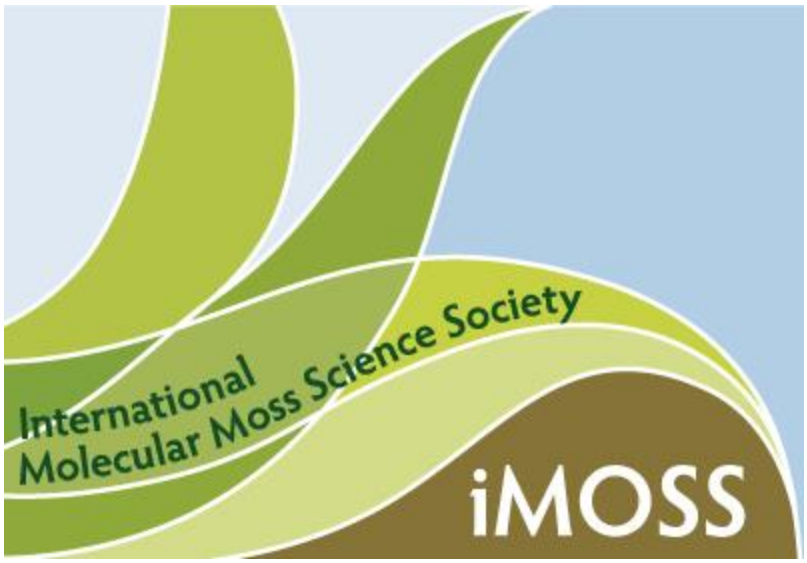

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Thursday, June 22, 2017

Start Time	End Time	Event
8:00 AM	9:00 AM	Breakfast, Registration Opens & Poster Set-Up
9:00 AM	9:05 AM	**Opening Remarks by** Stefan Rensing, University of Marburg
9:05 AM	10:20 AM	**Oral Session I: Metabolism** Chair: Luis Vidali, Worcester Polytechnic Institute
		9:05 AM – 9:30 AM Aruna Kilaru, East Tennessee State University *Novel Polyunsaturated Acylethanolamides and Their Role in* Physcomitrella Patens
		9:30 AM – 9:55 AM Stefanie Müller, Rheinische Friedrich-Wilhelms Universität Bonn *Probing the glutathione-linked Redox Regulation in Chloroplasts: Glutathione Reductase Mutants in* Arabidopsis *and* Physcomitrella
		9:55 AM – 10:20 PM Alison Roberts, University of Rhode Island *Biosynthesis of a Novel Mixed-linkage Arabinoglucan Cell Wall Polysaccharide in* Physcomitrella patens
10:20 AM	10:45 AM	Coffee Break
10:45 AM	12:05 PM	**Oral Session II: Cell Wall** Chair: Jean-Marc Neuhaus, University of Neuchatel
		10:45 AM – 11:10 AM Xingxing Li, University of Rhode Island *A Hetero-oligomeric Cellulose Synthesis Complex (CSC) Involved in the Secondary Cell Wall Deposition in* Physcomitrella patens
		11:10 AM – 11:35 AM Hiroyoshi Takano, Kumamoto University *Chloroplasts in* Physcomitrella patens *are Surrounded by a Peptidoglycan Wall*
		11:35 AM – 12:00 PM Magdalena Bezanilla, University of Massachusetts, Amherst *Evolution of a Fusion between the Exocyst and Actin Nucleation in the Mosses may Reveal a Direct Connection between Actin and Exocytosis*
12:00 PM	2:00 PM	Lunch Break – Boxed Lunch
2:00 PM	3:20 PM	**Oral Session III: Signaling and Development** Chair: Mark Estelle, University of California San Diego
		2:00 PM – 2:25 PM Noémie Fahr, University of Neuchatel *Function of RMR Proteins in the Moss Secretory Pathway*
		2:25 PM – 2:50 PM Shih-Long Tu, Academia Sinica

		Phytochrome Interacts with hnRNP to Silence Prespliceosome Activity and Modulate Alternative Splicing in Physcomitrella patens
		2:50 PM – 3:15 PM Thomas Kleist, Carnegie Institution for Science *Developmental Programming by Calcium and Phytohormone Signaling in Protonema of the Moss* Physcomitrella patens
3:15 PM	4:45 PM	**Coffee Break & Poster Session**
4:45 PM	6:00 PM	**Oral Session IV: Tip Growth** Chair: Magdalena Bezanilla, University of Massachusetts
		4:45 PM – 5:10 PM Luis Vidali, Worcester Polytechnic Institute *Conditional Genetic Screen Reveals a Novel Microtubule Depolymerizing-End-Associated Protein*
		5:10 PM – 5:35 PM Shu-Zon Wu, University of Massachusetts, Amherst *Actin and Microtubule Crosstalk Mediate Persistent Polarized Growth*
		5:35 PM – 6:00 PM Giulia Galotto, Worcester Polytechnic Institute *Unraveling Myosin XI function in* Physcomitrella patens *Tip Growth by Conditional Mutagenesis*
6:00 PM	8:00 PM	Business Meeting & Welcome Reception

Friday, June 23, 2017

Start Time	**End Time**	**Event**
8:00 AM	9:00 AM	Breakfast & Registration Opens
9:00 AM	10:20 AM	**Oral Session V: Development** Chair: Aruna Kilaru, East Tennessee State University
		9:00 AM – 9:25 AM Rabea Meyberg, University of Marburg *Fertility Variation of* Physcomitrella Patens *Ecotypes*
		9:25 AM – 9:50 AM Yukiko Kabeya, National Institute for Basic Biology *PpSBP Transcription Factors Negatively Regulate Stem Cell Formation in* Physcomitrella patens
		9:50 AM – 10:15 AM Kumudu N. Rathnayake, University of South Dakota *Desiccation Tolerance in the Moss* Physcomitrella patens*: Insights into Metabolic Pathway Alterations during Acquisition of Desiccation Tolerance*
10:15 AM	10:45 AM	Coffee Break
10:45 AM	12:25 PM	**Oral Session VI: Cytoskeleton & Growth** Chair: Peter Szovenyi, University of Zurich

		10:45 AM – 11:10 AM Laura Moody, University of Oxford *Identification of No Gametophores 1 (PpNOG1), A Novel Regulator of Three-dimensional Growth in* Physcomitrella patens
		11:10 AM – 11:35 PM Robert Orr, Worcester Polytechnic Institute *Chimeric Myosin XIs and Rab GTPases Uncover a Myosin XI-Mediated Transport Mechanism Conserved between* Physcomitrella patens *and* Arabidopsis *thaliana*
		11:35 PM – 12:00 PM Bascom Jr., University of Massachusetts, Amherst *Simultaneous Imaging and Functional Studies Reveal a Tight Correlation Between Cytosolic Calcium Levels and Actin Network Formation*
12:00 PM	2:00 PM	Lunch Break – Boxed Lunch
2:00 PM	3:20 PM	**Oral Session VII: Beyond *P. Patens*** Chair: Karen Hicks, Kenyon College
		2:00 PM – 2:25 PM Halina Pietrykowska, Adam Mickiewicz University in Poznan *Identification of the Liverwort-specific miRNAs in* Marchantia polymorpha *and Their Role in the Sexual Organs Development*
		2:25 PM – 2:50 PM Anthony Bortolazzo, University of Wisconsin-Madison *Conservation and Autoactivation of a Conserved Mycorrhizal Signaling Module in* Physcomitrella patens
		2:50 PM – 3:15 PM Peter Szovenyi, University of Zurich Anthoceros agresti*, A Model for Hornwort and Comparative Land Plant Biology*
3:15 PM	3:45 PM	Coffee break
3:45 PM	4:15 PM	Awards & Meeting Adjourns
4:15 PM	4:20 PM	Depart of Conference Dinner (Optional)

Speaker Abstracts

Oral Session I: Metabolism

June 22, 2017 | 9:00 AM – 10:15 AM

***Novel Polyunsaturated Acylethanolamides and Their Role in* Physcomitrella patens**

Aruna Kilaru, East Tennessee State University
Imdadul Haq, East Tennessee State University; Jedaidah Chilufya, East Tennessee State University; Shivakumar Devaiah, East Tennessee State University; Suhas Shinde, East Tennessee State University; Ruth Welti, Kansas State University

Anandamide (*N*-arachidonoylethanolamide, AEA), a 20C polyunsaturated (PU) *N*-acylethanolamine (NAE) influences many neurological functions in mammals. Although 20C PU-NAEs are considered unique to animals, they were recently discovered in early land plants but their metabolism and functions remain unknown. Comprehensive lipidomic analyses of *Physcomitrella patens* revealed not only abundance of arachidonic acid (AA, 20:4) and eicosapentaenoic acid (EPA, 20:5) but also their corresponding ethanolamides (AEA and EPEA, respectively). While moss showed increasing AA with development, 14% and 24% in protonemata and gametophyte tissues, respectively, EPA decreased from 7% in protonemata to ~1.3 % in gametophytes. An increase in 20:4- and decrease in 20:5-ethanolamides and their corresponding membrane precursors, phosphatidylethanolamides, also was observed during gametophyte development. Pharmacological studies revealed that AEA specifically inhibits polarized tip growth, which justifies the low endogenous levels of AEA in protonemata. To further determine the physiological relevance of these 20C PU-NAEs, a fatty acid amide hydrolase that catabolizes NAEs has been heterologously characterized. Furthermore, generation of metabolite mutants with altered NAE levels is underway. Overall, we identified two novel NAEs, AEA and EPEA in *Physcomitrella*, which may play an important role in regulation of moss growth and development, although the underlying mechanism is still unclear.

***Probing the Glutathione-linked Redox Regulation in Chloroplasts: Glutathione Reductase Mutants in* Arabidopsis *and* Physcomitrella**

Stefanie Müeller, Rheinische Friedrich-Wilhelms Universität Bonn
Sajid Khan Bangash, Rheinische Friedrich-Wilhelms Universität Bonn; Desiree Guetle, Albert-Ludwigs Universität Freiburg; Ralf Reski, Albert-Ludwigs Universität Freiburg; Andreas Meyer, Rheinische Friedrich-Wilhelms Universität Bonn

Redox regulation involves specific thiol switching in distinct subcellular compartments. Thus, many important metabolic processes in mitochondria and chloroplasts are under the control of Trx-linked thiol switches. In addition, these organelles represent reducing environments via the glutathione (GSH) linked redox-potential that is dependent on GSH abundance and oxidation state.

To date, concerning plastids, the crosstalk between Trx and GSH-linked redox potentials, as well as the impact of the GSH-linked redox potential on plant physiology and performance is still unresolved. We investigate the effects of a more oxidised glutathione redox-potential by characterising mutants with altered plastid glutathione reductase activity in both flowering and non-vascular model plants. Moreover, using the genetically-encoded biosensor redox-sensitive GFP (roGFP), we are able to monitor the GSH-linked redox-potential in life imaging, and to reveal the effects of distinct physiological conditions as well as tissues.

***Biosynthesis of A Novel Mixed-linkage Arabinoglucan Cell Wall Polysaccharide in* Physcomitrella patens**

Alison Roberts, University of Rhode Island
Jelle Lahnstein, University of Adelaide; Arielle Chaves, University of Rhode Island; Tess Scavuzzo-Duggan, University of Rhode Island; Andrew Lonsdale, University of Melbourne; Eric Roberts, Rhode Island College; Geoffrey Fincher, University of Adelaide; Vincent Bulone, University of Adelaide; Monika Doblin, University of Melbourne; Antony Bacic, University of Melbourne; Rachel Burton, University of Adelaid

Mixed-linkage glucan (MLG; β-1,3;1,4-glucan), an abundant cell wall polysaccharide in Poaceae, has been detected in ascomycetes, green algae, and seedless vascular plants, but not in either eudicots or non-commelinid monocots. The Poaceae MLG synthases are similar to cellulose synthases, but only distantly related to ascomycete MLG synthases; all are members of the glycosyl transferase 2 (GT2) family. No MLG synthases have been characterized in algae or seedless plants. However, we identified a predicted GT2 from the P. patens genome that is similar to a bona fide ascomycete MLG synthase. We tested the hypothesis that the P. patens GT2 is an MLG synthase by expressing it in Nicotiana benthamiana and testing for release of diagnostic oligosaccharides by enzymes that cleave MLG. One enzyme released unexpected oligosaccharides and further analysis revealed that they were derived from a novel mixed-linkage arabinoglucan (AG) polysaccharide. The 1KP transcriptome data includes sequences similar to the P. patens AG synthase from algae, bryophytes, lycophytes, and monilophytes, but not seed plants, raising the possibility that AG is widespread in early divergent plants. The similarity of AG synthases to MLG synthases from ascomycetes, but not those from Poaceae, suggests that AG and MLG have a common evolutionary history that includes loss in seed plants, followed by an independent origin of MLG in Poaceae. Supported by NSF IOS-1257047 and ARC COE in Plant Cell Walls CE110001007.

Oral Session II: Cell Wall
June 22, 2017 | 10:45 AM – 12:00 PM

***A Hetero-oligomeric Cellulose Synthesis Complex (CSC) Involved in the Secondary Cell Wall Deposition in* Physcomitrella patens**

Xingxing Li, University of Rhode Island
Alison Roberts, University of Rhode Island

Cellulose synthesis is catalyzed by plasma membrane Cellulose Synthesis Complexes (CSCs) that have been visualized by freeze fracture electron microscopy as rosette structures with 6-fold symmetry. In seed plants, CSCs are obligate hetero-oligomeric, consisting of three functionally distinct and non-interchangeable cellulose synthase (CESA) isoforms. Physcomitrella patens has rosette CSCs, but its seven CESAs are not members of the clades that comprise the functionally distinct subunits of the hetero-oligomeric seed plant CSCs. We know that PpCESA3 and PpCESA8 function redundantly in leaf midrib secondary cell wall deposition. However, it is not known whether the CSCs require other functionally distinct CESA subunits for activity and are therefore obligate hetero-oligomeric. Here we show that double ppcesa6/7KOs phenocopy the ppcesa3/8KO defects in leaf midrib secondary cell wall deposition. Real-Time quantitative PCR (RT-qPCR) analysis shows that expression of PpCESA3, PpCESA8, and PpCESA7 are co-regulated. Based on western blot analysis of isolated proteins, PpCESA3, PpCESA8, and PpCESA7 are all highly expressed in gametophores. Finally, Co-immunoprecipitation (Co-IP) shows that PpCESA3 and PpCESA8 can both interact with PpCESA6/7 in planta. These results indicate that cellulose microfibrils in the secondary cell walls of P. patens leaf midribs are synthesized by obligate hetero-oligomeric CSCs. This research was supported by National Science Foundation Award IOS-1257047.

Chloroplasts in* Physcomitrella patens *are Surrounded by a Peptidoglycan Wall

Hiroyoshi Takano, Kumamoto University
Takayuki Hirano, Kumamoto University; Koji Tanidokoro, Kumamoto University; Shinji Tadano, Kumamoto University; Hayato Ishikawa, Kumamoto University; Susumu Takio, Kumamoto University; Katsuaki Takechi, Kumamoto University

It is believed that the plastids in green plants lost peptidoglycan during their evolution. Nevertheless, the moss Physcomitrella patens has the genes required to generate peptidoglycan, and the knockout of these genes causes defects in chloroplast division, suggesting that a peptidoglycan system is present in moss chloroplasts. However, the existence of plastid peptidoglycan has not been demonstrated because wall-like structures could not be detected in the plastids of green plants. We visualized moss plastid peptidoglycan using a metabolic labeling method with click chemistry (Hirano et al. 2016). To incorporate a D-alanyl-D-alanine (DA-DA) analog with the ethynyl group into peptidoglycan, we isolated a homolog of the bacterial peptidoglycan-synthetic gene encoding D-alanine (D-Ala):D-Ala ligase (Ddl) from the P. patens genome (PpDdl) and generated a knockout line (ΔPpDdl) with a macrochloroplast phenotype. The addition of D-Ala-D-Ala to the medium suppressed the appearance of giant chloroplasts in ΔPpDdl, but not the addition of D-Ala or L-Ala-L-Ala. The ethynyl-DA-DA used in the click reaction also

complemented the mutant phenotype. Click reaction chemistry attached an azide-modified fluorophore to the ethynyl group. The results of click chemistry for the ΔPpDdl line complemented with ethynyl-DA-DA showed that moss chloroplasts are fully surrounded by peptidoglycan. Our findings strongly suggest that the plastids in basal green plants have a peptidoglycan wall.

Evolution of a Fusion between the Exocyst and Actin Nucleation in the Mosses May Reveal a Direct Connection between Actin and Exocytosis

Magdalena Bezanilla, University of Massachusetts, Amherst
Peter van Gisbergen, University of Massachusetts, Amherst; Shu-Zon Wu, University of Massachusetts, Amherst; Mingqin Chang, University of Massachusetts, Amherst; Kelli Pattavina, University of Massachusetts, Amherst; Madelaine Bartlett, University of Massachusetts, Amherst

P. patens contains For1F, an unusual essential gene that encodes for a protein with a novel domain configuration comprised of an N-terminal Sec10 domain and a C-terminal class I formin. Sec10 is one of eight subunits of the exocyst complex, a conserved complex that tethers secretory vesicles to the plasma membrane during exocytosis. Formins nucleate and elongate actin filaments. We tagged the For1F locus with three tandem copies of GFP and showed using immunoblots that a protein consistent with fusion of these two domains is expressed. Here, we show that loss of For1F reduces exocytosis. We find that For1F dynamically associates with Sec6, another exocyst subunit, and dissociation of Sec6/For1F requires actin. Paradoxically, we show that constitutive expression of either half of the fusion gene can rescue loss of For1F, suggesting that fusion of the two domains is not essential. Furthermore, constitutive expression of the paralogous Sec10b also rescues loss of For1F. Thus, we propose that exocyst complex function requires dynamic association with class I formins, linking exocytosis to actin polymerization. Using phylogenetic analyses, we show that the fusion gene is present in evolutionarily diverse mosses and most likely emerged after the early diverging mosses, Sphagnum and Takakia. While the fusion is not essential in protonemata, its continued presence in many moss species suggests that direct linkage of the exocyst and actin nucleation may have had selective advantages.

Oral Session III: Signaling and Development

June 22, 2017 | 2:00 PM – 3:15 PM

Function of RMR proteins in the Moss Secretory Pathway

Noémie Fahr, University of Neuchatel
Didier Schaefer, University of Neuchatel; Jean-Marc Neuhaus, University of Neuchatel

Targeting of proteins to the lytic vacuole have been extensively studied in flowering plants. So far, less in known about the traffic of proteins to the neutral/storage vacuole. A family of putative vacuolar receptors named RMRs (Receptor Membrane RING-H2) has been identified. They might be involved in

protein targeting to neutral vacuole but their role is still unclear. We aim to understand the mechanisms of action of RMRs in the experimental plant model, Physcomitrella patens.

P. patens has five RMR genes. Single, multiple and quintuple RMR knock-out mutants were produced but they do not show any obvious phenotype. The first part of this work was to create a library of fluorescent reporters to study the secretory pathway in moss. These markers were used to characterize the RMR mutants. A trafficking phenotype was observed with one reporter expressing a vacuolar sorting determinant from cardosin A. Vacuolar targeting of this reporter was disrupted in all the mutant lines.

RMRs are members of the PA-TM-RING family, which in animals have been found to act as E3 ubiquitin ligases (e.g. GRAIL in mammals). This suggested that plant RMRs could be E3 ligases as well. Ubiquitination may even play a role in protein trafficking to the vacuoles. Consequently, the second part of this project aimed to identify RMR partners by proteomic analysis. GST pull-down assays allowed the putative identification of some proteins interacting with RMRs.

***Phytochrome Interacts with hnRNP to Silence Prespliceosome Activity and Modulate Alternative Splicing in* Physcomitrella patens**

Shih-Long Tu, Academia Sinica
Chueh-Ju Shih, Academia Sinica; Hsiang-Wen Chen, Academia Sinica; Hsin-Yu Hsieh, Academia Sinica; Yu-Rong Chen, Academia Sinica; Tuan-Nan Wen, Academia Sinica

Alternative splicing (AS) is a widespread mechanism in eukaryotes that generate 2 or more mRNAs from the same pre-mRNA by using different splice sites. Splice site selection is largely influenced by splicing regulators that recognize regulatory cis elements to recruit spliceosomal components and initiate splicing reactions. Although the molecular mechanism of AS has been extensively studied in plants, whether and how it is modulated is still little elucidated. Genome-wide analyses have showed that light induces intensive AS in Physcomitrella patens and Arabidopsis thaliana. Photoreceptors especially the red/far-red sensing phytochrome directly participates in splicing regulation. We further found that Physcomitrella phytochrome 4 displays a red light-dependent interaction with a splicing regulator, the heterogeneous nuclear ribonucleoproteins (hnRNP) in vitro and in vivo. Red light promotes the phosphorylation of the hnRNP. The hnRNP also showed red light-stimulated, phytochrome-dependent binding with a U1 spliceosomal component, PRP39. Furthermore, PRP39 interacts with the U1 snRNP through one of its component U1-C. Global analyses demonstrated the involvement of PRP39 in light-mediated splicing regulation. These results suggest that phytochromes regulate AS through hnRNPs to interact with spliceosomes and control the activities of splicing machinery. We are also adopting a method to globally identify the RNA targets of the hnRNP. Details will be discussed.

Oral Session IV: Tip Growth
June 22, 2017 | 4:30 PM – 6:00 PM

Conditional Genetic Screen Reveals a Novel Microtubule Depolymerizing-End-Associated Protein
Luis Vidali, Worcester Polytechnic Institute
Xinxin Ding, University of Wisconsin, Madison; Leah Pervere, Worcester Polytechnic Institute; Carl Bascom, University of Massachusetts, Amherst; Jeffrey Bibeau, Worcester Polytechnic Institute; Sakshi Khurana, Worcester Polytechnic Institute; Allison Butt, Worcester Polytechnic Institute; Robert Orr, Worcester Polytechnic Institute; Patrick Flaherty, University of Massachusetts, Amherst; Magdalena Bezanilla, University of Massachusetts, Amherst

Our ability to identify essential genes that direct cell growth and division is limited because their loss often leads to lethality. A solution to this is to isolate conditional mutants where the phenotype is visible under restrictive conditions. Here, we capitalize on the haploid growth-phase of the moss Physcomitrella patens to identify conditional loss-of-growth (CLoG) mutants with impaired growth at high temperature. We used whole-genome sequencing of pooled segregants to pinpoint the lesion of one of these mutants (clog1) and validated the identified mutation by rescuing the conditional phenotype by homologous recombination. We found that CLoG1 is a novel and ancient gene conserved in plants. Fluorescent protein fusions of CLoG1 indicate it is microtubule associated with bias towards depolymerizing microtubule ends. By discovering a novel gene important for plant growth, our work demonstrates that P. patens is an excellent genetic system to study genes with critical function in plant development. Support was provided by PhRMA Foundation Informatics Grant 2013080079 to PF, NSF-MCB-1330171 to MB, and NSF-MCB-1253444 to LV.

Actin and Microtubule Crosstalk Mediate Persistent Polarized Growth
Shu-Zon Wu, University of Massachusetts, Amherst
Magdalena Bezanilla, University of Massachusetts Amherst

A dynamic actin cytoskeleton is essential for tip growth in mosses. Without actin, cells swell at their apexes and tip growth stops. Actively growing wild type cells have an accumulation of actin just below the cell tip. While this apical actin accumulation is persistent over long time periods, imaging with high temporal resolution reveals that this population of actin filaments is highly dynamic. Without microtubules, the apical actin accumulation loses the longer temporal persistence and instead stochastically forms and reforms in various regions of the cell, often leading to cell expansion at other sites.

In wild type cells, cytoplasmic microtubules are focused with their plus ends just below the apical actin accumulation. We find that in a mutant lacking all five myosin VIII genes, the microtubule focus is lost and the actin accumulation loses the long term persistence similar to loss of microtubules. Interestingly directional persistence is impaired in this mutant. We also find that myosin VIII localizes at the junction between the focused microtubule plus ends and the apical actin accumulation. These data suggest that

microtubules are required to stabilize apical actin and myosin VIII is the physical link between the two cytoskeletons.

Unraveling Myosin XI function in* Physcomitrella patens *Tip Growth by Conditional Mutagenesis
Giulia Galotto, Worcester Polytechnic Institute
Jeffrey Bibeau, Worcester Polytechnic Institute; Yen-Chun Liu, Worcester Polytechnic Institute; Pattipong Wisanpitayakorn, Worcester Polytechnic Institute; Erkan Tuzel, Worcester Polytechnic Institute; Luis Vidali, Worcester Polytechnic Institute

In the Physcomitrella patens protonemal stage, cells elongate by tip growth, a polarized growth dependent on actin cytoskeleton and cell wall secretion. The actin-associated motor myosin XI plays an essential role in tip growth. Moss plants where myosin XI genes have been silenced exhibit an altered morphology composed of round cells: the tip growth process is blocked; so, knockdown studies cannot be used to characterize the role of myosin XI. To overcome this limitation we developed conditional mutant moss plants in which myosin XI protein is temperature sensitive (TS). When TS plants are exposed at 32°C, myosin XI becomes non-functional. We used the TS mutant line to study the role of myosin XI in the tip growth process, in combination to actin deprivation through application of Latrunculin B. When the temperature is switched to 32°C, the cells undergo morphological changes visible within 3 hr: the zone behind the tip swells, and the extreme tip narrows. Both TS and control cells treated with Latrunculin B also exhibit a similar swelling phenotype. Furthermore, we observed that when TS plants are exposed to 32°C for 24 hr, a significant number of caulonema cells die, compared to the TS maintained at 20°C or the WT exposed to 32°C. These results suggest that myosin XI is involved in tip growth guiding exocytosis to the cell apex and determining the polarized morphology of the cell, and that myosin XI is important for caulonema cell survival. Supported by NSF-MCB-1253444.

Oral Session V: Development
June 23, 2017 | 9:00 AM – 10:15 AM

Fertility Variation of* Physcomitrella patens *Ecotypes
Rabea Meyberg, University of Marburg
Manuel Hiss, University of Marburg; Jens Westermann, University of Marburg; Lucas Schneider, University of Marburg; Rebecca Hinrichs, University of Marburg; Stefan A. Rensing, University of Marburg

This project focuses on the factors influencing sexual reproduction of Physcomitrella patens ecotypes Reute, Villersexel and different Gransden variants. We find that gametangiogenesis and ripening of the gametangia occur in a similar time frame and without any gross morphological differences. Yet, the sporophyte analysis revealed a severe and significant reduction of sporophytes per gametophore in the Gransden strains, one of which is close to sterile. Subsequent crossing experiments showed that Gransden strains were capable of developing sporophytes on up to 95% of the gametophores if fertilized

by Villersexel or Reute spermatozoids. This observation points out apparently recurring male fitness problems in the Gransden strains and is reinforced by a spermatozoid analysis revealing aberrant morphology and strongly reduced motility of Gransden spermatozoids. A comparative transcriptomic analysis between antheridia of the studied ecotypes is planned in order to gain detailed knowledge of expression differences and putative SNPs of genes expressed in the antheridia. In parallel, candidate genes were selected for their putative involvement in the process of sexual reproduction. They were validated by qPCR and analyzed regarding their genetic and epigenetic differences between ecotypes. Deletion mutants are being generated and will be analyzed. We aim to gain a understanding of factors influencing the efficiency of sexual reproduction the moss Physcomitrella patens.

***PpSBP Transcription Factors Negatively Regulate Stem Cell Formation in* Physcomitrella patens**

Yukiko Kabeya, National Institute for Basic Biology
Yohei Higuchi, ERATO, Japan Science and Technology Agency; Chaoyang Cheng, ERATO, Japan Science and Technology Agency; Yoshikatsu Sato, ERATO, Japan Science and Technology Agency; Yosuke Tamada, National Institute for Basic Biology; Mitsuyasu Hasebe, National Institute for Basic Biology

Stem cells are origins of most parts of a plant body and their activities should be properly regulated. Especially in differentiated cells, stem cell activity has to be repressed, although factors directly repress stem cell activity have not been reported in land plants. After leaf dissection in the moss Physcomitrella patens, differentiated leaf cells facing the cut change to protonema apical stem cells. Here, we show that Physcomitrella patens SQUAMOSA PROMOTER BINDING PROTEIN 4 (PpSBP4), PpSBP7, PpSBP8, and PpSBP12 genes negatively regulate the stem cell formation. The four PpSBP genes form a clade and their transcripts decreased after the leaf excision. Deletion of the four PpSBP genes facilitates the stem cell formation, while the induction of PpSBP4 retarded the process. As one of mechanisms to reduce the transcript amounts, we found that the repressive histone modification, H3K27me3 level on PpSBP genomic regions increased after the cut. Since HISTONE GENE REPRESSOR A1 (HIRA1) and HIRA2 function in the regulation of H3K27me3 levels in protonemata and are induced by leaf cut, we investigated H3K27me3 levels on PpSBP genes in hira double loss-of-function mutants and found the retardation of H3K27me3 depositions. With incorporating the negative regulation of PpSBPs by CRYPTOCHROME genes and the necessity of light for the stem cell formation, PpSBPs function as a connecting node of light signaling and histone modification pathways by wounding.

Desiccation Tolerance in the Moss* Physcomitrella patens*: Insights into Metabolic Pathway Alterations during Acquisition of Desiccation Tolerance

Kumudu N. Rathnayake, University of South Dakota
Karen L. Koster, University of South Dakota; Danny C. Alexander, Metabolon, Inc.; Bernard W. M. Wone, University of South Dakota

Physcomitrella patens does not typically survive rapid desiccation but becomes desiccation tolerant (DT) after exogenous abscisic acid (ABA) application, or very slow dehydration. To build a systems-level understanding of the acquisition of desiccation tolerance, we developed an acclimation protocol to

induce tolerance without exogenous ABA in both protonemata and gametophores of Physcomitrella. We then profiled the global metabolites of desiccation sensitive (DS), acclimated DT (Acc-DT), and ABA-treated DT (ABA-DT) protonemata and gametophores to find patterns of metabolic changes that relate to desiccation tolerance.

A total of 512 metabolites were characterized among the treatments. Endogenous ABA increased significantly in Acc-DT samples compared to DS samples, confirming ABA's important role in the acquisition of desiccation tolerance in Physcomitrella. Soluble sugars, nitrogen-rich amino acids, TCA intermediates, and some antioxidants accumulated in Acc-DT tissues, similar to ABA-DT moss. Glutathione pathway metabolites increased significantly in both Acc-DT samples compared to DS controls. Membrane lipids with polyunsaturated fatty acids increased significantly in Acc-DT protonemata during late acclimation, suggesting membrane remodeling. Our results indicate that both young and mature stages of Physcomitrella acquire desiccation tolerance through altered metabolic pathways, yet the responses to acclimation differed in many aspects from responses to exogenous ABA.

Oral Session VI: Cytoskeleton & Growth

June 23, 2017 | 10:45 AM – 12:00 PM

***Identification of No Gametophores 1 (PpNOG1), A Novel Regulator of Three-dimensional Growth in* Physcomitrella patens**

Laura Moody, University of Oxford
Steven Kelly, University of Oxford; Ester Rabbinowitsch, University of Oxford; Jane Langdale, University of Oxford

Multicellular eukaryotes exhibit 3-dimensional (3D) body plans that result from the elaboration of two or three growth axes. Although studies in a range of organisms have investigated the mechanisms underpinning the establishment of individual axes, we have little understanding of how 3D growth per se is initiated. In flowering plants the onset of 3D growth occurs within the earliest divisions of the fertilized zygote. As such, it is virtually impossible to genetically dissect the underlying mechanisms because mutants would be embryo lethal and a compromised switch to 3D growth would be difficult to distinguish from many other causes of lethality. In early divergent plant lineages such as the mosses, however, the production of 3D shoots is often preceded by an extended 2D growth phase.

Using UV-induced mutagenesis, we generated mutants in Physcomitrella patens that failed to initiate 3D growth and devised a novel strategy to map the causative mutations. The strategy is based on genome-wide bulk segregant analysis of progeny derived from somatic hybrids that were generated between polymorphic lines. This innovative approach enabled us to determine that a 3D-defective mutant phenotype was caused by the introduction of a premature termination codon in the No Gametophores 1 (PpNOG1) gene. PpNOG1 overexpression restored 3D growth to the mutant confirming correct gene identification.

***Chimeric Myosin XIs and Rab GTPases Uncover a Myosin XI-Mediated Transport Mechanism Conserved between* Physcomitrella patens *and* Arabidopsis thaliana**

Robert Orr, Worcester Polytechnic Institute
Fabienne Furt, Worcester Polytechnic Institute; Mary Munson, University of Massachusetts Medical School; Luis Vidali, Worcester Polytechnic Institute

The diverse morphologies observed across plants and animals require a coordinated asymmetry of intracellular components organized by the cytoskeleton. We leveraged the excellent tip growth and genetics of P. patens to answer the unknown question of how myosin XI associates with secretory vesicles to drive polarized growth. Here we exploited P. patens' only two functionally redundant myosin XIs, compared to the 13-member A. thaliana myosin XI family, to elucidate putative cargo-binding interfaces. We generated chimeric myosin XIs through fusions of the P. patens head domain with three isoforms of the cargo-binding tail domain of A. thaliana (K,E, and F) that vary in sequence similarity, subcellular localization, and known function. Chimeric myosin XIs K and E reconstituted polarized growth in moss in an RNAi complementation assay, whereas isoform F failed to complement. Following this result, we attempted to identify the heretofore unknown myosin XI vesicle receptor through an extensive yeast two-hybrid screen. This approach identified a RabE subfamily member (Sec4 in yeast, Rab8 in human) as a putative binding partner. Preliminary data with in-house directed yeast two-hybrid and bimolecular binding assays with purified myosin XI and RabE suggests a direct interaction. These results support the notion of sequence-level functional conservation of myosin XI transport across the plant kingdom. Supported by NSF-MCB-1253444

Simultaneous Imaging and Functional Studies Reveal a Tight Correlation Between Cytosolic Calcium Levels and Actin Network Formation

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Tip growing cells elongate in a polarized manner via focused exocytosis. In pollen tubes calcium and actin are vital components of a feedback loop regulating the deposition cell wall material. We investigated the calcium-actin interplay in the genetically tractable moss, Physcomitrella patens. To visualize cytosolic calcium, we used a genetically encoded cytosolic FRET probe, revealing a previously unreported fluctuating tip-ward gradient. Employing microfluidic devices to image protonemata, we showed that the calcium gradient is altered when growth is inhibited mechanically, pharmacologically, or in actin regulatory mutants, suggesting that calcium is downstream of growth changes. We simultaneously imaged cytosolic calcium and actin. In wildtype, the intensity and size of the tip-focused actin spot and cytosolic calcium levels are anticorrelated. Abolishing the calcium gradient with 0.1mM $LaCl_3$ results in an accumulation of actin at the tip, suggesting apical actin is tightly regulated by cytosolic calcium levels. In mutant plants with suppressed actin dynamics, apical actin is absent and thick actin bundles form on the subapical domain coincident with low cytosolic calcium levels, suggesting that with suppressed dynamics actin bundles are promoted in low calcium states. Together

these data indicate that low calcium promotes actin accumulation. Thus, P. patens presents an excellent system to genetically identify and dissect the actin-calcium feedback during tip growth.

Oral Session VII: Beyond P. Patens

June 23, 2017 | 2:00 PM – 3:15 PM

Identification of the Liverwort-specific miRNAs in Marchantia polymorpha *and Their Role in the Sexual Organs Development*

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MicroRNAs play a crucial role in eukaryotic gene expression regulation. At least ten conserved miRNA families have been reported to play a key role during flower development in higher plants. However, the knowledge about the role of microRNAs in liverwort sexual organ development is limited. We decided to study if there are microRNAs involved in sexual organs development and function in M. polymorpha. In our previous studies we identified 33 novel microRNA families in the liverwort P.endiviifolia. Analysis of the recent M. polymorpha sRNAs sequencing results revealed the presence of 13 miR families which were previously described as Pellia-specific. These families can be regarded as liverwort-specific. MiR8190 and miR8170 were highly expressed in archegoniophores and antheridiophores. miR8185 and miR8166 were present only in antheridiophores. Additionally, miR8163 and miR8181 were present at the lower level in the reproductive organs than vegetative thalli. Using degradome sequencing technique, new targets for these miRNAs were identified. The obtained results already suggest the involvement of these microRNAs in generative organs development/function. We obtained mutants OE-miR8185,miR8163 and the mutants with target mimicry for the miR8185. Analyses of these mutants with respect to sexual organ and sporophyte development will be discussed. This research is supported by NCN(UMO2014/13/N/NZ3/00321) and KNOW(No.01/KNOW2/2014).

Conservation and Autoactivation of a Conserved Mycorrhizal Signaling Module in Physcomitrella patens

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Arbuscular mycorrhizal (AM) interactions are controlled by a conserved plant signaling pathway inherited from the common ancestor of land plants. Many genes of this pathway are absent in lineages that have lost AM associations, such as the Brassicaceae. The calcium/calmodulin-dependent protein kinase (CCaMK) and its target Interacting Protein of DMI3 (IPD3), both required for AM colonization, are

among the genes most consistently lost in non-host lineages. Contrary to this, many mosses and some charophytes do not form AM-like associations but contain strong homologs of CCaMK and IPD3. We used biochemical and genetic approaches to study the function of this signaling module in non-mycorrhizal lineages. Yeast two-hybrid assays showed that one CCaMK and IPD3 maintain their interaction in Physcomitrella patens. Kinase assays with this P. patens CCaMK indicated it retained calcium- and calmodulin-dependent activity as described in angiosperms. This P. patens CCaMK gene rescued the symbiotic defects of a legume ccamk mutant. We expressed autoactive isoforms of CCaMK and IPD3 in P. patens. Activation of this module led to the development of drought-protective brachycytes, a well-characterized response to abscisic acid (ABA). These tissues contained elevated levels of ABA and Late Embryogenesis Abundant transcripts. The significance of the link between CCaMK/IPD3 and drought-responses in mosses, other land plants, and charophytes will be discussed.

Anthoceros agrestis*, A Model for Hornwort and Comparative Land Plant Biology*
Peter Szovenyi, University of Zurich

The monophyletic group of hornworts is believed to represent the immediate sister group of all vascular land plants. However, this traditional view is still debated and cannot be satisfactorily resolved owing to the lack of detailed knowledge on the general biology and genomic features of hornworts. Until now, advancement in this field was primarily hindered by the lack of genomic resources for a hornwort model species. Here we provide an update on the efforts towards a high-quality genome draft of the model hornwort, Anthoceros agrestis, and some of its relatives. We show that A. agrestis has a remarkably small genome, with few recent paralogs, which makes it approriate for genetic analysis. We also provide an overview of the A. agrestis gene space and a preliminary gene expression atlas which shed light on the regulation of morphological and developmental traits that are either shared with other embryophytes or unique to hornworts. Furthermore, we report our first achievements on the genetic transformation of A. agrestis using various techniques. Finally, we summarize our achievements and provide a list of issues that need to be resolved in the future.

Poster Abstracts

P1 *Role of Salicylic Acid as an Ancestral Hormone in the Regulation of Stress Responses in Plants*
Alexandra Castro, Facultad de Ciencias
Cecilia Ruibal, Facultad de Ciencias; Tomáš Pluskal, Whitehead Institute for Biomedical Research; Valentina Carballo, Whitehead Institute for Biomedical Research; Jing-Ke Weng, Whitehead Institute for Biomedical Research; Sabina Vidal, Facultad de Ciencias

Abiotic stress includes among others, water scarcity or excess, extreme temperatures and salinity. In recent years, several studies indicate the importance of salicylic acid (SA) in improving tolerance to abiotic stress in plants. Despite its importance in stress biology, the mechanisms involved in SA-mediated abiotic stress tolerance remain poorly understood. In addition, little is known about the

function of SA in primitive plants, and how conserved are the SA-mediantedresponses along the evolutionary timeline of plants. Mosses are basal land plants that have diverged from flowering plants at least 450 million years. Until now, there is no experimental evidence to support a role of SA in mosses. In this work, we used the moss Physcomitrella patens to assess the function of SA in plant responses to abiotic stress factors. Here we show that the endogenous levels of SA in Physcomitrella increase in response to heat and that treatment of wild type plants with SA increases thermotolerance. The mechanism used by SA to confer tolerance was explored through the generation and phenotypic evaluation of mutant lines carrying the salicylate hydroxylase (NahG) transgene that degrades SA. Our data show that NahG plants are more susceptible to heat than the WT. Furthermore, comparative transcriptome analysis revealed that SA treatment or heat exposure of WT or NahG plants results in the differential expression of several genes, many of which are involved in stress.

P2 ***A Member of the WCOR413 Protein Family in*** **Physcomitrella patens** ***is Involved in High Light and Low Temperature Stress Responses***

Cecilia Ruibal, Facultad de Ciencias, Universidad de la República
Luciana Fleitas, Facultad de Ciencias, Universidad de la República; Alexandra Castro, Facultad de Ciencias, Universidad de la República; Jorge Quezada, Facultad de Ciencias Farmacéuticas y Bioquímicas, Universidad Mayor de San Andrés; Sabina Vidal, Facultad de Ciencias, Universidad de la República

The moss Physcomitrella patens is highly tolerant to different abiotic stress factors and thus a model for studying mechanisms of stress tolerance in plants. In this study we describe the functional characterization of an abscisic acid up-regulated gene (PpCOR413A) from P.patens, encoding a protein belonging to the WCOR413 family.

These protein types were first described in wheat in relation to low temperature stress, but their precise function is still largely unknown. In silico analysis indicate the presence of several transmembrane domains (TrM) in their deduced amino acid sequence, and suggest a subcellular localization in plasma membrane or chloroplast thylakoid membrane. The genome of P.patens contains five genes encoding seven deduced WCOR413 proteins. Here we demonstrate that PpCOR413A, localizes in chloroplasts by using expression of PpCOR413A-GFP fusion proteins in different plant tissues. To assess the function of PpCOR413A in abiotic stress responses, we used gene targeting to generate disruption mutants of PpCOR413A gene.

Phenotypic characterization of the knockout mutants showed a severe reduction in plant growth under high light conditions. Furthermore, these plants exhibited significant alterations in various photochemical parameters, such as NPQ kinetics and PSII quantum yield, in response to high light or low temperatures. These results suggest that PpCOR413A plays a direct or indirect role in protection of the photosynthetic apparatus during stress.

P3 ***Four AUX/LAX*** **Physcomitrella patens** ***Homologues are Specifically Expressed, and Their Spatiotemporal Pattern Suggests Involvement in Developmentally Regulated Auxin Homeostasis***

Stefan Schwarzbach, Swedish University of Agricultural Sciences
Katarina Landberg, Swedish University of Agricultural Sciences; Eric Pederson, Stockholm University; Mattias Thelander, Swedish University of Agricultural Sciences; Eva Sundberg, Swedish University of Agricultural Sciences

The phytohormone auxin is an important signaling molecule that triggers differentiation and developmental switches in all land plants studied. Local peaks of auxin, necessary for developmental decisions, are largely mediated by local biosynthesis and polar transport. In Physcomitrella patens, just as in flowering plants, long PIN proteins mediate auxin efflux, and thus canalize the transport from production sites to sites where auxin is needed (Viaene et al., 2014; Bennett et al., 2014). As auxin influx carriers instead often are expressed where the actual auxin peak sites should be formed (Swarup et al., 2008), thereby revealing the spatiotemporal need of auxin peaks, we here aimed to characterize their expression domains in moss. We identified four closely related homologues to the Arabidopsis AUX/LAX auxin influx family and made transcriptional reporter lines of all four. The PpLAX genes form two gene pairs, PpLAXA and B, and PpLAX C and D. The first pair shows several expression sites in which they overlap, but also some in which they differ, while PpLAXC and D are more weakly expressed and limited to sites shared by all four genes. Overall, this expression pattern overlaps with sites known to need high auxin levels for developmental decisions, thus pointing towards a conserved function as influx carriers, which, however, still needs to be confirmed.

P4 ***Investigating the Genetic Pathways Controlling Meristematic Activity in Moss- and Hornwort Sporophytes***

Manuel Waller, University of Zürich
Alexander Kirbis, University of Zurich; Ana Marcela Florez-Rueda, University of Zurich; Ueli Grossniklaus, University of Zurich; Péter Szövényi, University of Zurich

Genetic pathways controlling meristematic activity in the Shoot Apical Meristem (SAM) of flowering plants have been a major research-interest for the past 25 years. The CLAVATA/WUSCHEL-pathway has been studied extensively in A. thaliana and is the best-known pathway involved in SAM maintenance today.

Orthologs of some components involved in this pathway were found in the genomes of bryophytes, specifically the hornwort Anthoceros agrestis and the moss Funaria hygrometrica. The sporophytes of those bryophytes do not exhibit an indeterminately active meristem comparable to the SAM of flowering plants, but they do show different meristematic regions. The Funaria-sporophyte forms an intercalary meristem, by which the seta grows until spore capsule maturation. Hornwort sporophytes, on the other hand, grow from a basal meristem. To uncover a possible ancestral relationship of different bryophyte-sphorophyte meristems and the SAM, we are investigating the genetic pathways controlling meristematic activity in the Funaria intercalary-meristem and the Anthoceros basal-meristem.

We are establishing transcriptional profiles of different tissues within the meristematic regions of Funaria- and Anthoceros-sporophytes using Laser-assisted Microdissection and RNA sequencing. We will

then investigate the role of potential meristem-controlling genes by generating knock-outs. Here we summarize our first results and their implication on the evolution of multicellular meristems in land plants.

P5 *Sex Chromosome Evolution and Linkage Mapping in* Marchantia polymorpha

Oliver Subotic, University of Zürich
Rachel Murray-Watson, University of Zürich; Filip Boskovic, University of Belgrade; Stuart McDaniel, University of Florida; Adam Payton, University of Florida; Elena Conti, University of Zürich; Peter Szovenyi, University of Zürich

In dioecious organisms, sex is frequently determined genetically in the form of sex chromosomes. In diploid organisms, sex chromosomes are often heteromorphic differing in physical size and gene content. This is explained by asymmetric heterozygosity and suppression of recombination in the hemizygous sex chromosome. In organisms with a haploid dominant life cycle, such as liverworts, both sex chromosomes are equally hemizygous and should therefore experience similar evolutionary forces and follow similar evolutionary trajectories. However, in liverworts, this is contradicted by the occurrence of highly heteromorphic sex chromosomes. We intend to use the emerging plant model system Marchantia polymorpha and closely related species M. paleacea, M. inflexa and M. quadrata as a model to gain insights into the evolution of sex chromosomes under haploid dioecy. We will make use of long-read sequencing platforms which are able to span highly repetitive regions in sex chromosomes and thus improving contiguity of their assemblies.

We set up a cross population in M. polymorpha and genotyped two parents and 90 progeny by ddRAD sequencing. We generated a high-density linkage map producing eight linkage groups likely corresponding to the eight autosomes. Using this linkage map we were able to assign scaffolds from the M. polymorpha draft genome to the corresponding linkage groups producing a chromosome -level assembly which will provide a useful resource for further research .

P6 *High-fidelity Digital Microscopic Observation of Entrapment on the Surface of* Aphanoregma patens *Leaves*

Yoshihiro Takikawa, Kindai University
Hirotoshi Sawada, Kindai University; Saimon Iwasaki, Kindai University; Teruo Nonomura, Kindai University; Koji Kakutani, Kindai University; Yoshinori Matsuda, Kindai University

The cormus (pre-gametophytic body) stage of the moss Aphanoregma (syn. Physcomitrella) patens dominates its life cycle. When the leaf surface of cormi is exposed to airborne fungal spores, the leaf surface is the site of an interaction against the fungal spores. However, little attention has been paid to the responses of the leaf surface to aerial microorganisms that land on the cormi. We discovered that A. patens can digest fungal pathogens inoculated on the leaf surface. For efficient digestion, the leaf surface must possess the ability to entrap aerial microorganisms that fall on the cormi. However, this ability has not been characterized. We investigated the entrapment ability of the A. patens leaf surface

using powdery mildew fungi as model microorganisms. Powdery mildews are fungal pathogens that infect many plants. The mature conidia of powdery mildews are readily dispersed by the wind. We inoculated conidia onto the leaves of A. patens using a glass needle under a high-fidelity digital microscope. The inoculated conidia adhered to the leaf surface. Moreover, a trace of adhered conidia was observed on the leaf surface after the conidia were removed from the leaf surface using a glass needle. This trace disappeared with a water-flow treatment using a micropipette. These results show that the leaf surface has the ability to trap organisms reaching the cormi.

P7 *ANGUSTIFOLIA Regulates Cell Expansion in Stems of Gametophores in* Physcomitrella patens

Hiroaki Nagase, Kumamoto University
Yoshikazu Hashida, Kumamoto University; Katsuaki Takechi, Kumamoto University; Tomoyuki Yabe, Kumamoto University; Tomomi Okita, Kumamoto University; Susumu Takio, Kumamoto University; Yoshikatsu Sato, Nagoya University; Mitsuyasu Hasebe, National Institute for Basic Biology; Hirokazu Tsukaya, University of Tokyo; Hiroyoshi Takano, Kumamoto University

Plant cells usually cannot move because of the cell wall, and plant development depends on the directions of cell division and growth. ANGUSTIFOLIA (AN), a plant homolog of CtBP/BARS with a plant-specific C-terminal region, regulates the width of leaves of Arabidopsis thaliana, by controlling the directional elongation of leaf cells. Four AN homologs (PpAN) were found in the Physcomitrella genome. While two PpAN proteins (1-1 and 1-2) are orthologous to AN, PpAN2-1 and 2-2 have no plant-specific C-terminus. In this study, we focused on the former two genes. To examine whether PpANs can complement Arabidopsis AN function, we induced PpAN1-1 or PpAN2-1 in Arabidopsis an-1 mutant. Recovery of leaf index (length to width ratio) indicates that each gene functions as AN does in Arabidopsis. To investigate their functions in Physcomitrella, spatiotemporal promoter activity of each gene was analyzed with transgenic lines harboring a DNA fragment with each promoter fused with uidA gene. In gametophores,GUS activity was mainly detected in stems for both genes. Concordantly, in double knockout mutants, stems became shorter and stouter than those of the wild type and leaves of the mutants were not distinguished from those of the wild type. We identified that cell growth was retarded in the longitudinal direction. To understand the molecular basis of cell growth, on-going investigation of tubulin dynamics with transgenic lines expressing GFP-tubulin fusion protein will be presented.

P8 *Developmental Programming by Calcium and Phytohormone Signaling in Protonema of the Moss* Physcomitrella patens

Thomas Kleist, Carnegie Institution for Science
Anthony Bortolazzo, University of Wisconsin, Madison; Adele Perera, University of California, Berkeley; Sheng Luan, University of California, Berkeley; Peggy Lemaux, University of California, Berkeley; Jean-Michel Ané, University of Wisconsin, Madison

Physcomitrella spores germinate to form a filamentous, branched network of protonemal cells, which provide a useful system to study interplay between plant development and the environment.

Chloronema, the initial cell type to form, give rise to caulonema, which bear 'buds' that develop into leafy gametophores. Auxin and cytokinin, respectively, promote these developmental transitions. Abiotic stress elicits developmental reprogramming and formation of brood cells, which act as stress-resistant asexual propagules that have been classically linked to abscisic acid (ABA) signaling. Calcium (Ca2+) has been implicated in signal transduction of each of these phytohormone-linked developmental pathways, yet it is unclear how specificity is achieved or maintained. To gain clues, we are using genetically encoded calcium indicators to document protonemal Ca2+ dynamics during development and in response to stress or phytohormone treatments. In parallel, we are using molecular genetics to identify responsible signaling components. We identified a Ca2+-dependent protein kinase and target transcription factor as positive regulators of brood cell formation and ABA signaling; however, loss-of-function analyses revealed that this module is not a core requirement for brood cell development. We discovered that ABA signaling is likewise sufficient but unnecessary for brood cell development; therefore, further work is needed to define molecular mechanisms of developmental (re)programming.

P9 *Effect of ABA on Chloroplast Division on the Moss,* Physcomitrella patens

Tomomichi Fujita, Hokkaido University
Pongthai Prapaporn, Hokkaido University; Hiroyoshi Takano, Kumamoto University; Yasushi Yoshioka, Nagoya University

Significance and mechanism of regulation of organelle numbers are still largely unknown. In the moss, *Physcomitrella patens*, abiotic stresses affect chloroplast division and morphology of chloroplasts. Previous data and also our observation showed that ampicillin, an inhibitor of peptidoglycan synthesis, inhibited chloroplast division and resulted in the appearance of a few of macrochloroplasts in the moss. The results suggest that the peptidoglycan synthesis positively regulates chloroplast division. Interestingly, chloroplast division in the bryophytes may be an intermediate between the cyanobacteria and the vascular plants, but the mechanisms are still unclear.

In this work we examined the relationship between ABA and peptidoglycan synthesis on chloroplast division of the moss, *P. patens*. We found that chloroplast numbers in the wild type increased under ABA plus ampicillin treatment. Moreover, ABA also increased the number of chloroplasts in the peptidoglycan knockout mutants and the mid-chloroplast FtsZ rings knockout mutant. The results suggest that ABA may act independently of peptidoglycan synthesis pathway and FtsZ to control chloroplast division. To prove our hypothesis and find a novel pathway of chloroplast division, observation of ABA signaling overexpression lines is in progress.

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