Plant Neurobiology 2008
4th International Symposium on
Plant Neurobiology
June 6-9 2008, Fukuoka, Japan


# Book of Abstracts

**The 4th International Symposium on Plant Neurobiology**

SOCIETY FOR Plant Neurobiology

北九州市立大学
THE UNIVERSITY OF KITAKYUSHU
International Collaboration Center for Environmental Research and Development

Faculty of Agriculture
Kyushu University

# PNB2008

## The 4th International Symposium on Plant Neurobiology

### Fukuoka, Japan (June 6-9 2008)

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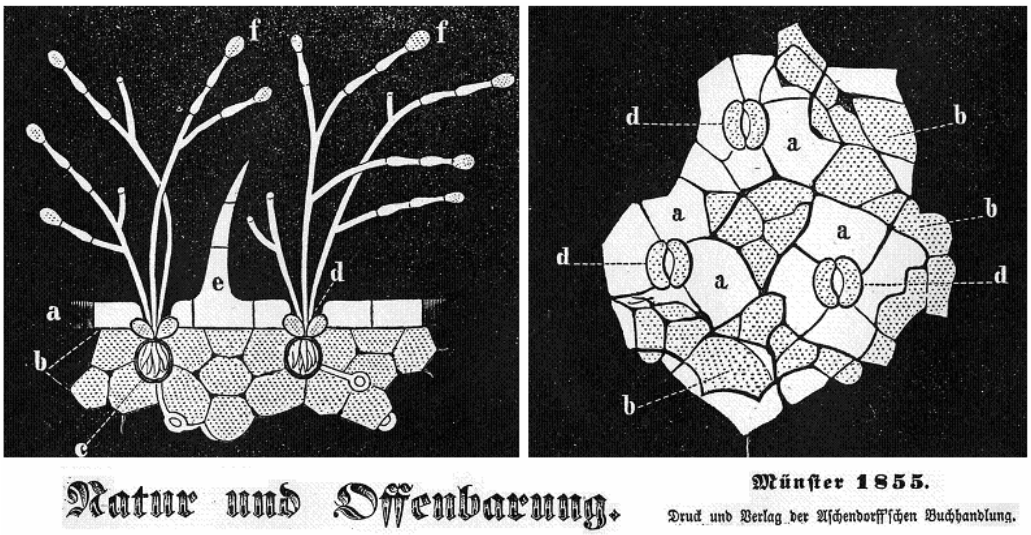


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# Contents

# Abstracts for Posters

# Abstracts for Satelite Session

# PNB2008 Programme

## *Friday, 6th of June, 2008*

## *Reception (Beer Party)*

10:00-17:00 Guests and participants from oversea will be guided from Fukuoka Airport to their hotels by student volunteers.

16:00- Registration (International hall in Kyushu University, Hakozaki Campus. Hall is opened for setting posters.)

19:00- Beer Party (Rokkakudo)

Detailed information (location) will be e-mailed to the registered participants.

## *Saturday, 7th of June, 2008*

## *(1) General Topics and Cell & Molecular Biology*

9:00-9:10 Opening Remark (Prof. Yuasa)

**SESSION 1**

| | | |
|---|---|---|
| 9:10-9:35 | Prof. BALUSKA, Frantisek | Plant Neurobiology from Evolutionary Perspective |
| 9:35-10:00 | Prof. MANCUSO, Stefano | Neuroid conduction in plant |
| 10:00-10:25 | Prof. INABA, Takehito | The role of plastid protein import in the |

| | | |
|---|---|---|
| | | regulation of nuclear gene expression |
| 10:25-10:40 | | Coffee break |

## SESSION 2

| | | |
|---|---|---|
| 10:40-11:15 | Prof. SHIMAZAKI, Ken-ichiro | Stomatal opening response by blue light |
| 11:15-11:40 | Dr. HARADA, Akiko | Blue light-dependent calcium signaling in higher plants |
| 11:40-12:05 | Prof. HOSON, Takayuki | Gravity and light signaling in growth regulation of stem organs |
| 12:05-12:30 | Dr. GEISLER, Markus | Regulation of auxin transport catalysts |
| 12:30-13:30 | | Lunch |
| 13:30-14:40 | | POSTER PRESENTATION |

## SESSION 3

| | | |
|---|---|---|
| 14:40-15:05 | Dr. SCHLICHT, Markus | D'orenone Blocks Polarized Tip-Growth of Root Hairs by Interfering with the PIN2-Mediated Auxin Transport Network in the Root Apex |
| 15:05-15:35 | Prof. HIRSCH, Ann (ICCERD/UoK-sponsored speech) | The importance of rhizobial attachment for successful legume nodulation and nitrogen fixation |
| 15:35-16:00 | Prof. SUZUKI, Akihiro | Control of root nodulation by the R:FR ratio |

| | | |
|---|---|---|
| 16:00-16:15 | | Coffee break |

**SESSION 4**

| | | |
|---|---|---|
| 16:15-16:50 | Prof. TAKAHASHI, Hideyuki | Mechanisms unique to hydrotropism in seedling roots |
| 16:50-17:15 | Prof. CASSAB, Gladys Iliana | Hydrotropism: root growth responses to water regulate root system architecture in Arabidopsis |
| 17:15-17:40 | Prof. LE DEUNFF, Erwan | Nitrate uptake responses to AVG and ACC treatments in relation to root elongation changes |
| 17:40-18:00 | Prof. YUASA, Takashi | Plant SNF1-related protein kinases and stress signaling |
| 18:00-19:00 | | Poster presentation continues |

## *Sunday, 8th of June, 2008*

## *(2) Electrophysiology and Long-distance signaling*

**SESSION 5**

| | | |
|---|---|---|
| 9:00-9:25 | Prof. SHIMMEN, Teruo | Position- and substratum-sensing in rhizoid differentiation of Spirogyra |
| 9:25-9:50 | Dr. OGATA, Koreaki | The double water film electrode characterized the electrical properties of the gap-junction in *Chara* as a function of time |
| 9:50-10:15 | Prof. BOUTEAU, François (ICCERD/UoK-sponsored | Anion channel activity is necessary to induce ethylene synthesis and Programmed Cell |

| | | |
|---|---|---|
| | speech) | Death in response to oxalic acid |
| 10:15-10:40 | Prof. TREBACZ, Kazimierz | Effects of thermoreceptor agonists on the membrane potential in plants |
| 10:45-10:55 | | Coffee break |

## SESSION 6

| | | |
|---|---|---|
| 10:55-11:30 | Prof. HEDRICH, Rainer (ICCERD/UoK-sponsored speech) | Ligand-gated Signal Transmission in Sensory Plant Cells |
| 11:30-11:55 | Prof. RONA, Jean-Pierre (ICCERD/UoK-sponsored speech) | Synergism between reactive oxygen species (ROS), calcium and ABA-induced cell depolarization in *Arabidopsis thaliana* suspension cells |
| 11:55-12:05 | | break |
| 12:05-12:30 | Prof. IIDA, Hidetoshi | Mechanosensitive Channel Candidates in Plants |
| 12:30-12:55 | Prof. UOZUMI, Nobuyuki | Membrane topogeneisi of voltage-dependent K channels |
| 12:55-13:20 | Prof. KUCHITSU, Kazuyuki | $Ca^{2+}$-ROS signaling network regulating stress responses, programmed cell death and development in plants |
| 13:20- | Lunch (Lunch box & Beer will be provided in front of bus) | |
| -18:40 | Short tour (Dazaifu shrine and Kyushu National Museum) | |
| 19:30-21:30 | Conference Dinner (Recent Hotel) | |

## *Monday, 9th of June, 2008*

## *(3) Sensory Biology, Chemistry and Ecology*

### SESSION 7

| | | |
|---|---|---|
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| 10:05-10:30 | Prof. ARIMURA, Gen-ichiro | Herbivore-Elicited Events in Legumes' Terpenoid Biosynthesis |
| 10:30-11:05 | Prof. TAKABAYASHI, Junji | Ecological functions of herbivore-induced plant volatiles |
| 11:05-11:20 | | Coffee break |

### SESSION 8

| | | |
|---|---|---|
| 11:20-11:45 | Prof. YOSHIOKA, Hirofumi | Molecular mechanisms of the radical burst in plant immunity |
| 11:45-12:10 | Dr. UMEMURA, Kenji | Disease defense response in rice plants induced by plant defense activators |
| 12:10-12:30 | Prof. KAWANO, Tomonori | Oxidative and calcium signaling in plants exposed to UV and photochemical oxidants |
| 12:30-12:55 | Dr. TAMAOKI, Masanori | Jasmonic acid and ethylene regulate selenite resistance in *Arabidopsis thaliana* |
| 12:55-13:55 | | Lunch |

SESSION 9

| | | |
|---|---|---|
| 13:55-14:20 | Prof. FUJIWARA, Toru | Regulation of transporters responsible for boron transport in response to boron conditions in the environment |
| 14:20-15:45 | Dr. BEILBY, Mary Jane | Mechanisms of salt sensitivity |
| 15:45-15:50 | | Closing Remark (prof. Kawano) |
| 17:00-21:00 | Sponsored Satellite Meeting at The University of Kitakyushu and Dinner in Kitakyushu City | |

## *Tuesday, 10th of June, 2008*

## *(4) Sponsored satellite session (at The Univ. Kitakyushu)*

| | | |
|---|---|---|
| 10:00-10:30 | Prof. RONA, Jean-Pierre | Abscisic acid-induced anion currents activation mediated by cyclic ADP-ribose / ryanodine receptor (RyR) in *Arabidopsis thaliana* suspensions cells |
| 10:30-11:00 | Prof. BOUTEAU, François | Thaxtomin A-induced defense responses in *Arabidopsis thaliana* cells require an early $Ca^{2+}$ influx |
| 11:00-11:20 | Prof. KAWANO, Tomonori | Similarity between plant redox enzymes and copper-bound prion protein |
| 11:20-11:40 | YOKAWA, Ken | Simulation of the signal transduction in artificial plant cells using NEURON: Inspired from the artificial retinal model |

# 1

# Plant Neurobiology from Evolutionary Perspective

Frantisek Baluska[1] and Mancuso Stefano[2]
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Plant Neurobiology has a focus on communication between adjacent cells in plant tissues via synaptic mode based on endocytosis and rapid vesicle recycling, between different root and shoot apices via rapid electrical signals - action potentials, as well as for inter-organismic plant-plant, plant-fungi, plant-bacteria, plant-animals communication. The latter is part of just emerging sensory ecology. Plants are using huge battery of volatiles for their communication, as well as manipulation. Besides volatiles signalling molecules, they synthesize numerous secondary metabolites, energy-rich and neuro-active compounds which are aimed to attract and manipulate animals and humans. The current dominant view is that all this happens only as by-product of metabolism, and that plant action potentials have no signalling roles, but represent some kind of evolutionary oddity. Plant neurobiology standpoint is that all these activities serve plants for their adaptation and survival.

Recently, Struik et al. (2008) questioned the validity of the plant neurobiology concept by arguing with the parsimony principle, also known as the ‘Ockham’s razor’, which suggests that the most plausible concept is that which is based on the simplest ideas and requires the lowest amount of assumptions. However, Francis Crick has commented on potential limitations of the Ockham's razor concept in biology (1988). Because biological systems are the products of (an on-going) natural selection, the mechanisms are not necessarily optimal in an obvious sense. He cautions: "While Ockham's razor is a useful tool in the physical sciences, it can be a very dangerous implement in biology. It is thus very rash to use simplicity and elegance as a guide in biological research."

In addition, evolution 'weeds out', or even 'do not allows' implementation, of any energetically costly processes which have no values and benefits for organisms adaptation and survival. Neuronal processes are extremely costly – for example, to run an action potential requires a lot of ATP. Their mere presence in plants should

serve as evidence that they have some essential roles. It is only our narrow-minded, unflexible, and animal-centric word-view which prevent us to grasp these issues in an open-minded fashion which would allow us to reveal the true communicative nature of plants. In order to interpret correctly genetic, molecular, and physiological data, we need to understand why such an unprecedent sensory complexity is needed for sessile plants. New concepts are needed, and new questions must be asked, for advancing our still rudimentary understanding of the communicative nature of sensory plants.

Crick FHC. 1988. What Mad Pursuit: A Personal View of Scientific Discovery. New York, New York: Basic Books

Struik PC, Yin X, Meinke H. 2008. Plant neurobiology and green plant intelligence: science, metaphors and nonsense. J Sci Food Agric 88:363-370.

# 2

# Neuroid conduction in plant

Stefano Mancuso[1], Elisa Masi [1], Marzena Ciszak[2], Giovanni Stefano[1], Luciana Renna[1], Elisa Azzarello[1], Camilla Pandolfi[1], Sergio Mugnai[1], Frantisek Baluska[3], and Tito Arecchi[4],

[1] Horticulture, Università di Firenze, Firenze, Italy; [2]INOA, Istituto Nazionale di Ottica Applicata, Firenze, Italy; [3]Zellbiologie der Pflanzen, Rheinische Friedrich-Wilhelms-Universität , Bonn, Germany; [4] Physics, Università di Firenze, Firenze, Italy

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The term neuroid conduction have originated with Parker (*The elementary nervous system*, 1919) who used it to describe the conduction of excitation in tissues of sponges, a group of animals without nervous system. Interestingly, at the time Parker wrote there was still no evidence that electrical signalling existed in sponges as this was demonstrated just 80 years after by Leys and Mackie (Nature, 1997). Nevertheless, the neuroid conduction, defined as the propagation of electrical events in the membranes of non-nervous, non-muscular cells have been demonstrated in many invertebrates as hydrozoans and tunicate, but also in the young stages of amphibian and lungfish. In carnivorous or sensitive plants as Dionea and Mimosa the spreading of the electrical signal has been described also as neuroid conduction (Mackie, 1970). Based on the results of our study we suggest that such kind of electrical transmission is a general characteristic of plants. We will show that the characteristic of the APs generated spontaneously in roots fulfil all the requirement normally associated to the neuroid conduction: a) they propagate in an all-or-none basis b) in non-nervous tissues c) going from cell to cell via plasmodesmata (in animal cells, via gap junction) and finally, d) decline rapidly in amplitude and velocity due to the flow of the current in all directions (compared with the one-directional conduction of nerves). Furthermore, the data recorded with a 60-channels Multi-Electrode-Array (MEA), revealed a vigorous and synchronised electrical activity in roots suggesting an intrinsic capacity of the cells of the root apex to generate functional electrical networks.

# 3

# The role of plastid protein import in the regulation of nuclear gene expression

Tomohiro Kakizaki, Institut [1], Hideo Matsumura, Institut [2], Katsuhiro Nakayama, Institut [1], Ryohei Terauchi, Institut[2], and Takehito Inaba [1]
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Email: tinaba@iwate-u.ac.jp

Plastids, such as chloroplasts, are a highly divergent group of organelles that provide essential metabolic and signaling functions within all plant cells. It is generally believed that plastids are originated from a unicellular photosynthetic bacterium inside a eukaryotic host cell. During evolution, most of the genes encoded by the bacterial ancestor have been transferred to the host nuclear genome. Therefore, the plastid biogenesis is reliant on the expression of nuclear-encoded plastid proteins and their import into plastids. One of the key cellular processes that coordinate the plastid protein import and the nuclear gene expression is the retrograde signaling from plastids to the nucleus. However, the molecular mechanism by which plastid regulates this process remains elusive. Using *ppi2* mutant lacking the Toc159 protein import receptor for photosynthetic proteins, we demonstrate that the expression of nuclear-encoded photosynthetic proteins are tightly coordinated with their import into plastids. Down-regulation of photosynthetic genes is also observed in the absence of other translocon components. Furthermore, the coordination of plastid protein import and the nuclear gene expression is likely to be mediated by a novel pathway that is distinct from GUN-ABI4. Comprehensive gene expression analysis of *ppi2* mutant identifies a number of potential signaling components involved in the retrograde signaling pathway. Based on these data, we will discuss a novel mechanism that coordinates the plastid protein import and the nuclear gene expression.

# 4

## Stomatal opening response by blue light

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E-mail: kenrcb@mbox.nc.kyushu-u.ac.jp

Stomata open in response to blue light. Recent investigations have demonstrated that phototropins (phot1, phot2) function as blue light receptors for varius responses, including chloroplast movement, stomatal opening, leaf flattening, leaf movement, and leaf positioning. Phototropin has been discovered as a blue light receptor for phototropism. In this talk, we will present our data on the signaling in stomatal guard cells in response to blue light and functional roles of phototropins. Since blue light perceived by phototropins results in activation of the plasma membrane $H^+$-ATPase in guard cells, we focused the signaling between phototropins and the $H^+$-ATPase. We showed that the type 1 protein phosphatase mediates the signaling between phototropins and the $H^+$-ATPase. Phototropins undergo autophosphorylation upon irradiation of blue light, however, physiological role of autophosphorylation remains unknown. We thus determined autophophorylation sites in phot1 by liquid chromatography tandem mass spectroscopy *in vivo*, and found eight phosphorylation sites of Ser and Thr residues. These located on the N-terminus, hinge region of LOV domain, kinase domain, and C-terminus. We substituted these Ser or Thr with Ala and investigated their roles after transformation of *phot1 phot2* double mutant with these *phot1* constructs. We indicate that phosphorylation sites in the activation loop of kinase domain have the essential role for all of the responses measured. We finally demonstrate that phot1 enhances plant growth under a weak light. The results suggest that a principal role of phototropin is an enhancement of photosynthesis under a weak light via efficient light capture.

# 5

## Blue light-dependent calcium signaling in higher plants

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Plants have several kinds of photoreceptors, which regulate growth and development. Recent investigations using *Arabidopsis thaliana* revealed that the newly found blue light receptor phototropins (phot1 and phot2) mediate plant movements and photomorphogenesis such as phototropism, chloroplast relocation, stomatal opening, rapid inhibition of hypocotyl elongation, and leaf expansion. Several physiological studies suggest that one of the intermediates in phototropin signaling is cytosolic $Ca^{2+}$. Studies using phototropin mutants have demonstrated that phototropins induce an increase in cytosolic $Ca^{2+}$ concentration and activate calcium-permeable channel. However, the function of $Ca^{2+}$ in the phototropin-mediated signaling process is largely unknown. I will present recent findings about phototropin-mediated calcium mobilization and the involvement of calcium in blue light-dependent plant responses.

Akiko Harada and Ken-ichiro Shimazaki (2007) “Phototropins and blue light-dependent calcium signaling in higher plants” *Photochemistry and Photobiology* 83: 102-111

# 6

# Gravity and light signaling in growth regulation of stem organs

Takayuki Hoson, Kouichi Soga, Saho Nakano, Makiko Nukada, Kazuyuki Wakabayashi
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Plants are surrounded by a variety of environmental stimuli. To respond and survive in the changeable environment, plants have to properly perceive each stimulus, and transform and transduce the perceived signal. We examined the possible signaling mechanism in suppression by hypergravity and light of stem growth.

Under hypergravity conditions, elongation growth of stem organs is suppressed via a decrease in the cell wall extensibility, which is brought about by modification of the metabolism of certain matrix polysaccharides, such as xyloglucans and 1,3,1,4-β-glucans, and modification of the cell wall environment, especially pH. Such growth suppression and cell wall modifications were similarly induced by basipetal- and acropetal-hypergravity. In the basal region cellulose, instead of matrix polysaccharides, was involved in the growth suppression. Thus, the upper growing region and the basal non-growing region may independently respond to hypergravity. Growth suppression and cell wall modifications by hypergravity occurred normally in stem organs of agravitropic mutants and in decapped roots. Also, hypergravity had no effects on growth or cell wall properties in the presence of lanthanum and gadolinium ions, blockers of mechanosensitive ion channels. Taken together, these results suggest that the gravity signal is perceived directly by mechanoreceptors in each cell, independent of gravitropism, and intercellular signal propagation along stem organs is not involved in growth suppression by hypergravity.

Light also suppresses elongation growth of stem organs by decreasing the cell wall extensibility. When only the basal region of azuki bean epicotyls was illuminated, elongation growth of the upper growing region was suppressed after a lag. The duration of the lag period was correlated with the distance between the growing region and illumination site, suggesting the presence of acropetal light signaling. Thus, the

signaling mechanism may be different between gravity and light, even if both similarly influence plant growth.

# 7

## Regulation of auxin transport catalysts

Aurelien Bailly [1], Valpuri Sovero [1], Stefano Mancuso [2], and Markus Geisler [1],
[1] Institute of Plant Biology, University of Zurich, Zurich, Switzerland, and [2] Department of Horticulture, University of Firenze, Sesto Fiorentino, Italy
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Active transport of the essential signaling molecule auxin is essential for plant physiology and development. Many aspects of these are controlled by cell-to-cell or polar auxin transport, which is primarily determined by auxin efflux complexes, characterized by PIN and ABCB (PGP/MDR) auxin exporters. Both types of proteins appear to act independently but perform specific interactions that determine the specificity and direction of auxin efflux.

Here, we summarize recent progress of ABCB interaction with immunophilin-like FKBP42, TWISTED DWARF1, which functions as a sensor in ABCB-mediated auxin transport. ABCB1-TWD1 interaction is disrupted by binding of synthetic and native auxin transport inhibitors, like NPA and quercetin, leading to inactivation of ABCB1. Contrary, IAA enhances ABCB1-TWD1 interaction resulting in activation of auxin transport and self-termination of its own signal.

Our data suggest that a combined action of transport and regulatory components forming an auxin efflux complex is needed for the establishment and control of asymmetric auxin fluxes.

# 8

# D'orenone Blocks Polarized Tip-Growth of Root Hairs by Interfering with the PIN2-Mediated Auxin Transport Network in the Root Apex

Markus Schlicht[1], Olga Šamajová[1], Doreen Schachtschabel[2], Stefano Mancuso[3], Diedrik Menzel[1], Wilhelm Boland[2], František Baluška[1*]
[1]Rheinische Friedrich-Wilhelms-Universität Bonn, Zellbiologie der Pflanzen, Bonn, Germany, [2]Max-Planck-Institut für chemische Ökologie, Jena, Germany, and [3]University of Florence, Florence, Italy.
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The $C_{18}$-ketone ((5*E*,7*E*)-6-methyl-8-(2,6,6-trimethylcyclohex-1-enyl)octa-5,7-dien-2-one) (D'orenone) has been postulated to be an early cleavage product of β-carotene *en route* to trisporic acids; these act as morphogenetic factors during the sexual reproduction of zygomycetes. Here we report that D'orenone blocks the highly polarized tip growth of root hairs at causing tip-growth to stop completely within a few minutes. Importantly, external auxin restores these effects of D'orenone on root hairs. Further analysis revealed that D'orenone lowers auxin concentration in trichoblasts via PIN2-mediated auxin efflux below critical levels essential for root hair growth. D'orenone increases specifically PIN2 protein abundance without affecting PIN2 transcripts, and that the PIN2 expression domain enlarges and shifts basipetally, resulting in more active auxin transport. Final evidence for PIN2 acting as the specific target of D'orenone is the observation that this compound does not interfere with the root hair growth in roots of null mutant lines.

# 9

## The importance of rhizobial attachment for successful legume nodulation and nitrogen fixation.

De Hoff, P.L.,[1] Suzuki, A.,[2] Fujishige, N.A.,[1] and A.M. Hirsch.[1]
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Legume nodule development has been extensively studied, especially with regard to rhizobial *nod* genes and the host's Nod Factor signal transduction pathway. However, little is known about rhizobial attachment to roots and entry into host tissues. Without attachment, rhizobia cannot colonize the root, and if the rhizobia cannot enter, they do not differentiate into nitrogen-fixing bacteroids.
Previously, we showed that transferring a soybean (SBL) or pea (PSL) lectin gene into *Lotus corniculatus* or *Medicago sativa* increased the attachment of rhizobia known to nodulate soybean or alfalfa (1, 2). Although binding was dependent on the presence of *nod* genes that produced the cognate Nod factor, the results strongly suggested that additional factors on legume roots influence rhizobial binding. We now have introduced the PSL or the alfalfa (MsLec1) lectin genes into Arabidopsis to determine whether *Rhizobium* binding to the roots of the transgenic lectin plant is enhanced. For *PSL*, Arabidopsis was transformed 1) with the vector alone, 2) with the vector carrying *PSL* with a mutated sugar-binding domain, and 3) with the vector carrying an intact *PSL*. The *PSL*-transformed roots were inoculated with *Rhizobium leguminosarum* bv. *viciae* Rlv128C53/ gfp or *Sinorhizobium meliloti* Rm1021/gfp. For *MsLec1*, Arabidopsis was transformed with 1) the vector alone or 2) with a construct carrying the vector plus *MsLec1*. The transformed roots were inoculated with wild-type, Nod$^-$ (Δ*nodD1ABC*), and

wild-type *S. meliloti* overexpressing the *nod* genes. Few Nod$^-$ *S. meliloti* cells bound to the roots confirming our recent results on the importance of the common *nod* genes for biofilm formation (3). In contrast, wild-type and Nod factor-overexpressing rhizobia attached very well to both Arabidopsis vector control roots and to roots carrying a mutated *PSL*. However, significantly reduced binding was observed on the transgenic Arabidopsis roots expressing either the wild-type PSL or MsLec1.


1. van Rhijn, P., Goldberg, R.B. and Hirsch, A.M. 1998. *Plant Cell*. 10:1233-1250.

2. van Rhijn, P., Fujishige, N.A., Lim, P.-O., and Hirsch, A.M. 2001. *Plant Physiol.* 125:133-144.

3. Fujishige, N.A., Lum, M.R., De Hoff, P.L., Whitelegge, J.P., Faull, K.F., and Hirsch, A.M. 2008. *Mol. Microbiol*. 67:504-515.

# 10

## Control of root nodulation by the R:FR ratio

*Akihiro Suzuki[1], Lalith Suriyagoda[1], Toshiki Uchiumi[2], Mikiko Abe[2], Masatsugu Hashiguchi[3], Ryo Akashi[3], Tatsuya Sakai[4], Sayaka Inada[4], Shusei Sato[5], Takakazu Kaneko[5], Satoshi Tabata[5] and Susumu Arima[1]
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Phytochrome (PHY) is a major light responsive molecule in plants, which regulates various shoot morphological functions through out the life cycle. Influence of phytochrome on root morphophysiology is less reported and the mediation on legume symbiosis is not documented.

We found two EMS mutants of *Lotus japonicus*, 01-0017 and 01-1428, which were having elongated shoot phenotype with pale green foliage and had elongated hypocotyls when germinate under red and white light conditions. Having these evidences, subsequent sequencing analysis revealed to have mutations in the *PHYB* gene of both lines. Contradictorily to elongated shoots, both mutants formed lesser number of nodules per plant compared to the wild type (WT).

Reciprocal and self-grafting experiments using *phyB* mutants showed that shoot genotype is responsible for the negative regulation of root nodulation. One possible reason for the suppression of root nodule formation in *phyB* mutants is due to limiting energy source (photosynthates), because PHYB is very important for development of photosynthetic organ. How about another reason? WT *L. japonicus* which was grown under continuous white light for 10 days was moved to high R:FR or low R:FR condition (uniform photosynthetically active radiation) and then nodulation test were carried out using *Mesorhizobium loti*. Surprisingly, the number of root nodules of low R:FR plants 28 days after inoculation was dramatically reduced compared with that of high R:FR plants. These results indicate that root nodule formation is one of the R:FR ratio perception reaction.

# 11

## Mechanisms unique to hydrotropism in seedling roots

Akie Kobayashi, Yutaka Miyazawa, Akiko Takahashi, Tomoko Kaneyasu, Nobuharu Fujii, and Hideyuki Takahashi
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We have demonstrated that roots of various plants display hydrotropism in response to moisture gradient, but it is interfered by gravitropic response on Earth. Interestingly, apparatus for sensing both gravity and moisture gradient appear to reside in the columella cells of the root cap. We recently showed that hydrotropic response easily overcome gravitropic response in Arabidopsis roots. Using the experimental system with Arabidopsis, we successfully isolated ahydrotropic mutants termed *mizu-kussei* (m*iz)*. Hydrotropic responses of *miz1* and *miz2* roots are impaired, whereas their gravitropic responses do not differ from that of the wild type. No morphological abnormalities are observed in *miz1* and *miz2* plants when compared with the wild type. These results imply that *MIZ1*> and *MIZ2* are essential for hydrotropism but not for gravitropism. *MIZ1* encodes a protein with a domain with unknown function that is highly conserved among land plant species, suggesting that *MIZ1* has evolved to play an important role in the adaptation to terrestrial environment. Moreover, *MIZ1* is expressed predominantly in the root columella cells, and thus we assume that this gene functions inside gravisensing cells of the roots. In Arabidopsis roots, although auxin response is required, the PIN-mediated auxin transport is unlikely required for the induction of hydrotropic response. Thus, there exist mechanisms unique to hydrotropism, which differentiate hydrotropism from gravitropism in seedling roots.

# 12

# Hydrotropism: root growth responses to water regulate root system architecture in Arabidopsis.

Manuel Saucedo, Georgina Ponce, Fatima Rasgado & Gladys I. Cassab.
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Roots are capable to construct perspective of their local space by sensing and regulating their growth orientation according to the environmental signals they face. By doing this, plants actively forage resources or avoid stresses from their environment. Hydrotropism allows roots to modify their growth direction in search of water overcoming their positive gravitropic response. The no hydrotropic response *nhr1* mutant of Arabidopsis lacks a hydrotropic response, and shows a stronger gravitropic response than that of wild type (wt) in a medium with a water potential gradient. Local application of abscisic acid (ABA) to seeds or root tips of *nhr1* increases root downward growth, indicating a critical role of ABA in tropisms. Wt roots germinated and treated with ABA in this system were strongly gravitropic, even though they had almost no starch amyloplasts in the root-cap columella cells. Hydrotropically stimulated *nhr1* roots, with or without ABA, maintained starch in amyloplasts, as opposed to those of wt. Thus, starch degradation in the wt might help the root to sustain osmotic stress and carry out hydrotropism instead of reducing gravity responsiveness. We have also developed a testing system for the isolation of putative *su*per *h*ydrotropic response (*suh*) mutants in Arabidopsis. *suh1* mutant roots continuously grew under water deficit for 10 days and reach the moderate water potential conditions present in the lower section of the Petri dish in contrast with wt roots, which only grew for 4 days. *suh1* mutant roots also modify their root system architecture according to the position of water availability in the medium and by the local addition of ABA. Furthermore, s*uh1* seedlings developed a deep and highly branched root system under the stress conditions of the test medium. We conclude that ABA and water stress are critical regulators of root tropic responses.

# 13

## Nitrate uptake responses to AVG and ACC treatments in relation to root elongation changes

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In *Brassica napus* seedlings changes of ethylene biosynthesis pathway by treatments with the ethylene precursor: aminocyclopropane carboxylic acid (ACC) and the ethylene biosynthesis inhibitor: aminoethoxyvinylglycine (AVG) modify elongation of exploratory root and root hair systems in a dose-dependent way. These physiological responses induced by ethylene to the root cells and their consequences in absorbing surfaces were questioned in relation to nitrate uptake and nitrate transporter genes expression. Treatments with high concentrations of ACC and AVG (10 uM) over five days revealed significant differences between root elongation, nitrate uptake capacities and nitrate transporter genes expression of BnNrt2.1 and BnNrt1.1. Although ACC increased the length and number of root hairs, the rate of N uptake and the transcript level of the nitrate transporter BnNrt2.1 were markedly reduced. In contrast, the decrease in root hairs length and number in AVG treated seedlings was over-compensated by an increase of nitrate uptake and BnNrt2.1 gene expression. These results demonstrated that root hair cells are not the only location of N absorption in the root and that BnNrt2.1 expression levels were more correlated to exploratory root system. Moreover, the changes of root elongation and nitrate uptake in AVG treated seedlings were not uniquely due to ACC synthase inhibition but certainly to an inhibition of a non-overlapping ethylene pathway. Indeed, the restoration of root elongation in AVG treated seedlings by 1mM L-glutamate suggested that AVG root elongation effects are mediated by inhibition of pyridoxal 5'-phosphate (PLP)-dependent enzymes of N metabolism instead of ACC synthase of ethylene pathway.

# 14

## Plant SNF1-related kinases and stress signaling

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Plants use complex mechanisms such as hormonal signaling, regulation of ion transport, and synthesis of osmolytes to survive under various environmental stresses. Recently, it has been reported that salinity, drought and ABA stimulate a set of plant specific protein kinases, SNF1-related kinases (SnRKs). Plant SnRK family was classified to three subgroups; 1) SnRK1, regulating nutrient metabolisms, 2) SnRK2, involving tolerance against salinity and drought, 3) SnRK3/Calcineurin B-like molecule(CBL) interacting Protein Kinase (CIPK), regulating ion transporters. SnRK2s and CIPKs are novel signal components regulating ion homeostasis and osmolyte production under osmotic stress.

A SnRK2 homolog (SlSnRK2C) cDNA was isolated from tomato Micro-Tom [1)]. Protein levels of SlSnRK2C appeared to increase specifically during young fruits while there was no difference in mRNA leveles of SlSnRK2C among several organs of tomato. SlSnRK2C is activated in response to salt stress and chilling when SlSnRK2 is expressed transiently in Nicotiana benthamiana by agroinfiltration [2)]. Involvement of the fruit-specifc SnRKs on regulation of sugar metabolisms under salt stress will be discussed.

A CIPK homolog (VuCIPK1) cDNA isolated from cowpea shows significant similarity to AtCIPK3 (86% amino acid identity), which is involved in $K^+$ transport. Immunoblot with an anti-VuCIPK1 specific antibody and an anti-CBL antibody showed that immunologically CIPK- and CBL-related polypeptides were preferentially associated with the membrane fractions. Immunoprecipitation assay with cowpea leaf extracts by anti-VuCIPK1 antibody showed that endogenous VuCIPK1 is autophosphorylated at the threonine residues in response to salt stress. These observations suggest that VuCIPK1 is an osmotic stress-activated kinase regulated by its phosphorylation status.

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# 15

# Position- and substratum-sensing in rhizoid differentiation of Spirogyra

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Filaments of Spirogyra are composed of tandem cylindrical cells. Some species of Spirogyra living in running water differentiate the terminal cell to be a rhizoid for anchoring. This differentiation can be easily induced by severing the algal filaments in the laboratory. Before starting the rhizoid differentiation, Spirogyra cells elongate via diffuse growth. When algal filament was severed, the terminal cell starts tip growth at its distal end. Rhizoid is formed only by the terminal cell, suggesting that the cell recognizes its own position before starting the differentiation. Distal end of the terminal cell becomes convex due to its high turgor pressure. When the cell turgor pressure was decreased by adding sorbitol to the external medium, rhizoid differentiation did not start. Upon sorbitol removal, the differentiation started. It is suggested that stretching of the plasma membrane of the distal end is responsible for the position-sensing by the terminal cell. Involvement of stretch-activated channel was suggested. Substratum plays an important role in the rhizoid differentiation. When algal filament did not attach to the substratum, the rhizoid differentiation did not start. Thus, mechanical stimulation of the substratum is necessary for starting the differentiation. In addition, we found that the properties of the substratum is responsible for morphology of rhizoids. On hydrophilic substratum, rod-shaped rhizoids were formed. On the other hand, rosette-shaped rhizoids were formed on the hydrophobic substratum. Involvement of protein phosphorylation in the signal processing was suggested.

# 16

# The double water film electrode characterized the electrical properties of the gap-junction in *Chara* as a function of time

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By scanning the double water film electrode unit (Ogata, 2000) along the length of *Chara* plant, the resistance (Rm) and capacitance (Cm) at 30Hz across the internode/node interface were studied as a function of time. Rm and Cm were $30*10^{-3}$ ohms $m^2$ and $1.5*10^{-1}$ Fs $m^{-2}$ at 20 C, respectively. The series resistance (Rs) of $8*10^{-3}$ohms $m^2$ could also be resolved simultaneously. Rm and Cm were strongly depending on the temperature and on a mechanical stimulus. The temperature depencency of Rm generally showed a significant hysteresis, but not for Rs. These observations will be discussed in relation to the dynamic properties of plsmodesma(ta), gap-junction(s) might be responsible for one of the cell-cell comunications.

# 17

# Anion channel activity is necessary to induce ethylene synthesis and Programmed Cell Death in response to oxalic acid

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Oxalic acid is thought to be a key factor of the early pathogenicity stage in a wide range of necrotrophic fungi. Studies were conducted to determine whether oxalate could induce programmed cell death in Arabidopsis thaliana suspension cells and to detail the transduction of the signalling pathway induced by oxalate. A. thaliana cells were treated with millimolar concentrations of oxalate. Cell death was quantified and ion flux variations were analysed from electrophysiological measurements. Involvement of anion channel and ethylene in the signal transduction leading to programmed cell death were determined by using specific inhibitor. Oxalic acid induced a programmed cell death displaying cell shrinkage and fragmentation of DNA into internucleosomal fragments with requirement for active gene expression and de novo protein synthesis, characteristic hallmarks of programmed cell death. Other responses generally associated with plant cell death, such as anion effluxes leading to plasma membrane depolarization, mitochondrial depolarization and ethylene synthesis, were also observed following addition of oxalate. Regarding our results, we propose a model in which oxalic acid activates an early anionic efflux which is a necessary prerequisite for the synthesis of ethylene and for the programmed cell death observed in A. thaliana cells.

# 18

## Effects of thermoreceptor agonists on the membrane potential in plants

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Effects of menthol and some other "cooling" compounds on the membrane potential in the liverwort Conocephalum conicum were examined. Conocephalum conicum belongs to philogenetically oldest terrestrial plants. It is an excitable plant - generates action potentials, APs, in response to different stimuli including depolarizing current, illumination, and cooling. The temperature increase evokes hyperpolarization, whereas cooling produces depolarization, which, when strong enough, leads to generation of AP. Two enantiomers of menthol: (-)-menthol and (+)-menthol were tested. In animal cold receptors - cation channels belonging to TRP family, (-)-menthol is almost four-times more effective than (+)-menthol in mimicking a response to cold - transient depolarization and a vanishing series of APs. In Conocephalum, in contrast to TRP receptors, (-)-menthol evokes concentration-dependent hyperpolarization of the membrane potential. At 0.01 mM concentration the response to (-)-menthol changed to depolarization and APs were occasionally registered. (+)-Menthol caused generation of APs when applied at concentration as high as 10 mM. More diluted (+)-menthol solutions produced hyperpolarization of the membrane potential. Capsaicin, which activates heat receptors in animals, but also is an agonist of Trpm8 cold receptor, evoked hyperpolarization in Conocephalum cells. Although homologs of TRP encoding genes have not been found in Arabidopsis thaliana, it occurs that their agonists are effective in Conocephalum.

# 19

# Ligand-gated Signal Transmission in Sensory Plant Cells

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The plant hormone abscisic acid (ABA) is involved in the transmission of environmental changes like drought-, saline-, and cold-periods into stress adaptation processes. Based on the timescale of the individual ABA evoked responses they have been subdivided into fast (membrane transport) and slow (transcription) signalling. In contrast to the latter process the fast ABA response - exemplified by half times of stomatal closure around 5-10 min - seem not involve gene activation. Instead, stomatal closure is accomplished by the release of potassium ions as well as the anions chloride and malate.

In search for ABA signalling intermediates the response of ion channels of guard cells in epidermal peels as well as guard cell protoplasts and vacuoles have been challenged with well-characterized modulators effective in signal transduction pathways of animal cells. Isolated, experimentally well controlled guard cell preparations, however, often lack communication with neighbouring cells, turgor or cytosolic components. In addition potential signalling components derived from mutants altered in ABA-induced stomatal closure. Current models, gained from observations on different screens, guard cell preparations, species or not even guard cells, trying to bridge the gap between the still unknown ABA-receptor and stomatal closure, are at least very complex.

To online record changes in ion fluxes across the plasma membrane of guard cells in intact plants, we have developed a method, based on multi-barreled microelectrodes introduced into the cytoplasm of these sensory motor cells in combination with spectrocopy. This approach proved suitable when exploring blue- and red light as well $CO_2$-signalling.

Using this online, *in planta* approach, we have been able to identify signalling

elements required for fast ABAinduced stomatal closure. A model on the ABA-based regulation of guard cell ion transport will be presented at the meeting.

# 20

# Synergism between reactive oxygen species (ROS), calcium and ABA-induced cell depolarization in *Arabidopsis thaliana* suspension cells

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Abscisic acid (ABA) is a plant hormone involved in multiple plant developmental processes. ABA promotes embryo dormancy and seed desiccation tolerance, elicits bud dormancy and is involved in the control of root growth. ABA also mediates some aspects of plant responses to certain environmental stresses such as drought, salinity and cold. In guard cells of several species, plasma membrane (PM) depolarization is one of the earliest responses monitored after ABA perception and it is shown that activation of PM anion channels plays a key role in the depolarization step (see, Verslues and Zhu, 2007; Wasilewska et al, 2008).

In A. thaliana suspension cells, PM depolarization was also observed in response to ABA (Jeannette et al., 1999). It results from the activation of PM anion channels and the reduction proton pumping (Brault et al., 2004). In addition, the ABA-signaling pathway is associated with $Ca^{2+}$ oscillations induced by reactive oxygen species (ROS). The aim of this work was to explore the mechanism behind the synergism between ROS, calcium and ABA-induced cell depolarization.

ABA-induced activation of anion currents and proton pumping reduction are both mediated by calcium signaling: in A. thaliana suspension cells, $Ca^{2+}$ is a second messenger involved in modulating rapid ABA responses promoting the cell depolarization like has been observed in guard cells. We show that upon ABA treatment ROS are rapidly generated and provoke transient calcium spikes.

PM $Ca^{2+}$ channel inhibitors 100 µM $La^{3+}$; 50 µM Fluspirilène; 50 µM Pimozide) which reduce ABA-induced frequency modulation of $[Ca^{2+}]_{cyt}$ increases were also found to reduce the ABA-induced (i) anion channel activation (ii) proton pumping activity (iii) RD 29a *and* RAB 18 genes expression (Brault et al., 2004; Zalejski et al., 2006).

Similar results were obtained with 5 mM EGTA a chelator of extracellular Ca2+ (Brault et al., 2004). This suggests that $[Ca^{2+}]_{cyt}$ increases we observed in response to ABA mainly resulted from the mobilization of extracellular calcium which generally occurs through calcium influx. This implied that plasma membrane calcium channels were involved.

ABA elicits a rapid and transient generation of ROS in suspension cells : reactive oxygen species (ROS) have emerged as second messengers of ABA in the activation of $Ca^{2+}$ -permeable channels. ROS are generated by the AtRBOH NADPH oxidases in guard cells upon ABA treatment and are found to mimic the ABA-induced stomatal closure. In A. thaliana suspension cells pre-incubated with 10 µM diphenyleneiodonium (DPI), an inhibitor of NADPH oxidase, the ROS production elicited by ABA was reduced by 80 %. These results indicate that ABA elicits a ROS production also in A. thaliana suspension cells.

$H_2O_2$ treatment induces an increase in $[Ca^{2+}]_{cyt}$ and participates to the ABA-induced plasma membrane depolarization : we used $H_2O_2$ because is a more stable ROS and can diffuse across PM through water channels. We determined the consequence of exogenous application of $H_2O_2$ on the cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$) in suspension cells. Variations of $[Ca^{2+}]_{cyt}$ were evaluated with a cell suspension prepared from leaves of A. thaliana transformed with the apoaequorin gene. Application of $H_2O_2$ on apoaequorin cell suspension triggers immediately a transient increase in $[Ca^{2+}]_{cyt}$ that reaches a maximum 1 min after the introduction of $H_2O_2$ and was dose-dependent from 0.1 mM to 1 mM $H_2O_2$. Indeed, after $[Ca^{2+}]_{cyt}$ increase promoted by external application of $H_2O_2$, we observed anion channel activation and proton pumping reduction as does ABA. In the presence of calcium channel inhibitor $La^{3+}$ (100 µM), the $[Ca^{2+}]_{cyt}$ increase provoked by $H_2O_2$ (1 mM) was abolished. Similar result was obtained when the extra-cellular calcium was chelated by EGTA (10 mM). These results support the idea that the calcium mobilized by $H_2O_2$ has an extra-cellular origin.

In conclusion, we show that ROS are involved in the ABA induce $Ca^{2+}$ signaling pathways at the plasma membrane. Taken together, the results presented here show that in *A. thaliana* cells, $[Ca^{2+}]_{cyt}$ oscillation signatures encode specific responses leading to

the anion channel activation and proton pumping reduction, including gene expression (RD29a). This indicates that ROS induced $Ca^{2+}$ increase is one central component of the signaling pathways leading to the plasma membrane depolarization induced by ABA.

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# 21

## Mechanosensitive Channel Condidates in Plants

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Mechanosensitive (MS) channels open a conductance pore in response to mechanical stresses, such as touch, gravity, and osmotic shock and thereby convert the stresses into an electrical or chemical signal. Animal and bacterial MS channels have been studied intensively and characterized considerably at the molecular level. The best-understood examples are the bacterial MS channels MscL and MscS. However, the molecular nature of plant MS channels has been unknown until recently, although their physiological roles have long been implicated in thigmotropism and gravitropism. Recently, ten MscS-like proteins have been identified in the genome of *Arabidopsis thaliana*, and two of them are reported to be present on the plastid envelope and control the size and shape of the plastid. Another candidate of plant MS channels, named Mca1, has been reported by us (Nakagawa *et al*., *PNAS* 104:3639-3644, 2007). Mca1 cDNA was isolated from an *Arabidopsis* cDNA library by functional complementation of a yeast *mid1* mutant defective in a putative MS channel component. Mca1 can indeed enhance $Ca^{2+}$ influx in yeast cells and is localized to the yeast plasma membrane as an integral membrane protein. In *Arabidopsis*, GFP-tagged Mca1 is also localized to the plasma membrane. Mechanical stress appears to activate Mca1. First, hypotonic shock increases $[Ca^{2+}]_{cyt}$ higher in *MCA1*-overexpressing *MCA1*ox) seedlings than in control ones. Second, *MCA1*ox roots accumulate $Ca^{2+}$ about 1.7-fold greater than wild-type roots. Third, the expression of *CML12* (*TCH3*), which is known to be induced by touch and $Ca^{2+}$, is increased in *MCA1*ox seedlings. Finally, primary roots of *mca1*-null seedlings fail to penetrate the harder, lower agar medium of two-phase agar medium from the softer, upper agar medium. These results suggest that Mca1 acts as a mechanosensitive $Ca^{2+}$ channel.

# 22

## Membrane topogeneisi of voltage-dependent K channels

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Several kinds of genes encoding K channels and K transporters have been identified in prokaryote and eukaryote. A simple K channel, which is found in almost any kinds of cells consists of membrane-pore-membrane (MPM) motif. HKT/Ktr-type transporters had been classified as a different group from K channels, but the recent study has revealed that they possess four MRM motifs in a single polypeptide. Plants contain two families of potassium ($K^+$) channel. One is the voltage-dependent (*Shaker*-type) $K^+$ channel family and the other is the TPK (two pore $K^+$) channel family. *Shaker*-type $K^+$ channels have a voltage sensing domain that controls the open and closed state of ion conducting pore. Membrane-embedded voltage-sensor domains in voltage-dependent K channels contain an impressive number of charged residues. We have studied the membrane topogensis of voltage sensor domains of plant KAT1 and *Drosophila* neuron Shaker B (Zhang et al. PNAS 104, 8263-8268, 2007). These results indicate that co-operative (‘post-translational’) integration of the voltage-sensor transmembrane segments is a property common to voltage-dependent channels and that a combination of hydrophobic and electrostatic forces involving S2, S3 and S4 controls the membrane insertion of the voltage sensor.

# 23

# $Ca^{2+}$-ROS signaling network regulating stress responses, programmed cell death and development in plants

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Ion fluxes including $Ca^{2+}$ and production of reactive oxygen species (ROS) are induced at the early step of defense signaling triggered by both biotic and abiotic stresses. To reveal the molecular mechanisms and physiological roles of stress-induced $Ca^{2+}$ mobilization, we established the retrotransposon-insertional knockout lines as well as the overexpressing lines of a putative voltage-gated $Ca^{2+}$ permeable channel, OsTPC1, in rice. The *Ostpc1* overexpressor showed enhanced sensitivity to a proteinaceous elicitor, whereas the elicitor-induced defense responses including activation of a MAP kinase and hypersensitive cell death were strongly suppressed in the knockout cells, which was rescued by expression of *Ostpc1* (Kurusu *et al. Plant J.* 2005). The gene expression profiles as well as changes in cytosolic $Ca^{2+}$ concentration induced by various stresses are being comparatively analyzed between the *Ostpc1* knockout and the wild type lines.

Plant respiratory burst oxidase homolog (rboh) proteins, have been implicated in ROS production in stress responses and during development. They have hydrophilic N-terminal regions containing two EF-hand motifs. By employing a heterologous expression system, we showed that ROS production by Arabidopsis thaliana rbohD and rbohC/RHD2 were induced by ionomycin, a $Ca^{2+}$ ionophore. This activation required a conformational change in the EF-hand region, as a result of $Ca^{2+}$ binding to the EF-hand motifs. AtrbohD was directly phosphorylated in vivo, and that this was enhanced by the protein phosphatase inhibitor calyculin A (CA). CA itself induced ROS production and dramatically enhanced the ionomycin-induced ROS production. These results suggest that $Ca^{2+}$ binding and phosphorylation synergistically activate the rboh-mediated

ROS-production that governs stress responses and development including root hair growth (Ogasawara *et al. JBC* 2008; Takeda *et al. Science* 2008).

# 24

## Herbivore-induced early and late responses in Plant-Insect Interactions

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Feeding herbivores elicit defence responses in the damaged plants, typically the emission of a blend of volatile organic compounds (VOCs) that mediates interactions with the parasites or enemies of the herbivore (indirect defenses). Other plants (additionally) respond with the secretion of extrafloral nectar that attracts ants as defenders. In the Lima Bean (Phaseolus lunatus) both indirect defense strategies are utilized simultaneously.

These and other defense responses are initiated by the mechanical damage as well as by the oral secretions (OS) of the herbivore. Using the black lipid membrane (BLM) technique, OS was analyzed with regard to their membrane activities. Transmembrane ion fluxes were generated by OS of eight different Lepidopteran larvae, which all displayed comparable ion channel-forming properties in artificial membranes.

The herbivore-linked reprogramming of the plant defense was additionally analyzed with microarrays comprising the whole genome of *A. thaliana*. In total about 5000 genes were either up- or down regulated, even after simple mechanical damage. By Principal Component analysis different treatments of leaves of *A. thaliana*, such as mechanical damage, feeding by a specialized insect (Diamond Back Moth), and a generalist herbivore (Beet Army Worm), could be clearly distinguished by a typical set of differently affected genes. Interestingly, the salivary secretions of the feeding insects seem to silence locally the gene expression in the damaged leaf, compared to the effect of mechanical wounding, but in distant leaves a significant reprogramming occurs that is not observed after the MecWorm treatment. The complexity of interactions with focus on the very early events will be discussed.

# 25

# Heivore-Elicited Events in Legumes' Terpenoid Biosynthesis

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Volatile terpenoids, the major products among the herbivore-induced plant volatiles in legumes, mediate interactions that attract herbivores' natural enemies and serve as signals to neighboring plants. In this study, we demonstrated cross-talk among the signaling components involving $Ca^{2+}$, jasmonic acid, and ethylene; together these are responsible for the formation of volatile terpenoids in Medicago truncatula and lima beans (*Phaseolus lunatus*). We describe that, like the cross-talk among stress-induced phytohormones, herbivore-sti¬mulated $Ca^{2+}$ transients also influence the blend of terpenoids, whose biosynthesis depends on the jasmonic acid (JA)/ethylene pathway in *M. truncatula.*

Likewise, we investigated the transcriptional mechanisms in Lima bean that underlie herbivory and diurnal responses involved in terpenoid formation. In order to investigate the effect of diurnal versus nocturnal damage on the signaling pathway for legumes' volatile emissions, we used MecWorm, a robotic device designed to reproduce tissue damage caused by herbivore attack. Lima bean leaves that were damaged by MecWorm during the photophase emitted maximum levels of monoterpenes [β-ocimene] and C6 volatiles [(Z)-3-hexenyl acetate] in the late photophase. Leaves damaged during the dark phase responded differently. JA accumulated locally in direct response to the damage and led to the immediate up-regulation of the β-ocimene synthase gene (PlOS) inde¬pen¬dent of the phase, that is, light or dark. In summary, damage-dependent JA levels directly control the expression level of PlOS, irrespective of light conditions. We discuss a new perspective on possible events (e.g., $Ca^{2+}$ signaling) leading to terpenoid biosynthesis.

# 26

# Ecological Functions of Herbivore-Induced Plant Volatiles

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In response to feeding by phytophagous arthropods, plants emit volatile chemicals. This is shown to be an active physiological response of the plant and the released chemicals are called herbivore-induced plant volatiles (HIPV). One of the functions of HIPV for the plant is to attract carnivorous natural enemies of herbivores. Depending on which plant and herbivore species interact, blends of HIPV show qualitative and/or quantitative variation. An intriguing question is whether this allows the natural enemies to discriminate between volatiles from plants infested by herbivore species that are either suitable or unsuitable as a food source for the natural enemy. Another question is, whether natural enemies can also recognize HIPV when two or more herbivore species that differ in suitability as a food source simultaneously attack the same plant species. Here, I will show that arthropod parasitoids can tell different HIPV blends apart in single-plant-single-herbivore systems and even in single-plant-multiple- herbivore systems. HIPV further mediate interactions between two plant individuals of the same/different species, and between plants and phytophagous arthropods. The resulting interaction networks mediated by HIPV would have important consequences in ecological community.

# 27

# Molecular Mechanisms of the Radical Burst in Plant Immunity

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Rapid production of nitric oxide (NO) and reactive oxygen species (ROS) has been implicated in the innate immunity in plants. There are many reports about complementary, synergistic and overlapping functions of NO and ROS in the defense responses. Recent advances provide the molecular mechanisms of NO and oxidative bursts in the defense responses. NOA1 (NO ASSOCIATED1; formerly named NOS1) and membrane-bound NADPH oxidase are believed to participate in the radical burst. Here we describe that two mitogen-activated protein kinase (MAPK) cascades, MEK2-SIPK and cytokinesis-related MEK1-NTF6, are involved in the induction of NADPH oxidase at the transcriptional level in *Nicotiana benthamiana*. On the other hand, NOA1-madiated NO burst is regulated by MEK2-SIPK cascade. Furthermore, we introduce the calcium-dependent protein kinase (CDPK) activates NADPH oxidase by the direct phosphorylation of its N-terminal region.

# 28

# Disease defense response in rice plants induced by plant defense activators

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Acquired disease resistance is known to be induced by specific chemical compounds, called plant defense activators. Among known plant defense activators, probenazole (Oryzemate<sup>®</sup> from Meiji Seika Kaisha, Ltd.) has been used as an agrochemical to control rice blast disease for over thirty years. Figures for recent years indicate it is applied in roughly 30% of all paddy fields in Japan. Our group focused on the defense responses in rice plants induced by probenazole to clarify the defense mechanism of the rice-blast fungus pathosystem. Previously, we isolated a novel rice phytoalexin as a phytocassane from rice plants, and PBZ1—a novel pathogenesis-related (PR) protein. In addition, we purified elicitor molecules from blast fungus. These were found to be cerebrosides, a type of sphingolipid. We have demonstrated that probenazole-induced disease resistance in rice leaves induced by drenching application shares several defense-related responses induced by spray-treatment with cerebroside, including MAP kinase activation, PR protein induction, and signal transduction via G proteins. Among unshared defense responses, treatment with cerebroside induced the production of active oxygen species and phytoalexin accumulation. Treatment with probenazole alone did not induce such phenomena, which may appear only with concurrent blast fungus inoculation. We have recently discovered that probenazole induces the accumulation of conjugated salicylic acid (SA) in rice leaves, whereas no induction of conjugated SA was observed following treatment with a cerebroside elicitor or blast fungus inoculation. Genetic analysis indicates that probenazole requires the accumulation of conjugated SA to exert its full effects, suggesting the existence of an SA-related defense signaling mechanism in rice plants.

We will present and discuss the results of our research on rice plant disease response.

# 29

# Oxidative and calcium signaling in plants exposed to UV and photochemical oxidants

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Recently, we are engaged to the monitoring of the seasonal changes in photochemical oxidants and their impacts on the living plants (development of localized cell death on the surface of leaves) in some monitoring sites including an isolated island in western Japan. Reported simulations suggested that the oxidants and their precursors are most likely brought from the highly industrialized areas in China. From both the microscopic celbiological and macroscopic environmental view points, the impacts of photochemical oxidatns (ozone and PAN) and exposure to ultra violet rays (UV-A and C) on induction of cell death in Nicotiana batacum L. and Arabidopsis thaliana (cells and plants) were examined. We observed that both UV and photochemical oxidatnts induce the cell death in plants via stimulating the oproduction of reactive oxygen species (ROS), calcium signaling (rapid and transient influx of $Ca^{2+}$) and protein kinase casecades. ROS scavengers and calcium chelators completely inhibited the development of photochemical oxidant- and UV-induced cell death. In cases of ozone and UV responses in ozone-sensitive tobacco cell line (Bel-W3) expressing aequorin gene, we observed the nonbiological ROS production and ROS-responsive increase in cytosolic $Ca^{2+}$ concentration. In case of ozone responses in Arabidopsis cells, drastic changes in anion channel current and membrane potential were shown to be associated with the induced cell death. In addition to such early signaling events, involvement of salicylic acid were also suggested by the use of cell suspensions derived from various mutants and transgenic lines of Arabidopsis, such as *npr1*, *cpr1*, *cpr5*, *sid2*, *NahG*, and *NPR*-overexpression. Our data may contribute for connecting the interests of enviomental researchers, biochemists and plant cell biologists.

# 30

## Jasmonic acid and ethylene regulate selenite resistance in *Arabidopsis thaliana*

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To better understand plant Se toxicity and resistance mechanisms, we compared the physiological and molecular responses of two Arabidopsis accessions, Col-0 and Ws-2, to selenite treatment. Measurement of root length demonstrated a clear difference between selenite-resistant Col-0 and selenite-sensitive Ws-2. Macroarray analysis showed more pronounced selenite-induced increases in mRNA levels of ethylene or jasmonic acid (JA) biosynthesis and -inducible genes in Col-0 than in Ws-2. Indeed, Col-0 exhibited higher levels of ethylene and JA. The selenite-sensitive phenotype of Ws-2 was attenuated by treatment with ethylene precursor or MeJA. Conversely, the selenite resistance of Col-0 was reduced in mutants impaired in ethylene- or JA-biosynthesis or signaling. Furthermore, the generation of reactive oxygen species (ROS) by selenite was higher in Col-0 than in Ws-2. Together these results indicate that JA and ethylene play important roles in Se resistance in Arabidopsis.

# 31

# Regulation of transporters responsible for boron transport in response to boron conditions in the environment.

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Boron is an essential micronutrient for plants and is also toxic in high concentrations. Boron homeostasis is important for plant survival. Homeostasis is mainly achieved by regulating transporters responsible in response to B condition in the environment. BOR1 is identified as the first boron transporter required for efficient xylem loading of B (Takano et al., 2002). Arabidopsis and rice have seven and four BOR1 or BOR1-like genes, all likely to encode efflux transporters, with different physiological functions and location within the cell. We also identified NIP5;1, a protein similar to aquaporin, as a transporter required for efficient B uptake (Takano et al., 2006). Expression of BOR1 and NIP5;1 are both upregulated under B deficient conditions but with different mechanisms. BOR1 accumulates in plasma membrane under low B conditions, and degraded through endocytosis under sufficient B supply (Takano et al., 2005). NIP5;1 is transcriptionally upregulated under low B conditions (Takano et al., 2006). With these knowledge in hands, we have successfully generated transgenic plants that are tolerant to low (Miwa et al., 2006) or high boron (Miwa et al., 2007).

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Takano et al., *Plant Cell 18, 1498-1509 (2006)*
Miwa et al., *Plant J. 46, 1084-1091 (2006)*
Miwa et al., *Science 318, 1417 (2007)*

# 32

## Mechanisms of salt sensitivity

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The current-voltage (I/V) technique was employed to resolve the response of ion transporters to salt stress in salt sensitive charophyte *Chara australis*. Cells were challenged by 50 mM NaCl/ 0.1 mM $Ca^{2+}$ medium after pre-treatment in artificial pond water (APW) adjusted to same osmolarity by sorbitol. At this $Ca^{2+}/N^{+}$ ratio the membrane potential difference (PD) depolarised to -100 mV within minutes. The background current with increased conductance became dominant in the I/V characteristics. The proton pump was inactivated. The PD of -100 mV is close to the excitation threshold and spontaneous repetitive action potentials (APs) were often observed. Each AP depletes the cell of $Cl^{-}$ and $K^{+}$. The resting PD continued to depolarise. The I/V characteristics became upwardly concave and were modelled by gradual opening of $H^{+}$ channels, with reversal PD, $E_H$, near zero. The presence of the background current kept the resting PD negative of $E_H$, causing cytoplasmic acidification. At this point the cell could still be rescued by replacing the saline medium by APW, which decreased the background conductance, closed the $H^{+}$ channels and reactivated the pump. However, continued exposure to saline medium depolarised the PD to very low level of -50 to -20 mV, where the outward $K^{+}$ rectifier channels were activated producing sustained $K^{+}$ efflux. This was the final step in the cell decline.

# P01

## Magnetic field and root development

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Land plants have developed various mechanisms for regulating their growth orientation according to the environmental signals they face. Magnetic field is an omnipresent accompaniment of Earthly life and hence plants have evolved with it as a background constant. The effects of magnetic field upon living organisms have been thoroughly documented, however, the mechanisms of action are still unclear. Genomic studies have revealed that several genes of the model plant *Arabidopsis thaliana* are regulated by the application of a high-density (19T) magnetic field (MF). One hundred and twelve genes out of 8000 showed an increase of 2.5 times in their expression compared with untreated plants. The genes induced are also induced by stresses such as heat, cold, drought and obstacles, as well as genes that code for ion transporters ($Cl^-$, $SO_4^-$ and $NH_4^-$). In the present study, we explored the effect of a fixed MF with magnets of 250, 850 and 1000 gauss in 4-day-old Arabidopsis seedlings treated for 8 days. MF affects the development of the primary root and differences were higher depending upon de strength of the MF. Furthermore, an alternating EMF of 8.5 gauss generated by Helmholtz device of 120 Hz applied to 4-day-old seedlings for 8 days and evaluated when seedlings were 12-days old indicated that both the length of primary roots and the number of lateral roots are significantly reduced. Apparently, MF reduced the effect of stresses in living organisms, and thus we also analyzed the effect of both MF and cadmium, a poisonous heavy metal, in root development. Interestingly, the addition of MF increased the cadmium effect on root growth instead of ameliorating it.

# P02

## Characteristics of the artificial electrical activity generated in plant roots under artificial changes in gravity

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Gravity-changing conditions revealed to generate strong and rapid responses in root of plants and many evidences lead to the hypothesis of an intrinsic capacity of the root apex to generate functional electrical networks.

The MEA (multi-electrode array) system is a new tool usually employed in research on animal electrogenic cells that has been recently and for the first time used on plant tissues by our Lab. The MEA approach allows to record extracellular signals from as many as 60 sites simultaneously, thus examining any distributed/synchronized electrical activity of whole cells and tissue, highlighting possibly the link between the electrical activity and the physiological response to external stimuli such as that of temporary gravity-changes conditions during an ESA parabolic flight campaign.

Signals from maize roots were recorded continuously during the flight at 20 KHz rate of sampling frequency. No previous data on the use of MEA system in microgravity condition have been ever reported in literature, neither in human/animal nor in plant physiology. For this reason, one of the main goal was to check the capability of the system to perform continuous monitoring of root electrical activity without troubles or crash during the flights.

Correlations between spikes and acceleration were analyzed to investigate the role of gravity changes in the onset of naturally occurring electrical spikes. Gravity data

from each experimental day were grouped together into classes, respectively 0g, 1g and 1.8 g. For each “g-class”, spike rate, ISI (inter-spike intervals) and grade of synchronization analysis were performed. Results showed a clear and detectable overall root electrical activity during each experiment, with differences in rates, ISI and synchronized events in correlation with gravity-changes.

As a conclusion, the trial was successful, with gravity changes proving to affect spike rate generated by maize root tips.

# P03

## Effect of Mitate Conglomerate on root and shoot growth of *Raphanus sativus* var. *radocula* with special notice to Cu in cultivation medium

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We have investigated the effect of Mitate Conglomerate, which is practically used as bedrock bath, on the growth and morphology of plants. Growth and mineral nutrition of shoot of *Raphanus sativus* var. *radicula* was estimated as the function of root mass and length. When plants were cultured in the Hoagland solution with grounded rock (Mitate Conglomerate or Serpentine, Silica sand; as control) on the hydroponic condition for fifteen days, the amount of Cu in leaves increased significantly when Mitate Conglomerate was added to solution ($p<0.05$). Hence, Cu is one of the important elements that affect the growth of the plant interacting with Mitate Conglomerate. Main root elongation increased when Mitate Conglomerate or Serpentine was added to the solution containing 0.1ppm of Cu. Main root elongation didn't differ significantly with increasing Cu concentration in the solution irrespective of rock type. Dry weight of root wasn't significantly different between rocks or Cu concentrations. Leaf-root ratio of dry weight decreased with increase in Cu concentration in the solution with Mitate Conglomerate. Consequently, mass growth of leaf to unit root mass was inhibited when Mitate Conglomerate was added in the solution under 0.1ppm of Cu concentration.

# P04

## Continuous GABA supply to the root affects nitrate uptake in *Brassica napus* seedlings

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In plants, GABA is a major free non protein amino acid metabolized mainly from glutamate and accumulated in response to various biotic and abiotic stresses. The function of GABA in plants as metabolite or signal is still a matter for debate[1]. Emerging literature suggests that GABA may function in plants as a potential modulator of ion transport and consequently mineral acquisition via putative GABA responsive receptors [2]. In this respect, recent data have shown that acute treatment of GABA up regulates transcription and activity of nitrate transporters in *Brassica napus* L. [3]. In order to quantify and to differentiate the signaling effect of GABA on nitrate uptake and its N nutritional effect via its g amino group, we have used $^{15}N$ labeled nitrate and GABA. Rape seedlings were submitted to a continuous application of GABA during five days on agarose gel. Our results show that the supply of 0.1 and 1mM GABA to the root did not affect elongation of exploratory root system compared to KN03 treated plants (control). However, 1mM GABA treatment decreased nitrate uptake. Similar results obtained with Glutamate suggested that GABA and glutamate act as important N sources and raised the question of a putative co-regulation of N uptake via organic or mineral sources in long term experiments.

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[2] Kinnersley AM, Turano FJ (2000) *Crit. Rev. Plant Sci*., 19: 479-509

[3] Beuve N, et al. (2004) *Plant Cell Env*., 27: 1035-1046

# P05

## Over-accumulation of GABA affects development of *Arabidopsis thaliana*

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Gamma-aminobutyric acid (GABA) is a non-protein amino acid found in all kingdoms, from bacteria to animals, known to accumulate in plants in response to a wide range of environmental *stimuli* [1]. Nevertheless, its roles are still unclear whereas it has been associated to several physiological processes. These role range from regulation of cytosolic pH and C/N balance, protection against oxidative stress, deterrence of insects, osmoregulation and anaplerotic role. In Mammals, GABA plays a major role in the central nervous system as a neurotransmitter and is involved in the establishment of brain networks [2]. The signalling role of GABA in plants has also been previously demonstrated, notably in developmental processes and metabolic regulation. Indeed, GABA is a key component in the growth and orientation of the pollinic tube [3], it is also involved in the down-regulation of the expression of several *14-3-3* genes [4]. Moreover, a negative correlation has been found between the GABA content of the phloem sap and the expression of nitrate transporters in *Brassica napus* [5].

To study the impact of GABA on *Arabidopsis thaliana* development, we used *pop2* mutants impaired in GABA catabolism as systems that over-accumulate the molecule when subjected to exogenous GABA treatment. Over-accumulation of GABA led to abnormal phenotype. Main physiological traits affected will be presented.

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[2] Represa A, Ben Ari Y (2005) *Trends Neurosci*., 28 : 278-283
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[5] Beuve N, et al. (2004) *Plant Cell Env*., 27: 1035-1046

# P06

## Reduced glutathione (GSH) regulates nitrate uptake in winter oilseed rape (*Brassica napus* L. cv Capitol)

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Many reports have shown that sulfate assimilation is well coordinated with the assimilation of nitrate and carbon. It is well known that the capacity to reduce nitrate is decreased in plants starved for sulfate. In this study, glutathione (GSH) has been proposed to be the signal as interconnecting N and S metabolism and more precisely, the signal peptide regulating $NO_3^-$ and $SO_4^{2-}$ uptake in winter oilseed rape (*Brassica napus* L.). Experiments have been undertaken to study the effect of an exogenous supply of GSH on HATS activity and the root growth in plants deprived or supplied with sulfate. Our results revealed a closed relationship ($R^2$=0.97) between the total N and the total S amounts in plants supplied with $SO_4^{2-}$ (N/S = 4.3). HATS activity is slightly decreased (approximately by 10 %) by a deprivation of sulfate during the first four days of deprivation. A supply of GSH (1 mM) decreases the HATS activity by 24 and 35% in plants supplied and deprived of sulfate, respectively. A supply of different concentrations of GSH has also been tested on the exploratory root system during seven days of treatment in agarose gel. Low concentrations of GSH (≤ 100 µM) increases the growth of lateral roots whereas a high concentration (1mM) limits the root growth. From all these data, it can be hypothesized that glutathione could regulated $NO_3^-$ uptake by exerting negative feedback on HATS activity or by controlling the exploratory root system in function of sulfate availability in soil.

# P07

## A roles of salicylic acid in UV-C induced cell death signaling

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UV-C damages the plants by targeting the DNA, photosynthetic apparatus, a wide variety of physiological processes and generation of reactive oxygen spices (ROS). Our previous study in tobacco cell cultures (*Nicotiana tabacum* L.; cell line, Bel-B and Bel-W3) were suggested that involvements of ROS and Ca signaling in UV-C-induced cell death. To be analyzed UV-C induced gene expression of Bel-W3 by RT-PCR showed that salicylic acid related gene expression induction. There, to elucidate a role of salicylic acid in UV-C-Induced cell death, the experiments were performed using *Arabidopsis thaliana* (Ecotype, Columbia) which are present many mutant and transgenic cells(*sid2*,*nah G*,*npr1*,*NPR OVER*,*cpr1*,*cpr5*).Date suggested that UV-C induced cell death is inducted by activation of salicylic acid signaling but SID2 and NPR1 is not related. But NPR OVER was suppressed UV-C induced cell death. There, we preformed analyzing of UV-C induced gene expression by RT-PCR in NPR OVER and *nah G*.

# P08

## Simple and sensitive bioassays for monitoring of night time ozone in the air using model plant seedlings

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We have developed a simple and sensitive bioassay model for monitoring of low concentration ozone in the air nusing model plant seedlings of Bel-B (ozone-tolerant, as the reference) and Bel-W3 (ozone-sensitive, as the probe) tobacco varieties. This system is shown to be responsive to low ozone exposure level (0.08 - 0.1 ppm*h). This assay system was applied for monitoring of night time ozone in the air generated through photochemical reactions during daytime in Japan and Korea.

# P09

## Expression Analysis of Trehalose Biosynthesis Related Genes in Tomato

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Trehalose, a nonreducing disaccharide in which two glucose molecules are connected in an alpha-1,1-glycosidic linkage, is considered to be an important osmoprotectant that has unique abilities that protect biomolecules from environmental stresses in many organisms, such as bacteria, fungi, lichens, algae and invertebrates. In the plant, since endogenous trehalose levels are very low, the role of trehalose biosynthesis genes, trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP), were not fully understood. The recent investigation has implicated these genes in important modulators of plant development, e.g. embryogenesis in Arabidopsis and formation of inflorescence architecture in maize. In this study, expression pattern of trehalose biosynthesis related genes in tomato development was investigated by RT-PCR with a set of primers designed from EST data base, MiBASE (http://www.kazusa.or.jp/jsol/microtom/index.html). The 11 trehalose biosynthesis related genes were found by homology analysis for Arabidopsis. As result of expression analysis by RT-PCR, *AtTPS*s and *AtTPPB* homolog showed ubiquitous expression. However, *AtTPPA* homolog expressed during germination, early seedling and fruit formation. In addition, the expression of *AtTPPA* homolog in the tomato suspension cultured cells was not observed, while almost *TPS* homologs and *AtTPPB* homolog were detected. These results indicated that *AtTPPA* homolog gene is involved in specifically early stage of tomato development and fruit formation.

# P10

## Plant mitochondrial porin regulates defense response against bacterial pathogen and Bax-induced cell death

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Mitochondrial porin, also called voltage-dependent anion channel (VDAC), is a major integral protein of the outer membrane. It is well documented that the protein plays an important role in apoptosis, a kind of programmed cell death, in mammalian system. However, little is known about the role of the plant counterpart during the process of plant-specific cell death such as pathogen-induced hypersensitive response (HR). To address this issue, we isolated three *VDAC* full-length cDNAs (*NtVDAC1-3*) from *Nicotiana tabacum* L. The deduced products, NtVDACs, share 78-85% identity and retain the conserved eukaryotic mitochondrial porin signature (MPS) distal to their C-terminal regions. Mitochondrial localization of three NtVDACs in plant cells was confirmed via a green fluorescent protein fusion method. We further evidenced that the MPS motif is partially responsible for this organelle targeting, because NtVDAC1$^{\Delta MPS}$, eliminating the MPS motif from the intact protein, localized not only to mitochondria, but also to other cellular spaces. After analysing these basic characteristics of the plant VDACs we addressed the main issue concerning pathogenesis relation. The *N. benthamiana* orthologues of *NtVDACs* were up-regulated by challenge with the non-host pathogen *Pseudomonas cichorii*, but not after challenge with the virulent pathogen *P. syringae* pv. *tabaci*. Both the pharmaceutical inhibition of VDAC and silencing of *NbVDACs* genes compromised the non-host resistance against *P. cichorii*. Involvement of NbVDACs in the mouse Bax-induced cell death was also suggested with a similar approach.

# P11

## Oxidative stress and distortion of calcium signaling by ions of group 13 elements in tobacco cells

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The group 13 metal elements including Aluminum (Al), Gallium (Ga) and Indium (In) were used for semiconductor industry. Recently, toxicity of the group 13 metal to animals and plants has been reported. On the other hand, Al is main factor which inhibit the plant growth in the acid soil. Previously study shown that Al was induced ROS such superoxide anion ($O_2^-$) generation and involve in calcium signaling response to various environmental stresses. Furthermore, many trace metal elements shown inhibition effects on Al induced $O_2^-$ generation in plant cells (not publish date).

In this study, we analysis the group 13 metal elements induced cell death, $O_2^-$ generation and change of cytosolic calcium concentration using tobacco cells (*Nicotiana tabacum* L.) expressing a $Ca^{2+}$-monitoring luminescent protein aequorin. Result in that all elements used here shown cell death and $O_2^-$ generation induction. Cell death induced by the group 13 metals was inhibited by ROS scavenger such as systeine and dimethylthiourea (DMTU) in any case. Whole Al and Ga were induced $[Ca^{2+}]_c$ increase and EGTA shown inhibit effects against Al and Ga induced cell death. In dose not induce $[Ca^{2+}]_c$ increase and EGTA did not show inhibit effect against In induced cell death. We also investigated the effects of some trace metal elements that inhibited the Al induced $O_2^-$ generation against Al induced cell death.

# P12

## Computational simulation of the plant cell responses to microbial physiologically active substances: principles and ongoing approaches

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NEURON, a well recognized free software, has been developed specifically for rapid simulation reflecting the status of the nerve cells using the equations used in the electrophysiological area. This approach enables the simulation of the cellular events by building a model based on the known or expected activities of ion channels, pumps, carriers and other biochemical components (or events) involved in the cell signaling. By using NEURON, various factors such as characteristics of ion channels and transporters, influx and efflux of ions, production and release of neurotransmitters and other biochemical events involved in cell signaling can be utilized as the parameters. Here, we would like to propose a use of NEURON simulation system applicable to the simulation of the time-dependent changes in cellular signal transduction status in plant cells challenged by various stimuli such as microbial components after defining the parameters based on the actual electrophysiological and biochemical data obtained through experiments (such as voltage-clumping). Here, we would like to describe a likely approach for constructing a reliable artificial plant cell in silico capable of simulating some of the cellular responses.

# P13

## Specific capture of phosphoprotein by immobilized Zirconium ion affinity chromatography

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The development of new efficient methods for highly specific enrichment of phosphorylated proteins and peptides is one of most active research fields in phosphorproteome analysis. Enrichment of phosphorylated proteins and peptides from complex peptides mixtures by immobilized metal affinity chromatography (IMAC) is a poplular way to perform phosphoproteome analysis. IMAC is originally based on the affinity of the phosphate group with metal ions (usually $Fe^{3+}$ or $Ga^{3+}$) immobilized on a choromatographic support. However the specificity of those IMAC adsorbents is still not high enough [1]. To characterize phosphoproteins more efficiently, it was necessary to develop novel affinity suface with higher selectivity for phosphoproteins.

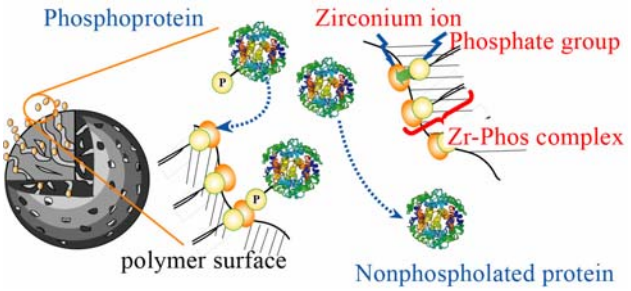
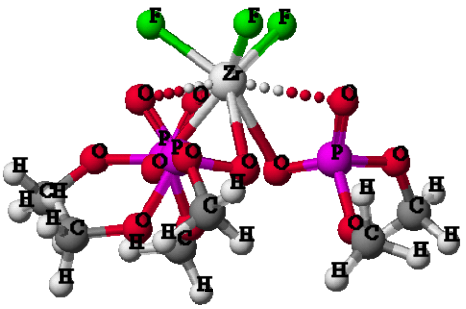



Fig.1 Immobilized Zr(IV) Affinity chromatography prepared by surface template polymerization

In this study, we focused that interaction between Zirconium phosphonate (Zr-Phos complex) and phosphate groups is strong [2]. Taking advatage of strong interaction, we have developed a novel IMAC adsorbent immobilized Zr(IV) on the polymer surface (Zr-Phos IMAC) by surface template polymerization with W/O/W emulsion [3] (Fig. 1). Phosphoric acid oleyl ester (DOLPA), sorbitan monooleate



(Span80), divinyl-benzene (DVB), polystyrene, and toluene were employed as the functional monomer, emulsion stabilizer, matrix-forming monomer, porogen, and diluent, respectively.

So far, we investigated conformation of Zr-Phos complex on the polymer surface by using fluoride ion [4]. Fig. 2 shows calculated molecular model for the F-Zr-Phosphate complex. Molar ratio of F-Zr-Phosphate complex is 3 : 1 : 3. So we expect that binding site with phosphorylated proteins and peptides exist enough on the polymer surface. Because of the multicoordination effect, the interaction between Zr-Phos complex and phosphopeptide is much stronger.

Fig.2 Calculated molecular model for the F-Zr-Phosphate complex by using MOPAC PM5 method

Model phosphoproteins were employed to investigated the performance of a novel Zr-Phos IMAC. The separation performance of the phosphoproteins (Phosphorylase a , phosphorylase b, $\beta$-casein, bovine serum albumin (BSA)) was evaluated by using electrophoretic. Phosphoproteins (Phosphorylase a, $\beta$-casein) sepalated from complex proteins mixtures at the following condition: Buffer solution (5 mL) is 0.1 M acetic acid/ NaOH, pH 3.1. The concentration of model phosphoproteins is 10 mg/L. We also discussed about phosphopeptide enrichment and MALDI-TOF MS analysis.

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# P14

## Quantum analysis of interaction between fungal polysaccharide and single polynucleotide

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β-1,3-D-glucans (polysaccharide) have been isolated from fungi as right-handed $6_1$ triple helices, in which the constituting three glucan chains are underpinned with one another by intermolecular hydrogen bonds. Among these polysaccharides, Curdlan is very simple structurally since it only contains linear 1,3-linked repeat units with no 1,6-linked side chain glucosyl units. Recently, we found that when the single chain of these is renatured together with a homo nucleic-acid [ex. poly (cytidylic acid): poly(C), poly (adenylic acid): poly(A), and poly (deoxyadenylic acid): poly(dA)], the polysaccharide and polynucleotide chains form a macromolecular complex, instead of forming the polysaccharide triple helix.

We examined the structure of the poly(C)/polysaccharide (poly(C)/Curdlan and poly(C)/Schizophyllan) complex by the semi-empirical molecular-orbital package (MOPAC).

# P15

## Molecular Dynamics Studies of Side Chain Effect on the Microbial Polysaccharides Triple Helix in Aqueous Solution

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β-1,3-D-glucans have been isolated from fungi as right-handed $6_1$ triple helices. They are categorized by the side chains bound to the main triple helix through β-(1→6)-D-glycosyl linkage. Indeed, since a glucose-based side-chain is water-soluble, the presence and frequency of glucose-based side-chains give rise to significant variation in the physical properties of the glucan family. Curdlan has no side-chains, self-assembles to form an water-insoluble triple helical structure while Schizophyllan, which has a 1,6-D-glucose side chain of every third glucose unit along the main chain, is completely water soluble. A thermal fluctuation in the optical rotatory dispersion is observed for the side chain, indicating probable co-operative interaction between the side-chains and water molecules. Our recent research reported on the formation of stoichiometric complexes of Schizophyllan with polynucleotides experimentally and computationally.

We simulated three kinds of β-1,3-D-glucan: Curdlan; a hypothetical glucan with a side-chain at every sixth glucose unit; and Schizophyllan. And we investigated how water molecules interact to glucan side chains and affect the glucan structure in aqueous solution at room temperature.

# P16

## A Dynamic Measurement Technique Characterizes the Turgor pressure Change in Characean Internodal Cells: the Hydraulic Conductivity of the Plasma Membrane is Isotropic and Independent of the External Hydrostatic Pressure

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Assuming that the magnitude of the peak-to-peak difference in strain $\triangle Sn$ caused by an external constant sinusoidal stress is proportionate to the turgidity of the material, I applied this method, a dynamic measurement technique, to characterize the relative turgor pressure P of cylindrical Characean internodal cells as a function of time. In contrast to the ordinary transient methods, the present method "vibro-method", is not only simpler than any of those introduced by other authors, but also enables the measurement of P without being invasive to the living cell, with the temporal resolution of 100msec. The cell length $l$ and diameter $d$ could be measured simultaneously in the present apparatus. The resolution estimated is 1μm or less. A rectilinear correlation was found between $l$ and $\triangle Sn$ within at least 5 sec immediately after a step change in external hydrostatic pressure $\pi_e$: the Young's modulus along the length of the cell can be assumed to be constant in a short term study. On the other hand, $d$ was found to be practically unchanged during this period, suggesting that the absolute volume of water flow across the membrane can be estimated by changes in $l$, $\triangle l$. Therefore, the velocity of water flow across the membrane $V$ can be explained thus: $V = d\triangle Sn / dt$. Consequently, because the change in intracellular hydrostatic pressure $\pi_i$ is negligibly small, the hydraulic conductivity of the plasma membrane $L_p$ can then be calculated as: $L_p = V / (\pi_i - \pi_e) = V / \triangle \pi$.

Results suggest there is no evidence to prove the plasma membrane itself is a

rectifier of water volume flow, but that the apparent rectification is due to the mechanical properties of the cell wall and/or to the change in water motive force across the membrane. Furthermore, there is no evidence to prove a change in $L_p$ in response to $\pi_e$ up to 1.5MPa within at least 5 sec immediately after the onset.

# P17

## The relationship between vegetational succession and water environmental change in a warm-temperate, volcanic peat mire in south-western Japan

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The relationship between vegetational succession and water environmental change was investigated in Tadewara mire, in a warm-temperate, volcanic peat mire in south-western Japan. Water chemical environment are monitored every month from August 2006 to July 2007 and changes in species abundance at the correspondence sites were investigated. Vegetational group classified by TWINSPAN did not correspondence sites grouped by water chemistry using cluster analysis. Then we tested the correlation between changes in species abundance and the corresponding environmental change within the investigation period.Abundance of *Moliniopsis japonica* (Hack.) Hayata show significant positive correlation *Sphagnum fimbriatum* Wils. in Hook. , *Hydrangea paniculata* Sieb. et Zucc. , and *Phragmites australis* (Cavanilles) trinius ex steudel. Abundance of *M. japonica* show significant negative correlation *S. palustre*. Abundance of *S. fimbriatum* show significant positive correlation *M. japonica*, *H. paniculata*, and *P. australis*. Abundance of *S. fimbriatum* show significant negative correlation *S. palustre*. Abundance of *H. paniculata* decreased at sites with low Mn concentration. Abundance of *S. fimbriatum* decreased at sites with low $Na^{+}$ concentration. Change in abundance *S. palustre* increased at sites with low $Mg^{2+}$ and low $Ca^{2+}$ concentration. *H. paniculata* decreased at sites with low pH, $Mg^{2+}$, $SO_4{}^{2-}$, TN, Fe and low Mn concentration.

# P18

## Hydrological Control of River Systems

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Many researches on hydrological control by vegetation have been reported. Over grown vegetation, however, had negative effect on river management because the vegetation is removed by high water level during the flooding, and consequently blocked the river channel. In this research, we evaluated the response of Pharagmites japonica Steudel community in the Iwatake River to flow rate and water table at various flooding condition, we measured the vegetation density and flow rate distribution in a P. japonica community, and then calculate the threshold of community resistance to increasing flow rate. In situ density of P. japonica was 76‐106 m-2, and column diameter was 0.4‐0.6 cm in the Iwatake River and the estimated threshold of vegetation resistance to flow rate and water table was 0.45‐0.6 ms-1 and 80－100 cm respectively.

# P19

## Effect of volcanic activity on mire vegetation in Tadewara mire, southwest Japan

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Peat stratigraphy and macrofossil composition of three – 210 cm, 270 cm, 420 cm peat cores obtained from Tadewara mire (Ooita) were investigated to clarify the relationship between volcanic activity and vegetational change with special reference to chemical deposition from volcano. Tow distinct horizons consist of volcanic glass were observed at 160 and 252 cm depth, however most layers contained volcanic glass implying frequent impact of volcanic activity on Tadewara mire. Distinct peak of sulfur content in soil core was found from depth at 110 cm by elemental analysis of peat soil. Content of carbon, nitrogen, and hydrogen decreased corresponding to the increase of sulfur. Dominant species of macrofossil community started to change from *Sphagnum* SPP. to *Phragmites australis* just corresponding to the increase of sulfur at 110 cm. By $^{14}C$ dating, increase in sulfur content started at 970±40 y.B.P and it just corresponded to the peak of volcanic activity of mount Kurotake near by Tadewara mire.

Thus we obtained the evidence that mire vegetation changed from ombrotrophic to minerotrophic community by sulfur deposition due to the volcanic activity.

# P20

## Inhibition of copper-induced calcium influx by prion-drived peptide in suspension-cultured tobacco cells

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Recently, we have been engaged to the designing of artificial peptides for protection of the plant cells from various metal stresses. Copper is known to induce an increase in cytosolic fee calcium concentration ($[Ca^{2+}]_c$) in cultured tobacco cells (Inoue et al. 2005). Since our recent reports suggested that the human prion protein-derived copper-binding peptide functions as the chelators and the catalysts of generating the reactive oxygen species such as superoxide anion radical (Kawano, 2007), we expected the effects of such peptides as modulators of copper toxicity. Here, we examined the effects of copper-binding peptides derived from prion on the action of copper in n tobacco BY-2 suspension culture expressing aequorin gene. Addition of copper resulted in rapid and transient increase in $[Ca^{2+}]_c$. When the cells were pre-treated with a prion-derived copper-binding peptide fragment (corresponding to the neurotxic region), the copper-induced $[Ca^{2+}]_c$ was effectively lowered. While the copper-sequestering action of prion neurotoxic peptideis likely attributed to the chelating activity of the neurotoxic peptide, other prion-derived copper-binding peptides (including those corresponding to octa-repeat region and helical region) were shown to be less active in sequestering of the copper action. Further examinations are required for designing the peptidic or biochemical agents for protection of plant cells from metal toxicity.

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# P21

## Possible use of green paramecia in development of photo-controlled micro-particle transport system

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*Paramecium bursaria* is known to harbor endosymbiotic algae in its cytoplasm, and responds to light stimuli to show photoaccumulation or photoavoidance as the consequences of phototactic cellular migration in either positive or negative manner. Early studies indicated that *P. bursaria* has rhodopsin-like protein as a photoreceptor in plasma membrane. On the other hand, calcium ion ($Ca^{2+}$) is known as one of the important regulatory elements for ciliary movements. However, the mechanism for photosensory signal transduction of *P. bursaria* is not fully understood to date. In this study, the migration by *P. bursaria* irradiated with ultraviolet lights (UV-A and UV-C) in fine capillary tube was demonstrated. Both UV-A and -C induced the negative phototaxis while UV-C is lethal and UV-A is not harmless. The UV-C-dependent phototaxis was performed in the presence of various pharmacological inhibitors. The data suggested that $Ca^{2+}$ signaling (sensitive to flunarizine, a T-type $Ca^{2+}$/$Na^{+}$ channel inhibitor but insensitive to tetrodotoxin, a $Na^{+}$ channel inhibitor) is required for the light-dependent cell migration. However, despite of expectation, the signaling mechanism is likely free from phosphorylation events (thus insensitive to KT5720, staurosporine and K252a) and G-protein-mediated sensing (thus insensitive to cholera toxin and pertussis toxin).

# P22

## Development of micro-particle transport system using galvanotactically migrating green paramecium

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It is well known that protozoan species including *Paramecium* species towards the cellular movements to anodic electrode when exposed to the electric field in the medium. This phenomenon is known as the galvanotaxis. We have developed a system for controlled transportation of the microparticles packed in the cells of a ciliate (*Paramecium bursaria*, pre-conditioned for maximizing the particle-loading capacity) migrating inside the capillary tubes (φ, 2 mm) by galvanotaxis. We succeed in controlled cell migration by galvanotaxis in the complexed routes made of fine capillaries. Since the demonstration was successful, we could conclude that *P. bursaria* has a potential to be used as one of the micro-biorobotic devices.

# P23

## Response to the presence of glucose depending on NDHD in Synechocystis sp. PCC6803

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Cyanobacteria have several growth characteristics and are able to grow under photoautotrophic, photomixotrophic and heterotrophic conditions. However, little is known about the mechanisms how cyanobacteria respond to the presence of glucose in their environment. Moreover, while glucose sensitive (GS) and glucose tolerant (GT) strains of *Synechocystis* sp. PCC 6803 are available, the mechanisms distinguishing their response to glucose are poorly understood.

*Synechocystis* sp. PCC6803 genome contains multiple copies of ndhD and ndhF genes. Analysis of these double mutants in a pair of homologous have been reported that there were functionally distinct multiple of NAD(P)H dehydrogenase complexes (NDH-1) in GT strains. One is classified into $CO_2$ uptake NDH-1, which include NdhD3 related to low $CO_2$ induced $CO_2$ uptake system and NdhD4 related to constitutive $CO_2$ uptake system. There were the distinct role between NdhD3 and NdhD4. On the other hand, the other is classified into cyclic electron transport and respiration NDH-1, which include NdhD1 or/and NdhD2. However, mechanisms of the response to glucose in these mutant cells have not been poorly clarified.

To clarify response to the presence of glucose depending on NDH-1 in *Synechocystis* sp. PCC6803, we have constructed each single disruption mutant in GS strains. Both ndhD1 and ndhD2 mutant shows higher sensitive to glucose than GS strains, but ndhD3 and ndhD4 mutant did not show significant differences. Analysises of these mutants are in progress.

# Satelite Session 1

## Abscisic acid-induced anion currents activation mediated by cyclic ADP-ribose / ryanodine receptor (RyR) in *Arabidopsis thaliana* suspensions cells

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The cytosolic $Ca^{2+}$ activity $[Ca^{2+}]_{cyt}$ controls essential and multiple animal and plant cellular processes, including the plant growth regulator abscisic acid (ABA) signal transduction. Increase of $[Ca^{2+}]_{cyt}$, is the major trigger for ABA-induced anion currents activation in *Arabidopsis thaliana* suspension cells. (Brault *et al*., 2004; Zalejski *et al*., 2006).

We previously shown that ABA-induce $H_2O_2$ production triggers an increase in $[Ca^{2+}]_{cyt}$ and participates to the fast depolarization of the plasmalemma, due to anion channel activation. $H_2O_2$ generation was shown to trigger the second of the two $[Ca^{2+}]_{cyt}$ increases observed in response to ABA, probably by promoting extracellular $Ca^{2+}$ entry since addition of EGTA impaired this $H_2O_2$ generated $[Ca^{2+}]_{cyt}$ increase. In addition, L, N and T-type $Ca^{2+}$ channel inhibitors, fluspirilene (50 µM), pimozide (50 µM) and EGTA (5mM) considerably altered ABA induced *RD29a* expression. These first results support the idea that the calcium mobilized by $H_2O_2$ can at least has an extra-cellular origin (Ghelis *et al*., 2000).

$[Ca^{2+}]_{cyt}$ is also critically affected by the release from intracellular stores (vacuole, reticulum) which are controlled by two major channel/receptor complexes, the inositol trisphosphate receptor ($IP_3R$) and the cADP-ribose/ryanodine receptor (RyR). Here, we report that ABA-Signaling mechanism implies the regulation of vacuolar $Ca^{2+}$ release.

$[Ca^{2+}]_{cyt}$ oscillation signatures may be controlled through the balance of processes of $Ca^{2+}$ influx and release into the cytosol against those of $Ca^{2+}$ sequestration and elimination.

The major approach to the study of the RyR's role has been to use the inhibitory effects of drugs effective on IP3-mediated $Ca^{2+}$ increase. Previous studies have indicated that the IP3-mediated $Ca^{2+}$ signal requires subsequent RyR activity. In *A. thaliana* suspension cells, we used two tonoplast $Ca^{2+}$ -receptor/channel inhibitors, (i) 8-bromo-cADP-ribose an antagonist of endogenous cADP-ribose, which induces $Ca^{2+}$ release from the stores through both ryanodine channel-receptor (RyR) and via mechanisms independent of RyR channels, and (ii) Dantrolene an agent which interacts with the ryanodine receptor (RyR) to modulate the channel function inhibiting one part of the vacuolar $Ca^{2+}$ efflux.

Both inhibitors, blocked the release of calcium from the vacuole. 8-bromo-cADP-ribose (100 μM) decrease the ABA-induced activation of anion channels (65%). Dantrolene (100μM) decrease the ABA-induced activation of anion channels (52%) and reduce the expression of ABA-responsive gene (reduction of 20% for *RD29a*).

The finding that 8-bromo-cADP-ribose and Dantrolene can at least partially block $[Ca^{2+}]_{cyt}$, increase induced anion efflux current in response to ABA, demonstrates that vacuolar $Ca^{2+}$ is also clearly involved in the ABA-signaling pathways.

In conclusion, we show that tonoplast cADPR/ryanodine receptor (RyR) is probably involved in the ABA induce $Ca^{2+}$ signaling pathways. In addition, our results suggest that ABA activates indirectly several $Ca^{2+}$ channels at the tonoplast and provide strong evidence that vacuolar $Ca^{2+}$ release is an important component in the ABA signal transduction pathway.

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# Satelite Session 2

## Thaxtomin A-induced defense responses in Arabidopsis thaliana cells require an early $Ca^{2+}$ influx

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The pathogenicity of various Streptomyces scabies isolates, involved in the potato scab disease, was correlated with the production of thaxtomin A. Since calcium is known as an essential second messenger associated with pathogen-induced plant responses and cell death, we investigate on Arabidopsis thaliana suspension cells, a convenient model to study plant-microbe interaction, whether thaxtomin A could induce a $Ca^{2+}$ influx rela]ted to cell death and to other putative plant responses. A. thaliana cells were treated with micro-molar concentrations of thaxtomin A. Cell death was quantified and ion flux variations were analysed from electrophysiological measurements, with the apoaequorine $Ca^{2+}$ reporter protein and by external pH measurement. Involvement of anion and calcium channels in the signal transduction leading to programmed cell death was determined by using specific inhibitors. Our data suggest that this toxin induces a rapid $Ca^{2+}$ influx and cell death in A. thaliana cell suspension. Moreover, our data provide strong evidence that the $Ca^{2+}$ influx induced by thaxtomin A is necessary to achieve this cell death and is a prerequisite to early thaxtomin A-induced responses: anion current increase, alkalization of the external medium and the expression of PAL a key enzyme of the phenylpropanoid pathway.

# Satelite Session 03

## Similarity between plant redox enzymes and copper-bound prion protein

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Recently, our group has shown that human prion-derived copper-binding motifs (including well characterized octarepeat region and helical region) may be good model for studying the neurotransmitter-dependent oxidative burst in which superoxide is generated while aromatic monoamines such as phenylethylamine and tyramine are oxidized in the presence of trace amount of hydrogen peroxide. The type of reaction revealed was shown to be very much similar to the reactions catalyzed by plant enzymes (peroxidases). This presentation focusses on the mechanisms how prion protein and plant enzymes catalyze the generation of reactive oxygen species. Lastly the consequence of reactions *in vivo* both in plant and animal systema will be discussed.

# Satelite Session 04

## Simulation of the signal transduction in artificial plant cells using NEURON: Inspired from the artificial retinal model

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Human retinal tissue has several nerve cell layers and realizes complicated signal processing by communicating within layers. Artificial retina, a bioengineering implement for re-construction of vision by stimulating degenerated-retinal nerve cells directly with electrode, has been studying in many places. However, limitation of experiment of human body prevents the progression of research and the development of these devices. One of solution is that a environment of dry-experimentation for exploring a optimized parameters such as electrical intensity, electrode position and cell responses has been conducted. Number of computational simulators for constructing a mathematical system to monitor the conditions of living cell or organisms has been developed. At the field of neurophysiology, a well recognized free software, NEURON has been highly exploited for calculating the parameters of the nerve cells based on the differential equations. Various factors such as characteristics of ion channels and transporters, influx and efflux of some kind of ions, production and release of neurotransmitters and other biochemical events involved in cell signaling can be utilized as the parameters. NEURON has definitely been used for construction the artificial retinal model. Here, we would like to propose a use of NEURON system for the simulation of cellular signal transduction in plant cells after defining the parameters based on the actual electrophysiological and biochemical data obtained through experiments.

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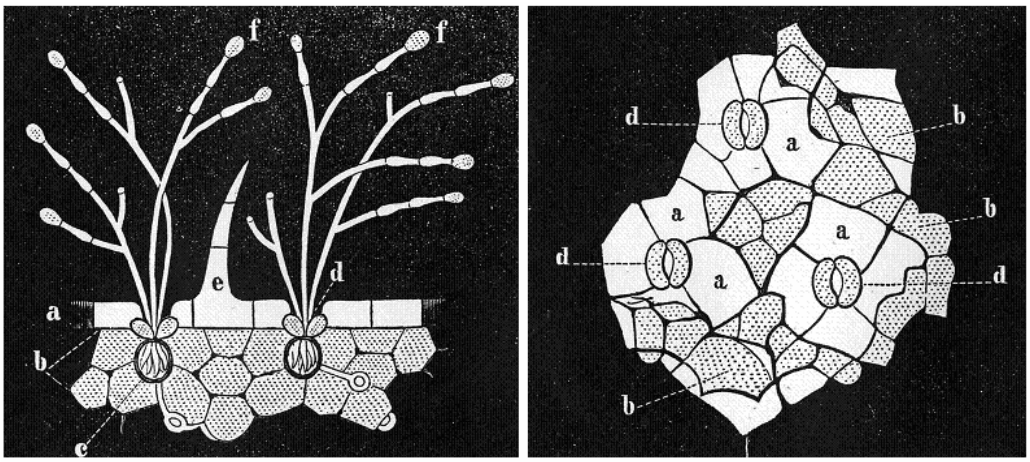
## The 4th International Symposium on Plant Neurobiology

### Fukuoka, Japan (June 6-9 2008)

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