CSRS Early Career Workshop
17 November 2015
Room E717/719

1000 -1005	Opening remarks by Keiko Sugimoto, Team Leader of Cell Function Research Team (RIKEN CSRS)
	Each slot includes 20-25 minutes presentation & 5-10 minutes Q&A time
1005 -1035	**Joanne Hepworth (Dean Lab, JIC) - FLC** **'Releasing FLC back into the wild - Measurement of temperature exposure and integration over time (MEXTIM)'**
1035 -1105	**Daniel Bouyer (Laboratory Vincent Colot, IBENS) - DNA methylation** **'Global DNA methylation reprogramming during plant embryogenesis'**
1105 -1135	**Souha Berriri (Kumar Lab, JIC) - H2A in immunity** **'Temperature modulation of plant immunity and role of H2A.Z'**
1145 -1155	Photo session (Entrance hall in the Main office bldg.)
1200 -1300	Lunch break (Party room in the RIKEN Cafeteria)
	Each slot includes 20-25 minutes presentation & 5-10 minutes Q&A time
1315 -1345	**Vladimir Nekrasov (Kamoun Lab, TSL) - CRISPR /CAS** **'Exploiting the CRISPR/Cas9 technology as a tool for targeted mutagenesis in plants'**
1345 -1415	**Martin Stegmann (Zipfel Lab, TSL) - PAMP receptor** **'Negative regulation of PAMP-triggered immunity by a protease/peptide/receptor module'**
1415 -1445	**Gildas Bourdais (Robatzek Lab, TSL) - endocytosis** **'Regulation of PRR localization and immunity by subcellular transport processes'**
1445 -1455	Break
1455 -1525	**OLIVER J. FURZER (Jones Lab, TSL) - NLR sensor** **'Exploring Brassicaceae NLR diversity with Resistance gene enrichment sequencing'**
1525 -1555	**Chih-Hang Wu (Kamoun Lab, TSL) - NLR sensor** **'Helper redundancy and specificity of NLR immune signaling in solanaceous plants'**
1555 -1600	Concluding remarks by Ken Shirasu, Group Director of Plant Immunity Research Group (RIKEN CSRS)

Joanne Hepworth
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ABSTRACT
Releasing FLC back into the wild - Measurement of temperature exposure and integration over time (MEXTIM)

FLOWERING LOCUS C (FLC) is a major regulator of development in Arabidopsis thaliana, as its expression pattern determines the life history of the plant by controlling the timing of the transition to flowering. In plants carrying active alleles of FLC and its activator FRIGIDA, FLC expression must be repressed by experiencing prolonged cold before flowering can occur, in a process called vernalisation. Vernalisation is a highly studied process that in standardised experiments has shed light on the genetic components required for FLC expression shutdown in response to cold, on the elements that generally control transcriptional processes in plants and on important biological questions such as how memory of environmental information can be stored quantitatively at the level of the gene locus. As a gatekeeper to reproduction, variation at the FLC locus is also under strong selection and variants are known that generate different responses to temperature.

However, although vernalisation allows plants to synchronise flowering with the end of winter, how do plants identify “winter”? In the field during autumn and winter temperatures can daily span twenty degrees, and require up to six weeks to average to a steady decline. In the MEXTIM project we are exploiting this experimentally well-characterised system to determine how organisms can measure and integrate temperature information over months to accurately recognise the passing seasons in time to complete their life cycle.

Daniel Bouyer
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ABSTRACT
Global DNA methylation reprogramming during plant embryogenesis

DNA cytosine methylation (5mC) is a chromatin modification that plays important roles in gene regulation and silencing of transposable elements in plants and mammals. However, in contrast to mammals, the extent to which DNA methylation is reprogrammed during the life cycle remains largely unknown in plants. Using Arabidopsis as a model, we show that DNA methylation is highly dynamic during embryonic and early postembryonic development. On the one hand, gene body methylation, which concerns 20‐30% of all genes and is restricted to CG sites, fluctuates at individual CGs, in agreement with the notion of competing activities between maintenance methylation and demethylation over gene targets. On the other hand, transposable elements and other repetitive sequences have consistently high CG methylation levels, whereas CHG and especially CHH methylation progressively increase during embryogenesis, to reach up to 100% at many individual CHH sites, and decrease rapidly after germination. Moreover, the progressive increase in CHG and CHH methylation in embryos mirrors the loss of DNA methylation at CG and CHG sites in the endosperm, suggesting transfer of information from the endosperm to the embryo, most likely in the form of sRNAs. Finally, impairing the embryo to seedling transition by through loss of PRC2 activity, results in the persistence of high CHH methylation levels after germination, specifically over sequences that are targeted by the RNA‐directed DNA methylation (RdDM) machinery. Collectively, our findings demonstrate

widespread RdDM activity specifically during embryogenesis and points to a critical role of early plant life in establishing or reinforcing silencing of transposable elements.

Souha Berriri
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ABSTRACT
Temperature modulation of plant immunity and role of H2A.Z

Climate change, most importantly increasing global temperatures associated with it poses a severe threat to global agriculture. Elevated temperatures cause increased susceptibility to pathogens resulting in increased severity and extended ranges of crop diseases. Climate change thus poses a huge threat to food security, and it is therefore vital that crop plants with sustainable climate-resilience are developed. In spite of its importance the phenomenon of temperature induced disease susceptibility is not sufficiently well understood at the molecular level. To gain insights into how temperature perception modulates plant immunity we are using a combination of genetic, molecular biology and biochemical approaches. Our study shows that elevated temperature affects PAMP-triggered immunity (PTI). Using RNA-seq analysis we found that genome-wide transcriptional reprogramming in response to Pathogen-Associated Molecular Patterns (PAMP) is dampened at high temperature. Gene expression regulation at elevated temperature was shown to be governed by chromatin dynamics mediated by the histone variant H2A.Z in Arabidopsis. We are therefore investigating its role as well as the chromatin remodeling complex SWR1, responsible for the replacement of H2A containing nucleosomes by H2A.Z in immunity. Results from these studies will be presented.

Vladimir Nekrasov
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ABSTRACT
Nodule development by global control

CRISPR/Cas9 is a rapidly developing genome editing technology that has been successfully applied in many organisms, including model and crop plants. Cas9, an RNA-guided DNA endonuclease, can be targeted to specific genomic sequences by engineering a separately encoded guide RNA with which it forms a complex. As only a short RNA sequence must be synthesized to confer recognition of a new target, CRISPR/Cas9 is a relatively cheap and easy to implement technology that has proven to be extremely versatile. We have developed a CRISPR/Cas9 system for plant applications where expression units are assembled together into a single construct using the Golden Gate cloning method. The system is modular and allows easy replacement of gene elements, such as Cas9 or sgRNA promoters, T-DNA selectable marker etc. In addition, it allows assembly of multiple sgRNAs into a single construct enabling multiplex gene editing in plants. We tested the system in the model plant Nicotiana benthamiana as well as in tomato (Solanum lycopersicum). Remarkably, in tomato, the CRISPR/Cas9 system proved to be particularly efficient and homozygous knockout mutants could be produced as the first generation transgenic plants. Together with other sequence-specific nucleases, CRISPR/Cas9 is a game-changing technology that is poised to revolutionise basic research and plant breeding.

Martin Stegmann
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ABSTRACT
Negative regulation of PAMP-triggered immunity by a protease/peptide/receptor module

Plants utilize surface-localized pattern recognition receptors (PRRs) to sense pathogen-associated molecular patterns (PAMPs) and trigger PAMP-triggered immunity (PTI). We performed a novel forward genetic screen in Arabidopsis thaliana to identify mutants affected in the regulation of PTI signalling. This screen was performed in the bak1-5 mutant background, which represents a unique allele of the PRR co-receptor BAK1 that is specifically impaired in PTI without pleiotropic phenotypes normally observed in bak1 null mutants. The screen resulted in the identification of 10 modifier of bak1-5 (mob) mutants which restore PTI responses. Here, we show that the mob6 mutant is affected in a subtilisin-like serine protease, which was previously described to be required for the cleavage-dependent release of endogenous RALF peptides. Interestingly, consistent with the mob6 phenotypes, mutants in two closely related RALF genes show increased PTI responses. Furthermore, overexpression of the RALF genes results in a strong inhibition of immunity. In addition, we show that application of the corresponding chemically synthesized processed peptides results in the impairment of PTI responses. These results suggest that the regulation of PTI signalling by the MOB6 protease is mediated via the processing of these pro-peptides. Furthermore, we have genetic and biochemical evidence for the receptor kinase required for perceiving these peptides, suggesting a novel protease/peptide/receptor module for the regulation of PTI signalling. This research is funded by grants from the Gatsby Foundation, European Research Council and the German research council.

Gildas Bourdais
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ABSTRACT
Regulation of PRR localization and immunity by subcellular transport processes

Sub-cellular transport processes regulate the localization of key components of the plant's immune system. Our main research focus has been how pattern recognition receptors (PRRs), the primary sensors of the plant's immune system, are transported through the cell and how subcellular transport control immune responses. PRRs are receptor kinases and receptor-like proteins that must be presented at the plasma membrane to recognize invading pathogens. We found that PRRs from different protein families (FLS2, EFR, PEPR1) are internalized in a ligand-induced and BAK1/SERK3 co-receptor dependent manner via a common endosomal pathway. Clathrin-mediated internalization, vesicle scission by dynamin-related proteins and transport to the vacuole are components of this endosomal pathway. Furthermore, our studies have revealed that inhibition of FLS2 endocytosis leads to enhancing bacterial infection through differential modulation of flg22-induced defences. One of these, the closure of stomata in response to flg22 is impaired but not stomatal closure triggered by abiotic stress. To identify molecular components specifically required for stomatal immunity, we performed a genetic screen using quantitative high-throughput life-cell imaging. This revealed additional components of subcellular transport involved in stomatal immunity regulation, including a Rab GTPase playing roles in vacuolar transport. Thus, our research indicates a link between the subcellular transport processes involved in PRR trafficking and defense.

OLIVER J. FURZER
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ABSTRACT
Exploring Brassicaceae NLR diversity with Resistance gene enrichment sequencing

In plants, Resistance genes (R genes) confer strong immunity against pathogens and pests such as bacteria, viruses, fungi, oomycetes and invertebrates. R genes typically encode nucleotide binding, leucine rich repeat proteins (NLRs). R gene enrichment sequencing, "RenSeq", is an RNA based DNA capture method to select the putative NLR encoding gene complement of a plant DNA sample prior to sequencing (Jupe et al, 2013, The Plant Journal). We have used RenSeq and Illumina based genetic mapping to identify functional R genes against various pathogens, in particular the "White Rust"-causing Albugo species that infect the Brassicaceae. We are in the process of identifying more genes, aiming to introduce transgenic R gene stacks to Brassicaceae crops. We also use RenSeq to investigate natural variation in R gene repertoires. NLR encoding gene clusters harbour extensive genetic diversity and are complex and difficult to resolve using Illumina reads. We are using RenSeq in combination with long-read PacBio sequencing to define the diversity of NLRs in wide-ranging Arabidopsis accessions and Brassicaceae species, and will be using this information to further our understanding of the evolutionary history of these fascinating genes.

Chih-Hang Wu
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ABSTRACT
Helper redundancy and specificity of NLR immune signaling in solanaceous plants

Both plants and animals rely on nucleotide-binding domain and leucine-rich repeat- containing (NLR) proteins to respond to invading pathogens and activate immune responses. An emerging concept of NLRs function is that "sensor" NLR proteins are paired with "helper" NLRs to mediate immune signaling. However, our basic knowledge of sensor/helper NLRs in plants remains limited. We aim to understand how the members in the NRC (NLR required for cell death) family, a helper NLR family, cooperate with the sensor NLRs to mediate immune signaling. We found that members in the NRC family are functionally redundant but display specificity to different sensor NLRs from the NRC-sister superclade, the phylogenetic superclade next to the NRC clade. Triple silencing of NRC2, NRC3 and NRC4 abolished the cell death mediated by Rx (a Potato virus X resistance protein), Bs2 (a Xanthomonas campestris resistance protein), Sw5b (a Tomato spotted wilt virus resistance protein) and R8 (a potato late blight resistance protein. However, silencing of NRC2 and NRC3 is sufficient to suppress Prf/Pto-(a bacterial speck resistance protein complex) mediated cell death, and silencing NRC4 is sufficient to suppress Rpi-blb2-(a potato late blight resistance protein) mediated cell death. To further understand how the specificity of NRC proteins is determined, we generated chimeric proteins of NRC3 and NRC4, and tested their signaling specificity. Surprisingly, swapping the leucine-rich repeats of NRCs altered the specificity, suggesting that the molecular determinates of specificity are in the LRR region. These results point out interesting phylogenetic perspectives of sensor/helper NLR evolution and provide new insight into how NLR proteins work together to provide disease resistance in solanaceous plants.