutamate does not induce any of the characteristic events of the defense pathways described above. Further, $[Ca^{2+}]_{cyt}$ elevation induced by cryptogein was shown to be suppressed by the glutamate receptor inhibitors tested, suggesting that cryptogein could activate a calcium channel of the GLR-like and also the GLR-dependent calcium influx. Interestingly, these inhibitors do not affect nuclear calcium variations induced by cryptogein. Furthermore, glutamate receptor inhibitors do not inhibit the calcium-dependent cell signalisation pathway activated by cryptogein. Thus, we are trying to understand the potential implication of the plant homolog GLRs in the activation of the defense mechanism of plants.

30. The calcium sensor CBL10 mediates salt tolerance by regulating ion homeostasis in Arabidopsis

WAADT, RAINER, ECKERT, CHRISTIAN, SCHUELTKE, STEFANIE & KUDLA, JÖRG
Institut fuer Botanik, Universitaet Muenster, Schlossplatz 4, 48149 Muenster, Germany

Calcium signals regulate a multitude of biological responses and cellular processes in eukaryotic organisms. An important level of regulation in calcium signalling is decoded by calcium binding proteins functioning as calcium sensors.
The calcium sensor CBL10 belongs to the family of calcineurin-B-like-proteins (CBLs) which specifically interact with a family of serine-threonine protein kinases designated as CBL-interacting protein kinases (CIPKs). CBL10 is predominantly expressed in the shoot and likely to be involved in mediating salt stress tolerance, as revealed by the hypersensitivity of a T-DNA insertion mutant. Ion content determination of plants cultivated under salt stress conditions indicated a reduced Na^+ and an increased K^+ content in *cbl10* plants compared to wild type. This identified *cbl10* as the first plant salt sensitive mutant with an enhanced K^+/Na^+ ratio under salt stress conditions. Localization studies of GFP fusion proteins suggest that CBL10 is localized to moving punctate structures (endosomes or PVC) and at the tonoplast. Yeast-two-hybrid and BiFC analyses identified the salt tolerance factor CIPK24 (SOS2) as predominant interaction partner and revealed CBL10/CIPK24 complex formation at the tonoplast.
Our analyses suggest that CBL10 and CIPK24 constitute a novel Ca^{2+}-regulated salt tolerance pathway that regulates the sequestration/compartmentalization of Na^+ into vacuoles of green tissues.

31. The dynamics of Ca^{2+} signalling in *Chlamydomonas* - rapid elevations in cytosolic and flagellar Ca^{2+} underlie flagellar excision.

WHEELER, GLEN L.[1], JOINT, IAN[1] & BROWNLEE, COLIN[2]
[1]*Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, UK*
[2]*The Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, UK*

Ca^{2+}-dependent signalling processes are important in many aspects of flagella function in the green alga, *Chlamydomonas.* Using biolistically-loaded calcium-responsive dyes, we have examined the spatio-temporal dynamics of cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) and flagella Ca^{2+} in single *Chlamydomonas* cells. We demonstrate that elevation of external Ca^{2+} ($[Ca^{2+}]_{ext}$) promoted very rapid spiking of $[Ca^{2+}]_{cyt}$ across the whole cell and in the flagella. We also detected very rapid apical-localised Ca^{2+} signalling events with an approximate duration of 500 ms. In addition, elevated $[Ca^{2+}]_{ext}$ led to flagellar excision in 91% of cells. 97% of deflagellation events coincided with a rapid elevation in $[Ca^{2+}]_{cyt}$ in the apical region of the cell, either in the form of a whole cell or an apical-localised increase. When deflagellation was induced by the acid-shock method, a single transient elevation in whole cell $[Ca^{2+}]_{cyt}$ (including the apical region) was observed in parallel with deflagellation. The $[Ca^{2+}]_{cyt}$ elevation was absent in the acid deflagellation deficient *adf1* mutant. Our results indicate that $[Ca^{2+}]_{cyt}$ elevations in the apical region play an underlying role in signalling deflagellation. Furthermore, analysis of the *Chlamydomonas* genome indicates the cellular mechanisms responsible for the generation of rapid Ca^{2+} elevations may differ substantially from those found in flowering plants.

Participants

** S=speaker abstract; P=poster abstract*

Participant	Email	Establishment	Abstract No. *	Research interests
Anna Anielska-Mazur	gabrys@mol.uj.edu.pl	Jagiellonian University	P1	intracellular movements, signal transduction, actin cytoskeleton
Seong Jin Baek	sjb257@cam.ac.uk	University of Cambridge	P23	calcium signalling pathway, circadian clock
Oliver Batistic	oliver.batistic@uni-muenster.de	University of Münster	P2	calcium signalling calcium decoding calcium binding proteins CBL CIPK lipid modification
Gideon Baum	gbaum@protalix.com	Protalix biotherapeutics		
Michael Berridge	michael.berridge@bbsrc.ac.uk	Babraham Institute	Plenary 1	calcium oscillations, signal transduction
Yann Boursiac	boursiac@unr.edu	University of Nevada Reno	P4	calcium signalling, calcium reporter, calcium pumps, environmental stresses, programmed cell death, hydraulic regulation, reactive oxygen species, flowering transition
Janet Braam	braam@rice.edu	Rice University	P9	calcium binding proteins
Christian Brière	briere@scsv.ups-tlse.fr	CNRS/University of Toulouse	P5	calcium signalling transcription regulation mathematical modelling
Colin Brownlee	cbr@mba.ac.uk	Marine Biological Association	S3.3, P3, P11,P14,P24	calcium signalling, development, algae, membrane, channels
Ward Capoen	ward.capoen@bbsrc.ac.uk	John Innes Centre		nodulation symbiosis Nod factor signalling calcium signalling ion channels
Julia Davies	jmd32@cam.ac.uk	University of Cambridge	P7, P11, P24	channel, root, NADPH oxidase, reactive oxygen
J Allan Downie	allan.downie@bbsrc.ac.uk	John Innes Centre	S4.5, P17	calcium signalling during nodulation
Lyudmila Dubovskaya	dubovsk@mail.ru	National Academy of Sciences of Belarus	P8	signalling pathways, stress responses, stomatal aperture, cGMP, nitric oxide, hydrogen peroxide, calcium ions
Hillel Fromm	hillelf@post.tau.ac.il	Tel Aviv University	S2.2	calmodulin, transcription regulation, abiotic stress

Halina Gabrys	gabrys@mol.uj.edu.pl	Jagiellonian University	P1	chloroplast movements, cytoskeleton, light, organelle movements, signal transduction
Jean-Philippe Galaud	galaud@scsv.ups-tlse.fr	University Paul Sabatier	S3.5, P13	calcium sensors, calmodulin and calmodulin like proteins environmental stress
Simon Gilroy	sgilroy@wisc.edu	University of Wisconsin	S3.1, P15	signal transduction, calcium, tropisms, root hair, phospholipase, reactive oxygen species
Alice Harmon	harmon@ufl.edu	University of Florida	S4.3	CDPKs, cysteine metabolism, oil bodies, Arabidopsis, soybean, abiotic stress
Alistair Hetherington	Alistair.Hetherington@bristol.ac.uk	University of Bristol	S1.4, P8	guard cells, sphingolipids, CDPKs, CIPK, calmodulin
Katharine Hubbard	keh35@cam.ac.uk	University of Cambridge	P9, P23	
Megumi Iwano	m-iwano@bs.naist.jp	Nara Institute of Science and Technology		Ca^{2+} dynamics and Ca^{2+} signalling pathway in plant reproduction
Marc Knight	m.r.knight@durham.ac.uk	Durham University	S1.2, S2.2, P6	reactive oxygen species ROS, aequorin, calcium signature, low temperature, light
Heather Knight	p.h.knight@durham.ac.uk	Durham University	S1.2, S2.2	abiotic stress, low temperature, light, starvation
Jörg Kudla	jkudla@uni-muenster.de	Universitity of Münster	S4.2, P2, P30	
Anuphon Laohavisit	al355@cam.ac.uk	University of Cambridge	P11, P24	annexin, calcium, electrophysiology, root hair
Benedicte Le Martret	bene.le-martret@laposte.net	NUI Maynooth		Calcium, chloroplast transformed tobacco plants, ROS
Yunjuan Liang	yun-kuan.liang@bristol.ac.uk	University of Bristol		Arabidopsis, stomata, signal transduction, network
George Littlejohn	g.r.littlejohn@ex.ac.uk	University of Exeter	S1.5, P12	FRET, calcium signalling, *A. thaliana*, plant expression systems.
John Love	j.love@ex.ac.uk	University of Exeter	S1.5, P12, P18, P19	calcium, signalling, flowering, transcriptomics, the circadiome
Jianzhong Ma	majz@lut.cn	Lanzhou University of Technology		Ca^{2+}, kinase, protein interactin, ABA, signal transduction
Enid MacRobbie	eam1@cam.ac.uk	University of Cambridge		guard cell, signalling, ABA, ion fluxes

Christian Mazars	mazars@scsv.ups-tlse.fr	CNRS/University of Toulouse	S1.3, P5	nuclear and calcium signalling in plants, sphingolipids
Martin McAinsh	m.mcainsh@lancaster.ac.uk	Lancaster University	S2.3, P3, P8, P20	guard cell calcium signalling, abiotic stress, ozone ABA signal specificity
Paul McCabe	paul.mccabe@ucd.ie	University College Dublin	S4.1, P16	programmed cell death, development, cell signalling
Deirdre McLachlan	d.mclachlan@bristol.ac.uk	University of Bristol	P14	stomata, microalgae, environmental stimuli, intracellular imaging
Elizabeth Molony	libby.molony@ucd.ie	University College Dublin	S4.1, P16	programmed cell death, calcium signalling, stress response, apoptosis,
Gabriele Monshausen	monshausen@wisc.edu	University of Wisconsin-Madison	S3.1, P15	plant growth, tip growth, auxin, Calcium, pH, ROS, fluorescence microscopy, ion imaging
Giulia Morieri	giulia.morieri@bbsrc.ac.uk	John Innes Centre	S4.5, P17	role of calcium influx and calcium spiking in legume-rhizobia symbiosis
Kate Morrissey	klm27@bath.ac.uk	University of Bath	P6	plant disease resistance, signalling, MAMPs, molecular biology
Andrew Murphy	a.j.murphy@ex.ac.uk	University of Exeter	S1.5, P18, P19	the circadiome, calmodulin binding affinities, calcium signalling.
Carl Ng	carl.ng@ucd.ie	University College Dublin		sphingolipids, stomatal guard cell signalling, abiotic stress
Ralf Oelmüller	b7oera@uni-jena.de	Friedrich Schiller University, Jena	P28	Piriformospora indica- arabidopsis symbiotic system and role of calcium
Giles Oldroyd	giles.oldroyd@bbsrc.ac.uk	John Innes Centre	S2.1, S4.5, P17	Nod factor signalling, symbiosis, signal transduction, calcium, genetics
Michael Panagopulos	michael.panagopulos@bristol.ac.uk	University of Bristol		guard cell, stomata, sphingosine-1-phosphate, transcriptomics, signal transduction
Edgar Peiter	ep5@york.ac.uk	University of York	S1.1	
Helen Pinfield-Wells	h.pinfield-wells@lancaster.ac.uk	*New Phytologist*		
Jon Pittman	jon.pittman@manchester.ac.uk	University of Manchester	S2.3, P20	calcium transport, calcium/proton exchanger CAX, calcium ATPase, vacuolar calcium, calcium signalling, calcium tolerance, Arabidopsis, abiotic stress
Roman Pleskot	pleskotr@vscht.cz	Institute of Chemical Technology	P21	phospholipase D, actin cytoskeleton, plant signalling, pollen tube, root hairs

B.W. Joe Poovaiah	poovaiah@wsu.edu	Washington State University	S4.4	calcium/calmodulin-mediated signalling
Natalie Poulter	NSP118@bham.ac.uk	University of Birmingham	P22	self-incompatibility in poppy pollen, the cytoskeleton, signalling, programmed cell death.
Alain Pugin	alain.pugin@dijon.inra.fr	INRA 1088/CNRS 5184/ University of Bourgogne	P29	
Margaret Pullen	M.L.Pullen@durham.ac.uk	Durham University		cellular pattern development, reactive oxygen signalling, cell division control, plant signal transduction
Raoul Ranjeva	ranjeva@scsv.ups-tlse.fr	CNRS Castanet Tolosan	S1.3, P5, P29	
Benoit Ranty	ranty@scsv.ups-tlse.fr	UMR 5546 CNRS/UPS	S3.5, P13	calcium sensors, calmodulin, calmodulin-binding proteins, protein-protein interaction, biotic/abiotic stress, Arabidopsis
Fiona Robertson	fr233@cam.ac.uk	University of Cambridge	P23	calcium signalling, circadian rhythms, cADPR, microarrays, systems analysis
Tina Romeis	romeis@zedat.fu-berlin.de	FU Berlin		CDPK, calcium, signal transduction, biotic / abiotic stress
Helen Rushton	helen.rushton@plants.ox.ac.uk	Oxford University	S1.2	calcium regulated gene expression and specificity of calcium signalling
Elizabeth Ruszala	Lizzy.Ruszala@bris.ac.uk	University of Bristol		stomata, evolution, signal transduction in lower plants
Dale Sanders	ds10@york.ac.uk	University of York	S1.1	
Julian Schroeder	julian@biomail.ucsd.edu	UC San Diego	S3.4	
Karin Schumacher	karin.schumacher@uni-tuebingen.de	University of Tübingen	P10	V-ATPase, Ca2/H-antiport, Ca2-signatures
Cecile Segonzac	cecile.segonzac@tsl.ac.uk	The Sainsbury Laboratory		calcium-dependent protein kinases biotic stress signalling
Bjorn Sieberer	bjorn.sieberer@toulouse.inra.fr	INRA-CNRS	P25	plant cell biology, live-cell microscopy, FRET, calcium-signalling, cameleon calcium sensors, plant-microbe interaction
Holly Slater	h.slater@lancaster.ac.uk	*New Phytologist*		

Ashish Srivastava	ashish@barc.gov.in	Bhabha Atomic Research Centre	P26	transcript profiling, calcium signalling, Q PCR
Martin Steer	martin.steer@ucd.ie	University College Dublin		pollen tubes, calcium, exocytosis, endocytosis, tip growth
Jongho Sun	sun@bbsrc.ac.uk	John Innes Centre		calcium spiking, Nod factor, Nodulation
Sarah Swanson	swanson.sj@gmail.com	Univeristy of Wisconsin, Madison		hormone and mechanical cell signalling
Seiji Takeda	seiji.takeda@bbsrc.ac.uk	John Innes Centre	P27	arabidopsis, reactive oxygen species ROS, root hair tip growth, calcium, phosphorylation
Patrice Thuleau	thuleau@scsv.ups-tlse.fr	CNRS		calcium signalling, sphingolipid, cell death
Tony Trewavas	trewavas@ed.ac.uk	University of Edinburgh	Plenary 2	
Bekir Ülker	bekir.ulker@durham.ac.uk	Durham University		biotic and abiotic stress signalling
Jyothilakshmi Vadassery	jvadassery@ice.mpg.de	Fredrick Schiller University, Jena	P28	calcium response symbiotic interaction in arabidopsis
Parul Vatsa	parul.vatsa@dijon.inra.fr	UMR INRA 1088 - CNRS 5184 - Université de Bourgogne	P29	calcium, plant defenses, cell signalling, glutamate receptor, elicitor
Rainer Waadt	raina@uni-muenster.de	Institut fuer Botanik	P30	Ca sensors, kinases, abiotic stress, vacuole, membrane transport
Alex Webb	alex.webb@plantsci.cam.ac.uk	University of Cambridge	S3.2, P9, P11, P23, P24	
Glen Wheeler	glw@pml.ac.uk	Plymouth Marine Laboratory	S3.3, P31	calcium signalling algae imaging flagella
Tou Cheu Xiong	Tou-Cheu.Xiong@ucd.ie	University College Dublin	P5	calcium signalling, nucleus, tobacco BY-2 cells, guard cell, sphingolipid, *Arabidopsis thaliana*