The 2nd NIBB-MPIPZ Joint Symposium

Plant Science Communications 2010

November 16th (Tue) - 18th (Thu), 2010
Okazaki Conference Center
Aichi, Japan

Preface

Dear Colleagues:

We are pleased to announce that the joint symposium on "Plant Science Communications 2010" will be held from November 16th to 18th, 2010 at the Okazaki Conference Center, Okazaki, Japan. In 2009, the National Institute for Basic Biology (NIBB) in a mutual effort to promote research in the field of plant sciences, joined hands with the Max Planck Institute for Plant Breeding Research (MPIPZ) in a new initiative aimed at stimulating academic and scholarly exchange. Also, NIBB has joined hands with the Singapore's Temasek Life Sciences Laboratory (TLL) in an academic cooperation program. While continuing to expand the cooperative program with the JSPS Machida Plant Meristems Project at the leading edge of plant development research in Japan, NIBB aims at expanding the boarders of the plant sciences community.

This international joint symposium supported by NIBB, MPIPZ, TLL and Machida Plant Meristems Project, is dedicated to providing opportunities to create new networks of plant scientists.

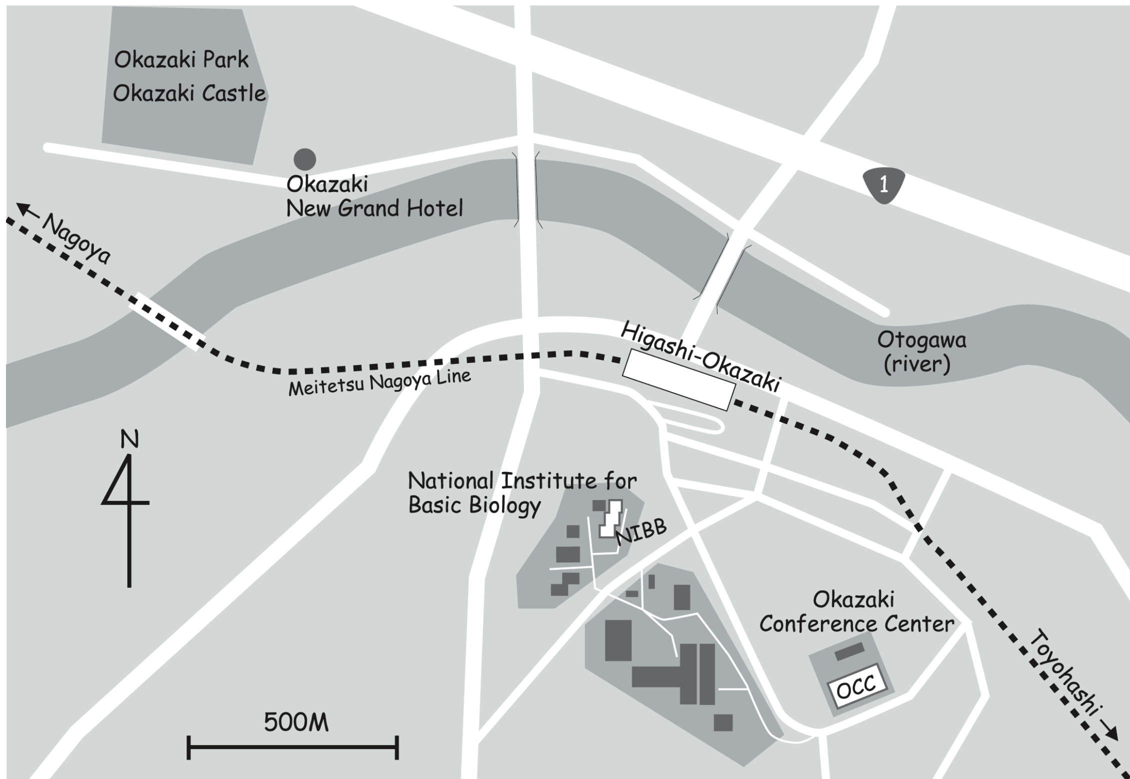
The organizing committee would like to express our sincere thanks to all participants and we intend to provide you with an exciting and unforgettable scientific experience.

Best regards,

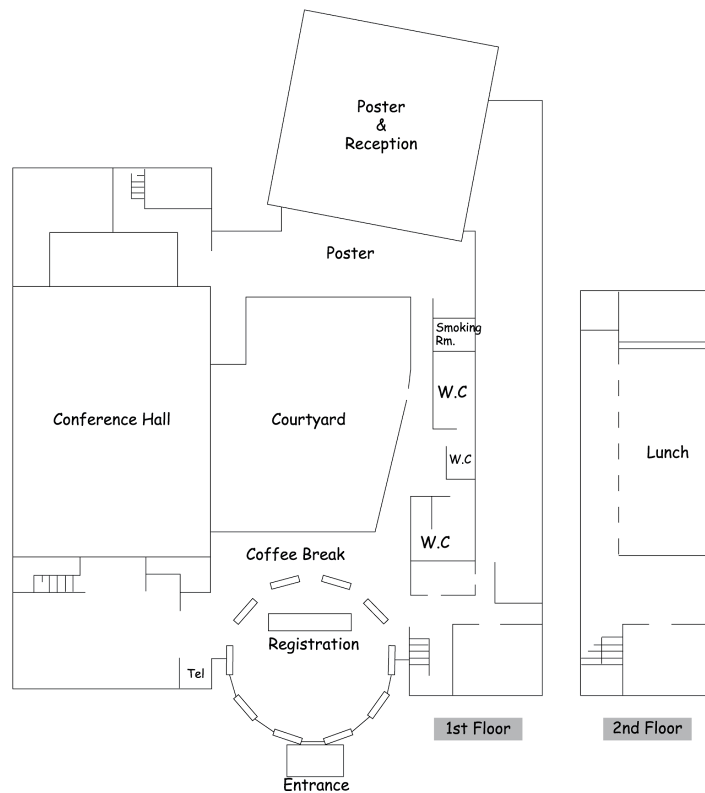
Masayoshi Kawaguchi
Mitsuyasu Hasebe
Kiyotaka Okada

Organizers, Plant Science Communications 2010
National Institute for Basic Biology

Okazaki MAP



Okazaki Conference Center (OCC)



Program

November 16th, Tuesday

09:00-09:10 **Opening Remarks**
Masayoshi Kawaguchi NIBB, Japan

Session 1

Chair: Masayoshi Kawaguchi

09:10-09:20 **Introduction of NIBB**
Kiyotaka Okada Director-General, NIBB, Japan

09:20-10:00 **Keynote Lecture**
"Biogenesis and Functional Differentiation of Plant Peroxisomes"
Mikio Nishimura NIBB, Japan

10:00-10:30 "Plant-Unique Endosomal Trafficking Pathways Regulated by ARA6"
Takashi Ueda Univ. of Tokyo, Japan

10:30-10:45 *Coffee Break*

Session 2

Chair: Mitsuyasu Hasebe

10:45-10:55 **Introduction of MPIPZ**
Maarten Koornneef MPIPZ, Germany

10:55-11:25 "Natural Variation in Arabidopsis Reveals Gene Functions and Signatures of Selection"
Maarten Koornneef MPIPZ, Germany

11:25-11:55 "RNA-seq Analysis in Tomato and Its Wild Relatives"
José Jiménez-Gómez MPIPZ, Germany

11:55-13:20 *Lunch*

Session 3

Chair: Mikio Nishimura

13:20-13:30 **Introduction of TLL**
Toshiro Ito TLL, Singapore

13:30-14:00 "Developmental Timing Control by Chromatin Remodeling"
Toshiro Ito TLL, Singapore

14:00-14:30 "The Battle of Histone Marks: Transposons Cause Natural Variation of Adjacent H3K27me3 Domains in *Arabidopsis thaliana*"
Franziska Turck MPIPZ, Germany

14:30-15:00 “Secreted Peptide Signals Required for Maintenance of Root Stem Cell Niche in *Arabidopsis*”

Yoshikatsu Matsubayashi Nagoya Univ., Japan

15:00-15:20 *Coffee Break*

Session 4

Chair: Yoshikatsu Matsubayashi

15:20-15:30 **Introduction of JSPS Machida Plant Meristems Project**

Yasunori Machida Nagoya Univ., Japan

15:30-16:00 “Cyclin-Dependent Kinases of Plants Control Activity of the MAPK Cascade That Is Required for Cytokinesis and Affects Asymmetric Cell Division”

Yasunori Machida Nagoya Univ., Japan

16:00-16:30 “Molecular Mechanisms of the Integration of Flowering Signals”

Hao Yu TLL, Singapore

16:30-17:00 “Proteasome Activity Profiling and Imaging Elucidates Mechanisms and Biological Roles of the Bacterial Nonribosomal Effector SylA”

Renier van der Hoorn MPIPZ, Germany

17:00-17:20 *Coffee Break*

Session 5

Chair: Yuji Hiwatashi

17:20-17:50 “Maintenance of Vascular Stem Cell Activity by TDIF Peptide”

Hiroo Fukuda Univ. of Tokyo, Japan

17:50-18:20 “A Supercomplex of Supercomplexes Driving Cyclic Electron Flow in Photosynthesis”

Jun Minagawa NIBB, Japan

18:20-18:30 “Application of Bioimage Data in Plant Organelle Research: Construction of the Plant Organelles Database 2 (PODB2) and Their Use for Computer-Assisted Analysis”

Shoji Mano NIBB, Japan

18:30- *Dinner & Poster Session*

November 17th, Wednesday

09:00-09:45 Lab Tour 1

09:45-10:30 Lab Tour 2

10:30-10:50 Move to OCC

Session 6

Chair: Kiyoshi Tatematsu

10:50-11:10	"Molecular Analysis of Seed Dormancy QTL *DOG1* in *Arabidopsis thaliana*"
	Kazumi Nakabayashi MPIPZ, Germany
11:10-11:30	"Mechanisms of ER Body Formation and Its Possible Function in *Arabidopsis thaliana*"
	Kenji Yamada NIBB, Japan
11:30-11:50	"Laser Capture Microdissection of the *Arabidopsis* Shoot Apical Meristem to Identify Genes Involved in the Floral Transition"
	Stefano Torti MPIPZ, Germany
11:50-12:10	"SSA Controls a Leaf Adaxial-Abaxial Polarity"
	Koichi Toyokura NIBB, Japan
12:10-12:20	Take photos
12:20-13:30	*Lunch*

Session 7

Chair: Jun Minagawa

13:30-14:10	**Keynote Lecture**
	"Extreme Parasitism, Functional Tradeoffs, and Dollo's Law of Irreversibility"
	Paul Schulze-Lefert MPIPZ, Germany
14:10-14:40	"Molecular Recognition of Rice *R* gene *Xa10* by TAL Effector AvrXa10"
	Zhongchao Yin TLL, Singapore
14:40-15:10	"Receptor Quality Control and Signaling from MAMP Recognition to Robust Immune Activation in Plants"
	Yusuke Saijo MPIPZ, Germany
15:10-15:25	*Coffee Break*

Session 8

Chair: Takuya Suzaki

15:25-15:55	"Regulation of Mycorrhizal Phosphate Uptake"
	Marcel Bucher Univ. Cologne, Germany
15:55-16:25	"Molecular Analysis of Symbiotic Systems in Leguminous Plants"
	Naoya Takeda NIBB, Japan

16:25-16:55 “The Molecular Regulation of Seed Dormancy in Arabidopsis”

Wim Soppe MPIPZ, Germany

16:55-17:10 *Coffee Break*

Session 9

Chair: Atsushi Hoshino

17:10-17:40 “Asymmetries of DNA Methyltransferases Activity between Male and Female Gametes Cause Imprinting in the Arabidopsis Endosperm”

Frederic Berger TLL, Singapore

17:40-18:10 “Regulation of Epidermal Cell Pattern through Three Secretory Peptides ”

Tatsuo Kakimoto Osaka Univ., Japan

18:10-18:40 “Mechanism of Cell Partitioning as Revealed by Live Cell Imaging”

Takashi Murata NIBB, Japan

18:40- Reception

November 18th, Thursday

Session 10

Chair: Kazuo Tsugane

09:00-09:30 “Regulation of Flowering by the FT Florigen and Its Antagonist TFL1”

Takashi Araki Kyoto Univ., Japan

09:30-10:00 “Control of the Floral Transition by Histone H3 Lysine-4 Methylation and Demethylation”

Yuehui He TLL, Singapore

10:00-10:20 *Coffee Break*

Session 11

Chair: Yosuke Tamada

10:20-10:50 “Seasonal Flowering in Annual and Perennial Plants”

George Coupland MPIPZ, Germany

10:50-11:20 “Structure and Function of Rice Hd3a Florigen”

Ko Shimamoto NAIST, Japan

11:20-11:50 “Sustaining the Plant Circadian Clock”

Seth J. Davis MPIPZ, Germany

11:50-12:00 **Closing Remarks**

George Coupland MPIPZ, Germany

POSTER PRESENTATION

P01
"Positive Autoregulation of *KNOX* Genes in Rice"
Katsutoshi Tsuda (NIG, Japan)

P02
"Leaflet Patterning on Compound Leaf"
Momoko Ikeuchi (Univ. of Tokyo, NIBB, Japan)

P03
"RPK2 Mediates CLV3 Signal in Arabidopsis"
Shinichiro Sawa (Kumamoto Univ., Japan)

P04
"*WOX1* and *PRS* Gene Functions during Leaf Development"
Miyuki Nakata (NIBB, Japan)

P05
"General and Specific Roles of Ribosomes in Leaf Development"
Gorou Horiguchi (Rikkyo Univ., Japan)

P06
"Transcriptional Regulation of Cell Expansion in Arabidopsis"
Christian Breuer (RIKEN PSC, Japan)

P07
"Auxin-Inducible LBD/ASL Members Regulate Lateral Root Formation"
Tatsuaki Goh (Kobe Univ., Japan)

P08
"Chloroplast Development Affects Leaf Adaxial and Abaxial Domain Sizes in Arabidopsis."
Toshiaki Tameshige (NIBB, Japan)

P09
"Identifying Gene(s) Essential for the Initiation of Embryogenesis"
Tomokazu Kawashima (TLL, Singapore)

P10
"AP2 Transcription Factors Determine Stem Cell Identity in the Moss *Physcomitrella patens*"
Tsuyoshi Aoyama (NIBB, Japan)

P11
"Genetic Framework of Leaf Blade Flattening in Unifacial Leaves"
Takahiro Yamaguchi (NIBB, Japan)

P12
“Regulatory Mechanisms of Initiation of a Sporophyte-Like Stem Cell in *Physcomitrella patens* CURFY LEAF Deletion Mutants”
Yuji Hiwatashi (NIBB, Japan)

P13
“TONSOKU Repress Cell Cycle Checkpoint Activity Dependent on ATR”
Takamasa Suzuki (Nagoya Univ., Japan)

P14
“Pattern Formation of Plant Leaf Venation”
Hirionori Fujita (NIBB, Japan)

P15
“miR165/166 Might Regulate Non-Cell-Autonomously the Expression Domains of HD-Zip III in Arabidopsis Leaf Primordia.”
Kiyoshi Tatematsu (NIBB, Japan)

P16
“Identification of a Novel Transcription Factor Regulating Cuticle Development in Arabidopsis.”
Yoshimi Oshima (AIST, Japan)

P17
“IR Laser Mediated Gene Induction in a Single Pericycle Cell of Arabidopsis”
Hiroko Urawa (NIBB, Japan)

P18
“Mitogen-Activated Protein Kinase Regulated by the CLAVATA Receptors Contributes to the Shoot Apical Meristem Homeostasis”
Shigeyuki Betsuyaku (Univ. of Tokyo, Japan)

P19
“Dual Repression of Expression of ETT/ARF3 and ARF4 Genes by ASYMMETRIC LEAVES2 (AS2) and AS1 for Establishment of the Leaf Polarity in *Arabidopsis thaliana*”
Chiyoko Machida (Chubu Univ., Japan)

P20
“Elongator Proteins Affect the Establishment of Leaf Polarity in *Arabidopsis thaliana*”
Shoko Kojima (Chubu Univ., Japan)

P21
“Genome-Wide and Expression Analyses of Potential G-quadruplex-forming Sequences in *Arabidopsis thaliana*”
Ayami Nakagawa (Chubu Univ., Japan)

P22
"Arabidopsis TERMINAL FLOWER 1 Plays a Negative Role in Flowering Time and Inflorescence Development through the Transcriptional Repression"
Shigeru Hanano (RIBS Okayam, Japan)

P23
"The Gene Activation Cascade for Flowering by Short Day Signal in Chrysanthemum"
Atsushi Oda (NIFS, Japan)

P24
"Digital Gene Expression Profiling of a Reprogramming, a Process from Differentiated Leaf Cells to Pluripotent Stem Cells, in the Moss *Physcomitrella patens* by Feasible 5'-end Sequencing of mRNAs"
Tetsuya Kurata (NAIST, Japan)

P25
"Temperature-Dependent Transposition of Tam3 in Antirrhinum is Performed by Control of Nuclear Import of Transposase"
Yuji Kishima (Hokkaido Univ., Japan)

P26
"Epigenetic Regulation of Flower Variegation in the Morning Glory"
Atsushi Hoshino (NIBB, Japan)

P27
"Transposition and Target Preferences of an Active Nonautonomous DNA Transposon nDart1 and Its Epigenetic Regulation in Rice."
Kazuo Tsugane (NIBB, Japan)

P28
"Genome-Wide Analyses of mRNA Expression and Histone H3 Methylation in *Physcomitrella patens* curly leaf Mutant"
Yosuke Tamada (NIBB, Japan)

P29
"A Genetic Dissection of DNA De-methylation Using Arabidopsis Endosperm"
Diana Buzas (NAIST, Japan)

P30
"Fe-Hydrogenase Deficiency Impaired Expression of Imprinted Gene FWA in the Endosperm."
Miyuki Nakamura (NAIST, Japan)

P31
"Genetic Analysis of the Mutants of Rice Three DNA Methyltransferase Genes, *OsDRM2*, *OsDRM-like* (*OsDRM-L*), and *OsCMT3a* Generated by Homologous Recombination-Mediated Gene Targeting"
Satoru Moritoh (NIPS, Japan)

P32
"HS3 (HEAVY SEED3) Regulates Transcriptional Activity of Chloroplast Genome in Seedling and Embryo of Arabidopsis"
Masatake Kanai (NIBB, Japan)

P33
"Regulation of ROS-Producing Activity of an Arabidopsis NADPH oxidase, AtrbohF, by Binding of Ca^{2+} and Phosphorylation."
Sachie Kimura (Tokyo Univ. of Sci., Japan)

P34
"Exploiting Ragi (*Eleusine coracana*) for Identifying Novel Genes Associated with Salinity Tolerance"
Kishor Shedage (Ehime Univ., Japan)

P35
"Novel Small Open Reading Frames Which Regulate Plant Immunity"
Rebecca Lyons (RIKEN, Japan)

P36
"Using Small Molecules to Investigate Plant Immunity"
Ivana Saska (RIKEN, Japan)

P37
"Genetic Analysis of Nodulation and an Application of Heat-Shock Mediated Gene Induction System to *Lotus japonicus*"
Takuya Suzaki (NIBB, Japan)

P38
"Nod Factor/Nitrate-responsive *CLE* Genes Induce Systemic and HAR1-Dependent Regulation of Nodulation"
Satoru Okamoto (NIBB, Japan)

P39
"KLV Mediates the Long-Distance Negative Control of Nodulation in *Lotus japonicus* and Interacts with HAR1"
Hikota Miyazawa (NIBB, Japan)

P40
"Plant-Specific APM9 Is Essential for Peroxisome Biogenesis in Arabidopsis"
Shino Goto (NIBB, Japan)

P41
"Identification and Characterization about Arabidopsis Aberrant Peroxisome Morphology Mutants Exhibiting Defects in Peroxisome Biogenesis"
Shoji Mano (NIBB, Japan)

P42
"Analysis of Trans-Golgi Network (TGN) Dynamics in Plants"
Tomohiro Uemura (Univ. of Tokyo, Japan)

P43
"Proteomic Identification of Novel Factors Regulating Peroxisomal Protein Import in Arabidopsis"
Songkui Cui (NIBB, Japan)

P44
"A Defect of Autophagy Causes the Accumulation of Catalases with Low-Activity in Peroxisomes in *Arabidopsis thaliana*."
Michitaro Shibata (NIBB, Japan)

P45
"Quantitative Proteome Analysis of Peroxisomal Transition in Soybean Cotyledons"
Atsushi Nakai (NIBB, Japan)

P46
"Light-Dependent Interaction among Peroxisomes, Mitochondria, and Chloroplasts in Photosynthetic Tissue of Arabidopsis"
Kazusato Oikawa (NIBB, Japan)

P47
"The MAP Kinase MPK4 Is Required for Cytokinesis in *Arabidopsis thaliana*"
Ken Kosetsu (Nagoya Univ., Japan)

November 16th, 2010

Biogenesis and Functional Differentiation of Plant Peroxisomes

Mikio NISHIMURA[1,2], Makoto HAYASHI[1,2], Shoji MANO[1,2], Maki KONDO[1]
Kazusato OIKAWA[1], Shino GOTO[1,2], Atsushi NAKAI[1,2], Songkui CUI[1,2]
and Michitaro SHIBATA[1,2]

[1]Department of Cell Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan;
[2]Department of Basic Biology, School of Life Science, The Graduate University of Advanced Studies (SOKENDAI), Okazaki 444-8585, Japan

Peroxisomes in higher plant cells are known to differentiate in function depending on the cell type. By a comprehensive survey of peroxisomal genes in the entire *Arabidopsis* genome, we found 286 candidates of peroxisomal genes. They included all genes for peroxisomal enzymes reported up to date, and most of them encoded functionally unknown proteins in peroxisomes. Analyses of gene expression profiles revealed that peroxisomal differentiation is caused by the expression of specific genes that are induced in specific organs in addition to constitutively expressed genes. The clustering analyses suggested that basic foundations of peroxisome were β–oxidation (1), H_2O_2 degradation, branched chain amino acid catabolism and some unknown functions, and that peroxisomes in seedlings, cotyledons, leaves and roots were differentiated by organ-specific expressed genes.

To understand biogenesis and functional differentiation of peroxisomes, we isolated a number of *Arabidopsis* mutants having aberrant morphology (*apm*) and different function of (*ped*) peroxisomes. In this presentation, we identified the components responsible for peroxisomal biogenesis and function from the analyses of these mutants. We show the mechanism on biogenesis (2) and dynamic features (3) of plant peroxisomes including direct interaction of peroxisomes and other organelles by visualization of peroxisomes in the cell.

References:

1. Arai, Y., M. Hayashi and M. Nishimura (2008) Plant Cell 20, 3227-3240.
2. Singh, T., S. Mano, M. Hayashi, Y. Arai, S. Goto and M. Nishimura (2009) Plant J. 60, 488-498.
3. Kamigaki, A., M. Kondo, M. Hayashi and M. Nishimura (2009) Plant Cell Physiol. 50, 2034-2046.

Plant-Unique Endosomal Trafficking Pathways Regulated by ARA6

Takashi Ueda

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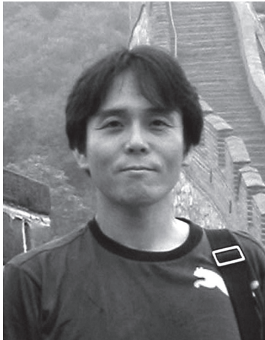

All eukaryotic cells comprise various single membrane-bound organelles, which are interconnected by the trafficking system mediated by vesicular and/or tubular membrane carriers. This system, membrane traffic, plays fundamental roles in various cellular functions including maintenance of organelle identity, transport and retention of organelle resident proteins, and intra- and inter-cellular signaling. Rab GTPases and SNARE molecules are evolutionary conserved key players regulating tethering and fusion steps between donor and target membranes. Recent comparative genomics suggested that the diversification of membrane trafficking pathways in eukaryotic cells was achieved by paralogous gene expansion of organelle identity molecules such as Rabs and SNAREs. In addition to the increase in the net number of these genes, plants also seem to have acquired new Rab and SNARE genes tailored to fulfill plant-unique functions.

ARA6, an *A. thaliana* RAB5-related small GTPase with remarkable structural characteristics, is a good example of the plant-unique regulators of membrane traffic. ARA6 was localized on a subpopulation of endosomes, which indicated that ARA6 is associated with some endosomal function. In addition to *ARA6*, plants also harbor orthologs of animal *RAB5*, but it was unknown why plants evolved two distinct types of RAB5 and how they differed functionally. Our genetic, biochemical, and cell biological analyses have been revealing their interesting functions, which indicates that land plants have evolved quite unique post-Golgi trafficking pathways.

Natural Variation in Arabidopsis Reveals Gene Functions and Signatures of Selection

Rubén Alcázar, Ana V. García, Ilkka Kronholm, Juliette de Meaux, Jane Parker, Matthieu Reymond and Maarten Koornneef

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Natural variation in plants, even in model species such as *Arabidopsis thaliana* allows the identification of the function of genes in addition to mutant approaches. This variation in nature represents genetic variation that might reflect adaptation to local environments, although some of this variation results from the demographic history etc. A QTL (Quantitative Trait Loci) approach is commonly used to decipher genetic differences between accessions. To address whether the genes identified are under natural selection, molecular population genetic as well as ecological experiments can be carried out. The occurrence of novel phenotypes in the progeny of inter-accession crosses can, in most cases, be explained by epistatic interactions between specific alleles from the two parents. An example of this phenomenon concerns the molecular genetic basis of lines with reduced fitness segregating in specific mapping populations at low temperature. We have revealed that some allele combinations between genes involved in plant immunity underlies the observed phenotypes. This identified a novel component of the plant immune system but also identified signatures of local adaptation at one of the loci involved. Different alleles at this locus provide contrasted thresholds for pathogen resistance, which suggests that the adaptive fine-tuning of adequate defense responses has the potential to promote the emergence of incompatibilities between genotypes of one species, which can lead to post-zygotic isolation barriers.

References:

1. Alcázar et al., (2009) PNAS. 106: 334-339.
2. Alcázar et al., (2010) Nature Genetics. in press.

RNA-seq Analysis in Tomato and Its Wild Relatives

José M Jiménez-Gómez[1,3], Dan Koenig[1], Ravi Kumar[1], Seisuke Kimura[1], Jie Peng[2], Neelima Sinha[1] and Julin N Maloof[1]

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Wild tomato species are native to diverse habitats in South America and show wide morphological and ecological diversity that has proven useful in breeding programs. The current high throughput sequencing technologies have the ability to characterize genome-wide genetic variation in sequences, expression profiles and other molecular events with phenotypic effect. It is becoming clear that the information generated using these technologies together with the abundant information available in the databases allow for novel integrative approaches that can help us identify genes and pathways that are responsible for the phenotypic variation found in nature.

We have used RNA-seq to sequence cultivated tomato (*Solanum lycopersicum*) and three wild relatives: *S. pennellii*, *S. habrochaites* and *S. pimpinellifolium*, generating up to 50x -150x coverage of their transcriptome. Since only a draft of the genome sequence of cultivated tomato is currently available, it was required to combine well-established methodologies with custom protocols to effectively obtain relevant information from the short reads generated from the wild species. Our approaches have allowed us to infer genome-wide polymorphisms, as well as species-specific expression differences and alternative splicing events. I will present the rationale behind the bioinformatic protocols used for our analysis, and the results obtained in sequence diversity and expression divergence. In addition, we are now integrating the functional and evolutionary information obtained to identify candidate genes underlying phenotypic variation between tomato and its wild relatives.

Developmental Timing Control by Chromatin Remodeling

Bo Sun[1], Zemiao He[1], Eng-Seng Gan[1] and Toshiro Ito[1, 2, 3]

[1]Temasek Life Sciences Laboratory 117604, Singapore; [2]National University of Singapore 117534, Singapore; [3] PRESTO, Japan Science and Technology Agency, Japan

Unlike shoots, flowers have determinate structures. In flower development, stem cell activity is terminated after a proper number of floral organs are created. The negative feedback pathway of the stem cell determinant WUSCHEL (WUS) and the floral homeotic protein AGAMOUS (AG) plays a major role for the meristem determinacy control in *Arabidopsis*. AG is the evolutionary conserved C class gene in the genetic ABC, and encodes a MADS domain transcription factor. *AG* is directly induced by the homeodomain protein WUS functioning cooperatively with other transcriptional factors in the center of floral primordia at stage 3 of flower development, and then AG starts to specify reproductive organ identities. *WUS* and *AG* are co-expressed for about two days. When floral buds reach at stage 6, AG represses *WUS* expression to terminate floral stem cell activity. Thus, AG integrates stem cell regulation with floral patterning events, and the strict timing regulation of each genetic pathway is essential for coordinated growth and differentiation. We recently showed that *KNUCKLES (KNU)*, a C2H2-type zinc finger protein is a direct target of AG, mediating the repression of *WUS*. The main topic of my talk will be on how AG controls *KNU* expression at the right developmental timing. We show that the *KNU* locus contains the repressive histone mark H3Lys27 tri-methylation (H3K27me3) and that the AG-dependent removal of the mark is followed by *KNU* activation, which happens in a cell-cycle dependent manner. We further show that AG binding sites contain the landing site for a Polycomb Group (PcG) protein necessary for the maintenance of H3K27me3. These results indicate that the competition of AG with the PcG leads to dilution of H3K27me3 during cell division. I will present our model on how the transcription factor AG controls a specific target in a proper developmental timing through chromatin remodeling to coordinate stem cell growth and floral differentiation.

The Battle of Histone Marks: Transposons Cause Natural Variation of Adjacent H3K27me3 Domains in *Arabidopsis thaliana*

Franziska Turck, Julia Reimer, Heiko Schoof, Xue Dong, Ulrike Göbel and Julia Engelhorn

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Natural variation is the summary of phenotypic changes caused by small sequence difference between accessions of the same species. So far, little is known about the epigenetic effects of these sequence differences. Epigenetic effects that cause natural variation cannot be detected on the sequence level but require additional analysis of stable chromatin modifications.

Two histone marks are implicated in stable, epigenetic gene repression in *Arabidopsis thaliana*, the meiotically inherited H3K9me2 mark enriched in heterochromatin and the Polycomb Group associated H3K27me3 mark capable to ensure mitotically stable gene repression. The H3K27me3 pathway is important for the proper regulation of many key developmental regulators in time and space and therefore crucial for plant development. Our group uses ChIP-chip and ChIP-Seq techniques to study cross-talk between the two chromatin repression pathways. We found that H3K9me2 and H3K27me3 are mutually exclusive and therefore often occur in sharply delimited adjacent locations. Most natural variation of the H3K27me3 mark detected between the Col and Ler accessions can be explained by shifted borders with adjacent H3K9me2 regions. Interestingly, only in half of the cases, the inheritance of the marks remains allelic whereas the others cases show a disappearance of the H3K27me3 mark in F1 hybrids of Col and Ler. In the future we will assess whether these epigenetic changes remain stable in segregating populations.

Secreted Peptide Signals Required for Maintenance of Root Stem Cell Niche in *Arabidopsis*

Yo Matsuzaki, Mari Ogawa-Ohnishi, Ayaka Mori and Yoshikatsu Matsubayashi

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Secreted peptides are now recognized as important members of intercellular signals that coordinate and specify cellular functions in plants. Some of the secreted peptide hormones undergo complex post-translational modifications that are mediated by specific enzymes which recognize multiple target peptides. Because such modifications are generally critical for the functions of individual peptide hormones, the presence of novel peptide hormones should be revealed through phenotypic analysis of the mutants of post-translational modification enzymes.

Tyrosine sulfation is a post-translational modification that has been found in several peptide hormones in plants. This modification is mediated by tyrosylprotein sulfotransferase (TPST). In loss-of-function mutant of *TPST* (*tpst-1*), root meristematic activity is considerably decreased and root stem cells are not maintained. Because known tyrosine sulfated peptide hormones did not recover these defects of *tpst-1*, we speculated that an as-yet undiscovered tyrosine-sulfated peptide(s) regulates root meristematic activity and maintenance of the stem cell niche in *Arabidopsis*.

To identify this peptide signal, we searched the *Arabidopsis* genome for genes likely to encode sulfated peptide(s), determined their mature peptide structures and tested their activities to recover defects of *tpst-1* mutant. We identified a family of functionally redundant homologous peptides that are secreted, tyrosine-sulfated, and expressed mainly in the stem cell area and the innermost layer of central columella cells. We named these peptides root meristem growth factors (RGF). RGFs are required for maintenance of the root stem cell niche and transit amplifying cell proliferation in *Arabidopsis*. RGF1 defines expression levels and patterns of the stem cell transcription factor PLETHORA mainly at the post-transcriptional level. The RGFs function independently of the auxin pathway. Our results reveal that secreted peptide signals play a crucial role in root meristem development.

Reference:

1. Matsuzaki Y. *et al*. *Science* **329,** 1065-1067 (2010)

Cyclin-Dependent Kinases of Plants Control Activity of the MAPK Cascade That Is Required for Cytokinesis and Affects Asymmetric Cell Division

Yasunori Machida[1], Michiko Sasabe[1], Véronique Boudolf[3], Lieven De Veylder[3], Dirk Inzé[3] and Chiyoko Machida[2]

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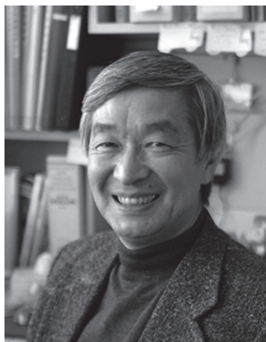

Duplicated chromosomes and cytoplasm are precisely distributed to two daughter cells during cytokinesis, which is spatially and temporally controlled. In plants, cytokinesis is achieved by the formation of the cell plate, which occurs in phragmoplast. A pathway that includes the NACK1 kinesin-like protein (KLP) and the MAP kinase (MAPK) cascade has been shown to play a key role in the control of cytokinesis in tobacco cells (1, 2, 3, 4). Mutations in genes for some of the components exhibit abnormality of asymmetric cell division. The MAP kinase cascade consists of NPK1 MAPKKK, NQK1 MAPKK and NRK1/NTF6 MAPK. We designated this signal process as the NACK-PQR pathway (3), which is conserved in *Arabidopsis thaliana* (5, 6). Previous studies have shown that the although both NACK1 KLP and NPK1 MAPKKK are accumulate before metaphase in M phase, NACK1 enhances activity of NPK1 via the interaction of these proteins only after metaphase (2, 7), which results in activation of the whole kinase cascade and eventually stimulates the expansion of phragmoplast and the formation of cell plates. How the interaction between NACK1 KLP and NPK1 MAPKKK can be regulated during the progression of M phase, however, remains largely unknown. We show here that tobacco cyclin-dependent kinases (CDKs) phosphorylate specific sequences in NPK1 and NACK1 *in vitro* and *in vivo* before metaphase. We have also shown that the phosphorylation interferes with the interaction between NACK1 and NPK1. The CDKs appear to repress in the early M phase activation of NPK1 MAPKKK and that dephosphorylation of NPK1 and NACK1 after metaphase is crucial for activation of the NACK-PQR pathway and cytokinesis. Plants have two distinct CDKs, CDKa and CDKb. We have investigated roles of these CDKs in phosphorylation of NPK1 and NACK1. Results of these experiments will also be presented.

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Molecular Mechanisms of the Integration of Flowering Signals

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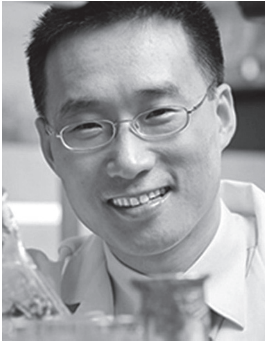

In *Arabidopsis*, multiple flowering genetic pathways act in response to developmental cues and environmental signals converge on the transcriptional regulation of two major floral pathway integrators, *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), which in turn activate floral meristem identity genes to initiate the generation of floral meristems. The expression of *FT* and *SOC1* is directly regulated by a key repressor complex that consists of two MADS-box transcription factors, *FLOWERING LOCUS C* (*FLC*) and *SHORT VEGETATIVE PHASE* (*SVP*). In the last two decades, intensive investigations have progressively unraveled the underlying mechanisms of the integration of flowering signals in *Arabidopsis*. This talk will focus on our recent advances in understanding this key developmental process.

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Proteasome Activity Profiling and Imaging Elucidates Mechanisms and Biological Roles of the Bacterial Nonribosomal Effector SylA

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Protein activities change tremendously during plant-pathogen interactions but much of this functional information can not be retrieved from studies on transcriptomes, proteomes or even post-translational modifications since protein activities are regulated by a multitude of post-translational mechanisms that include interactions with other proteins and micro environments. The Plant Chemetics lab studies protein activities during plant-pathogen interactions using activity-based protein profiling (ABPP). ABPP employs small molecule probes that react with the active site of protein classes in an activity dependent manner. The labeling is covalent and irreversible, facilitating the detection and identification of the labelled proteins. Using different probes, we are able to detect activities of the proteasome, Cys and Ser proteases, lipases, acyltransferases. As an example, probes for the proteasome are presented. Selective proteasome probes display and image the activities of all three catalytic subunits of the proteasome in extracts and in living plants. This method is used to show that Syringolin A (SylA), a small molecule proteasome inhibitor from the plant pathogen Pseudomonas syringae pv. syringae, targets two of the three catalytic subunits of the host proteasome. Imaging revealed that SylA accumulates in the host nucleus. Host proteasome inhibition also occurs during infection with SylA-producing strains. Studies with a SylA-deficient strain uncovered that SylA suppresses host cell death and effector-triggered immune responses and promotes distant colonization by suppressing salicylic acid-mediated signalling and other immune responses.

Maintenance of Vascular Stem Cell Activity by TDIF Peptide

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In the vascular meristem, procambium and cambium, as vascular stem cells, differentiate into xylem and phloem cells, while they proliferate. Using a zinnia xylogenic cell culture, we have identified TDIF (TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR), which is a CLE (CLAVATA3 (CLV3)/EMBRYO SURROUNDING REGION-related) peptide modified by hydroxylation but not by glycosylation (Ito et al., 2006). In Arabidopsis hypocotyls, TDIF is secreted from the phloem and perceived in procambial cells through its receptor, TDR (TDIF RECEPTOR)/PXY (PHLOEM INTERCALATED WITH XYLEM) belonging to the leucine-rich repeat receptor-like kinase family (Hirakawa et al., 2008). The TDIF-TDR signal prevents procambial cells from differentiating into xylem vessel cells and promotes self-renewal of procambial cells. These two processes, inhibition of commitment to differentiation and self-renewal are two essential characters of stem cell maintenance. Further analysis revealed that WOX4 is a target transcription factor of the TDIF-TDR signaling pathway, however WOX4 is involved in promotion of procambial cell proliferation but not in suppression of xylem cell differentiation from procambial cells (Hirakawa et al., 2010). In the shoot apical meristem (SAM), the CLV3-CLV1-WUS signaling pathway regulates stem cell fates to maintain the size of SAM. The components of the CLV3-CLV1-WUS and the TDIF-TDR-WOX4 signaling pathways are similar but their regulation mechanisms underlining stem cell maintenance differ. Based on these data, we discuss the regulation of stem cell fates in plant meristems.

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A Supercomplex of Supercomplexes Driving Cyclic Electron Flow in Photosynthesis

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Photosynthetic light reactions establish electron flow in the chloroplast's thylakoid membranes, leading to the production of the ATP and NADPH that participate in carbon fixation. Two modes of electron flow exist—linear electron flow (LEF) from water to $NADP^+$ via photosystem (PS) II and PSI in series and cyclic electron flow (CEF) around PSI. Although CEF is essential for satisfying the varying demand for ATP, the exact molecule(s) and operational site are remained elusive. This issue is now addressed directly in the green alga *Chlamydomonas reinhardtii*. In this unicellular alga, the electron flow shifts from LEF to CEF upon preferential excitation of PSII, which is brought about by an energy balancing mechanism between PSII and PSI (state transitions). Here, we isolated a protein supercomplex composed of PSI with its own light-harvesting complex (LHCI), the PSII light-harvesting complex (LHCII), the cytochrome b_6f complex (Cyt *bf*), ferredoxin (Fd)-NADPH oxidoreductase (FNR), and the integral membrane protein PGRL1 from *C. reinhardtii* cells under PSII-favoring conditions. Spectroscopic analyses indicated that upon illumination, reducing equivalents from downstream of PSI were transferred to Cyt *bf*, while oxidized PSI was re-reduced by reducing equivalents from Cyt *bf*, indicating that this supercomplex is engaged in CEF. Thus, formation and dissociation of the PSI-LHCI-LHCII-FNR-Cyt *bf*-PGRL1 supercomplex not only controlled the energy balance of the two photosystems, but also switched the mode of photosynthetic electron flow.

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Session 5-3

Application of Bioimage Data in Plant Organelle Research: Construction of the Plant Organelles Database 2 (PODB2) and Their Use for Computer-Assisted Analysis

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Technical advances in imaging procedures generated a variety of information on a large-scale basis in the field of plant biology. These large data sets are deposited in databases, and used in further analyses. The Plant Organelles Database 2 (PODB2; http://podb.nibb.ac.jp/Organellome) was launched in 2006 and provides imaging data of plant organelles, protocols for plant organelle research, and external links to relevant websites. The unique feature of PODB2 is that researchers in plant biology produced and directly contributed all of the imaging data and protocols presented. Using PODB2, users are readily able to examine organelle dynamics. As image and movie data contain valuable and significant information such as size, length and velocity, we are currently adopting a mathematical morphology approach to perform statistical analysis of plant organelle dynamics.

Moreover, a new website, Plant Organelles World (http://podb.nibb.ac.jp/Organellome/PODBworld/en/index.html), which is based on PODB2, was recently launched as an educational tool to engage members of the non-scientific community such as students and school teachers. Plant Organelles World is written in layman terms, and technical terms were avoided where possible. We would appreciate contributions of data from all plant researchers to enhance the usefulness of PODB2 and Plant Organelles World.

In order to make researchers find new insights from bioimage data, and make users understand plant organelles easily via more visually ‘fun’ interface, we are developing a system to show the visualized organelles, cells and tissues as 3 dimensional computer graphics using methods developed in 4-Dimensional Digital Universe (4D2U) project. In this symposium, we would like to introduce recent progress of PODB2 including some movies, which were generated by 4D2U project.

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November 17th, 2010

Molecular Analysis of the Seed Dormancy QTL *DOG1* in *Arabidopsis thaliana*

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DELAY OF GERMINATION 1 (DOG1) was identified as a major determinant of natural variation for seed dormancy between the accessions L*er* and Cvi of *Arabidopsis thaliana*. The gene was cloned and encodes a protein of unknown function. *DOG1* is predominantly expressed in seeds. Mutant alleles of *DOG1* are completely non-dormant implicating that *DOG1* is absolutely required for the induction of seed dormancy.

It has been shown that ABA is necessary to induce and maintain seed dormancy and ABA deficient mutants are known to be non-dormant. The relation between DOG1 and the ABA pathway in dormancy induction still largely remains to be analyzed. We found an increased abundance of *DOG1* transcript in the *aba1* mutant, despite its non-dormant phenotype. A near isogenic line, having the Cvi allele of *DOG1* in L*er* background, fails to induce seed dormancy when combined with *aba1*. Based on these, we hypothesized that ABA might be necessary to stabilize the DOG1 protein. Immunoblot analysis, however, ruled out this idea since DOG1 protein was detected in the *aba1* mutant. This indicates that DOG1 employs the ABA pathway for its function to induce dormancy. Combined with analyses using mutants related to ABA, we are trying to understand more about the relation between DOG1 and ABA during dormancy induction in *Arabidopsis*.

Mechanisms of ER Body Formation and Its Possible Function in *Arabidopsis thaliana*

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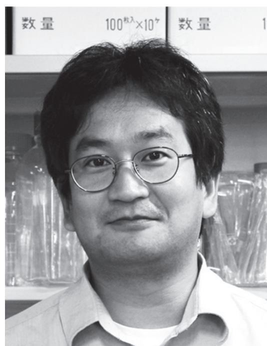

The endoplasmic reticulum (ER) body is an ER structure that is specific to the Brassicales (1,2). ER bodies constitutively appear in seedlings and root, and they accumulate a large amount of the β-glucosidase PYK10 (3). In mature leaves, no ER body is appeared but induced after wounding or jasmonate treatment (4). This suggests that ER body is responsible for defense against pathogens and herbivores (5,6). In Arabidopsis, a basic-helix-loop-helix transcription factor, NAI1, regulates the expression of PYK10 and ER body protein, NAI2 (7,8). We identified two genes encoding membrane proteins, termed membrane of ER body 1 (MEB1) and MEB2, by microarray analysis of *nai1*. MEB1 and MEB2 are specifically localized in ER body by NAI2 dependent manner. Interestingly, co-expression of NAI2 and PYK10 is necessary and sufficient for ER body formation in the epidermal cells of onion (*Allium cepa*), which is a non-Brassicales plant and does not normally form ER bodies. NAI2 physically interacts with PYK10 to form dense matrix of ER bodies and with MEB1 and MEB2 to form ER body membrane. Our findings indicate that NAI2 is necessary and sufficient for ER body formation by the accumulation of matrix and membrane protein of ER body. We are now trying to identify ER body functions against pathogens using Arabidopsis mutants that lack ER bodies.

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Laser Capture Microdissection of the *Arabidopsis* Shoot Apical Meristem to Identify Genes Involved in the Floral Transition

Stefano Torti[1], Fabio Fornara[1], Coral Vincent[1], Fernando Andrés Lalaguna[1], Ulrike Göbel[1], Heiko Schoof[1] and George Coupland[1]

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Flowering in plants is regulated by different genetic pathways. Photoperiod, or day-length, is a major environmental stimulus controlling flowering in *Arabidopsis thaliana*. In long-day photoperiod (LD), a gene cascade is activated in the leaf and floral transition occurs at the shoot apical meristem (SAM). Upon exposure to LD, transcription of *FLOWERING LOCUS T* (*FT*) is activated in leaves, and the FT protein moves to the SAM where it triggers the floral transition. FT is thought to interact with FD, a bZIP transcription factor, to activate target genes, such as the MADS-box transcription factors *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*SOC1*), *APETALA1* (*AP1*) and *FRUITFULL* (*FUL*). *TWIN SISTER OF FT* (*TSF*) is the closest paralog of *FT*, and it also promotes flowering.

In order to identify novel genes in the SAM responding to the photoperiodic induction we performed a global gene expression analysis by means of next-generation sequencing. The tissue used consisted of SAM specifically collected through laser microdissection, in the early stages of the floral transition upon shift from short-day (SD) to LD. Among the genes that were found to be differentially expressed, a subset of candidates was tested by *in situ* hybridisation in wild-type apices. Some of those genes were confirmed to be up-regulated during the floral transition at the SAM, and also novel spatial expression patterns were identified. Additionally, some candidate genes were tested for their expression in *ft tsf* double mutant, revealing whether their expression kinetics depends or not on FT/TSF function.

A few examples of those genes are described, together with their preliminary functional characterization.

SSA Controls a Leaf Adaxial-Abaxial Polarity

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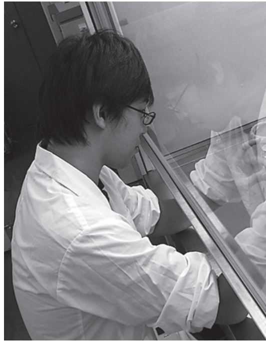

Asymmetric organization along the adaxial-abaxial axis in leaf is regulated by several putative transcription factors. They are expressed in a polarized manner along the adaxial-abaxial axis to confer the asymmetric characters to cells according to its position. Their polarized expression suggest s that there is pre-existing polarity along the adaxial-abaxial axis, however, it is unclear what regulates the polarized gene expression. To understand the upstream mechanism, we isolated *enlarged fil expression domain1* (e*nf1*) mutant, in which the robustness of the *FIL* expression pattern was disrupted. *ENF1* gene encoded SUCCINIC SEMIALDEHYDE DEHYDROGENASE, known to catalyze succinic semialdehyde (SSA) to succinate. *gabat1* mutant, lacking enzyme transaminating gamma-amino butyric acid to SSA, increased the *FIL* expression domain and suppressed *enf1* mutant. Exogenous application of SSA on leaf initial induced adaxial characters on the abaxial side. GABAT1 is expressed in the L1 layer at the central zone of SAM, whereas ENF1 is expressed outside of SAM. Our study suggests that the maintenance of SSA is necessary to establish the polarity along the adaxial-abaxial axis.

Extreme Parasitism, Functional Tradeoffs, and Dollo's Law of Irreversibility

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Powdery mildews are phytopathogens whose growth and reproduction are entirely dependent on living plant cells. The molecular basis of this lifestyle, obligate biotrophy, remains unknown. I will present the genome analysis of barley powdery mildew, *Blumeria graminis* f.sp. *hordei* (*Bgh*), and a comparison with those of two powdery mildews pathogenic on dicotyledonous plants. These genomes display massive retrotransposon proliferation, genome size expansion and gene losses. The missing genes encode enzymes of primary and secondary metabolism, carbohydrate-active enzymes and transporters, probably reflecting their redundancy in an exclusively biotrophic lifestyle. Among the over 250 candidate effectors of pathogenesis identified in the *Bgh* genome very few (<15) define a core set conserved in all three mildews, suggesting that most effectors represent species-specific adaptations.

Molecular Recognition of Rice *R* gene *Xa10* by TAL Effector AvrXa10

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Transcription activator-like (TAL) type III effectors of *Xanthomonas* spp contribute to pathogenesis by targeting to host gene promoters and activating host gene expression. In this study, we report the isolation of rice bacterial blight resistance gene *Xa10* and the characterization of the molecular recognition between *Xa10* and TAL type III effector AvrXa10 from *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The *Xa10* gene was isolated from *Xa10*-containing rice line by positional cloning and genetic transformation. *Xa10* encodes an unknown transmembrane protein. The expression of the *Xa10* gene was specifically induced by *Xoo* strains that harbor the *AvrXa10* gene. Mutation of the nuclear localization signal (NLS) motifs in AvrXa10 or deletion of the transcription activation domain (AD) at its C-terminal region abolished its function for *Xa10* activation. A 17-bp *AvrXa10 box* was identified at *Xa10* promoter by candidate approach. The *AvrXa10 box* was recognized by AvrXa10 in *Nicotiana benthamiana* in transient assay and this recognition activated reporter gene expression. The interaction of the *AvrXa10 box* and AvrXa10 was further confirmed *in yeast* by yeast-one-hybrid assay and *in vitro* by electromobility shift assay (EMSA). The identification of molecular recognition between *Xa10* and AvrXa10 provides us opportunity to engineer new resistance specificity for broad-spectrum and durable resistance to bacterial diseases caused by *Xanthomonas* spp.

Receptor Quality Control and Signaling from MAMP Recognition to Robust Immune Activation in Plants

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The importance of microbe-associated molecular pattern (MAMP)-triggered immunity (MTI) has been accepted as a functional basis for different layers of plant immunity. MTI is conferred by pattern recognition receptors (PRRs) that recognize MAMPs, non-self structures conserved in a class of microbes, and then trigger a diverse set of defense-related outputs. Important unsolved questions involve (1) how plants link initial MAMP detection to robust immune activation; (2) how plants distinguish pathogens from non-pathogens that share MAMPs; and (3) how plants fine-tune and coordinate immune activity with other physiological processes. Our genetic screens have revealed a collection of Arabidopsis "*priority in sweet life*" (*psl*) mutants that de-repress sucrose-induced anthocyanin accumulation in the presence of the bacterial MAMPs elf18 and flg22. Some of *PSL* genes identify components in an ER N-glycocylation pathway that is essential for the biogenesis of the elf18 receptor EFR but not of the flg22 receptor FLS2. Remarkably, weakly dysfunctional *psl* alleles are partially and differentially impaired in post-recognition signalling of EFR, providing a genetic tool to selectively perturb a subset of EFR-triggered outputs. In an ER *glucosidase IIa* allele, designated *rsw3*, EFR-triggered immunity collapses despite WT-like EFR accumulation and co-activation of ROS spiking, MAPKs, ethylene generation, and callose deposition in response to elf18. We found that EFR-triggered activation of defense-related genes is impaired in a late, but not early, phase in *rsw3* plants. This points to the existence of two separate phases in MTI and the importance of sustained activation of EFR signalling as a critical step in mounting robust immunity. We have explored molecular processes affected in *rsw3* plants, with an aim at mechanisms that determine the transition from initial MAMP detection to robust immune activation. In light of the above three questions in MTI, I will discuss possible molecular principles with which plants thrive in a microbe-rich environment.

Regulation of Mycorrhizal Phosphate Uptake

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Research on mycorrhizas has become one of the important areas in contemporary biological and environmental sciences, ranging from fundamental aspects to applications in agriculture and in the restoration of disturbed ecosystems. Arbuscular mycorrhizas are the most common soil-based symbiosis. In these ecologically and agriculturally important associations between plants and fungi, the host plant derives mainly phosphorus from the fungus, which in turn benefits from plant-based photosynthetic sugars. Our major interests are the molecular and biochemical mechanisms involved in phosphate uptake at mycorrhizal interfaces. In our current model, transcriptional regulation of mycorrhiza-specific phosphate transport includes phosphate repressible lysophospholipid signaling which merges at *cis*-elements in plant phosphate transporter genes.

Molecular Analysis of Symbiotic Systems in Leguminous Plants

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Arbuscular mycorrhizal symbiosis (AMS) is a mutualistic plant-microbe interaction between land plants and *Glomeromycota* fungi[1]. The fungal symbiont provides water and inorganic materials, mainly phosphate, to the host plant. In return, the host gives photosynthetic products to the symbiont. This nutrient exchange is performed through symbiotic structure formed in the host root. The AM fungi attach epidermal cell of the host root and penetrate into the root. The intraradical hyphae run between the cortical cells and form symbiotic organs; arbuscule and vesicle. The AM fungi also expand hyphae into the soil and facilitate absorbance of water and nutrients from the rhizosphere. These nutrients are transferred in the fungal cell to the arbuscules, where the material exchange is performed through bacterial and plant membrane expressing various transporters. Similar symbiosis system is seen in other plant-microbe interaction; root nodule symbiosis (RNS) in legumes. Host plant forms a symbiosis organ called 'nodule' in the root. In the nodule, bacterial symbiont; rhizobium fixes atmospheric nitrogen and provides nitrogen source to the host. These symbiotic nutrient supplies are of great advantage for plant survival on the land, studies for utilization of the biological function have been conducted.

We studies molecular mechanism of AM and RNS systems in legume model plant *Lotus japonicus*. Recent studies revealed the evolutionally younger RNS was established by integrating AM signaling factors into its own system. The shared symbiosis factors constitute a common symbiotic signaling pathway called 'Common Sym Pathway' (CSP)[2]. These symbiosis factors were genetically isolated from RNS mutants and analyzed the functions in RNS. The findings in RNS studies can apply to AMS studies through the CSP, which contributes to elucidation of AMS system. At present, molecular analysis of AMS system largely depends on the sharing of the knowledge with RNS because molecular biology and genetics tools for AM analysis are absolutely lacking. Thus we established AMS gene maker *SbtM1* which is specifically induced during AMS[3]. Using this gene maker, we revealed that hyphal elongation in the root is controlled by symbiosis signaling of the host.

We also analyzed physiological changes in the host root cells. Ca spiking is a periodic Ca ion oscillation induced in response to both AMS and RNS signals in the host root cells. Bioimaging technique visualized the dynamics of Ca ion in the host cells and we investigate how the general second messenger confers symbiosis specific function and activates the symbiosis signaling pathways.

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The Molecular Regulation of Seed Dormancy in Arabidopsis

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Plants are sessile organisms whose survival depends on their adjustment to seasonal changes. The timing of germination has a crucial role in this adjustment, as it determines the start of the life cycle. Germination timing is controlled by seed dormancy, which prevents a viable seed from germinating under favourable conditions. Dormancy induction in Arabidopsis occurs during the maturation of seeds in the silique and is strongly determined by abscisic acid levels. Seed dormancy can be released by low temperatures (stratification) and dry storage (after-ripening).

To reveal the molecular mechanisms that determine the induction and release of seed dormancy, we analysed and cloned mutants with reduced dormancy levels. The underlying genes could be divided in two groups. The first group consists of genes that are upregulated during seed maturation, but expressed in all plant tissues. Several of these genes have a role in transcription initiation and elongation. The second group consists of genes with a seed-specific expression. The gene *DELAY OF GERMINATION 1* (*DOG1*) belongs to this group and is probably a key regulator of dormancy. DOG1 protein levels in the seed correlate with dormancy levels. *DOG1* is alternatively spliced and we have shown that DOG1 function requires binding between its different protein isoforms.

In this seminar, I will present our present understanding of the molecular mechanism of seed dormancy in Arabidopsis and focus on the roles of transcription elongation factors and DOG1.

Asymmetries of DNA Methyltransferases Activity between Male and Female Gametes Cause Imprinting in the Arabidopsis Endosperm

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Imprinted genes are expressed predominantly by one parental allele. In plants, imprinted genes are expressed in endosperm. The concept emerged that DNA demethylation occurs in the central cell genome but not in sperm cells, leading to inheritance of a transcriptionally active maternal allele while the paternal allele remains silent, resulting in an imprinted status. Demethylation in the central cell relies both on the inactivation of MET1 transcription and the expression of the DNA demethylase DEMETER. Hence imprinting has so far been linked exclusively to cytosine methylation on CG contexts, which depend on MET1 activity.

The RNA dependent DNA methylation (RdDM) pathway causes de novo methylation of cytosine residues in any context and is involved in silencing transposons, participates to the maintenance of constitutive heterochromatin around centromeres, as well as the silencing of transgenes. We report that the RdDM pathway is active only in male gametes but not in the central cell. This asymmetry is sufficient to cause imprinting. We present the case of an imprinted gene controlled by the RdDM pathway and extrapolate further generalization of the mechanism.

Regulation of Epidermal Cell Pattern through Three Secretory Peptides

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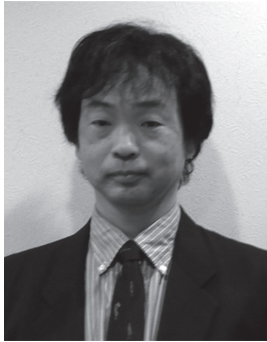

During leaf epidermal development, a protodermal cell first makes a fate decision of whether to be a meristemoid mother cell (MMC) or diffrentiates into a general epidermal cell (pavement cell). A MMC undergoes asymmetric cell division forming the meristemoid, which is the precursor of guard cells. The asymmetric cell division plane is regulated so that new meristemoids are not formed in contact with the previously formed maristemoids, guard cells, and guard cells. This regulation ensures that stomata are not formed in contact. MMC is the sourse of both guard cells and pavement cells, thus the epidermal cell number depends on the number of MMCs formed.

We demonstrate that MMCs and meristemoids produce a small secretory peptide EPIDERMAL PATTERING FACTOR 2 (EPF2), which inhibits formation of MMCs from the protodermal cells. This feedback loop plays a critical role in regulation of density of epidermal cells, including stomata and non-stomata. EPF1, a homolog of EPF2, is expressed in the meristemoid, guard mother cell, and young guard cells. EPF1 regulates the positioning of the plane of asymmetric cell division forming the meristemoid, thus is the regulator of stomatal positioning. Stomagen, also a homologue of EPF1 and EPF2, is expressed in the inner cells of leaf primordia, and acts on the protoderm of leaf primordia to promote formation of stomatal lineage. Our results support a model in which Stomagen antagonizes EPF1 and EPF2 at the TMM/ERs containing putative receptor complex. We reconstructed the signaling pathway in *Nicotiana benthamiana*. Our results suggested that EPF1 and EPF2 de-stabilize SPCH, which is a transcription facor directing the protodermal cells to enter the stomatal lineage. This destabilization depends on the MAPK and proteasome. By contrast, Stomagen stabilized SPCH.

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Mechanism of Cell Partitioning as Revealed by Live Cell Imaging

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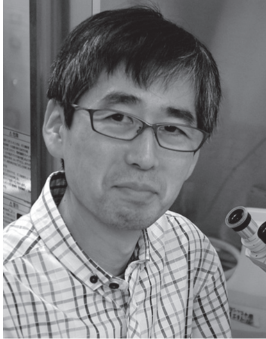

Cell division is essential for plant growth and development. At the final step of cell division, the new cell wall, called the cell plate, separates the parental cell into two. The cell plate initially forms between the daughter nuclei and then expands towards cell periphery until it fuses with the parental cell walls. The molecular mechanism how the cell plate expands towards the cell periphery is essentially unknown, although many factors that are involved in the process have been identified (1). We are trying to address the mechanism by live cell imaging.

The cell plate develops inside of a special structure called the phragmoplast. The phragmoplast contains many microtubules. The microtubules are considered to transport the vesicles that contain precursors of a cell wall (1). Because assembly of the new cell wall occurs at the margin of a disk-shaped cell plate, structure of the phragmoplast is ring-shaped (2). Translocation of the phragmoplast towards the cell periphery is the driving force of the cell plate expansion. Pharmacological studies have shown that cycles of microtubule polymerization/depolymerization are involved in the phragmoplast translocation (3).

We analyzed direction of individual microtubule growth and lifespan of microtubules in the phragmoplast. We found that elongating microtubules localize throughout the phragmoplast, and direction of the elongation varies among microtubules. On the other hand, we found that microtubule lifespan is biased: Microtubules that localizes on the completed cell plate is more rapidly depolymerized than those in the region without the cell plate. We propose that driving force of the phragmoplast translocation is elongation of microtubules from inside to outside of the phragmoplast, and direction of the translocation is determined by difference of microtubule stability. The molecular mechanism how microtubule stability decreases on the completed cell plate should be examined in future.

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Session 10-1

Regulation of Flowering by the FT Florigen and Its Antagonist TFL1

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In many plants, initiation of flowering is controlled by photoperiod perceived in leaves by the action of circadian clock. Long-distance signal, called florigen, is generated in leaves and transported via phloem to the shoot apex where it triggers flower development. In a long-day plant Arabidopsis, a clock-controlled transcriptional regulator CONSTANS (CO) plays a key role in regulation of flowering by photoperiods. *FLOWERING LOCUS T* (*FT*) and its closest paralog, *TWIN SISTER OF FT* (*TSF*), which encode proteins of the PEBP (phosphatidylethanolamine binding protein) family, are direct regulatory targets of CO. They are expressed in the phloem tissues of cotyledons, leaves, and hypocotyls with a peak in the late evening. FT protein (possibly TSF protein as well) is a major component of the florigen and is transported to the shoot apex and acts together with a bZIP transcription factor FD to activate transcription of target genes such as floral meristem identity gene *APETALA1* (*AP1*) in subsets of cells in the shoot apical meristem. Another *FT* paralog, *TERMINAL FLOWER 1* (*TFL1*), on the other hand, is expressed in the inner cells of the shoot apical meristem. TFL1 protein acts as a short-distance signal via cell-to-cell movement to regulate flowering in an antagonistic manner with FT in part through competition for interaction with FD.

Recent progress in our understanding of the action of FT and its antagonist TFL1 in the shoot apical meristem will be presented.

Control of the Floral Transition by Histone H3 Lysine-4 Methylation and Demethylation

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Histone H3 lysine-4 (H3K4) methylation is associated with transcribed genes in eukaryotes. In *Drosophila* and mammals, both di- and tri-methylation of H3K4 are associated with gene activation. In contrast to animals, In *Arabidopsis*, H3K4 trimethylation, but not mono- or di-methylation of H3K4, has been implicated in transcriptional activation. H3K4 methylation is catalyzed by the H3K4 methyltransferase complexes known as COMPASS-like in mammals. We have found that *Arabidopsis* relatives of the three core structural components of the mammal COMPASS-like complexes form a nuclear structural subcomplex during vegetative and reproductive development, that can associate with multiple putative H3K4 methyltransferases. Functional disruption of this subcomplex in Arabidopsis causes a decrease in genome-wide H3K4 trimethylation, but not in di- or mono-methylation. Furthermore, we found that this structural subcomplex, together with an H3K4 methyltransferase, forms a functional COMPASS-like complex that deposits H3K4 trimethylation at a crucial Arabidopsis floral repressor, *FLOWERING LOCUS C* (*FLC*), and *FLC* homologs to activate these gene expression, leading to the inhibition of floral transition. In addition, we have previously found that several putative H3K4 demethylases act in partial redundancy to mediate H3K4 demethylation in *FLC* and repress its expression, leading to the acceleration of flowering. These findings together provide concrete evidence that H3K4 methylation is dynamically regulated and plays an important role for the control of developmental-regulatory gene expression in plants.

Seasonal Flowering in Annual and Perennial Plants

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We are comparing the biochemical mechanisms by which plants synchronise their life cycles to the changing seasons. *Arabidopsis thaliana* is a model annual plant, which flowers only once in a life cycle that typically lasts for a few months. We have studied the regulation of transcription factors that promote flowering specifically in response to the long day lengths of summer. We have shown that this response depends on two layers of regulation involving circadian-clock control of transcription and protein degradation that is controlled by light. As a perennial species, we have used *Arabis alpina*, which is a close relative of *A. thaliana*. This species lives for many years, flowers each year and absolutely requires exposure to winter temperatures for flowering. We have shown how specific transcription factors involved in temperature response are differently regulated between these annual and perennial species, and that this enables the perennial species to flower each year. Evolution of the regulation of these genes involves changes in chromatin regulation. We are exploring natural variation in this process in *Arabis alpina* and how vernalization response intersects with the age of the shoot to influence flowering time. I will discuss the mechanisms that confer these seasonal patterns of flowering and how they have evolved.

Structure and Function of Rice Hd3a Florigen

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We have previously demonstrated that Hd3s protein is a rice florigen based on the analysis of transgenic rice plants expressing *Hd3a pro:: Hd3a-GFP*. Since then we found that rice has another florigen RFT1, encoded by a gene highly homologous to *Hd3a*, and that it mainly functions under LD conditions. We also showed that rice does not flower when both florigen genes functionally defective, suggesting that rice absolutely requires florigen for flower induction.

The main focus of our recent research is to study how rice Hd3a florigen functions to induce flowering. One approach is to understand the localization of rice Hd3a florigen in the apical meristem after transition to the flowering stage. We study this by using transgenic rice expressing *Hd3a pro:: Hd3a-GFP.* The second approach is to identify interactors of Hd3a and anayze their functions during floral induction. We found several interactors by yeast two hybrid screens and are now studying their functions and interactions with Hd3a. Recent results will be presented.

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Sustaining the Plant Circadian Clock

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The circadian clock is an endogenous molecular mechanism that anticipates daily environmental changes and optimizes the timing of developmental, physiological, and metabolical processes. Mathematical models have been generated to explain various molecular-expression data, and we used a genetic approach to test these models. We found strong support in explaining mutants whose clocks run too fast. But these models could not explain the behavior of mutants with an arrested clock. Here I will report on molecular-genetic, systems, cell-biological, and biochemical approaches that collectively explain a repressive complex required to "sustain" the oscillator. With a new model on the circadian system, we have started to explore how light-signaling enters the clock. This new hypothesis will be explored.

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