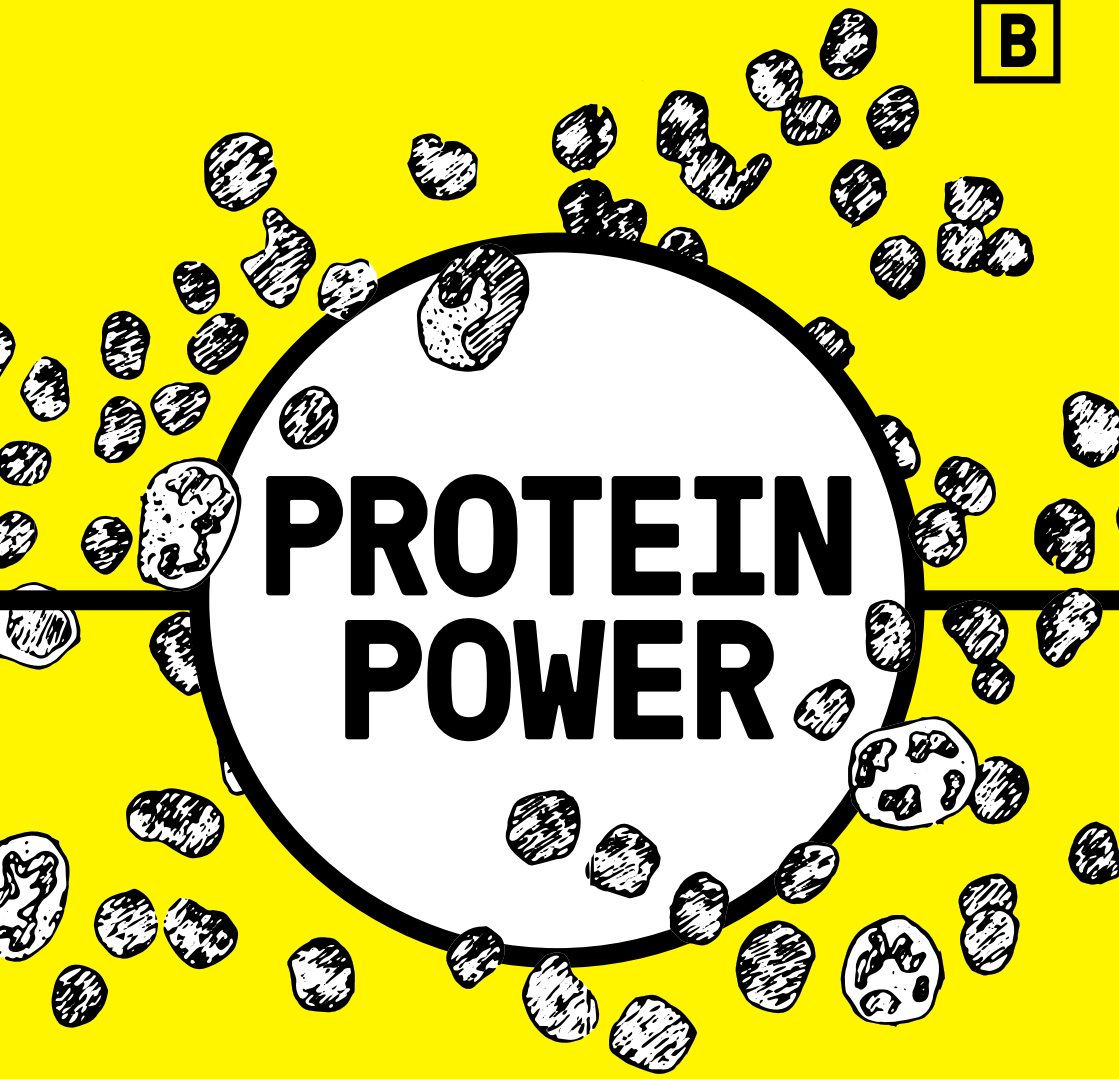
PROGRAMME AND ABSTRACT BOOK

FROM PROTEOME TO PHENOTYPE: ROLE OF POST-TRANSLATIONAL MODIFICATIONS

11 – 13 DECEMBER 2017
UNIVERSITY OF EDINBURGH,
UK



SOCIETY FOR EXPERIMENTAL BIOLOGY

FROM PROTEOME TO PHENOTYPE: ROLE OF POST-TRANSLATIONAL MODIFICATIONS

ORGANISED BY:
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GARNET
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CYRIL ZIPFEL
THE SAINSBURY LABORATORY, UK

IN COLLABORATION WITH:

GARNet

DELEGATE INFORMATION

BADGES

Participants are required to wear name badges at all times for proof of registration, security purposes and catering identification.

CATERING

Lunch and refreshments during the symposium are included in your registration fee and will be served in the breakout area.

CERTIFICATE OF ATTENDANCE

Delegates requiring a certificate of attendance should visit the SEB registration desk on their departure.

VENUE

John McIntyre Conference Centre
Edinburgh First, Pollock Halls
18 Holyrood Park Road, Edinburgh EH16 5AY

The scientific sessions will be taking place in the Pentland room and the catering and posters will be in the breakout area.

GARNET WORKSHOP ON PLANT PROTEOMICS

If you have been selected to attend the plant proteomics workshop on 13 December, you will have received further information by email. Please visit the SEB registration desk if you require information or have any queries.

WI-FI INTERNET ACCESS

Internet access is available during the symposium and will be complimentary. Log in details will be available at the registration desk.

POSTER SESSION

The poster session will be taking place in the breakout area between 18:30 – 20:00 on Monday 11 December 2017. Poster presenters are invited to hang their poster on their arrival (Velcro will be provided) and are asked to remove their posters by 16:00 on Tuesday 12 December. Any posters left behind will be disposed of.

LIABILITY

Neither the Society for Experimental Biology nor the University of Edinburgh will accept responsibility for damage or injury to persons or property during the symposium. Participants are advised to arrange their own personal health and travel insurance.

PHOTOGRAPHY

No photographs are to be taken of the speakers and their slides during the symposium.
**Please note: The SEB will be taking photos during the event for promotional purposes. If you have any concerns, please visit the SEB registration desk.*

REGISTRATION

The registration desk will be open during the hours of the symposium and a SEB staff member will be on hand during the refreshment and lunch breaks should you require any assistance.

TWITTER

We're looking to increase the conversation at the meeting using Twitter so please get tweeting!
Follow the conversation **#SEBPTM17**
SEB – **@SEBiology**
GARNet – **@GARNetweets**

PROGRAMME

MONDAY 11 DECEMBER

09:00 REGISTRATION

09:25
Welcome

Cyril Zipfel
Steven Spoel
Cell symposium co-organisers

09:30
PLENARY LECTURE
Jesper Olsen
University of Copenhagen, Denmark
Dissecting cell signalling networks by quantitative phosphoproteomics
CS17.1

10:15 REFRESHMENT BREAK/POSTERS

SESSION 1 DYNAMIC PHOSPHORYLATION

CHAIR: CYRIL ZIPFEL

10.45
Cyril Zipfel
The Sainsbury Laboratory, United Kingdom
Phosphocode-dependent functional dichotomy of the common co-receptor BAK1 in plant signalling
CS17.2

11:15
Gitta Coaker
University of California Davis, United States
The PBL13 kinase negatively regulates the ROS burst in the absence of pathogen perception
CS17.3

11:45
John Christie
University of Glasgow, United Kingdom
Engineering the function of Phototropin receptor kinases
CS17.4

12:15
Kris Vissenberg
University of Antwerp, Belgium
The auxin-regulated kinase *ERULUS* controls cell wall composition during root hair tip growth in *Arabidopsis thaliana*
CS17.5

12:30
Cornelia Klose
Institute of Biology 2, University of Freiburg, Germany
Dynamic regulation of phytochrome B activity by phosphorylation
CS17.6

12:45 LUNCH/POSTERS

SESSION 2 MECHANISMS AND ACTION OF PROTEIN SUMOYLATION

CHAIR: ARI SADANANDOM

14:00
Ari Sadanandom
Durham University, United Kingdom
Rice SUMO protease *Overly Tolerant to Salt 1* targets the transcription factor, OsbZIP23 to promote drought tolerance in rice
CS17.7

14:30
Andreas Bachmair
Max F. Perutz Laboratories, University of Vienna, Dept. Biochemistry and Cell Biology, Austria
SUMO conjugation - chain formation and links to phosphorylation
CS17.8

PROGRAMME

15:00
L Maria Lois
CRAG, Spain
Deciphering SUMO conjugation *in vivo*: multiple regulatory layers controlling plant growth
CS17.9

15:30
Mark Kwaaitaal
Molecular Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Netherlands
SUMOylation orchestrates formation of nuclear bodies that overlap with COP1 photobodies
CS17.10

15:45
Justyna Labuz
Jagiellonian University, Poland
Sumoylation of phototropins in *Arabidopsis thaliana*
CS17.11

16:00 REFRESHMENT BREAK/POSTERS

SESSION 3 EMERGING PTMS SUMOYLATION

CHAIR: PIERS HEMSLEY

16:30
Piers Hemsley
University of Dundee, United Kingdom
S-acylation in plants - greasing membrane protein function?
CS17.12

17:00
Markus Wirtz
Heidelberg University, Germany
N-terminal acetylation - a prevalent protein modification that likes to surprise
CS17.13

17:30
Stéphane Ravanel
CEA, Plant Cell Physiology laboratory - Grenoble, France
Protein lysine methylation in plants: from proteome to phenotype...the quest for the Holy Grail
CS17.14

18:00
Doris Lucyshyn
University of Natural Resources and Life Sciences Vienna, Austria
O-Glycosylation of nuclear and cytosolic proteins in plants
CS17.15

18:15
Amanda Chaplin
University of Essex, United Kingdom
Glycated peptides triggers H_2O_2 signalling in *Arabidopsis thaliana*
CS17.16

18:30 POSTER SESSION

20:00 DINNER
LOCATION: SOUTH HALL COMPLEX

PROGRAMME

TUESDAY 12 DECEMBER

08:45 REGISTRATION

09:00
PLENARY LECTURE
Ron Hay
University of Dundee, United Kingdom
SUMO-SIM networks regulate the assembly of dynamic protein complexes
CS17.17

09:45 REFRESHMENT BREAK/POSTERS

SESSION 4 UBIQUITIN-MEDIATED PROTEASOME SYSTEM

CHAIR: STEVEN SPOEL

10:15
Steven Spoel
University of Edinburgh, United Kingdom
Transcriptional regulation by dynamic ubiquitination
CS17.18

10:45
Luz Irina Calderon-Villalobos
Leibniz Institute of Plant Biochemistry (IPB), Germany
Auxin-programmed ubiquitylation as a master signal integration system
CS17.19

11:15
Emmanuelle Graciet
Maynooth University, Ireland
Regulation of plant/pathogen interactions by the N-end rule pathway
CS17.20

11:45
Barbara Korbei
University of Natural Resources and Life Sciences Vienna, Austria
Ubiquitin binding and conjugation is important for the function and localisation of TOL proteins
CS17.21

12:00
Marco Trujillo
Leibniz Institute for Plant Biochemistry, Germany
Phosphorylation and ubiquitination regulate the Exo70B2 subunit of the exocyst – a convergence point of immune signalling and autophagy
CS17.22

12:15 LUNCH/POSTERS

SESSION 5 REDOX-BASED PTMs IN STRESS AND DEVELOPMENT

CHAIR: GARY LOAKE

13:30
Gary Loake
University of Edinburgh, United Kingdom
Control of plant immune function through S-nitrosylation
CS17.23

14:00
Frank van Breusegem
VIB Ghent, Belgium
Oxidative stress signalling in plants. Towards the proteome and beyond
CS17.24

14:30
Daniel Gibbs
University of Birmingham, United Kingdom
The N-end rule pathway couples PRC2 activity to oxygen availability in flowering plants
CS17.25

PROGRAMME

15:00
Eva-Esther Rudolf
Helmholtz Zentrum München, Germany
Post-translational modification of histones: Nitric oxide modulates chromatin structure
CS17.26

15:15
Capilla Mata-Perez
University of Edinburgh, United Kingdom
Nucleoredoxin 1 (NRX1) selectively rescues plant immunity
CS17.27

15:30 REFRESHMENT BREAK/POSTERS

SESSION 6 TECHNOLOGICAL ADVANCES IN PTM DETECTION

CHAIR: ALEX JONES

16:00
Alex Jones
University of Warwick, United Kingdom
Developing mass spectrometry based proteomic methods to identify and quantify protein carbonylation in plants
CS17.28

16:30
Frank Menke
The Sainsbury Laboratory, United Kingdom
Monitoring phosphorylation and ubiquitination in pattern recognition receptor signalling
CS17.29

17:00
Katja Baerenfaller
SIAF, Switzerland
On-site inspection of ubiquitylation
CS17.30

17:30
Greg Vert
I2BC CNRS, France
Proteasome-independent roles of lysine63-linked polyubiquitin chains: Insight from sensor-based proteomics
CS17.31

17:45
Ive De Smet
VIB, Belgium
The dynamic phosphoproteome allows identifying novel abiotic stress signalling components
CS17.32

18:00 END OF SYMPOSIUM

POSTER SESSION MONDAY 11 DECEMBER

Gregory MacNeill
University of Guelph, Canada
Post-translational modifications regulate starch branching enzyme 2.2 in Arabidopsis
CS17.33

Yang Do Choi
Seoul National University, Korea (South)
OsSGT1 regulates root development and growth in rice
CS17.34

Mansoor Azeem Siddiqui
International Centre for Genetic Engineering and Bio-technology, India
Protein S-Palmitoylation regulates invasion and apical organelle secretion in human malaria parasite, *Plasmodium falciparum*
CS17.35

Arsheed Sheikh
School of Life Sciences, University of Warwick, United Kingdom
Mitogen Activated Protein Kinases (MAPKs) activation post effector recognition in tomato
CS17.36

Lam Dai Vu
VIB-UGent Center for Plant Systems Biology, Belgium
Unravelling phosphorylation-mediated signalling in plants at high ambient temperature
CS17.37

Hailong Guo
The Sainsbury Laboratory, United Kingdom
Interplay between phosphorylation and acetylation in regulating RPS4/RRS1 immune receptor pair
CS17.38

Marita Anggarani
Institute of Plant Molecular Biology, Academia Sinica, Taiwan
Impaired proteasome function in Arabidopsis *rad23abcd* mutant alters auxin homeostasis
CS17.39

Ram Nivas Ahirwar
Institute of Plant Molecular Biology, Academia Sinica, Taiwan
Arabidopsis deubiquitinase OTU5 is involved in flowering by regulating major repressors *FLC/MAF4/MAF5*
CS17.40

Maura Di Martino
University of Warwick, United Kingdom
Combinatorial transcriptional regulation of the plant defence response
CS17.41

Jasmine Pham
The James Hutton Institute, United Kingdom
Identification of host membrane proteins that are involved in plant-aphid interactions
CS17.42

Inês Luís
Instituto de Tecnologia Quimica e Bioiogica Antonio Xavier - Universidade NOVA de Lisboa (ITQB-UNL), Portugal
On the quest for posttranslational modifications governing phosphoenolpyruvate carboxylase life cycle
CS17.43

Luis Lightbourn
Lightbourn Research Institute, Mexico
Transcriptomic profiling of Capsicum annuum in response to high-intensity UV irradiation reveals stress defence signalling
CS17.44

POSTER SESSION MONDAY 11 DECEMBER

Sara Alegre García
University of Turku, Finland
PP2A-B'γ controls methylation of indole glucosinolates and modulates methionine metabolism in Arabidopsis
CS17.45

Stuart Sullivan
University of Glasgow, United Kingdom
Correlation between NPH3 phosphorylation status, localisation and phototropic enhancement
CS17.46

Michael Skelly
University of Edinburgh, United Kingdom
Dynamic ubiquitination determines NPR1 transcriptional coactivator activity
CS17.47

Joanna Landymore
University of Sheffield, United Kingdom
Understanding the role of Nitrate reductases NIA1 and NIA2 under abiotic stresses
CS17.48

Dionne Turnbull
University of Dundee, United Kingdom
What 'R' you doing here? Investigating the role of S-acylation in plant disease resistance proteins
CS17.49

Young Hee Joung
Chonnam National University, Korea (South)
Functional analysis of CYP707A70 gene (ABA 8'-hydroxylases) from Hot Pepper *(Capsicum annuum)* in transgenic tobacco and tomato
CS17.50

Doris Lucyshyn
University of Natural Resources and Life Sciences Vienna, Austria
The role of O-Glycosylation in plant developmental transitions
CS17.51

Kyle Bender
The Sainsbury Laboratory, United Kingdom
Characterising the role of ELONGATION FACTOR TU RECEPTOR (EFR) protein kinase activity and phosphorylation in pattern-triggered immunity
CS17.52

Hannes Vanhaeren
VIB Center for Plant Systems Biology, Belgium
The role of the peptidase DA1 and the E3-ligase BIG BROTHER in regulating cell proliferation and senescence in plants
CS17.53

Nelson Serre
University Grenoble Alpes, France
Lysine methylation of non-histone proteins is involved in the response of Arabidopsis plants to a stress induced by cadmium
CS17.54

Upendo Lupanga
University of Dundee, United Kingdom
Dynamic S-acylation in plants
CS17.55

Dae Sung Kim
The Sainsbury Laboratory, United Kingdom
A downy mildew effector imposes host susceptibility by modulating the activity of a host RING E3 ligase required for immunity
CS17.56

POSTER SESSION MONDAY 11 DECEMBER

Eirini Kaiserli
University of Glasgow, United Kingdom
Understanding the role of sumoylation in regulating light signalling components in plants
CS17.57

Hui Dong
John Innes Centre, United Kingdom
Control of growth by phosphorylation and ubiquitylation of DA1 peptidase
CS17.58

Giorgio Perrella
University of Glasgow, United Kingdom
Understanding the role of sumoylation in regulating light signalling components in plants
CS17.59

Anne Marie Labandera
University of Calgary, Canada
The *Arabidopsis thaliana Rhizobiale*-like phosphatase 2 is a novel D-group Mitogen Activated Protein Kinase (MAPK) tyrosine-specific PPP-family protein phosphatase
CS17.60

Mirko Pavicic
University of Helsinki, Finland
Genomic and phenomic screens for flower related RING type Ubiquitin E3 ligases in Arabidopsis
CS17.61

Mhairi Davidson
IMCSB University of Glasgow, United Kingdom
Understanding the role of sumoylation in regulating light signalling components in plants
CS17.62

Luis Lightbourn
Lightbourn Research Institute, Mexico
Role of post-translational modifications that regulates signalling from G-proteins in Capsicum annuum
CS17.63

Noriyuki Suetsugu
University of Glasgow, United Kingdom
RPT2 and NCH1 are key factors for the blue-light receptor phototoropin-signalling pathway in land plants
CS17.64

Clara Williams
VIB, Belgium
Repression of 'LURP' regulon by the F-BOX protein and JA receptor, COI1
CS17.65

Hannah Tedds
University of Birmingham, United Kingdom
VERNALIZATION2 is an oxygen- and nitric oxide-regulated substrate of the N-end rule pathway of proteolysis
CS17.66

Rumana Keyani
COMSATS Institute of information technology Islamabad, Pakistan
Nucleoredoxin guards against oxidative stress by protecting antioxidant enzymes
CS17.67

Charlotte Hurst
Dundee University at the James Hutton Institute, United Kingdom
S-acylation: What the FLS2 is going on?
CS17.68

POSTER SESSION MONDAY 11 DECEMBER

Alberto Campanaro
Durham University Biosciences Department, United Kingdom
SUMO proteases OTS1 and 2 control filament elongation through a DELLA-dependent mechanism
CS17.69

Jose Monreal
University of Sevilla, Spain
Post-translational modifications (PTMs) in plant proteins: Phosphoenolpyruvate carboxylase (PEPC) as a case of study
CS17.70

Beatriz Orosa
Department of Biosciences, Durham University, United Kingdom
Transcriptional analysis of OTS SUMO protease mutants reveals new signalling nodes that promote biotic stress tolerance in plants
CS17.71

Dhiya Zawawi
University of Sheffield, United Kingdom
Expression-functional studies of phosphoenolpyruvate carboxylase kinase (PEPCK)
CS17.72

Fenella Croley
Durham University, United Kingdom
Fifty Shades of SUMO: its role in immunity and at the fulcrum of growth-defense balance
CS17.73

Rebecca Gwyther
Durham University, United Kingdom
Exploiting protein modification systems to boost crop productivity: SUMO in the focus
CS17.74

Lucas Frungillo
University of Edinburgh, United Kingdom
Protein S-nitrosylation in immune-induced hormonal networks
CS17.75

Maiju Laurila
University of Dundee, United Kingdom
Discovering de-S-acylating enzymes in Arabidopsis
CS17.76

Lisa Asseck
ZMBP Tuebingen, Germany
IP-MS identifies a membrane receptor of the Arabidopsis GET pathway
CS17.77

Rashid Alijani Ardeshir
Khoramshar University of Marine Science and Technology, Iran
CYP1A gene expression as a basic factor for fipronil toxicity in Caspian kutum fish
CS17.78

Nicola Leftley
University of Nottingham, United Kingdom
Dissecting the molecular mechanism regulating lateral root hydropatterning
CS17.79

Baris Uzilday
Ege University, Turkey
Disruption of N-linked protein glycosylation alters polyamine oxidase signalling pathway in *Arabidopsis thaliana*
CS17.80

POSTER SESSION MONDAY 11 DECEMBER

Mariia Stanovova
Lomonosov Moscow State University, Russia
Role of proteasomes in non-specific immune response of marine annelids
CS17.81

Linda Millyard
Durham University, United Kingdom
Ubiquitination in wheat defence against Septoria fungus
CS17.82

Bruna Marques dos Santos
University of Copenhagen, Denmark
An Omics approach to investigate the effect of climate change in *Eucalyptus*
CS17.83

Adnan Khan Niazi
CABB University of Agriculture Faisalabad, Pakistan
Self-protection of Arabidopsis cytosolic malate dehydrogenase against oxidative stress
CS17.84

Thomas DeFalco
The Sainsbury Laboratory, United Kingdom
Identifying and characterising substrates of the immune kinase BIK1
CS17.85

Adnan Khan Niazi
CABB University of Agriculture, Faisalabad, Pakistan
The siRNA suppressor RTL1 is redox-regulated through glutathionylation of a conserved cysteine in the double-stranded-RNA-binding domain
CS17.86

Hanna Hõrak
University of Sheffield, United Kingdom
Improvement of plant water use efficiency via modification of stomatal signalling pathways
CS17.87

Mark Bailey
University of Birmingham, United Kingdom
Characterisation of the N-terminal Acetylation branch of the N-end rule pathway of protein degradation in *Arabidopsis thaliana*
CS17.88

Maria Derkacheva
The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom
Dynamic interplay between phosphorylation and ubiquitination during plant receptor kinase-mediated immunity
CS17.89

Sebastien Lambertucci
Royal Holloway University of London, United Kingdom
Identification of barley exta-haustorial membrane proteins in the haustoria of the obligate biotrophic fungal pathogen, *Blumeria graminiss f.sp hordei*
CS17.90

Hansjörg Stampfl
Center for Health Bioresources, Austrian Institute of Technology, Austria
Phosphorylation-mediated stress signalling and redox regulation
CS17.91

POSTER SESSION MONDAY 11 DECEMBER

Laura Moody
University of Oxford, United Kingdom
Identification of *No Gametophores 1 (PpNOG1)*, a novel regulator of three-dimensional growth in *Physcomitrella patens*
CS17.92

Heather Grey
University of Edinburgh, United Kingdom
Proteasome-associated HECT-type ubiquitin ligase activity is required for plant immunity
CS17.93

Becky Morrell
Durham University, United Kingdom
Insecticidal fusion proteins: How does the orientation of the toxin to the carrier affect toxicity to insects?
CS17.94

Hirofumi Nakagami
Max Planck Institute for Plant Breeding Research, Germany
Phosphoproteomic approach to survey molecular components of plant immune system
CS17.95

Frederica Theodoulou
Rothamsted Research, United Kingdom
N-terminal peptide enrichment as a tool for studying targeted protein degradation pathways and proteolytic events
CS17.96

Kevin Goslin
Maynooth University, Ireland
Investigating the degradation of *Arabidopsis* RIN4 fragments after cleavage by the *Pseudomonas syringae* effector AvrRpt2
CS17.97

FROM PROTEOME TO PHENOTYPE: ROLE OF POST-TRANSLATIONAL MODIFICATIONS

CS17.1 DISSECTING CELL SIGNALLING NETWORKS BY QUANTITATIVE PHOSPHOPROTEOMICS

MONDAY 11 DECEMBER 2017 **09:30**

JESPER V OLSEN (UNIVERSITY OF COPENHAGEN, DENMARK)

@ JESPER.OLSEN@CPR.KU.DK

Mass Spectrometry based quantitative phosphoproteomics is emerging a powerful technology for global analysis of cellular signalling networks. In particular tyrosine phosphorylation (pTyr) is of great importance in eukaryotic cells due to its crucial role in regulating intracellular signalling networks controlling cell fate decisions such as proliferation, migration, differentiation, cell cycle progression and apoptosis. We employed quantitative phosphoproteomics to delineate receptor tyrosine kinase (RTK) signalling dynamics activated by different ligands leading to differential cellular outcomes. We quantified thousands of pTyr events as a function of ligand and stimulation time and revealed RTK-specific regulation of pTyr sites on key adaptor and signalling molecules, which fine-tune cell migration and proliferation. These results, based on a multidisciplinary approach, which combines quantitative phosphoproteomics and functional assays, identify ligand-dependent mechanisms for the control of RTK signalling and for the specification of long-term cellular outcomes.

CS17.2 PHOSPHOCODE-DEPENDENT FUNCTIONAL DICHOTOMY OF THE COMMON CO-RECEPTOR BAK1 IN PLANT SIGNALLING

MONDAY 11 DECEMBER 2017 **10:45**

CYRIL ZIPFEL (THE SAINSBURY LABORATORY, UNITED KINGDOM)

@ CYRIL.ZIPFEL@TSL.AC.UK

Plants rely on cell surface-localized receptors kinases for their survival. Plant receptor kinases form ligand-induced complexes with shape-complementary co-receptors, which are required for their activation. The best-characterised plant co-receptor is BAK1 (also called SERK3), which controls immunity, as well as several aspects of growth and development, through association with leucine-rich repeat receptor kinases. The mechanisms by which BAK1 control the activation of these receptors is however still mostly unknown. Here, we report key regulatory events controlling the function of BAK1 and more generally leucine-rich repeat receptor kinases. Through a combination of phospho-proteomics and targeted mutagenesis, we identified five phosphosites that are required for BAK1 immune function in *Arabidopsis thaliana*. The inability to phosphorylate on any of these residues abolish cellular immune outputs induced by the bacterial pathogen-associated molecular pattern flg22, and leads to impaired anti-bacterial immunity. Strikingly, these phosphosites are yet not required for BAK1/SERK-dependent brassinosteroid-regulated plant growth. Beyond revealing a critical role for BAK1 C-terminal tail phosphorylation, we also identified a conserved phosphorylated tyrosine residue that is required for the function of BAK1 and potentially that of ~80% of Arabidopsis leucine-rich repeat receptor kinases. Our results thus suggest a phosphocode-based dichotomy of BAK1 functions in plant signalling, and provide novel insights into receptor kinase activation, which have broad implications to our understanding of how plants grow, develop and respond to their changing environment.

CS17.3 THE PBL13 KINASE NEGATIVELY REGULATES THE ROS BURST IN THE ABSENCE OF PATHOGEN PERCEPTION

MONDAY 11 DECEMBER 2017 **11:15**

GITTA COAKER (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), DONGHYUK LEE (UNIVERSITY OF CALIFORNIA DAVIS, UNITED STATES), DANIEL LIN (DONALD DANFORTH PLANT SCIENCE CENTER, UNITED STATES)

@ GLCOAKER@UCDAVIS.EDU

Plant surface localized immune receptors, termed pattern recognition receptors, recognize conserved microbial features, resulting in pattern-triggered immunity. Reactive oxygen species (ROS) play pivotal roles in PTI as signalling molecules. The NADPH oxidase RBOHD is a major source of ROS production in Arabidopsis and RBOHD is positively regulated by N-terminal phosphorylation through multiple kinases. Recently, we identified and characterised the PBL13 kinase. PBL13 exhibits a unique C-terminal repeat region and knockout lines exhibit an enhanced ROS burst and increased RBOHD abundance. PBL13 is able to phosphorylate RBOHD's C-terminus at conserved residues. C-terminal phosphorylation acts to negatively regulate RBOHD enzymatic activity and protein accumulation. Data will also be presented highlighting the interplay between C-terminal phosphorylation and ubiquitination of RBOHD. Taken together, these data indicate that PBL13 acts to appropriately regulate RBOHD activity in the absence of pathogen perception.

CS17.4 ENGINEERING THE FUNCTION OF PHOTOTROPIN RECEPTOR KINASES

MONDAY 11 DECEMBER 2017 **11:45**

JOHN CHRISTIE (UNIVERSITY OF GLASGOW, UNITED KINGDOM)

@ JOHN.CHRISTIE@GLASGOW.AC.UK

Phototropins (phot1 and phot2) are plasma membrane-associated protein kinases that function to regulate a wide range of responses in plants. These include chloroplast relocation movements, leaf positioning and expansion, stomatal opening and phototropism, all of which influence a plant's photosynthetic competence by improving the efficiency of light capture, reducing photodamage, and regulating gas exchange between leaves and the atmosphere. Research over the last two decades has improved our understanding of how these autophosphorylating kinases are activated, how they are regulated and how they initiate signalling from the plasma membrane. Here, we outline several engineering strategies aimed at modulating phototropin receptor function in plants. These include approaches aimed at altering the light-regulation of phototropin kinase activity, the outcome of which could lead new possibilities for altering plant growth through changes in photosynthetic performance. We also describe how recently developed optogenetic tools are being used to manipulate phototropin-mediated changes in ion transport, especially K^+. Finally, we show how the activity of phototropin kinases can be successfully engineered to accommodate non-natural ATP analogues. This chemical-genetic approach now offers new possibilities to track and identify substrates targets for the phototropins and other related AGCVIII kinases.

CS17.5 THE AUXIN-REGULATED KINASE *ERULUS* CONTROLS CELL WALL COMPOSITION DURING ROOT HAIR TIP GROWTH IN *ARABIDOPSIS THALIANA*

MONDAY 11 DECEMBER 2017 **12:15**

KRIS VISSENBERG (UNIVERSITY OF ANTWERP, BELGIUM), SÉBASTJEN SCHOENAERS (UNIVERSITY OF ANTWERP, BELGIUM), DARIA BALCEROWICZ (UNIVERSITY OF ANTWERP, BELGIUM), GORDON BREEN (UNIVERSITY OF BRISTOL, UNITED KINGDOM), KRISTINE HILL (CENTRE FOR PLANT INTEGRATIVE BIOLOGY, UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), MALGORZATA ZDANIO (UNIVERSITY OF ANTWERP, BELGIUM), GRÉGORY MOUILLE (INRA VERSAILLES, FRANCE), TARA J HOLMAN (CENTRE FOR PLANT INTEGRATIVE BIOLOGY UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), JAESUNG OH (CENTRE FOR PLANT INTEGRATIVE BIOLOGY, UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), MICHAEL WILSON (CENTRE FOR PLANT INTEGRATIVE BIOLOGY, UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), NATALIA NIKONOROVA (VIB/GHENT UNIVERSITY, BELGIUM), LAM DAI VU (VIB/GHENT UNIVERSITY, BELGIUM), IVE DE SMET (VIB/GHENT UNIVERSITY, BELGIUM), RANJAN SWARUP (CENTRE FOR PLANT INTEGRATIVE BIOLOGY, UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM), WINNOK DE VOS (UNIVERSITY OF ANTWERP, BELGIUM), ISABEL PINTELON (UNIVERSITY OF ANTWERP, BELGIUM), DIRK ADRIAENSEN (UNIVERSITY OF ANTWERP, BELGIUM), CLAIRE GRIERSON (UNIVERSITY OF BRISTOL, UNITED KINGDOM), MALCOLM J BENNETT (CENTRE FOR PLANT INTEGRATIVE BIOLOGY, UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM)

KRIS.VISSENBERG@UANTWERPEN.BE

Root hair (RH) growth is an auxin-regulated process dependent on localised synthesis, secretion and modification of RH tip cell wall (CW). Direct targets of auxin signalling in RHs and a direct link between auxin and RH CW signalling are still lacking. We identified *ERULUS (ERU)*, an auxin-induced Arabidopsis receptor-like kinase whose transcription is directly regulated by ARF7 and ARF19 transcription factors. ERU belongs to the *Catharanthus roseus* RECEPTOR-LIKE KINASE 1-LIKE (CrRLK1L) subfamily of putative CW sensor proteins. Visualisation of the fluorescent ERU-GFP fusion protein revealed that ERU is expressed only in RH cells and localised to the apical RH plasma membrane. ERU regulates CW composition in RHs and modulates pectin dynamics through negative control of pectin methylesterase (PME) activity. Mutant *eru* RHs accumulate de-esterified homogalacturonan (HG) and exhibit aberrant pectin Ca^{2+} binding site oscillations and increased PME activity. Up to 80% of the *eru* RH phenotype is rescued by pharmacological supplementation with a PME inhibiting catechin extract. ERU transcription levels are altered in specific CW-related RH mutants, suggesting it is a target for feedback regulation. Loss of ERU alters the phosphorylation status of FERONIA (FER) and H^+-ATPase 2 (AHA2), regulators of apoplastic pH during cell growth. Furthermore, both AHA2 and ERU are differentially phosphorylated in response to auxin. We conclude that ERULUS is a key auxin-controlled regulator of CW composition and pectin dynamics during RH tip growth, possibly through control of CW pH via FER and AHA2.

CS17.6 DYNAMIC REGULATION OF PHYTOCHROME B ACTIVITY BY PHOSPHORYLATION

MONDAY 11 DECEMBER 2017 **12:30**

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Phytochromes are dimeric red/far-red light absorbing photoreceptors that can photo-interconvert between two conformations: Pr and Pfr whereby the amount of the Pfr form in the nucleus is critical for physiological activity of phytochromes. In addition to photoconversion, Pfr can spontaneously revert back to Pr in a light independent thermal relaxation process called dark reversion. For phytochrome B (phyB), the dominating phytochrome in light grown plants, dark reversion is an essential mechanism determining its modes of action: it enables phyB to act as dynamic light quality-/ quantity- and temperature sensor to gradually control photomorphogenic development depending on the light and temperature conditions. Phytochromes including phyB are phosphoproteins, primarily phosphorylated at their N-terminus and have been assigned with autophosphorylation- as well as kinase activity. I will present our latest findings about dynamic phosphorylation of phyB and discuss how phosphorylation modulates phyB mediated responses to light and temperature. Particular emphasis will be on the molecular mechanism how phosphorylation alters phyB activity by modulating the dark reversion process and in what way this can be related to the observed effects of phyB phosphorylation on physiological responses.

CS17.7 RICE SUMO PROTEASE *OVERLY TOLERANT TO SALT 1* TARGETS THE TRANSCRIPTION FACTOR, OSBZIP23 TO PROMOTE DROUGHT TOLERANCE IN RICE

MONDAY 11 DECEMBER 2017 **14:00**

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Conjugation of SUMO (Small Ubiquitin-like Modifier) protein to cellular targets is emerging as a very influential protein modification system. Once covalently bound SUMO conjugation can change the stability or functionality of its cognate target proteins. SUMO protease can rapidly reverse SUMO conjugation making this modification system highly dynamic. A major factor in the variation of SUMO-target function is the balance between the conjugated/de-conjugated forms. The mechanistic role of these regulatory SUMO proteases in mediating stress responses has not been defined in any crops. In this study, we reveal the role of the SUMO protease, OsOTS1 in mediating tolerance to drought in rice. *OsOTS1* depleted transgenic plants accumulate more ABA and exhibit more productive agronomic traits during drought whilst *OsOTS1* overexpressing lines are drought sensitive but ABA insensitive. Drought and ABA treatment stimulates the degradation of OsOTS1 protein indicating that SUMO conjugation is an important response to drought stress in rice achieved through down-regulation of OTS1/2 activity. We reveal that OsOTS1 SUMO protease directly targets the ABA and drought responsive transcription factor OsbZIP23 for de-SUMOylation affecting its stability. *OsOTS*-RNAi lines show increased abundance of OsbZIP23 and increased drought responsive gene expression while *OsOTS1* overexpressing lines show reduced levels of OsbZIP23 leading to suppressed drought responsive gene expression. Our data reveals a mechanism where rice plants govern ABA dependant drought responsive gene expression by controlling the stability of OsbZIP23 by SUMO conjugation through manipulating specific SUMO protease levels.

CS17.8 SUMO CONJUGATION – CHAIN FORMATION AND LINKS TO PHOSPHORYLATION

MONDAY 11 DECEMBER 2017 **14:30**

ANDREAS BACHMAIR (MAX F. PERUTZ LABORATORIES, UNIVERSITY OF VIENNA, DEPT. BIOCHEMISTRY AND CELL BIOLOGY, AUSTRIA), KONSTANTIN TOMANOV (MAX F. PERUTZ LABORATORIES, UNIVERSITY OF VIENNA, DEPT. BIOCHEMISTRY AND CELL BIOLOGY, AUSTRIA), ELLA NUKARINEN (UNIVERSITY OF VIENNA, DEPT. OF ECOGENOMICS AND SYSTEMS BIOLOGY, AUSTRIA), LILIAN NEHLIN (MAX F. PERUTZ LABORATORIES, UNIVERSITY OF VIENNA, DEPT. BIOCHEMISTRY AND CELL BIOLOGY, AUSTRIA), WOLFRAM WECKWERTH (UNIVERSITY OF VIENNA, DEPT. OF ECOGENOMICS AND SYSTEMS BIOLOGY, AUSTRIA)

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Modification of substrates by the small ubiquitin-related modifier SUMO occurs usually by covalently linking one SUMO moiety to the substrate. However, SUMO chains have also been detected, and SUMO ligases have been identified that promote SUMO chain formation (PIAL1 and2 in Arabidopsis; Tomanov et al., 2014, Plant Cell 26, 4547-4560). SUMO chains can be formed even without ligase, by an *in vitro* system consisting of SUMO, SUMO activating enzyme SAE and SUMO conjugating enzyme SCE. We found that Lys to Arg changes in the amino-terminal region of SCE can either increase, or decrease SUMO chain synthesis. The SCE mutations investigated have a similar effect when chain formation is enhanced by PIAL ligases, suggesting mechanistic similarity for SUMO chain synthesis with and without ligase. A substrate was identified that is decorated with a SUMO chain in the *in vitro* system consisting of SAE and SCE, whereas other substrates tested in the same reaction receive a single SUMO moiety only. SCE can therefore select

substrates for either mono-sumoylation, or for addition of a SUMO chain.

Mutant plants with defects in SUMO conjugation were compared to WT plants regarding phosphoprotein content. Some 50 phosphoproteins could be identified that had significantly changed abundance, implying that their occurrence is influenced by SUMO conjugation (Nukarinen et al., 2017, Plant Journal 91, 505-517). Interestingly, these proteins frequently contained predicted SUMO attachment sites, and SUMO interaction motifs. They may thus be nodes that integrate sumoylation and phosphorylation signals, to affect cellular responses.

CS17.9 DECIPHERING SUMO CONJUGATION *IN VIVO*: MULTIPLE REGULATORY LAYERS CONTROLLING PLANT GROWTH

MONDAY 11 DECEMBER 2017 **15:00**

L. MARIA LOIS (CRAG, SPAIN), ABRAHAM MÁS (CRAG-CENTER FOR RESEARCH IN AGRICULTURAL GENOMICS, SPAIN), LAURA CASTAÑO-MIQUEL (CRAG-CENTER FOR RESEARCH IN AGRICULTURAL GENOMICS, SPAIN)

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Posttranslational modification with Small Ubiquitin-related MOdifier (SUMO) regulates essential protein functions in eukaryotes. In plants, genetic studies have established a role for SUMOylation in plant development and environmental stress responses. However, the molecular mechanisms through which SUMO regulates those biological processes are poorly understood. Another key question that remains to be answer is whether SUMOylation of protein targets is only dependent on substrate accessibility or, in addition, other regulatory mechanisms modulate the spatiotemporal capacity of the plant to conjugate SUMO.

In the last years, our group has contributed to highlight the complexity of the Arabidopsis SUMO system in terms of the number and the biochemical properties of its components. SUMO isoforms have diverged to the extent of displaying biochemical and biological differences. Other members, such as the E1 activating enzyme and SUMO proteases, also play an important regulatory role conferring specificity to the system. These aspects and current advances in E1 regulation will be discussed in greater detail.

CS17.10 SUMOYLATION ORCHESTRATES FORMATION OF NUCLEAR BODIES THAT OVERLAP WITH COP1 PHOTOBODIES

MONDAY 11 DECEMBER 2017 **15:30**

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Plant growth is strongly controlled by the PIF transcription factors during the dark/light cycle. The same growth pathway is also activated when plants experience high temperatures. The last process is called thermomorphogenesis. Increased ambient temperatures also compromise plant immune responses. The SUMO E3 ligase SIZ1 directly coordinates both processes, suggesting a novel role for SIZ1-dependent SUMOylation in the crosstalk between growth and immunity. Firstly, loss of SIZ1 function leads to a constitutive immune response. Secondly, SIZ1 stability is controlled by the E3 Ubiquitin Ligase Constitutive Photomorphogenesis 1 (COP1), while at the same time COP1 is a SUMO substrate. In the dark and at high temperatures COP1 relocalizes from the cytoplasm to nuclear bodies (NBs), called photobodies. The general notion is that these photobodies signify sites of degradation of COP1 substrates. Remarkably, we found that (i) COP1 co-localizes with SIZ1 in NBs. COP1 targeting to these bodies does not depend on its SUMOylation state, albeit COP1 and SUMO bodies strongly overlap. We also found that (ii) mutations that disrupt SUMO conjugation activity abrogate the formation of SUMO-bodies, but not COP1 bodies. Likewise, SUMO-body formation appears not to depend on the interaction between SIZ1 and COP1. We will present cell biology data on the functional overlap between COP1 and SUMO bodies.

CS17.11 SUMOYLATION OF PHOTOTROPINS IN *ARABIDOPSIS THALIANA*

MONDAY 11 DECEMBER 2017 **15:45**

JUSTYNA M LABUZ (JAGIELLONIAN UNIVERSITY, POLAND), DOMINIKA JAGIELLO FLASINSKA (JAGIELLONIAN UNIVERSITY, POLAND), OLGA SZTATELMAN (INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS PAS, POLAND), PAWEL HERMANOWICZ (JAGIELLONIAN UNIVERSITY, POLAND), ANETA BAZANT (JAGIELLONIAN UNIVERSITY, POLAND), AGNIESZKA K BANAS (JAGIELLONIAN UNIVERSITY, POLAND), WOJCIECH STRZALKA (JAGIELLONIAN UNIVERSITY, POLAND), HALINA GABRYS (JAGIELLONIAN UNIVERSITY, POLAND)

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Phototropins (phots) are plant UV-A/blue light photoreceptors which fine tune plant photosynthetic efficiency by controlling growth responses and organellar movements. The phototropin N-terminal part contains two LOV photosensory domains. The C-terminus consists of a serine-threonine kinase, which autophosphorylates the photoreceptor upon light exposure. Here we show that two phototropins of *Arabidopsis thaliana*, phot1 and phot2, may also be modified by SUMO (small ubiquitin-like modifier). BIFC experiments suggest that both phototropins interact with SUMO1, SUMO2 and SUMO3 *in planta*. The results of Y2H analysis reveal that these interactions occur mainly through their N-terminal parts. Y2H analysis also indicates that phot1 interacts with MMS21, a SUMO E3 ligase. Phot2 shows a stronger interaction with MMS21, than with SIZ1 SUMO E3 ligase. Both phot1 and phot2 N-terminal parts are modified by SIZ1 and MMS21 when expressed in an *Arabidopsis* sumoylation system reconstituted *in bacteria*. To analyze the significance of phototropin sumoylation, two phototropin-controlled reactions: phototropism in etiolated seedlings and chloroplast movements in leaves have been characterized in mutants: *sum1, sum2, sum3, sum5, siz1* and *mms21*. Chloroplast movements in *Arabidopsis* leaves are not altered after both light pulses and continuous light treatments. By contrast, the phototropic bending of seedlings is diminished in *siz1* and *mms21* mutant background upon very weak blue light exposure. This effect may be attributed to the modulation of phototropin1 activity. The analysis of phototropin levels in mutants of the sumoylation pathway reveals no differences in both *Arabidopsis* seedlings and leaves regardless of light conditions.

CS17.12 S-ACYLATION IN PLANTS – GREASING MEMBRANE PROTEIN FUNCTION?

MONDAY 11 DECEMBER 2017 **16:30**

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S-acylation is a lipid based post-translational modification of proteins thought to alter a proteins physical properties leading to alterations in how a protein interacts with the membrane environment. It is unique amongst lipid modifications as it is reversible; this lends it the ability to act as a regulatory mechanism. We have shown that ~40% or the plant membrane proteome is S-acylated and that S-acylation affects both integral and peripheral membrane proteins. The study of S-acylation is still in its infancy but we have found that S-acylation is key for the function of two plant-defining groups of proteins; the cellulose synthase complex (CSC) and receptor-like kinases (RLKs). We recently demonstrated that the CSC, a hetero-18-mer, contains ~100 S-acyl groups and is the most heavily S-acylated protein complex ever described. We will discuss the roles and implications of S-acylation in CSC function. Receptor-like kinases are responsible for perception of almost all extracellular physical stimuli in plants. The S-acylation state of RLKs changes upon ligand biding and we will discuss our findings in the context of RLK signalling regulation. We will also detail our work on defining the dynamic S-acyl proteome to increase understanding of how this enigmatic modification regulates membrane protein function.

CS17.13 N-TERMINAL ACETYLATION – A PREVALENT PROTEIN MODIFICATION THAT LIKES TO SURPRISE

MONDAY 11 DECEMBER 2017 **17:00**

MARKUS WIRTZ (HEIDELBERG UNIVERSITY, GERMANY), ERIC LINSTER (HEIDELBERG UNIVERSITY, GERMANY), IWONA STEPHAN (HEIDELBERG UNIVERSITY, GERMANY), PAVLINA MIKLANKOVA (HEIDELBERG UNIVERSITY, GERMANY), MONIKA HUBER (HEIDELBERG UNIVERSITY, GERMANY), WILLY BIENVENUT (INSTITUTE FOR INTEGRATIVE BIOLOGY OF THE CELL CNRS, FRANCE), THIERRY MEINNEL (INSTITUTE FOR INTEGRATIVE BIOLOGY OF THE CELL CNRS, FRANCE), CARMELA GIGLIONE (INSTITUTE FOR INTEGRATIVE BIOLOGY OF THE CELL CNRS, FRANCE), RUEDIGER HELL (HEIDELBERG UNIVERSITY, GERMANY)

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N-terminal acetylation (NTA) is among the most common protein modifications in eukaryotes. Its frequency increases with organismal complexity and reaches up to 80% in the human cytosolic proteome. In yeast NTA is tightly linked to ubiquitin-mediated proteasome degradation, but its significance in higher eukaryotes is still enigmatic. NTA has been shown to affect protein-protein interaction, subcellular localization and stability of N-terminal a-helices in a limited number of examples. Six N-terminal acetyltransferases (NatA-NatF) catalyze the transfer of the acetyl group from acetyl-CoA to the N-terminus of cytosolic proteins in higher eukaryotes. Here, we characterise the plant NatA complex and reveal evolutionary conservation of NatA biochemical properties within higher eukaryotes. Based on the identified substrate specificity, NatA targets up to 40% of the cytosolic plant proteome. The combined application of quantitative proteomics, transcriptomics and metabolomics on NatA depleted plants uncovers specific and essential functions of NatA for development, biosynthetic pathways and stress responses. Hitherto, NTA was believed to be constitutive in eukaryotes. We show that NTA decreases significantly after drought stress in plants and identify the phytohormone abscisic acid as an efficient and prompt regulator of NatA abundance in plants. Transgenic down-regulation of NatA promotes the drought stress response program and results in strikingly drought resistant plants. In addition, NatA controls the plant immune response by regulating the stability of the Nod-like immune receptor SNC1. We propose that imprinting of the proteome by NatA is an important, hormone-regulated switch for control of abiotic and biotic stress responses.

CS17.14 PROTEIN LYSINE METHYLATION IN PLANTS: FROM PROTEOME TO PHENOTYPE...THE QUEST FOR THE HOLY GRAIL

MONDAY 11 DECEMBER 2017 **17:30**

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Protein methylation is a very diverse, important and widespread post-translational modification affecting many cellular processes in eukaryotes. Methylation occurs primarily on the side chain of lysine and arginine residues, playing structural roles and contributing to the regulation of protein function. Lysine methylation has received considerable attention in the case of histone proteins due to its role as a key regulator of chromatin state and gene regulation. During the last 15 years, lysine methylation of non-histone proteins emerged as a very common modification and has been extensively studied in yeast and animal cells. In plants, our knowledge in this field is much more fragmentary. Here, we review historical and recent advances in the identification of lysine methylproteins in photosynthetic organisms together with the characterization of the enzymes involved in the deposition of methyl marks. Also, we discuss the current knowledge and future challenges about the role of protein lysine methylation in regulating molecular and cellular functions in plants.

CS17.15 O-GLYCOSYLATION OF NUCLEAR AND CYTOSOLIC PROTEINS IN PLANTS

MONDAY 11 DECEMBER 2017 **18:00**

DORIS LUCYSHYN (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES VIENNA, AUSTRIA), BIRGIT WIMMER (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES VIENNA, AUSTRIA), EVA LIEBMINGER (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES VIENNA, AUSTRIA), NICOLE NEUMAYER (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES VIENNA, AUSTRIA), ISABELLA ZANGL (UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES VIENNA, AUSTRIA)

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O-Glycosylation of nuclear and cytosolic proteins is a very common post-translational modification (PTM), contributing to the complexity in the function and regulation of proteins. In contrast to other types of glycosylation, a single sugar residue - either N-acetylglucosamine (GlcNAc) or fucose - is attached to serine or threonine residues of a high number of very diverse proteins. O-GlcNAc modification is well characterised in animals, where it is essential for development and decisively involved in a range of signalling events, often affecting protein interactions and competing with other O-linked PTMs. While animals carry only one O-GlcNAc-transferase (OGT), plants have two structurally related O-glycosyltransferases, competing for the same targets: the O-GlcNAc transferase SECRET AGENT (SEC) and the protein O-fucosyltransferase SPINDLY (SPY) show high sequence homology, but their activity can have opposite effects on their target proteins. This mechanism of counteracting O-glycosyltransferases seems to be specific for plants. Its importance for plant development is apparent from genetic analysis, as a double knockout of the two enzymes is embryonic lethal. However, clearly more research is necessary to identify more targets and establish which cellular and developmental processes involve this modification, in order to understand the molecular mechanisms underlying O-glycosylation in plants. So far also the techniques to study this type of glycosylation are limited. We are currently working on these questions using a combination of different approaches, including identification of targets of SPY and SEC, as well as interacting proteins.

Supported by the Austrian Academy of Sciences ÖAW and the Austrian Science Fund FWF.

CS17.16 GLYCATED PEPTIDES TRIGGERS H_2O_2 SIGNALLING IN *ARABIDOPSIS THALIANA*

MONDAY 11 DECEMBER 2017 **18:15**

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Plants suffer stress in a multitude of ways. Reduced water availability, increased temperature and light intensity are some of the challenges they may face. Elucidating the mechanisms underpinning the plants response during these conditions will lead to a significant improvement in their performance and is thus of interest. One such response that may be employed by the plants under these stress conditions is the glycation of proteins. Glycation is a non-enzymatic reaction between sugars and proteins yielding the formation of early and advanced glycation end products (AGEs). AGEs cross-link and have been shown to inhibit protein function in humans and are a major contributor in diabetes. AGEs also play essential roles in signalling cascades by switching on defence pathways through binding to specific receptors. These potentially deleterious compounds are well characterised in animal systems but limited information is available in plants. We have identified using a LCMS proteomics based approach the glycated proteome in *Arabidopsis thaliana*. These results revealed 165 target proteins across various aged plants. To analyse the effect of stress on glycation, high-light, heat and drought stressors have been applied and have revealed a handful of unique peptides that become targeted for glycation under these stressors. To determine whether glycated peptides can create a stress signalling response, glycated BSA peptides were used in conjunction with roGFP (orp1) *Arabidopsis* plants to monitor H_2O_2 production. Glycated BSA peptides trigger a clear H_2O_2 response compared with non-glycated BSA peptides. We concluded that glycation is an important mechanism in plants under stress.

CS17.17 SUMO-SIM NETWORKS REGULATE THE ASSEMBLY OF DYNAMIC PROTEIN COMPLEXES

TUESDAY 12 DECEMBER 2017 **09:00**

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Small ubiquitin-related modifier (SUMO) conjugation pathway is composed of SUMO paralogues, an E1 activating enzyme, and the E2 conjugating enzyme (UBC-9). The specificity and dynamic nature of this modification is achieved by SUMO specific E3 ligases and SUMO-specific isopeptidases. System wide proteomic analysis in a range of biological settings has identified many SUMO proteins, although sites of attachment were unknown. We thus developed a specific peptide enrichment strategy that enables the identification of SUMO acceptor lysine residues on a proteome-wide scale. Having validated this approach in human cell lines we imported this system into Caenorhabditis elegans to address the role of SUMO modification in meiosis. In oocyte meiosis, a multi-protein ring complex (RC) localized between homologous chromosomes, promotes chromosome congression through the action of the chromokinesin KLP-19. While some RC components are known, the mechanism of RC assembly has remained obscure. We show that SUMO E3 ligase GEI-17/PIAS is required for KLP-19 recruitment to the RC and proteomic analysis identified KLP-19 as a SUMO substrate in vivo. In vitro analysis revealed that KLP-19 is efficiently sumoylated in a GEI-17 dependent manner, while GEI-17 undergoes extensive auto-sumoylation. GEI-17 and another RC component, the kinase BUB-1, contain functional SUMO Interaction Motifs (SIMs) allowing them to recruit SUMO modified proteins, including KLP-19, into the RC. Thus dynamic SUMO modification and the presence of SIMs in RC components generate a SUMO-SIM network that facilitates assembly of the RC. Our results highlight the importance of SUMO-SIM networks in regulating the assembly of dynamic protein complexes.

CS17.18 TRANSCRIPTIONAL REGULATION BY DYNAMIC UBIQUITINATION

TUESDAY 12 DECEMBER 2017 **10:15**

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Gene expression plays a pivotal role in the development of eukaryotic cells and their response to the environment. Failure to precisely program cellular gene expression often has pathological or deleterious consequences. In plants, hormone-responsive transcriptional programs are controlled by nuclear E3 ubiquitin ligases that function as both hormone receptors and as transcription cofactors. The plant immune hormone salicylic acid (SA) is perceived by a nuclear Cullin3-RING ubiquitin ligase (CRL3), resulting in the reprogramming of thousands of genes to prioritise immune responses over normal cellular growth functions. SA-induced CRL3 ubiquitinates the indispensable transcription coactivator NPR1, resulting in its proteasome-mediated degradation. Surprisingly, turnover of NPR1 coactivator is necessary for the activation of its target genes, suggesting that transcription may require continuous delivery of fresh NPR1 coactivator to gene promoters. To better understand how ubiquitination orchestrates NPR1-dependent transcriptional reprogramming, we used genetic and biochemical approaches to investigate the role of additional ubiquitin-modifying enzymes. Here we present evidence that these enzymes control transcriptional activity of NPR1 through dynamic processive ubiquitination and enable the proteasome to function as a transcriptional amplifier.

CS17.19 AUXIN-PROGRAMMED UBIQUITYLATION AS A MASTER SIGNAL INTEGRATION SYSTEM

TUESDAY 12 DECEMBER 2017 **10:45**

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Auxin sensing is the archetype of a drug-like compound that act as interfacial molecule prompting recognition of degrons in protein targets, and stabilizing E3-target associations. Hence, auxin triggers SCFTIR1/AFBs-AUX/IAAs interactions and auxin receptor assembly, which ultimately results in AUX/IAA ubiquitylation and turnover. As there are 6 TIR1/AFBs and 29 AUX/IAA proteins in Arabidopsis, distinct TIR1/AFB-AUX/IAA co-receptor pairs differentially perceive auxin and constitute various auxin sensors in vivo. We explore how the SCFTIR1 E3 ligase catalyses transfer of ubiquitin from the active site cysteine of an E2 to specific residues in AUX/IAAs, and ensures ubiquitin chain extension. Thus, we are investigating what confers AUX/IAA diversity and specificity, namely AUX/IAA commitment and entry into the degradation pathway. By using biochemical and biophysical approaches and the power of molecular population genetics and proteomics, we carefully dissect auxin receptor formation, auxin binding, target ubiquitylation and degradation. We have also identified paramount features, including disordered hot spots, for target discrimination and processing. We pinpoint defining signatures for auxin sensing and postulate structural features in AUX/IAA proteins, which probably guide their interactions and consequently downstream gene regulation. The integrative approach advances our knowledge of writing and reading the ubiquitin code on target proteins in plants, and provide mechanistical insights into how hormonal signals are interpreted in ubiquitylation and proteolysis ensuring fitness, plasticity and survival.

CS17.20 REGULATION OF PLANT/PATHOGEN INTERACTIONS BY THE N-END RULE PATHWAY

TUESDAY 12 DECEMBER 2017 **11:15**

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The ubiquitin-dependent N-end rule pathway relates the stability of a protein to the identity of its N-terminal residue. Some N-terminal residues are stabilising and result in proteins with a long half-life, while other so-called destabilising residues may lead to rapid degradation of a protein when made N-terminal (e.g. following protease cleavage). In the model plant *Arabidopsis thaliana*, the N-end rule pathway has been shown to regulate various developmental processes and controls flooding tolerance through the degradation of several Ethylene Response Factors (ERFs). Using a pathogen susceptibility screen and *Arabidopsis* mutants for different components of the N-end rule pathway, we demonstrate that this protein degradation pathway positively regulates plant defences against a wide range of bacterial and fungal pathogens with different lifestyles. Transcriptomics analyses further indicate that the arginylation branch of the N-end rule pathway regulates the timing and potentiates the amplitude of the defence program against the model pathogen *Pseudomonas syringae*. Importantly, *Arabidopsis* mutant plants for the arginylation branch are also affected for their response to the immune hormone jasmonic acid and for the production of secondary metabolites with known functions in plant defence, thus suggesting a potential mechanism for a role of the N-end rule pathway in the regulation of plant/pathogen interactions. Finally, genetics approaches revealed the substrate proteins responsible for the susceptibility phenotype.

CS17.21 UBIQUITIN BINDING AND CONJUGATION IS IMPORTANT FOR THE FUNCTION AND LOCALISATION OF TOL PROTEINS

TUESDAY 12 DECEMBER 2017 **11:45**

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To be able to respond quickly and accurately to changing environmental conditions plasma membrane (PM) protein abundance and localisation are under tight regulation. In higher plants, the endocytic adaptor protein family of the TOL proteins, which contain two conserved ubiquitin binding domains (UBDs), functions at the PM in the recognition of ubiquitinated cargo destined for degradation. Ubiquitin receptors, containing UBDs, are frequently subjected to a regulatory mechanism termed coupled monoubiquitination, whereby their own ubiquitination affects their ability to bind to ubiquitin in trans. We found a portion of endogenous TOL6 as well as Venus-tagged reporter constructs of various TOLs to be ubiquitinated in plants. To assess the importance of ubiquitin binding as well as ubiquitination of the TOL proteins, we constructed non functional-UBD containing as well as constitutively ubiquitinated TOL constructs. With these TOL constructs, we performed *in vitro* ubiquitin binding studies and could successfully show a substantial decrease in ubiquitin binding. Accordingly, we designed *in vivo* TOL constructs to analyze the effect in plants, where we found a shift in the subcellular localisation from the PM to the cytosol, when compared to the endogenous TOLs. Furthermore, these TOLs lost their ability to fully rescue a higher order mutant plant line with a very pleiotrophic phenotype. The localization and function of the TOLs is therefore potentially regulated by coupled mono ubiquitination thereby adding another level to the fine-tuning in the degradation of PM proteins and thus adaptation in higher plants.

Funding: Austrian Science Fund (FWF) and Austrian Academy of Sciences (ÖAW).

CS17.22 PHOSPHORYLATION AND UBIQUITINATION REGULATE THE Exo70B2 SUBUNIT OF THE EXOCYST – A CONVERGENCE POINT OF IMMUNE SIGNALLING AND AUTOPHAGY

TUESDAY 12 DECEMBER 2017 **12:00**

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The exocyst is a conserved hetero-octameric complex mediating early tethering during exocytosis. Its Exo70 subunit plays a critical role as a spatiotemporal regulator by mediating numerous protein and lipid interactions. However, a molecular understanding of the exocyst function remains challenging. We show that Exo70B2, locates to the plasma membrane and transits through a BFA-sensitive compartment, reflecting its canonical function in secretion. We had previously shown that Exo70B2 is ubiquitinated by the E3 ligase PUB22 and degraded during PAMP-triggered immunity, potentially to dampen immune responses. We will show that treatment with the salicylic acid (SA) defence hormone analogue Benzothiadiazole (BTH), or the immunogenic peptide flg22, induced Exo70B2 transport into the vacuole via two ATG8-interacting motifs (AIMs). We also show that Exo70B2 is phosphorylated near the AIMs by the mitogen-activated protein Kinase 3 (MPK3) and mimicking phosphorylation enhances ATG8 interaction. Finally, Exo70B2 phosphonull lines are hypersensitive to BTH and displayed enhanced effector-triggered immunity (ETI). We propose a molecular mechanism, in which Exo70B2 phosphorylation may be required to control immune responses by synergistically regulating the interaction with ATG8 and ubiquitination to divert Exo70B2 from the secretory into the autophagic pathway.

CS17.23 CONTROL OF PLANT IMMUNE FUNCTION THROUGH S-NITROSYLATION

TUESDAY 12 DECEMBER 2017 **13:30**

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Changes in redox status are a conspicuous feature of immune responses in a variety of eukaryotes, but the associated signalling mechanisms are not well understood. In plants, attempted microbial infection triggers the rapid synthesis of nitric oxide (NO) and a parallel accumulation of reactive oxygen intermediates (ROIs). In this context, we are exploring S-nitrosylation, the addition of an NO moiety to a protein cysteine thiol to form an S-nitrosothiol, which is emerging as a key regulator of the plant defence response, controlling ROI synthesis, the accumulation of the immune activator, salicylic acid, hypersensitive cell death and defence at the cell wall.

CS17.24 OXIDATIVE STRESS SIGNALING IN PLANTS. TOWARDS THE PROTEOME AND BEYOND

TUESDAY 12 DECEMBER 2017 **14:00**

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In plants, alterations of reactive oxygen species (ROS) levels cause fluctuations of the redox balance and hence can affect many aspects of cellular physiology. ROS levels are controlled by a diversified set of antioxidant systems that allow the maintenance of redox status. Perturbations of these ROS levels can lead to transient or permanent changes in the redox status. This feature is exploited by plants in different stress signalling mechanisms. Understanding how plants sense ROS and transduce these stimuli into downstream biological responses is still a major challenge. Previous transcriptome-centered analyses, provided us first insights in the regulatory networks that govern the oxidative stress response. Now, tailoring various proteomics technologies allowed us to assess oxidative stress dependent changes at the posttranslational level. These efforts will allow a better understanding of how cells interpret the oxidative signals that arise from developmental cues and stress conditions.

CS17.25 THE N-END RULE PATHWAY COUPLES PRC2 ACTIVITY TO OXYGEN AVAILABILITY IN FLOWERING PLANTS

TUESDAY 12 DECEMBER 2017 **14:30**

DANIEL J GIBBS (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), HANNAH TEDDS (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), MARK BAILEY (UNIVERSITY OF BIRMINGHAM, UNITED KINGDOM), MICHAEL HOLDSWORTH (UNIVERSITY OF NOTTINGHAM, UNITED KINGDOM)

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The polycomb repressive complex 2 (PRC2) controls epigenetic gene repression in eukaryotes. Although many functions for PRC2 are known, mechanisms regulating PRC2 specificity and signal-responsiveness are poorly understood. In the flowering plant *Arabidopsis thaliana*, the PRC2 subunit VERNALIZATION2 (VRN2) accumulates in cold temperatures, where it regulates the epigenetic memory of winter to coordinate flowering in the spring, a process termed vernalization. A mechanism for this environmentally determined stabilisation has remained elusive. Here we show that the stability of VRN2 is controlled by the Arg/N-end rule pathway, a conserved division of the ubiquitin proteasome system that targets proteins for degradation based on the nature of their N-terminus. This pathway was previously shown to coordinate oxygen and nitric oxide sensing in plants, through controlling the stability of ERFVII transcription factors. Similarly to ERFVIIs, degradation of VRN2 is dependent on its Met-Cys-initiating N-terminus (N-degron), which links its stability to oxygen availability, and restricts its accumulation under normoxic (oxygen-replete) and non-vernalizing (warmer) conditions. We found that vernalization induces a hypoxia-like state that facilitates VRN2 stabilisation and function, providing a mechanism for its cold responsive accumulation and activity. Although PRC2s are widely conserved in eukaryotes, VRN2 orthologues in animals and early evolving plants lack Met-Cys N-degrons, prompting us to investigate how VRN2 was coopted to the N-end rule pathway during angiosperm evolution. Our phylogenetic and biochemical studies reveal that a latent internal gas-sensitive N-degron was exposed in VRN2 following gene-duplication and N-terminal truncation of an ancient orthologue, which facilitated neofunctionalisation of PRC2 in flowering plants.

CS17.26 POST-TRANSLATIONAL MODIFICATION OF HISTONES: NITRIC OXIDE MODULATES CHROMATIN STRUCTURE

TUESDAY 12 DECEMBER 2017 **15:00**

EVA-ESTHER RUDOLF (HELMHOLTZ ZENTRUM MÜNCHEN INSTITUTE OF BIOCHEMICAL PLANT PATHOLOGY, GERMANY), ALEXANDRA AGEEVA-KIEFERLE (HELMHOLTZ ZENTRUM MÜNCHEN INSTITUTE OF BIOCHEMICAL PLANT PATHOLOGY, GERMANY), ALEXANDER MENGEL (HELMHOLTZ ZENTRUM MÜNCHEN INSTITUTE OF BIOCHEMICAL PLANT PATHOLOGY, GERMANY), IGNASI FORNE (LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN PROTEIN ANALYSIS UNIT, GERMANY), RÜDIGER HELL (RUPRECHT-KARLS-UNIVERSITÄT HEIDELBERG CENTRE FOR ORGANISMAL STUDIES, GERMANY), AXEL IMHOF (LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN PROTEIN ANALYSIS UNIT, GERMANY), MARKUS WIRTZ (RUPRECHT-KARLS-UNIVERSITÄT HEIDELBERG CENTRE FOR ORGANISMAL STUDIES, GERMANY), JÖRG DURNER (HELMHOLTZ ZENTRUM MÜNCHEN INSTITUTE OF BIOCHEMICAL PLANT PATHOLOGY, GERMANY), CHRISTIAN LINDERMAYR (HELMHOLTZ ZENTRUM MÜNCHEN INSTITUTE OF BIOCHEMICAL PLANT PATHOLOGY, GERMANY)

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Post-translational modifications (PTM) are vital in epigenetic processes, because they allow a cell to quickly reply to internal or external appeals. Acetylation and methylation of lysine residues are well studied PTMs that plays a key role in regulation of gene expression through histone modifications. Several studies demonstrate the impotence of histone deacetylases (HDACs) that remove an acetyl group from histones during plant development and biotic or abiotic stress response. We demonstrated that nitric oxide (NO), an important plant signalling molecule, is involved in the regulation of histone acetylation by inhibiting HDACs activity. Genome-wide H3K9/14ac profiles in Arabidopsis seedlings were generated by ChIP-sequencing and NO-dependent changes were quantified thereby identifying genes which display putative NO-regulated histone acetylation. Functional classification of these genes revealed that many of them are involved in the plant defence response and the abiotic stress response. Moreover, we have evidence that NO is involved in regulation of histone methylation. Interestingly, the level of the methyl group donor S-adenosylmethionine is increased in plants with enhanced endogenous levels of the physiological NO donor S-nitrosoglutathione. Since perturbation of the methyl donor supply is supposed to affect DNA and histone methylation, the imprinting of histone H3 by methylation marks was quantified in these plants. Immunological detection of H3K9me2 and a LC-MS profiling approach demonstrated independently a significant global increase in this modification. Taken together, our data imply a new role of NO in regulation of chromatin structure in plants.

CS17.27 NUCLEOREDOXIN 1 (NRX1) SELECTIVELY RESCUES PLANT IMMUNITY

TUESDAY 12 DECEMBER 2017 **15:15**

CAPILLA MATA-PEREZ (INSTITUTE OF MOLECULAR PLANT SCIENCES, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF EDINBURGH, UNITED KINGDOM), SOPHIE KNEESHAW (INSTITUTE OF MOLECULAR PLANT SCIENCES, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF EDINBURGH, UNITED KINGDOM), STEVEN H SPOEL (INSTITUTE OF MOLECULAR PLANT SCIENCES, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF EDINBURGH, UNITED KINGDOM)

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Cellular redox changes play important roles in eukaryotic immune responses. Upon pathogen attack, host cells produce large quantities of reactive oxygen and nitrogen species, of which the signal molecule nitric oxide (NO) plays particularly important roles. Intracellular NO signals mainly by modifying cysteine residues, thereby forming S-nitrosothiols (SNO). SNO modifications have been shown to alter the activity, conformation, localisation and function of signalling proteins that harbour them. In plant cells, excessive protein-SNO accumulation has been linked to pathogenesis and impaired immunity, indicating that cellular mechanisms to remove protein-SNO are vital to cellular survival. It was recently reported that the evolutionary conserved oxidoreductase, Thioredoxin-h5 (TRXh5), plays a key role in directly removing protein-SNO during plant immunity. TRXh5 acts as a selective protein denitrosylase exhibiting preferential activity towards protein-SNO that are formed from free NO over those generated by the NO donor S-nitrosoglutathione (GSNO). Thus, an enzyme that directly reduces protein-SNO derived from GSNO has not yet been reported. Here we

show that the pathogen-inducible TRX superfamily member, Nucleoredoxin 1 (NRX1), was unable to restore immunity in mutants that display excessive accumulation of free NO, but rescued the immune-compromised phenotype of mutants that exhibit elevated levels of GSNO. Accordingly, overexpression of NRX1 partially restored gene expression programs induced by the immune hormone salicylic acid. Whilst it is still unclear if NRX1 can act directly as a protein-SNO reductase, collectively, these findings highlight that NRX1 and TRXh5 regulate distinct branches of and hence provide novel specificity to protein-SNO signalling in plant immunity.

CS17.28 DEVELOPING MASS SPECTROMETRY BASED PROTEOMIC METHODS TO IDENTIFY AND QUANTIFY PROTEIN CARBONYLATION IN PLANTS

TUESDAY 12 DECEMBER 2017 **16:00**

ALEX JONES (UNIVERSITY OF WARWICK, UNITED KINGDOM), GEORGINA CHARLTON (UNIVERSITY OF WARWICK, UNITED KINGDOM), JOHN SINCLAIR (SYNGENTA, UNITED KINGDOM), PETER KILBY (SYNGENTA, UNITED KINGDOM)

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Both biotic and abiotic stresses result in the generation of Reactive Oxygen Species (ROS) within cells. A ROS burst plays a fundamental role in stress response signalling, but also damages proteins and lipids through the uncontrolled action of free radicals. Protein carbonylation is an irreversible and harmful oxidative protein modification. The identification of many protein modifications is well established; the main limitations being the abundance of modified peptides in complex mixtures and the interpretation of peptide fragmentation patterns. The identification of carbonylated peptides has lagged behind other methods, due to their low abundance and complex secondary modifications. Most methods to identify carbonylation require enrichment and derivatisation. We are evaluating the use of a classic carbonyl-reactive compound, 2, 4-Dinitrophenylhydrazine compared to aminoxy Tamdem Mass Tags (ThermoScientific) and the enrichment of low abundance carbonylated peptides from complex mixtures such as plant leaves.

CS17.29 MONITORING PHOSPHORYLATION AND UBIQUITINATION IN PATTERN RECOGNITION RECEPTOR SIGNALLING

TUESDAY 12 DECEMBER 2017 **16:30**

FRANK MENKE (THE SAINSBURY LABORATORY, UNITED KINGDOM)

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Pattern recognition receptors (PRRs) play a key role in the first line of defence in both plant and animal innate immunity. PRR binding of their cognate ligand triggers a signalling network that ultimately results in an immune response. How activated PRR are connected to downstream defence activation has largely remained an open question, but it is evident that changes in phosphorylation play a major role and changes in ubiquitination play an emergent role. We have used quantitative phosphoproteomics approaches (Benschop et al., 2007; Mithoe et al., 2012) to identify key players in PRR signalling. Some of the earliest changes in phosphorylation, immediately downstream of activated PRRs, were identified on BIK1, RBOHD and MKKK7 (Kadota et al 2014; Mithoe et al 2016). Our initial analysis using a ubiquitin-remnant enrichment approach suggests that key components of PRR signalling also rapidly become ubiquitinated. Using targeted proteomics we are now measuring temporal changes in both phosphorylation and ubiquitination on components required in the PRR signalling network. Ongoing work on in vivo measurements of these post translation modification on PRR signalling components will be presented.

CS17.30 ON-SITE INSPECTION OF UBIQUITYLATION

TUESDAY 12 DECEMBER 2017 **17:00**

KATJA BAERENFALLER (SIAF, SWITZERLAND), SEBASTIAN GOMEZ PETERSEN (ETH ZURICH, SWITZERLAND), JULIA SVOZIL (ETH ZURICH, SWITZERLAND), PHILLIP IHMOR (ETH ZURICH, SWITZERLAND)

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Protein degradation and turnover are important cellular processes that ensure that at any time the adequate quantity of proteins are present in the proper location and in the required activity state. To get a better understanding for the role of protein turnover in protein homeostasis and in the response to internal or external cues we had enriched for ubiquitylated proteins in leaf mesophyll, epidermal and vascular tissues and found that almost all proteins in the aliphatic glucosinolate biosynthesis pathway were ubiquitylated in vasculature. Following this up we investigated whether constant protein degradation might serve to keep defence protein levels low as long as they are not required. As changing protein stability is a rapid post-transcriptional mechanism to change protein levels, we assessed changes in transcription, translation and protein levels, as well as in protein turnover using a dynamic SILAC approach in response to treatment with flagellin 22. Interestingly, we observed that upregulation at transcriptional and translational level was sometimes counteracted by increased protein turnover, leading to constant protein levels. As protein polyubiquitylation is the main mechanism to target proteins to degradation by the ubiquitin 26S-proteasome system (UPS), we were interested in determining the sites of ubiquitin attachment. After we got an unpleasant surprise applying a commonly used search strategy to identify the ubiquitin footprint, we inspected its fragmentation properties in more detail and keep on optimising the strategy to enrich for peptides with ubiquitin footprint.

CS17.31 PROTEASOME-INDEPENDENT ROLES OF LYSINE63-LINKED POLYUBIQUITIN CHAINS: INSIGHT FROM SENSOR-BASED PROTEOMICS

TUESDAY 12 DECEMBER 2017 **17:30**

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Plants have evolved sophisticated strategies to constantly monitor and respond to ever changing environmental conditions. This is achieved in part by sophisticated regulatory mechanisms using the post-translational modification ubiquitin. Ubiquitin modifications exist under many different forms in eukaryotic cells. Except for lysine-48-linked polyubiquitin chains, which mediate proteasome-dependent degradation, our knowledge about all other subtypes of post-translational modifications involving ubiquitin is still scarce. Over the past few years, my lab has been deconstructing the networks and roles of the second most abundant and yet poorly-characterised ubiquitin form: the lysine(K)-63 polyubiquitin chains. We have initiated the identification of the machinery driving the formation of K63 polyubiquitin chains and have isolated the Arabidopsis proteome specifically modified with K63-linked chains using a combination of interactome and K63 polyubiquitin sensor-based proteomics proteomics. This offers a unique insight into the biological functions of K63 polyubiquitination in protein translation, metabolism, trafficking processes between cellular compartments, etc. We have notably characterized the prominent role of K63 polyubiquitination in plant cell surface protein endocytosis and its contribution to plant growth and stress responses using the BRI1 brassinosteroid receptor and the IRT1 metal transporter. We have uncovered that IRT1 is differentially ubiquitinated in response to changes in environmental conditions to regulate its partitioning in the cell. We have also demonstrated that K63 polyubiquitination of IRT1 is sequentially acquired during endocytosis, by extension of monoubiquitin moieties attached to IRT1 at the cell surface into K63-linked polyubiquitin chains in endosomes, via a cascade of E3 ubiquitin ligases.

CS17.32 THE DYNAMIC PHOSPHOPROTEOME ALLOWS IDENTIFYING NOVEL ABIOTIC STRESS SIGNALLING COMPONENTS

TUESDAY 12 DECEMBER 2017 **17:45**

IVE DE SMET (VIB, BELGIUM), LAM DAI VU (VIB, BELGIUM), NATALIA NIKONOROVA (VIB, BELGIUM), SHANSHUO ZHU (VIB, BELGIUM), ANGELS DE LUIS BALAGUER (NORTH CAROLINA STATE UNIVERSITY, UNITED STATES), ROSS SOZZANI (NORTH CAROLINA STATE UNIVERSITY, UNITED STATES), BRIGITTE VAN DE COTTE (VIB, BELGIUM), LISA VAN DEN BROECK (VIB, BELGIUM), DIRK INZÉ (VIB, BELGIUM), KRIS GEVAERT (VIB, BELGIUM)

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To fully understand plant growth and development (also under environmental stress conditions), we need to identify novel signalling components and require insight in the underlying network. Abiotic stress conditions, such as drought and temperature, trigger signalling cascades in the plant. However, very little is known about the early signalling steps, immediately following stress perception. In this context, the reversible post-translational modification phosphorylation impacts on all aspects of cellular signalling and activity. Here, I will present data on how our phosphoproteomics workflow allowed exposing novel factors in osmotic and temperature stress signalling. I will show our progress on associated loss-of-function phenotypes (on the developmental and molecular level), and touch upon the functional relevance of specific phosphorylation signatures. Furthermore, our temporal data set following a temperature trigger allowed reverse engineering a static network in silico, connecting regulatory kinases with their potential substrates.

POSTER SESSION MONDAY 11 DECEMBER

CS17.33 POST-TRANSLATIONAL MODIFICATIONS REGULATE STARCH BRANCHING ENZYME 2.2 IN ARABIDOPSIS

MONDAY 11 DECEMBER 2017

GREGORY J MACNEILL (UNIVERSITY OF GUELPH, CANADA), IAN J TETLOW (UNIVERSITY OF GUELPH, CANADA), MICHAEL J EMES (UNIVERSITY OF GUELPH, CANADA)

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Starch is an important, osmotically inert carbon store in higher plants and a major component of the human diet. Transient starch is produced and degraded in chloroplasts over the diurnal cycle. Biosynthesis occurs through the coordinated activity of multiple classes of enzymes. Starch synthases polymerize ADP-glucose into α-glucan chains, while starch branching enzymes (SBE) introduce branch points to the growing glucan, and debranching enzymes modify glucan architecture. SBEs form phosphorylation-dependent complexes with other starch biosynthetic enzymes. Two functional isoforms of SBE exist in Arabidopsis, of which SBE2.2 accounts for most of the measurable activity. Recombinant SBE2.2 can be phosphorylated by soluble plastid extracts on residues Ser^{290} and Ser^{301}. A putative protein-protein interaction domain, conserved across all class II SBEs, has also been identified. Site-directed mutagenesis is being used to alter this conserved domain, Ser^{290} and Ser^{301}, and a C-terminal Cys residue to investigate their importance in catalysis and the formation of heteromeric complexes *in vitro*. The *in vivo* relevance of these post-translational modifications is being investigated by functional complementation of Arabidopsis *sbe* null lines with wt and mutated SBE2.2. Effects on starch biosynthesis and structure will be determined. This research is significant for its potential applications to crop production and targeted manipulation of starch structure.

CS17.34 OsSGT1 REGULATES ROOT DEVELOPMENT AND GROWTH IN RICE

MONDAY 11 DECEMBER 2017

YANG DO CHOI (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), TAE YOUNG UM (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH)), GEUPIL JANG (SEOUL NATIONAL UNIVERSITY, KOREA (SOUTH))

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Targeted degradation of protein is mediated by SCF complex composed of SKP1-Cullin-F-box protein. Suppressor of G2 allele of skp1, SGT1, is an E3 ligase complex-binding protein. It has been shown to be involved in plant development and immunity. Here we reports results of the functional study for OsSGT1 in rice. It is strongly expressed in roots than in other tissues such as shoot base and leaves. The root-specific expression pattern of OsSGT1 became decreased along age, indicating that is expressed in young roots at the higher activity of cell division. Root tips including apical meristems showed higher enrichment of OsSGT1 transcripts than upper part, suggesting that is involved in root growth. OsSGT1 expression in roots was also regulated by exogenous treatment of cytokinin which play a pivotal role in root development. Cytokinin increased OsSGT1 expression in dosage-dependent manner, suggesting its potential role in root growth in downstream of cytokinin. To understand the role of OsSGT1 in rice root development, we generated root-specifically overexpressing transgenic rice (pSR3-OsSGT1) and RNA interference rice (RNAi-OsSGT1). Overexpression of OsSGT1 induced the distinct root morphology. It developed longer and large number of roots suggesting that OsSGT1 expression is positively correlated with root development. Agronomic trait analysis of these transgenic plants showed increase rice productivity as well as root growth. This indicates that OsSGT1 is involved in the regulation of rice root development and growth. Target proteins regulated by the OsSGT1 is also discussed.

CS17.35 PROTEIN S-PALMITOYLATION REGULATES INVASION AND APICAL ORGANELLE SECRETION IN HUMAN MALARIA PARASITE, *PLASMODIUM FALCIPARUM*

MONDAY 11 DECEMBER 2017

MANSOOR AZEEM SIDDIQUI (INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIO-TECHNOLOGY, INDIA), SHAILJA SINGH SINGH (JAWAHARLAL NEHRU UNIVERSITY, INDIA), PAWAN MALHOTRA (INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIO-TECHNOLOGY, INDIA), CHETAN E CHITNIS (INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIO-TECHNOLOGY, INDIA)

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Protein S-palmitoylatiom is the only reversible post translational lipid modification known in eukaryotic cells. The recently published global palmitome for malaria parasite shows that over 400 proteins are S-palmitoylated in malaria parasite making it one of the most abundant post translational modification after phosphorylation. Inhibition of protein S-palmitoylation in parasite has been known to inhibit red blood cell invasion but little is known about the possible mechanisms. Here we propose that S-palmitoylation is necessary for microneme release and invasion related signalling in malaria parasite, *Plasmodium falciparum*. Inhibition of DHHC palmitoyl acyl transferases or depalmitoylases by small molecule inhibitors affects the parasite's ability to invade red blood cells by affecting vesicle (apical organelles) release or exocytosis in the parasite.

CS17.36 MITOGEN ACTIVATED PROTEIN KINASES (MAPKS) ACTIVATION POST EFFECTOR RECOGNITION IN TOMATO

MONDAY 11 DECEMBER 2017

ARSHEED H SHEIKH (SCHOOL OF LIFE SCIENCES, UNIVERSITY OF WARWICK, UNITED KINGDOM), VARDIS NTOUKAKIS (SCHOOL OF LIFE SCIENCES, UNIVERSITY OF WARWICK, UNITED KINGDOM)

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The major virulence strategy of the phytopathogenic bacteria Pseudomonas syringae pv. tomato DC3000 is to secrete effector proteins into the tomato cells to target the immune machinery. Effectors like AvrPto and AvrPtoB are recognised by intracellular effector-recognition complex composed of the NBLRR protein Prf and the Pto kinase. Following P+1 loop disruption and transphosphorylation the Pto/Prf complex dissociate leading to downstream signalling through mitogen-activated protein kinases (MAPKs). Hence, the Pto/Prf complex is a sophisticated molecular trap for effectors and provides an excellent model to study the mechanism of MAPKs activation. In the current study we sought to investigate the mechanism of MAPKs activation post Pto/Prf recognition of AvrPto/AvrPtoB effectors. We identify 14-3-3 proteins as part of the Pto/Prf resistance complex acting as regulators of MAPKs activation. 14-3-3 proteins could act as a bridge for signal transduction from activated Pto/Prf complex to downstream MAPK cascade in ETI responses.

CS17.37 UNRAVELLING PHOSPHORYLATION-MEDIATED SIGNALLING IN PLANTS AT HIGH AMBIENT TEMPERATURE

MONDAY 11 DECEMBER 2017

LAM DAI VU (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM), MARIA ANGELS DE LUIS BALAGUER (PLANT AND MICROBIAL BIOLOGY DEPARTMENT NORTH CAROLINA STATE UNIVERSITY, UNITED STATES), INGE VERSTRAETEN (INSTITUTE OF SCIENCE AND TECHNOLOGY, AUSTRIA), ROSANGELA SOZZANI (PLANT AND MICROBIAL BIOLOGY DEPARTMENT NORTH CAROLINA STATE UNIVERSITY, UNITED STATES), KRIS GEVAERT (VIB-UGENT CENTER FOR MEDICAL BIOTECHNOLOGY, BELGIUM), IVE DE SMET (VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY, BELGIUM)

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High temperature has numerous effects on plant growth and development. Our knowledge on thermal sensing and response mechanisms in plants has been increasing in the past years, mostly through genetics and by focusing on transcriptional changes and associated networks. However, many missing links in the thermal signalling cascades are likely governed by various post-translational modifications.

Here, I will report our latest findings on early changes in protein phosphorylation in the dicot *Arabidopsis thaliana* and the monocot *Triticum aestivumupon* increasing ambient temperature. Starting from a quantitative phosphoproteome, I identified thermoresponsive phosphoproteins, referred to as TOTORO/TARGET OF TEMPERATURE (TOT) candidates. The dynamic changes in phosphorylation status of TOTs at different stages of the response revealed potential networks of interplaying protein kinases, phosphatases and their targets. These are linked to diverse cellular processes, providing us an entry point to gain mechanistic insight into the regulation of distinct pathways by high temperature and the integration of those various signals in a global response. I will also present data on the functional characterisation of some central regulators of thermomorphogenesis in plants. For this, I focus on temperature-triggered growth responses, such as hypocotyl elongation and upward bending of petioles, and downstream molecular and biochemical responses in loss-of-function lines. Further functional characterisation will be supported by protein-protein interaction studies as well as *in vitro* and *in vivo* biochemical assays.

CS17.38 INTERPLAY BETWEEN PHOSPHORYLATION AND ACETYLATION IN REGULATING RPS4/RRS1 IMMUNE RECEPTOR PAIR

MONDAY 11 DECEMBER 2017

HAILONG GUO (THE SAINSBURY LABORATORY, UNITED KINGDOM), YAN MA (THE SAINSBURY LABORATORY, UNITED KINGDOM), ZANE DUXBURY (THE SAINSBURY LABORATORY, UNITED KINGDOM), JAN SKLENAR (THE SAINSBURY LABORATORY, UNITED KINGDOM), FRANK MENKE (THE SAINSBURY LABORATORY, UNITED KINGDOM), JONATHAN D. G. JONES (THE SAINSBURY LABORATORY, UNITED KINGDOM)

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Pathogens secrete effector proteins into plant cells to promote disease. In turn, plants evolved disease resistance genes (R genes) that confer specific recognition of pathogen effectors and activate a strong defence response known as effector-triggered immunity (ETI), which includes rapid transcriptional reprogramming and programmed cell death at sites of infection. Most plant R genes cloned to date encode immune receptors called NLRs, with a conserved nucleotide-binding (NB) domain and a C-terminal leucine-rich repeat (LRR) domain. The adjacent, divergently transcribed, *Arabidopsis Resistance to Ralstonia Solanacearum 1 (RRS1)* and *Resistance to Pseudomonas Syringae 4 (RPS4)* genes encode TIR-NLR proteins that function together to recognize two bacterial effectors, PopP2, an acetyl-transferase from *Ralstonia solanacearum* and AvrRps4, a coil-coiled protein from *Pseudomonas syringaepv.pisi.* The RRS1-R allele in *Arabidopsis* accessions Nd-1 and Ws-2 confers AvrRps4 and PopP2 recognition, whereas Col-0 allele of RRS1 (RRS1-S) confers AvrRps4, but not PopP2, recognition. We previously reported that AvrRps4 interacts with, and PopP2 acetylates, the RRS1 WRKY domain, resulting in activation of the RPS4/RRS1 complex and subsequent defence activation. Here we show that WRKY domain and its neighboring domain DOM6 of RRS1-R, but not RRS1-S, is phosphorylated *in vivo.* Multiple phosphorylated residues of RRS1-R were identified using immunoprecipitation and mass spectrometry (IP-MS). Among those phosphosites, we found that phosphorylation of RRS1-R at Ser 1296 is essential to PopP2, but not AvrRps4, recognition. Further studies are needed to dissect the molecular basis of phosphorylation of RRS1 and to address the coordinated regulation between phosphorylation and acetylation in mediating defence activation.

CS17.39 IMPAIRED PROTEASOME FUNCTION IN ARABIDOPSIS *RAD23ABCD* MUTANT ALTERS AUXIN HOMEOSTASIS

MONDAY 11 DECEMBER 2017

MARITA ANGGARANI (INSTITUTE OF PLANT MOLECULAR BIOLOGY, ACADEMIA SINICA, TAIWAN), YA-LING LIN (INSTITUTE OF PLANT MOLECULAR BIOLOGY, ACADEMIA SINICA, TAIWAN), HONGYONG FU (INSTITUTE OF PLANT MOLECULAR BIOLOGY, ACADEMIA SINICA, TAIWAN)

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Ubiquitylated substrate recognition during ubiquitin/proteasome-mediated proteolysis (UPP) is mediated directly by the proteasome subunits RPN10 and RPN13 and indirectly by ubiquitin-like (UBL) and ubiquitin-associated (UBA) proteins such as RAD23, DDI1 and DSK2. How the ubiquitylated substrate distinctly targeted to proteasome remains unclear. It has been reported that RAD23 quadruple knock out mutant is lethal and RAD23B is more important within the family. However, we are able to obtain the quadruple mutant thereof valuable for further functional characterisation. We found the phenotypes of *rad23b-1* mutant are not like the previously described. The quadruple mutant plants exhibited shorter root length, abnormal male and female gamete. The *rad23abcd* mutant has larger cell size in both epidermal and mesophyll cells of cotyledon which is the signature of proteasomal mutants. IAA-Asp increased and IAA-Glu decreased in *rad23abcd* as compared to wild type, while no alteration observed on the IAA and oxIAA level. IAA-Asp and IAA-Glu synthesis were regulated by GH3s genes, governed by the ARFs and AUX/IAA-targeted by proteasome. The qPCR analysis indicated alteration of these gene expressions in the *rad23abcd* compared to wild type. The *rad23abcd* mutant also has ABA and glucose sensitivity during early germination stage and sensitive to UV-C treatment. This data supports our previous finding that there is no substrate specificity for the ubiquitylated subtrates targeted to the proteasome. In addition, we provide evidence that the phenotypes observed in the *rad23abcd* mutant, and likely for other proteasomal mutants, were caused by Aux/IAA impaired degradation resulting in auxin homeostasis alteration.

CS17.40 ARABIDOPSIS DEUBIQUITINASE OTU5 IS INVOLVED IN FLOWERING BY REGULATING MAJOR REPRESSORS *FLC/MAF4/MAF5*

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The reversible ubiquitylation reaction is catalysed by different classes of deubiquitinases (DUBs), including ovarian tumor domain (OTU)-containing DUBs. Based on distinct phylogeny, substrate binding, cleavage specificities, and mutant phenotypes suggest the twelve Arabidopsis thaliana OTU deubiquitinases participate in different functions. The null *otu5-1* displayed pleiotropic phenotypes including early flowering, reduced organ size, plant height and reduced ovule numbers. Transcriptomics analyses indicate downregulation of major flowering repressors *FLC*, and *MAF4-5* is responsible for *otu5-1* early flowering. Nuclear fractionation, transient GFP expressions in protoplast and MNase treatment of chromatin revealed that OTU5 is enriched in nucleus and associated with chromatin. The qChIP analyses on *FLC* and *MAF4-5* revealed reduction of activation histone mark H3K36me3 and increment of repression histone marks H3K27me3/H3K9me2 and H2Bub1 in *otu5-1* indicate its involvement in transcriptional regulation on *FLC* and *MAF4-5*. qChIP results confirmed the OTU5 association and its regulatory function on FLC, and MAF4-5. Catalytically inactive OTU5 was unable to compliment the defective growth phenotypes and early flowering of *otu5-1*, indicating the importance of enzymatic function of OTU5. Size-exclusion chromatography indicated OTU5 likely to function in large complex. Interestingly, *otu5/arp6-/-* double mutant shows synergistic phenotypes with severe developmental defects including seed abortion, curly leaves, and short statured plants. The ChIP results did not show alteration of H2A.Z at *FLC*, *MAF4* and *MAF5* between *otu5-1* and wild type Col-0, suggested OTU5 might function in different pathway. OTU5-associated proteins and the underlying mechanism need to be elucidated by isolating complex and further characterisation.

CS17.41 COMBINATORIAL TRANSCRIPTIONAL REGULATION OF THE PLANT DEFENCE RESPONSE

MONDAY 11 DECEMBER 2017

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Transcriptional reprogramming plays a significant role in the defence of plants against pathogen infection. This defence response involves a complex and highly sophisticated regulatory circuitry in which transcription factors (TFs) play a key role in determining the specificity of the response to different pathogens. We have obtained high-resolution temporal transcriptome data following biotic and abiotic stress treatments in Arabidopsis, and have generated regulatory network models of the TF interactions underlying these responses. Such models predict widespread combinatorial transcriptional regulation of many known defence genes and pathways, providing a mechanism for generating complex transcriptional profiles, and thus fine-tuning the plant's response. Combinatorial regulation can also occur by variations in the composition of multi-subunit TFs. NF-Y TFs directly bind CCAAT boxes in target gene promoters as trimers of A, B and C subunits, and can act as positive or negative regulators of transcription. In Arabidopsis a multi-gene family encodes each subunit of the complex and many of these subunits change in expression during plant stress responses. A putative trimer (NF-YA2, NF-YB2, NF-YC2) has been identified. We have shown that NF-YA2 expression levels determine accumulation of JA and that the nf-ya2 mutant displays significantly increased susceptibility to Botrytis cinerea. Furthermore, BiFC assays in N. benthamiana revealed that NF-YB2 and NF-YC2 are able to heterodimerise in planta, and this interaction was confirmed in Arabidopsis expressing tagged NF-YC2. We are now investigating whether this NF-YB2/C2 dimer binds the NF-YA2 subunit, and, if so, the regulatory function of the resulting NF-Y trimer during defence against B.cinerea.

CS17.42 IDENTIFICATION OF HOST MEMBRANE PROTEINS THAT ARE INVOLVED IN PLANT–APHID INTERACTIONS

MONDAY 11 DECEMBER 2017

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Aphids are agriculturally important phloem-feeding pests which cause loss in many crops worldwide through damage caused by feeding, and by acting as vectors for transmission of plant viruses. These insects use specialised mouthparts (stylets) to puncture and probe leaf tissue in search of the phloem, from which they feed.

As the aphid probes the leaf tissue, a gel saliva is secreted that forms a protective sheath around the stylet. Staining of the gel saliva coupled with microscopy reveals the stylet path, showing extracellular movement through the tissue with probe sites in the leaf cells along this path. In addition, watery saliva is also produced, which contains effector proteins involved in manipulating host cellular processes to aid infestation.

Given the close proximity of the stylet to the plant cell surface, the secretion of effectors, and the role of membrane proteins in plant defences, we aim to identify membrane proteins that are involved in immunity or susceptibility towards aphid infestation. To achieve this, we have enriched for membrane proteins from whole protein extracts of Arabidopsis rosette and flowering tissue, with and without aphid infestation. Mass spectrometry and protein quantification (using MaxQuant and Perseus software) was used to identify proteins that were up- or down- regulated upon aphid infestation. To confirm the importance of these proteins in plant-aphid interactions, Arabidopsis knockout mutants of these proteins will be tested for resistance/susceptibility to aphid infestation. From this work, we hope to characterise the membrane proteins and the cellular processes involved in the plant-aphid interaction.

CS17.43 ON THE QUEST FOR POSTTRANSLATIONAL MODIFICATIONS GOVERNING PHOSPHOENOLPYRUVATE CARBOXYLASE LIFE CYCLE

MONDAY 11 DECEMBER 2017

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C4-plants possess a more efficient photosynthetic system, mainly due to its ability to concentrate CO_2 around RuBisCO. A key player in this CO_2-concentrating mechanism is the enzyme phosphoenolpyruvate carboxylase (PEPC). PEPC uses the solubilised atmospheric CO_2 to carboxylate phosphoenolpyruvate, producing oxaloacetate that will be the first carrier in the CO_2-concentrating mechanism. In C4-leaves, PEPC is a very abundant protein and its total amount varies little along the photoperiod. Thus, PEPC has to be tightly regulated to address the different carbon fixation needs along the day. Posttranslational modifications (PTMs), frequently, tune protein activity and/or stability. In PEPC, it is described that the phosphorylation of a serine residue at the N-terminal is important to diminish its allosteric inhibition by malate. However, C4-plants that are unable to phosphorylate this serine residue display normal photosynthetic capacity. This suggests that PTMs regulating PEPC are not yet described. To discover novel PEPC PTMs we collected leaves from maize, a C4-plant, during the photoperiod. We used an LC-MS/MS-based approach to analyse maize leaves proteome and identify putative-phosphorylated and -ubiquitinated residues. SWATH-MS analyses were performed to assess the variation of putative PTMs along the day. Dephosphorylation assays, followed by LC-MS/MS analyses, are being conducted to validate new PTMs. These results are the first step into the characterisation of the regulatory mechanisms modulating PEPC activity and stability. This characterisation will establish the basis to uncover regulators behind the C4-metabolic pathways, which are still mostly unknown.

CS17.44 TRANSCRIPTOMIC PROFILING OF CAPSICUM ANNUUM IN RESPONSE TO HIGH-INTENSITY UV IRRADIATION REVEALS STRESS DEFENCE SIGNALLING

MONDAY 11 DECEMBER 2017

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Ultraviolet (UV) radiation augmentation can restrain growth of plants, including some important crops, resulting in severe reduction in yield. This stress will increase in the near future because of global climate change, according to reports from the Intergovernmental Panel of Climate Change. Therefore, understanding UV irradiations stress responses is now thought to be one of the most important topics in plant science. However, UV-B stress signalling mechanisms in plants including Capsicum annuum remain poorly understood. Molecular biological analyses have allowed us to draw a picture of UV stress responses in plants, and determination of the transcriptome has had a significant impact on this research field. Therefore, the purpose of this work was to explore the transcriptome dynamics of Capsicum plants in response to UV-B stress. We used a RNA-sequencing method to perform gene-expression profiling of the transcripts under UV-B treatment. The radiation promoted the acclimation of Capsicum to UV by regulating the expression of genes with functions in UV protection and also by inducing the accumulation of phenolic compounds. Furthermore, most representative transcripts related to biological pathways, including antioxidant enzymes, G proteins, primary and secondary metabolism, and transcription factors. Interestingly, some of the genes involved in secondary metabolism. Transcriptome profiling highlights possible signalling pathways and molecules for future research. These results opened up ways of exploring the molecular mechanisms underlying the effects of UV-B irradiation on Capsicum and have great implications for further studies.

CS17.45 PP2A-B'γ CONTROLS METHYLATION OF INDOLE GLUCOSINOLATES AND MODULATES METHIONINE METABOLISM IN ARABIDOPSIS

MONDAY 11 DECEMBER 2017

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In a constantly changing environment plants need to have a rapid and adjustable system for sensing and responding to the external cues. Stress-induced reprogramming of primary metabolism and production of secondary metabolites are essential defence mechanisms that prevent pathogens and pests from colonizing host plant tissues. Defence response in plants is under the control of the cytoplasmic regulatory network and tightly connected with biosynthesis and recirculation of amino acids, which provide precursors for a variety of secondary compounds. In cruciferous Brassicaceae family plants like Arabidopsis thaliana, methionine metabolism is tightly linked with the biosynthesis of aliphatic methionine-derived and indole tryptophan-derived glucosinolates (GSL), major secondary compounds that confer resistance against insect herbivores and microbial pathogens. In response to biotic stress, indole GSL undergo hydroxylation and transmethylation reactions, and the resulting modified glucosinolates display diverse biological functions. We have shown that regulatory B'γ of protein phosphatase 2A (PP2A-B'γ) physically interacts with INDOLE GLUCOSINOLATE METHYLTRANSFERASEs (IGMTs) and specifically controls the methylation of indole glucosinolates and formation of 4-methoxy-indol-3-yl-methyl glucosinolate (4MO-I3M) in Arabidopsis leaves. Proteomic and metabolomics approaches revealed that PP2A-B'γ is required to control the abundance of oligomeric protein complexes functionally linked with activated methyl cycle and the transmethylation capacity of leaf cells. Our results highlight a key role for PP2A-B'γ in controlling cross-communicating metabolic cycles in methionine metabolism, which has a significant impact on plant resistance to biotic stress.

CS17.46 CORRELATION BETWEEN NPH3 PHOSPHORYLATION STATUS, LOCALISATION AND PHOTOTROPIC ENHANCEMENT

MONDAY 11 DECEMBER 2017

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Phototropism is the re-orientation of plant growth towards directional light and is important to promote light capture and early seedling growth. Traditionally, dark-grown (etiolated) seedlings are used to study phototropism, although light-grown (de-etiolated) seedlings retain phototropic responsiveness. Phototropin 1 (phot1) is a blue light-activated serine/threonine kinase acting as the main photoreceptor mediating hypocotyl phototropism in Arabidopsis. It is generally accepted that phototropic curvature ultimately arises from the establishment of a lateral auxin gradient across the hypocotyl. However, how phot1 coordinates this auxin redistribution is still unknown. Mutants lacking the phot1-signalling component Non-Phototropic Hypocotyl 3 (NPH3) are aphototropic and fail to show lateral auxin accumulation in response to phototropic stimulation. Thus, elucidating the function of NPH3 is key to understanding how phototropism is established. We and others have found that NPH3 is rapidly de-phosphorylated in response to phot1 activation, which in turn, induces its re-localisation from the plasma membrane. Here we show that the localisation of NPH3 correlates with its phosphorylation status. We show that a gradient of NPH3 re-localisation is detectable across the upper hypocotyl following phototropic stimulation. Compared to etiolated seedlings, de-etiolated seedlings show enhanced kinetics of phototropic curvature. This enhancement correlates with reduced levels of NPH3 de-phosphorylation and re-localisation. Phosphorylation is therefore important for regulating NPH3 function. Identification of the in vivo phosphorylation sites of NPH3 is now required to assess how dynamic changes in its phosphorylation modulates plant growth responses to light.

CS17.47 DYNAMIC UBIQUITINATION DETERMINES NPR1 TRANSCRIPTIONAL COACTIVATOR ACTIVITY

MONDAY 11 DECEMBER 2017

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Pathogen recognition by plants leads to accumulation of the immune hormone salicylic acid (SA), which causes dramatic reprogramming of the transcriptome to prioritise immune responses. These large-scale changes in gene expression are dependent on the SA-responsive transcription coactivator, NPR1. The coactivator activity of NPR1 has previously been shown to be regulated by ubiquitination and proteasomal degradation. Our recent data suggests that ubiquitination of NPR1 is a multi-step process in which initial ubiquitination activates NPR1, while processive ubiquitin chain extension mediated by the E4 ligase, UBE4, leads to its deactivation. Importantly, this establishes a window of opportunity for NPR1 to activate target genes and establish disease resistance. We have now discovered that in addition to the extension of ubiquitin chains, their trimming by deubiquitinases (DUBs) is also critical for NPR1 coactivator activity. Accordingly, pharmacological inhibition and genetic mutation of the proteasome-associated DUBs, UBP6 and UBP7, led to loss of both NPR1-mediated gene expression and immunity in Arabidopsis. Our data suggest that opposing activities of UBE4 and UBP6/7 ensure that the transcriptional window of opportunity for NPR1 coactivator remains flexible, allowing adjustment according to immune demand.

CS17.48 UNDERSTANDING THE ROLE OF NITRATE REDUCTASES NIA1 AND NIA2 UNDER ABIOTIC STRESSES

MONDAY 11 DECEMBER 2017

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Nitrogen is an essential plant nutrient taken up by the roots in the form of nitrates from the soil. Nitrate and drought stress are intrinsically linked. When a plant is under drought stress, nitrate uptake through the roots drops due to lack of water. An important enzyme in the process of nitrogen assimilation is nitrate reductase. Nitrate reductase reduces nitrate into nitrite and is involved in the reduction of nitrite to the signalling molecule of nitric oxide (NO). Nitric Oxide (NO) is implicated through oxidation of substrates for ubiquitination through the N-end rule pathway. This has been shown to be involved in multi-stress tolerance, in particular stomatal closure, through stress related transcription factors. Nitrate reductase is encoded for by two genes; *NIA1* and *NIA2*. In order to understand the implications of variable nitrate supply on stomatal aperture, understanding which gene (*NIA1* or *NIA2*) is involved under differing stress responses is critical. There is conflicting information on the role of the enzyme nitrate reductase in stomatal closure. I have identified single mutants to isolate and examine using genetic resources including fluorescent tagging to see where and when the gene is being expressed. There is also the indication of control of stabilisation by sumoylation. Individual roles and characteristics of NIA1 and NIA2 need to be identified and how they change in response to stress, in particular stomatal aperture responses.

CS17.49 WHAT 'R' YOU DOING HERE? INVESTIGATING THE ROLE OF S-ACYLATION IN PLANT DISEASE RESISTANCE PROTEINS

MONDAY 11 DECEMBER 2017

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Plants are constantly surrounded by potential pests and pathogens, threatening the health of the plant and the security of our food production. Unable to move to escape challenge, plants must defend themselves in situ, and possess a complex, multi-layered innate immune system. A crucial aspect of the plants immune response is the recognition of specific pest and pathogen 'effector proteins' - secreted molecules that manipulate plant processes to promote the pest or pathogens lifestyle, increasing disease potential. Effector recognition is facilitated by plant resistance (R) proteins, largely belonging to the nucleotide-binding leucine-rich repeat (NB-LRR) family, analogous to immune receptors found in animal systems. The modular structure, and interaction between domains, enables effector recognition and subsequent activation of these R-proteins, but the mechanisms of activation and downstream immune signalling remain poorly understood.

Recent work on a group of R-proteins from potato, required for resistance to the notorious potato late blight pathogen Phytophthora infestans, has revealed that several of these undergo S-acylation - a reversible fatty acid based post-translational modification. S-acylation is particularly known for its role in membrane anchoring and localisation but, due to its reversibility, is also linked to regulating aspects of protein function such as activation, trafficking, and protein-protein interaction. Ongoing investigation has shown that S-acylation occurs at multiple sites, in multiple domains, within R proteins, suggesting a complex role for this dynamic post-translational modification in plant R-protein function. Our latest data on the functional consequences of R-protein S-acylation during plant defence responses will be presented.

CS17.50 FUNCTIONAL ANALYSIS OF CYP707A70 GENE (ABA 8'-HYDROXYLASES) FROM HOT PEPPER *(CAPSICUM ANNUUM)* IN TRANSGENIC TOBACCO AND TOMATO

MONDAY 11 DECEMBER 2017

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Cytochrome P450 (P450 or CYP) monooxygenases in plants have important roles in the biosynthesis of plant hormones, defence related chemicals, and diverse secondary metabolites. We isolated CYP707A70 gene, which were encoded Abscisic acid 8'-hydroxylase from a cDNA library of hot pepper (Capsicum annuum L. cv. Bukang). The abscisic acid (ABA) is one of the important phytohormone that regulates many phygioiological processes in plant. The major ABA catabolic pathway is oxidative degradation to 8'-hydroxy ABA In order to understand the roles of the CYP707A70 gene in hot pepper, the gene expression patterns in various hot pepper tissues were analysed. The CYP707A70 gene was highest expressed in leaves. The expression level of gene was increased during the growth phase of the fruit but decreased in maturation. In flower, the relative transcript level of CYP707A70 was mainly expressed in ovary. To demonstrate the function of the CYP707A70 genes in plant, this gene was cloned into plant expression vector and transformed into tobacco (Nicotiana tabacum cv. Xanthi NC) and tomato (Solanum lycopersicum cv. Micro-Tom). The CYP707A70 over-expression transgenic tobacco plants showed the wilting phenotype. Furthermore, transgenic tobacco plants showed that down regulated seed formation and pollen viability compare to non-transgenic tobacco.

CS17.51 THE ROLE OF O-GLYCOSYLATION IN PLANT DEVELOPMENTAL TRANSITIONS

MONDAY 11 DECEMBER 2017

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Plants undergo several developmental transitions in the course of their life cycle, marked by specific morphological changes such as altered leaf morphology, the formation of trichomes and the onset of flowering. The timing of transition from juvenile to adult phase is regulated by the amount of available sugar accumulating during plant growth. This process is mediated by balancing the ratio between miRNA156 and miRNA172, thus regulating the downstream miRNA156 target genes, a family of SPL (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE) transcription factors. In Arabidopsis, mutants in the protein O-fucosyltransferase SPINDLY (SPY) show accelerated transition from the juvenile to the adult phase, as well as early flowering. O-fucosylation is a posttranslational modification and closly connected to O-GlcNAcylation. In this type of O-glycosylation, either a single fucose or N-acetylglucosamine (GlcNAc) is O-linked to side chains of serine- or threonine residues of nuclear and cytosolic proteins. In the current working model O-fucosylation and O-GlcNAcylation compete for the same targets, with counteracting functional effects. Given the observed early transition phenotypes of spy-mutants, we are currently investigating the role of O-glycosylation in the regulation of miRNA156 and miRNA172, potentially in response to the accumulation of sugar in growing tissues. Preliminary data suggest that spy-mutants indeed show altered levels of these miRNAs. Moreover, we are testing if O-glycosylation also affects the function of SPLs. These experiments will contribute to our understanding of the molecular mechanisms of O-glycosylation in the regulation of plant development.

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CS17.52 CHARACTERISING THE ROLE OF ELONGATION FACTOR TU RECEPTOR (EFR) PROTEIN KINASE ACTIVITY AND PHOSPHORYLATION IN PATTERN-TRIGGERED IMMUNITY

MONDAY 11 DECEMBER 2017

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Detection of pathogenic microbes by plants is mediated through perception of pathogen-associated molecular patterns (PAMPs) by plasma membrane (PM)-localised pattern recognition receptors (PRRs). PAMP perception elicits a battery of downstream responses, including reactive oxygen production, calcium influxes and MAPK activation, collectively known as pattern-triggered immunity (PTI). One of the most well characterised PRRs in Arabidopsis is the leucine-rich repeat receptor kinase (LRR-RKs) EFR (ELONGATION FACTOR TU RECEPTOR), which functions co-ordinately with the co-receptor BAK1 (BRI1-ASSOCIATED RECEPTOR-LIKE KINASE 1; also an LRR-RK) to detect the bacterial PAMP EF-Tu (or its derived peptide elf18). Perception of elf18 by EFR leads to heterodimerization and subsequent activation of the BAK1 protein kinase domain by an as yet unknown mechanism, leading to downstream signalling events. EFR is phosphorylated *in vivo,* presumably by BAK1, and is an active protein kinase (PK) itself, but whether EFR protein kinase activity is required for immunity is unknown. In order to assess the role of EFR PK activity in immunity we have generated a series of kinase inactive EFR mutants and are testing their capacity to activate PTI responses in transgenic *Arabidopsis thaliana*. Preliminary analyses indicate that EFR PK activity is dispensable for at least some branches of PTI. Additionally, our studies aim to identify the sites of EFR phosphorylation, the PKs that catalyse these events, and the functional importance of EFR phosphorylation for PTI. Collectively, these studies will provide insight into the molecular details of how immune receptor activation controls the downstream processes responsible for disease resistance.

CS17.53 THE ROLE OF THE PEPTIDASE DA1 AND THE E3-LIGASE BIG BROTHER IN REGULATING CELL PROLIFERATION AND SENESCENCE IN PLANTS

MONDAY 11 DECEMBER 2017

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How plants grow from a small seed to their final stature appeals to the imagination of many people. Uncovering the molecular mechanisms that determine plant organ growth and size is therefore a challenging and fascinating research topic. The E3-ligase BIG BROTHER (BB) and the peptidase DA1 work in concert to restrict cell proliferation and leaf longevity in Arabidopsis. Mutants of these genes produce larger organs that contain more cells whereas overexpression reduces growth. Recent findings have demonstrated the importance of this pathway for yield in crop plants, such as maize and Canola. Molecularly, BB activates the latent peptidase DA1 by ubiquitination in a novel activation-repression mechanism. Subsequently, the activated DA1 destabilises positive regulators of growth, such as UBP15 and the transcription factors TCP14 and TCP15. In addition, DA1 cleaves BB in a negative feedback loop. The C-terminal fragment of BB is recognized and ubiquitinated by the N-end rule E3 Ubiquitin ligase PROTROLYSIS1 and marked for proteasomal degradation. These events result in a unidirectional transition from cell proliferation to cell expansion. Recently, we have found that two additional ubiquitin-specific proteases, UBP12 and UBP13, interact with DA1 in vivo. These de-ubiquitinating enzymes might play an important role in plant growth and development by de-activating DA1 and thus promoting cell division.

CS17.54 LYSINE METHYLATION OF NON-HISTONE PROTEINS IS INVOLVED IN THE RESPONSE OF ARABIDOPSIS PLANTS TO A STRESS INDUCED BY CADMIUM

MONDAY 11 DECEMBER 2017

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Protein methylation on lysine residues is emerging as an important and widespread post-translational modification affecting many cellular processes in eukaryotes. However, methylation of non-histone proteins is still a poorly known process in plants. To improve our knowledge about this modification, we analysed the dynamics of lysine methylation on non-histone proteins in plants exposed to the toxic metal cadmium. We conducted our study using *Arabidopsis thaliana* and *Arabidopsis lyrata* plants, which are sensitive to cadmium, and with *Arabidopsis halleri* plants,which have the ability to tolerate and hyper-accumulate cadmium. First, we analysed the patterns of lysine methylation using a combination of western blot and mass spectrometry analyses. We showed that some methylproteins are differentially methylated in response to cadmium stress. Then, we analysed the expression of genes coding protein lysine methyltransferases and demethylases in different transcriptomic datasets related to cadmium stress. We showed that transcriptional regulation of these genes had little impact during response and adaptation to a stress induced by cadmium. Last, we developed a screening procedure to analyse the cadmium tolerance of *A. thaliana* mutants for genes coding methyltransferases. We identified and characterised two mutants that are either more tolerant or more sensitive to cadmium than wild-type plants. Together, our results show that the fine-tuning regulation of non-histone proteins by lysine methylation has a role in the response of *Arabidopsis* plants to cadmium stress.

CS17.55 DYNAMIC S-ACYLATION IN PLANTS

MONDAY 11 DECEMBER 2017

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With the discovery that a large proportion of the plant proteome is S-acylated, it has become apparent that this form of lipid modification on cysteines is an important post-translational modification that may have a vital role in plant growth and development. S-acylation was initially thought to act as a membrane anchor for soluble proteins but the fact that S-acylation is reversible and also occurs on integral membrane proteins, suggests that it is not just a means of anchoring proteins to membranes but may have a role in regulating important aspects of a protein such as function, localisation, activity and stability. With the aid of stable isotope labelling with amino acids in cell culture (SILAC) and CLICK chemistry with alkyne fatty acids, we aim to identify proteins that undergo dynamic changes in S-acylation as part of their regular cell function.

CS17.56 A DOWNY MILDEW EFFECTOR IMPOSES HOST SUSCEPTIBILITY BY MODULATING THE ACTIVITY OF A HOST RING E3 LIGASE REQUIRED FOR IMMUNITY

MONDAY 11 DECEMBER 2017

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Pathogen effectors subvert host immunity by manipulating host physiology for the benefit of the pathogen. *Hyaloperonospora arabidopsidis* contains ~140 effector candidates carrying a signal peptide and an RxLR motif. HaRxL62 promotes plant susceptibility and is highly expressed during infection. Using IP-mass spec, we identified RING type E3 ligase (RING62) as an HaRxL62 interactor. RING62 is single copy in Arabidopsis, but well conserved in dicots as well as monocots. From previous studies, RING62 interacts in yeast two hybrid with RCD1, TCP14, TCP21, SAG102 and JAZ3. RING62 shows *in vivo* and *in vitro* auto-ubiquitination activity in an ATP-dependent manner. To reveal the effect of HaRxL62 on E3 ligase activity of RING62, we monitored E3 ligase activity after adding HaRxL62 *in vitro* as well as *in vivo*. E3 ligase activity seemed to increase in the presence of HaRxL62. However, RING62 self-association was not affected in the presence of HaRxL62. ring62 mutants show compromised resistance against *Hpa* and *Pst* DC3000. To reveal the regulatory network between HaRxL62 and RING62, we conducted yeast two hybrid screening using HaRxL62 or RING62 as a bait, respectively. TCP 3, 7, 9 and 15 interact with both HaRxL62 and RING62, whereas additional TCPs such as TCP 10, 13 and 23 were detected only in RING62 screening. Our goal is to elucidate how HaRxL62 elevates virulence by rendering RING62 hyperactive, so that HaRxL62 perturbs the balance of turnover and accumulation of its targets to suppress plant immune responses.

CS17.57 UNDERSTANDING THE ROLE OF SUMOYLATION IN REGULATING LIGHT SIGNALLING COMPONENTS IN PLANTS

MONDAY 11 DECEMBER 2017

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Light is an essential informational signal that promotes plant growth and development. Photoreceptors, light signalling components and transcriptional regulators have been observed to translocate into the nucleus and form highly dynamic subnuclear structures known as photobodies. The composition, mode of formation and molecular function of photobodies remain elusive. Posttranslational modification of proteins represents a regulatory mechanism that underpins several cellular processes. The reversible attachment to nuclear proteins of the ~100-amino-acid protein small ubiquitin-like modifier (SUMO) controls a broad spectrum of cellular activities such as gene expression, chromatin remodelling, signal transmission, nuclear trafficking and protein stabilisation. TANDEM ZINC-FINGER-PLUS3 (TZP) is a light signalling component that promotes hypocotyl elongation and flowering in plants. TZP interacts with the red-light receptor phytochrome B (phyB) in small, distinct, transcriptionally active nuclear bodies. Recently, TZP was identified as a SUMO conjugate in proteomic analysis for proteins bound to a SUMO1 variant. Furthermore, light-reversible sumoylation was recently shown to negative regulate the signalling state of phyB. To understand the role of TZP sumoylation sites we employed a site directed mutagenesis approach and mutated the different lysines. Two site prediction algorithms were used to identify these sumoylation sites and SUMO interacting motifs (SIM) in TZP protein sequence. Preliminary data show that sumoylation sites are important for localisation and affect the size of TZP photobodies: as the number of sumoylation sites disrupted increases, there are fewer and larger photobodies. Overall, these findings support the role of sumoylation in light signalling.

CS17.58 CONTROL OF GROWTH BY PHOSPHORYLATION AND UBIQUITYLATION OF DA1 PEPTIDASE

MONDAY 11 DECEMBER 2017

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The characteristic sizes and shapes of plant organs are established by the interplay of cell proliferation and cell growth. These peptidases, DA1 and DAR1, cleave proteins that promote growth, such as members of the TCP family and UBP15, which de-ubiquitylates histones. A key question is how the activities of DA1 and DAR1 are regulated during organ growth. We have shown that receptor-like kinases (RLK) called TMK1 and TMK4, together with the co-receptor BAK1, phosphorylate DA1in vitro, and that DA1 is phosphorylated in vivo. Genetic analyses of quadruple tmk1 tmk4 da1 dar1loss-of-function mutants show that the strongly reduced growth of double tmk1 tmk4 mutants can be recovered to almost wild-type growth by loss of DA1and DAR1function. This suggested that lowered TMK1 and TMK4 activity reduces growth through DA1 and DAR1 activity. We identified two clusters of phosphorylated amino acids in functionally conserved regions of DA1 using LC-MS/MS. Phosphomimetic substitutions of phosphoserine and phosphothreonine decreased DA1 peptidase activity. This provides a plausible mechanism for TMK1 and TMK4 promoting growth by reducing DA1 activity. Thus, a dynamic balance of phosphorylation and ubiquitylation of DA1 may contribute to establishing final organ sizes.TMK4/BARK1mutants have reduced sensitivity to auxin, and it interacts with BAK1, a co-receptor of the brassinosteroid (BR) receptor BR1. This provides an opportunity to understand how growth regulators such as auxin and BR might control DA1 and influence organ size.

CS17.59 UNDERSTANDING THE ROLE OF SUMOYLATION IN REGULATING LIGHT SIGNALLING COMPONENTS IN PLANTS

MONDAY 11 DECEMBER 2017

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Light is an essential informational signal that promotes plant growth and development. Photoreceptors, light signalling components and transcriptional regulators have been observed to translocate into the nucleus and form highly dynamic subnuclear structures known as photobodies. The composition, mode of formation and molecular function of photobodies remain elusive. Posttranslational modification of proteins represents a regulatory mechanism that underpins several cellular processes. The reversible attachment to nuclear proteins of the ~100-amino-acid protein small ubiquitin-like modifier (SUMO) controls a broad spectrum of cellular activities such as gene expression, chromatin remodelling, signal transmission, nuclear trafficking and protein stabilisation. TANDEM ZINC-FINGER-PLUS3 (TZP) is a light signalling component that promotes hypocotyl elongation and flowering in plants. TZP interacts with the red-light receptor phytochrome B (phyB) in small, distinct, transcriptionally active nuclear bodies. Recently, TZP was identified as a SUMO conjugate in proteomic analysis for proteins bound to a SUMO1 variant. Furthermore, light-reversible sumoylation was recently shown to negative regulate the signalling state of phyB. To understand the role of TZP sumoylation sites we employed a site directed mutagenesis approach and mutated the different lysines. Two site prediction algorithms were used to identify these sumoylation sites and SUMO interacting motifs (SIM) in TZP protein sequence. Preliminary data show that sumoylation sites are important for localisation and affect the size of TZP photobodies: as the number of sumoylation sites disrupted increases, there are fewer and larger photobodies. Overall, these findings support the role of sumoylation in light signalling.

CS17.60 THE *ARABIDOPSIS THALIANA RHIZOBIALE*-LIKE PHOSPHATASE 2 IS A NOVEL D-GROUP MITOGEN ACTIVATED PROTEIN KINASE (MAPK) TYROSINE-SPECIFIC PPP-FAMILY PROTEIN PHOSPHATASE

MONDAY 11 DECEMBER 2017

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The study of tyrosine phosphorylation in plants has been largely neglected due to the lack of classic tyrosine kinases and underrepresentation of tyrosine phosphatases compared to humans. However, advanced phosphoproteomics studies have revealed that the abundance of phospho-tyrosine residues in plants parallels humans. This strongly suggests that in plants tyrosine phosphorylation is as important as in humans, yet we have limited knowledge about the players responsible of these events. The *Arabidopsis thaliana Rhizobiale*-like phosphatase 2 (AtRLPH2) is a novel protein phosphatase not found in mammals which, according to bioinformatics analysis, clusters with the serine/threonine specific phospho-protein phosphatase (PPP) group. Here, we demonstrate that AtRLPH2 surprisingly behaves like a tyrosine phosphatase. AtRLPH2 is inhibited by the specific tyrosine phosphatase inhibitor, orthovanadate and dephosphorylates phospho-tyrosine substrates presenting essentially no activity towards phospho-serine/threonine residues. This shows for the first time that a member of the plant PPP-family of phosphatases has the capability to dedicate its activity solely toward phospho-tyrosine. Additionally, we elucidated AtRLPH2 crystal structure in presence and absence of tungstate (as phospho-mimic) at 1.8 and 2.0 Å, respectively. This provided detailed mechanistic insight into its mode of action. AtRLPH2's structure along with biochemical validation, revealed the necessary conditions its substrates need to possess making Mitogen Activated Protein Kinases (MAPKs) perfect candidates. Last, to identify AtRLPH2 endogenous substrates, a phosphoproteomics study was performed by comparing phospho-tyrosine peptides from wild type and two *atrlph2* knockout plant lines. AtRLPH2 was found and validated to be a D-group specific MAPK tyrosine phosphatase.

CS17.61 GENOMIC AND PHENOMIC SCREENS FOR FLOWER RELATED RING TYPE UBIQUITIN E3 LIGASES IN ARABIDOPSIS

MONDAY 11 DECEMBER 2017

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Flower development and flowering time control integrate endogenous and environmental signals to promote vegetative to reproductive growth transition. The molecular networks involved are complex and incorporate different pathways and modes of signal transduction. In plants, ubiquitin mediated protein degradation pathway has been proposed to be an important mode of signalling in flowering control. To thoroughly study the role of ubiquitin pathway in the molecular regulation of flowering we took a reverse genetic approach to identify flower related Ubiquitin Proteasome System components. The RING type ubiquitin E3 ligase family was chosen as targets of the genomic screen, because of its large member number and versatile roles. To this aim, the whole list of Arabidopsis RING E3 ligases was retrieved and curated from the Arabidopsis genome v11. Using Genevestigator data sets, RING genes were grouped in different flower organs and over developmental stages categories from bolting to mature siliques, according to their relative differential expression. Known flowering regulators were identified in these categories through literature search and representative mutants were purchased for functional analysis by growth and morphological phenotyping. To this end, a workflow was established for high throughput phenotypic screening of growth, morphology, and flowering of nearly a thousand Arabidopsis plants in one experimental round.

CS17.62 UNDERSTANDING THE ROLE OF SUMOYLATION IN REGULATING LIGHT SIGNALLING COMPONENTS IN PLANTS

MONDAY 11 DECEMBER 2017

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Light is an essential informational signal that promotes plant growth and development. Photoreceptors, light signalling components and transcriptional regulators have been observed to translocate into the nucleus and form highly dynamic subnuclear structures known as photobodies. The composition, mode of formation and molecular function of photobodies remain elusive. Posttranslational modification of proteins represents a regulatory mechanism that underpins several cellular processes. The reversible attachment to nuclear proteins of the □100-amino-acid protein small ubiquitin-like modifier (SUMO) controls a broad spectrum of cellular activities such as gene expression, chromatin remodelling, signal transmission, nuclear trafficking and protein stabilisation. TANDEM ZINC-FINGER-PLUS3 (TZP) is a light signalling component that promotes hypocotyl elongation and flowering in plants. TZP interacts with the red-light receptor phytochrome B (phyB) in small, distinct, transcriptionally active nuclear bodies. Recently, TZP was identified as a SUMO conjugate in proteomic analysis for proteins bound to a SUMO1 variant. Furthermore, light-reversible sumoylation was recently shown to negatively regulate the signalling state of phyB. To understand the role of TZP sumoylation sites we employed a site directed mutagenesis approach and mutated the different lysines. Two site prediction algorithms were used to identify these sumoylation sites and SUMO interacting motifs (SIM) in TZP protein sequence. Preliminary data show that sumoylation sites are important for localisation and affect the size of TZP photobodies: as the number of sumoylation sites disrupted increases, there are fewer and larger photobodies. Overall, these findings support the role of sumoylation in light signalling.

CS17.63 ROLE OF POST-TRANSLATIONAL MODIFICATIONS THAT REGULATES SIGNALLING FROM G-PROTEINS IN CAPSICUM ANNUUM

MONDAY 11 DECEMBER 2017

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The multiple roles played by G proteins demonstrate the importance of the study and characterisation of these proteins in plants with agricultural and food importance, such as Capsicum annuum. A better understanding of the regulation of signal transduction mediated by G-proteins will require the identification of post-translational modifications of these proteins. Given the discussion above, the objectives of this research project were to conduct the characterisation of the G protein alpha subunit (Gα) from Cucumis sativus. The numerous biological functions of Gα are highly dependent upon specific post-translational modifications that guide their subcellular localisation and interaction with regulators and effectors. Several important functional sites, such as receptor-binding sites, effector binding sites, GTP-binding sites, important sites for maintaining the structural stability of the examined protein and post-translational modified sites were identified. Modifications of their carboxyl termini include the addition of palmitate to cysteine residues; the incorporation of this lipid favours the interaction of Gα with its membrane. Secondly, the amino-terminal region of Gα is susceptible to the addition of myristic acid, this modification contributes to the interaction between Gα molecules and cell membrane. To the best of our knowledge, this study is of the first investigations in which the Gα from Cucumis sativus has been analysed; thus, the findings of this study are highly significant.

CS17.64 RPT2 AND NCH1 ARE KEY FACTORS FOR THE BLUE-LIGHT RECEPTOR PHOTOTOROPIN-SIGNALLING PATHWAY IN LAND PLANTS

MONDAY 11 DECEMBER 2017

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Plant photoreceptor kinases known as the phototropins (phots) mediate various blue light responses including phototropism, chloroplast movement, stomatal opening, and leaf flattening and thus facilitate the light capture for photosynthesis. In *Arabidopsis thaliana*, two phot-interacting proteins NONPHOTOTROPIC HYPOCOTYL3 (NPH3) and ROOT PHOTOTROPISM2 (RPT2), that belong to NPH3/RPT2-like (NRL) BTB/POZ domain protein family, mediate phototropism and leaf flattening. Recently, we found that RPT2 and another NRL protein NRL PROTEIN FOR CHLOROPLAST MOVEMENT1 (NCH1) are essential for the chloroplast accumulation response. The RPT2/NCH1 ortholog from the liverwort *Marchantia polymorpha*, MpNCH1, is also essential for chloroplast accumulation in this organism, indicating that the RPT2/NCH1 subfamily is an essential signalling factor for this response in land plants. However, NCH1 and RPT2 do exhibit different functions in that NCH1 is specific to chloroplast movement, whereas RPT2 additionally regulates phototropism and leaf flattening in Arabidopsis. To investigate the functional differences between RPT2 and NCH1, we have performed promoter swapping analysis between Arabidopsis RPT2 and NCH1 and also expressed MpNCH1 in the *rpt2nch1* double mutant of Arabidopsis. Irrespective of which promoter was used, RPT2, but not NCH1, could mediate phototropism, indicating that RPT2 and NCH1 are not functionally equivalent in the regulation of phototropism. Furthermore, MpNCH1 could partially rescue the phototropic defects of *rpt2nch1* seedlings. These latter findings suggest that NCH1 has lost the ability to regulate phototropism after the separation of RPT2 and NCH1 during seed plant evolution.

CS17.65 REPRESSION OF 'LURP' REGULON BY THE F-BOX PROTEIN AND JA RECEPTOR, COI1

MONDAY 11 DECEMBER 2017

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Plants, as sessile organisms, have evolved to coordinate spatial and temporal signals to allow them to internally anticipate a myriad of stresses. They optimize their chance of survival through intersecting hormonal networks that enable them to correctly amplify or dampen specific defence responses. For instance, upon mechanical wounding the plant responds by producing the conserved plant hormone jasmonate (JA). JA acts to coordinate the recruitment of the JAZ repressor proteins by the Skp, Cullin, F-Box complex, marking them for ubiquination and subsequent degradation by the 26S proteasome system. Much remains to be elucidated about how hormones relay these signals to increase plant fitness. Interestingly, we have discovered that COI1 can repress the salicylic acid (SA) regulated genes in the 'LURP regulon', which are required for defence against oomycetes, in a manner independent of the JA pathway. Better understanding of how COI1 modulates this response, and conversely, how COI1 itself is modulated (post-translationally), will uncover novel JA-independent roles of COI1 and help in our molecular understanding of how plants coordinate defence against pathogens.

CS17.66 VERNALIZATION2 IS AN OXYGEN- AND NITRIC OXIDE-REGULATED SUBSTRATE OF THE N-END RULE PATHWAY OF PROTEOLYSIS

MONDAY 11 DECEMBER 2017

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The N-end rule pathway of proteolysis targets proteins for destruction based on the nature of their N-terminus. We have shown that the N-end rule pathway in Arabidopsis regulates the 'Methionine-Cysteine (MC)-initiating' protein-VERNALIZATION2 (VRN2). VRN2 functions to coordinate cold-responsive flowering and has several other key developmental roles. VRN2 is one of three plant homologues of the Drosophila protein SUPPRESSOR OF ZESTE12 (Su(z)12), which functions as part of the polycomb repressive complex2 (PRC2), a conserved eukaryotic complex that regulates the epigenetic silencing of genes through depositing the H3K27me3 repressive mark to chromatin. Here we provide in vitro and in vivo evidence that VRN2 is a physiological substrate of the N-end rule pathway. VRN2 is stabilised under hypoxia and NO-limited conditions and post-translational accumulation of VRN2 during vernalisation is linked to its regulation by the N-end rule. One hypothesis to explain VRN2 stabilisation is that cold-induced VERNALIZATION INSENSITIVE3 (VIN3) shields the MC terminus to prevent it becoming a target for degradation by the E3 ligase PROTEOLYSIS6 (PRT6). However, in vitro and via inducible VIN3 transgenic lines VRN2 is still degraded in the presence of VIN3. Additionally, the destabilising N-terminus of VRN2 likely arose following gene duplication and N-terminal truncation of an ancient homologue of EMBRYONIC FLOWER2 (EMF2), providing new insight into how proteins can become co-opted to the N-end rule pathway during evolution to provide new functions. Finally, we have found that EMF2c in Barley is also a substrate of the N-end rule pathway and may represent a functional homologue of VRN2.

CS17.67 NUCLEOREDOXIN GUARDS AGAINST OXIDATIVE STRESS BY PROTECTING ANTIOXIDANT ENZYMES

MONDAY 11 DECEMBER 2017

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Cellular accumulation of reactive oxygen species (ROS) is associated with a wide range of developmental and stress responses. Although cells have evolved to use ROS as signalling molecules, their chemically reactive nature also poses a threat. Antioxidant systems are required to detoxify ROS and prevent cellular damage, but little is known about how these systems manage to function in hostile, ROS-rich environments. Here we show that during oxidative stress in plant cells, the pathogen-inducible oxidoreductase Nucleoredoxin 1 (NRX1) targets enzymes of major hydrogen peroxide (H_2O_2)-scavenging pathways, including catalases. Mutant nrx1 plants displayed reduced catalase activity and were hypersensitive to oxidative stress. Remarkably, catalase was maintained in a reduced state by substrate interaction with NRX1, a process necessary for its H_2O_2-scavenging activity. These data suggest that unexpectedly H_2O_2-scavenging enzymes experience oxidative distress in ROS-rich environments and require reductive protection from NRX1 for optimal activity.

CS17.68 S-ACYLATION: WHAT THE FLS2 IS GOING ON?

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Receptor-Like Kinases (RLK) conduct extracellular signals across the plasma membrane into cells, allowing cells to respond and adapt to environmental changes. FLS2, the most widely-studied plant RLK, is the receptor for the bacterial protein flagellin and we have found that FLS2 is S-acylated. S-acylation is a reversible and dynamic post-translational protein modification whereby fatty acids are added to cysteine residues, but the effects of S-acylation on protein function are largely unknown. Here, using FLS2 as a model, we describe the effects of S-acylation on RLK function.

In plants treated with bacterial flagellin levels of S-acylated FLS2 rapidly increase. To identify when during FLS2 signalling S-acylation is occurring, mutants in FLS2 signalling pathway components were tested for flagellin-mediated increases in FLS2 S-acylation. Loss of components required for activation (co-receptor BAK1) or attenuation (E3 ubiquitin ligases PUB12/13) prevented the flagellin-mediated increase in FLS2 S-acylation. However, mutants in endocytic processes (DYNAMIN-RELATED PROTEIN 2B) not only showed a wild-type increase in FLS2 S-acylation but accumulated S-acylated FLS2. This suggests that S-acylation occurs after ubiquitination but before endocytosis of activated FLS2 and we hypothesise that S-acylation is required for promoting endocytosis of activated receptor. Initial characterisation of FLS2 mutants that cannot undergo flagellin-mediated S-acylation suggest that S-acylation is required for FLS2 mediated MAPK activation, gene expression and growth inhibition in Arabidopsis.

The site of S-acylation within FLS2 is conserved throughout the RLK superfamily. Based on our data, we propose that S-acylation is an entirely novel regulatory factor affecting RLK function.

CS17.69 SUMO PROTEASES OTS1 AND 2 CONTROL FILAMENT ELONGATION THROUGH A DELLA-DEPENDENT MECHANISM

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During fertilisation, stamen elongation needs to be synchronised with pistil growth. The phytohormone gibberellic acid (GA) promotes stamen growth by stimulating the degradation of growth repressing DELLA proteins. DELLA accumulation is negatively regulated by GAs through the ubiquitin-proteasome system. In Arabidopsis thaliana, a proportion of DELLAs is also conjugated to the small ubiquitin-like modifier (SUMO) protein, which stabilizes DELLAs. Increased DELLA levels occur in the SUMOprotease-deficient OVERLY TOLERANT TO SALT 1 and 2 (ots1 ots2) double mutants, especially under salt stress conditions. Here, we show that OTS genes play a redundant role in the control of plant fertility under non-stress conditions. Mutants of ots1 ots2 display reduced fertility compared with the wild type, owing to reduced stamen elongation. Stamen growth, pollination rate and seed production are restored in ots1 ots2 della mutants, thus linking OTS1 function to the control of DELLA activity in the context of filament elongation. OTS levels appear to be developmentally regulated as OTS1/2 transcript upregulation during stamen development overlaps with GAs accumulations. We propose that OTS genes enable synchronisation of stamen development by facilitating DELLA degradation at a specific developmental stage.

CS17.70 POST-TRANSLATIONAL MODIFICATIONS (PTMS) IN PLANT PROTEINS: PHOSPHOENOLPYRUVATE CARBOXYLASE (PEPC) AS A CASE OF STUDY

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Phosphoenolpyruvate carboxylase (PEPC) (EC 4.1.1.31) is a ubiquitous cytosolic enzyme that catalyses the irreversible β-carboxylation of PEP in the presence of HCO_3- to yield oxaloacetate and Pi. PEPC plays a crucial role in C_4 and CAM photosynthesis, where it catalyses the initial fixation of atmospheric CO_2. PEPC also fulfils several important non-photosynthetic functions including supporting carbon-nitrogen interactions, seed formation and germination, fruit ripening, guard cell metabolism, root malate excretion, or provision of malate for N_2-fixing bacteroids. Due to its pivotal roles in plant metabolism, PEPC protein is tightly regulated by post-translational modifications (PTMs). PEPC is phosphorylated in a conserved Ser residue producing an activation of the enzyme. C_4 PEPC is inhibited by anionic phospholipids and presumably recruited to the membrane, in a manner independent from the phosphorylation state of the enzyme. Regulatory PEPC monoubiquitination has been demonstrated in castor oil seeds, developing proteoid roots of harsh hakea, and in lily pollen. Recently we have showed that PEPC from sorghum seeds, and in sorghum roots under ammonium toxicity, is monoubiquitinated and that this process is inhibitory. In addition, photosynthetic PEPC is S-nytrosilated and/or carbonylated under salt stress conditions. Carbonylation inactivates C_4 PEPC while nytrosilation has little impact on its activity but holds back carbonylation. Moreover, recent results suggest that photosynthetic PEPC can be tyr-nitrated in a specific residue yielding an inhibited enzyme. Interestingly, most of these PTMs can coexist in the same protein. The high variety of modifications makes PEPC an ideal model for the study of PTMs in plants.

CS17.71 TRANSCRIPTIONAL ANALYSIS OF OTS SUMO PROTEASE MUTANTS REVEALS NEW SIGNALLING NODES THAT PROMOTE BIOTIC STRESS TOLERANCE IN PLANTS

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Posttranslational modification of proteins by small ubiquitin-like modifier (SUMO) is emerging as a key regulator of plant stress responses. In plants, SUMOylation has been implicated in response to environmental stresses. The covalent attachment of SUMO to target proteins can affect these proteins in different ways: altering their stability, sub-cellular localisation or activity. SUMO is removed from their target conjugates by SUMO proteases. Two of these SUMO proteases are OVERLY TOLERANT TO SALT 1 and 2 (OTS1 and OTS2), which are localised in the nucleus and act redundantly to control salt stress response in Arabidopsis. To help to bring understanding to previously observed ots1/2 phenotypes. We carried out an RNA-seq experiment on Arabidopsis thaliana wildtype and ots1/ots2 double mutant plants in basal condition and in response to Salicylic Acid (SA) and Jasmonic Acid (JA) treatment.

We found that the transcriptional response initially is composed of a core set of multi-stress responsive genes and becomes increasingly stress specific as time progresses. Furthermore we report the identification of many of the core genes differentially regulated in ots1/2 background. Finally, since SUMOylation is a post-transcriptional modification, we looked for enriched TF sites in the promoters of the differentially expressed genes to find SUMOylated transcription factors. Several TF sites were enriched among our differentially expressed genes and we have experimentally proved that they are SUMOylated in vivo. Our results provide mechanistic insight into the phenotypes observed in SUMO proteases defective Arabidopsis mutants and also contribute to understanding the role of SUMO in response to stress.

CS17.72 EXPRESSION-FUNCTIONAL STUDIES OF PHOSPHOENOLPYRUVATE CARBOXYLASE KINASE (PEPCK)

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In the C_4 photosynthesis pathway, atmospheric CO_2 enters mesophyll cells and converted to bicarbonate (HCO^{3-}). Together HCO^{3-} and phosphoenolpyruvate are carboxylated by phosphoenolpyruvate carboxylase (PEPC) to produce oxaloacetate (OAA). The OAA is further converted into malate to release CO_2 for fixation by RuBisCo in the Calvin Cycle. PEPC plays a major role in increasing the concentration of CO_2 to the point needed to saturate RuBisCo thus inhibiting photorespiration. PEPC activity is controlled by reversible phosphorylation at serine residue located close to the N-terminal and this reaction is catalysed by phosphoenolpyruvate carboxylase kinase (PEPCK). The mechanism of how PEPCK phosphorylate PEPC with ATP and how phosphorylated PEPC acts as a carboxylase enzyme is still unknown. Thus, the current goal of the research is to obtain biologically active PEPCK and validate whether it can phosphorylate PEPC or not. Therefore, DNA fragment encoding C_4 PEPCK from Panicum queenslandica was cloned in-frame into pET-44a over-expression plasmid. PEPCK was over-expressed in E. coli Rosetta and then purified by His6-affinity chromatography. The in vitro phosphorylation assay was carried out by incubating 0.1 µg/µl PEPC with 0.02µg/µl PEPCK with or without ATP for 120 min at 30°C and it was found that PEPC was phosphorylated by PEPCK from Pro-Q® Diamond phosphoprotein gel stain. Further works will be involve separating phosphorylated PEPC from non-phosphorylated PEPC and determination of the sensitivity of phosphorylated PEPC to be inhibited by malate and activation by glucose-6-phosphate by kinetic assay. The PEPCK structure also will be visualised at the atomic level through crystallography.

CS17.73 FIFTY SHADES OF SUMO: ITS ROLE IN IMMUNITY AND AT THE FULCRUM OF GROWTH-DEFENCE BALANCE

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The sessile nature of plants requires them to cope with an ever-changing environment. Effective adaptive responses require sophisticated cellular mechanisms at post-transcriptional and -translational levels. Post-translational modification by Small Ubiquitin-like Modifier (SUMO) proteins is emerging as a key player in these adaptive responses. SUMO conjugation can rapidly change the overall fate of target proteins by altering their stability or interaction with partner proteins or DNA. SUMOylation entails an enzyme cascade that leads to the activation, conjugation and ligation of SUMO to lysine residues of target proteins. In addition to their SUMO processing activities, SUMO proteases also possess de-conjugative activity capable of cleaving SUMO from target proteins providing reversibility and buffering to the pathway. These proteases play critical roles in maintaining SUMO machinery in equilibrium. We hypothesise that SUMO proteases provide the all-important substrate specificity within the SUMO system. Furthermore, we provide an overview of the role of SUMO in plant innate immunity. SUMOylation also overlaps with multiple growth promoting and defence-related hormone signalling pathways and hence is pivotal for maintaining the growth-defence balance. This review aims to highlight the intricate molecular mechanisms utilized by SUMO to regulate plant defence and stabilise the growth-defence equilibrium.

CS17.74 EXPLOITING PROTEIN MODIFICATION SYSTEMS TO BOOST CROP PRODUCTIVITY: SUMO IN THE FOCUS

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The posttranslational modification system is important in enabling plants to adapt to their environment. During the processes of domestication and breeding, plants were selected for various yield and adaptational characteristics. The posttranslational modifier SUMO is known to have a role in the regulation of a number of these characteristics. Using bioinformatics, we mined the genomes of cereal and Brassica crops and their non-crop relatives *Arabidopsis thaliana* and *Brachypodium dystachon* for ULP SUMO proteases sequences. We discovered the SUMO system in cereal crops is disproportionately elaborate in comparison to *B. dystachon*. We use this data to propose deSUMOylation as a mechanism for specificity in the SUMO system.

CS17.75 PROTEIN S-NITROSYLATION IN IMMUNE-INDUCED HORMONAL NETWORKS

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Plant immunity relies on sophisticated hormonal signalling networks tightly associated with intracellular redox changes. In response to the hemibiotrophic bacterial pathogen *Pseudomonas syringae (Psm)*, the phytohormone salicylic acid (SA) mediates defence responses by coordinating fluctuations in the total glutathione (GSH) pool and a marked burst in reactive oxygen/nitrogen species production. To promote virulence, *Psm* hijacks phytohormone signalling by secreting the phytotoxin coronatine, a highly active jasmonate (JA) analogue that counteracts SA signalling. However, it remains unknown if cross talk between the SA and JA signalling pathways impacts the effectiveness of reactive oxygen/nitrogen species, including nitric oxide (NO). NO is extensively involved in shaping hormonal signalling during immunity. NO bioactivity is mediated by protein S-nitrosylation, *i.e.* the covalent attachment of a NO moiety to reactive thiol groups of proteins, forming a protein-SNO modification. Recently, the denitrosylation activity of S-nitrosoglutathione reductase (GSNOR) and thioredoxin-*h5* (TRX-h5) has been reported to determine fate and amplitude of SA-mediated immune responses by impacting specific branches of protein-SNO signalling. By using genetic and biochemical approaches, here we provide evidence that *Psm*-produced coronatine targets specific protein-SNO branches in an unexpected fashion to wear down the host immune system and promote disease. We propose a model in which disease resistance/susceptibility is determined by a molecular battle between host and pathogen to offset the equilibrium of interchangeable NO donor species that ultimately determines specificity in the NO-mediated post-translational control of plant immunity.

CS17.76 DISCOVERING DE-S-ACYLATING ENZYMES IN ARABIDOPSIS

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S-acylation is the only reversible lipid-based post-translational modification of proteins. Even though 24 protein acyl transferases - enzymes that add acyl groups onto proteins - are identified and verified in Arabidopsis, the identities and numbers of any plant de-S-acylating enzymes remain unknown. More than 500 proteins have been suggested to be S-acylated in plants and a still unexplored number of these are likely to be de-S-acylated as means of regulating localisation and function. It is therefore very probable that one or more families of plant de-S-acylating enzymes await their discovery. De-S-acylating enzymes from mammals and Toxoplasma have previously been identified by inhibitor-based approaches using competitive activity-based protein profiling (cABPP). By combining cABPP with quantitative SILAC proteomics we are identifying serine hydrolases in plants that are sensitive to known inhibitors of de-S-acylation. We have shown that we can deliver these inhibitors into plants and that they can inhibit plant serine hydrolase activities in vivo. Furthermore, several de-S-acylating inhibitors can cause an abnormal root hair phenotype when applied to growing roots.

CS17.77 IP-MS IDENTIFIES A MEMBRANE RECEPTOR OF THE ARABIDOPSIS GET PATHWAY

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Type II tail-anchored membrane (TA) proteins are involved in diverse cellular processes such as cell signalling, vesicle trafficking and protein translocation. Their correct biogenesis including membrane insertion, however, is a challenging task for the cell: Lack of N-terminal signal sequences prevents insertion via the co-translational SRP/SEC61 pathway while at the same time the C-terminal transmembrane domain necessitates chaperone action to prevent aggregation in the hydrophilic environment of the cytosol. Such TA proteins are post translationally targeted into the ER membrane via the Guided Entry of Tail-anchored Proteins (GET) pathway that was previously described in mammals and yeast.

Recently, we identified several GET orthologues in *Arabidopsis thaliana* through *in silico* analyses, including one of the two membrane receptors, *At*GET1, and found a role of the GET pathway in root hair elongation. Additionally, direct *in planta* interaction analysis using immunoprecipitation mass spectrometry (IP-MS) of *At*GET3a-GFP-expressing lines identified a potential candidate for the elusive *At*GET2 receptor that we named *At*GET3a-interacting protein 1 (GIP1). This protein is predicted to possess three transmembrane domains (TMDs) similar to ScGET2 and the mammalian orthologue CAML - a feature that survived the evolutionary divergence of the amino acid sequence. We provide here evidence that this gene of unknown function might indeed code for the previously unidentified *At*GET2.

CS17.78 CYP1A GENE EXPRESSION AS A BASIC FACTOR FOR FIPRONIL TOXICITY IN CASPIAN KUTUM FISH

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The aim of this study was to assess the effects of fipronil insecticide on the Caspian kutum fish at different levels of biological organisations and to find possible relationship between these biomarkers. Different doses of fipronil (65, 130 and 200 mg/kg) were intraperitoneally administered to the fish for 2 weeks. After 7 and 14 days of exposure, alterations in organ-somatic index, tissue and DNA structure, oxidative stress and CYP1A gene expression in gill, liver, brain and kidney were studied. Determination of these parameters in the liver showed that the degree of tissue change (DTC), comet tail, superoxide dismutase (SOD) and relative CYP1A mRNA expression increased mostly in a time dependent manner whereas in the kidney increased mostly in a dose dependent manner. These parameters in the gill increased more in time and dose dependent manner. Apart from the changes in CYP1A expression and oxidative stress, no alterations were observed in the brain. Multiple regression analysis showed that the CYP1A had the most correlation with the organ-somatic index (R^2 = 0.76) and comet tail (R^2 = 0.89) in the liver, and with DTC (R^2 = 0.93) and oxidative stress (R^2 = 0.87) in the kidney. Generally, this study showed that CYP1A gene expression can be considered as one basic factor for fipronil toxicity in this fish. However, other possible factors also should be considered for future research.

CS17.79 DISSECTING THE MOLECULAR MECHANISM REGULATING LATERAL ROOT HYDROPATTERNING

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Lateral roots (LR) contribute considerably to the architecture of the root system. The hormone auxin tightly controls the regulation of LR formation in response to environmental signals. For example, roots have the ability to distinguish between wet and dry micro environments in the soil and adapt the positioning of lateral roots accordingly. This concept is referred to as LR hydropatterning and is a novel adaptive mechanism for controlling root branching. When growing vertically down an agar plate, Arabidopsis thaliana roots are also exposed to an asymmetric distribution of water that causes a meniscus to form around the primary root (PR) circumference. LRs develop preferentially on the side of the PR in contact with water, rather than the side exposed to air. It has been revealed that the transcription factor AUXIN RESPONSE FACTOR 7 *(ARF7)* is essential for LR hydropatterning. In contrast to wild type, *arf7* loss of function mutants do not exhibit greater LR emergence towards the side of the PR in contact with moisture. Ectopic expression of *ARF7* can rescue *arf7* LR hydropatterning, implying that *ARF7* regulates LR hydropatterning via a post-transcriptional mechanism. One promising post-transcriptional mechanism that may control LR hydropatterning involves protein SUMOylation (a **S**mall **U**biquitin-like **Modifier**), since the SUMO mutant *ots1 ots2* phenocopies the *arf7* LR hydropatterning defect. *ARF7* is a target for SUMO modification, containing several SUMOylation sites including one within its DNA binding domain. Expressing wild type *ARF7* and a non-SUMOylated

version in *arf7* revealed that *ARF7* controls LR hydropatterning via SUMOylation.

CS17.80 DISRUPTION OF N-LINKED PROTEIN GLYCOSYLATION ALTERS POLYAMINE OXIDASE SIGNALLING PATHWAY IN *ARABIDOPSIS THALIANA*

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N-linked glycosylation occurs in endoplasmic reticulum (ER) and is one of the important post translational modifications that are needed for proper folding of glycoproteins. However, heavy protein load in ER lumen can prevent proper glycosylation. This can be mimicked by disruption of N-glycosylation with an antibiotic named tunicamycin. Accumulation of unfolded proteins in ER lumen is called ER stress, which is perceived by a complex gene network and their induction triggers unfolded protein response (UPR), increasing protein folding capacity. Polyamines are low molecular weight molecules that function in growth and stress response in plants. Putrescine, spermidine and spermine are important polyamines and among them spermidine is known to induce a key transcription factor bZIP60 involved in ER stress response. However, information on effects of disruption of N-linked protein glycosylation on polyamine metabolism in plants is not known. Polyamine oxidases (PAO) catalyse the oxidation of spermidine and spermine and participate in triggering of stress signalling. Due to this, in this work we have investigated the response of different PAO genes (PAO1-PAO5) to tunicamycin treatment in *Arabidopsis thaliana.* Among these genes only the expression of *PAO1* and *PAO5* responded to tunicamycin treatment. In further experiments, response of *pao1, pao5* and *pao1/pao5* double mutant to tunicamycin was evaluated in terms of induction of UPR. For this, expressions of *bZIP28, bZIP17, bZIP60, BiP1, BiP3,CNX, ERO1, HRD1, DER1, SEL1, UBC32*, which are involved ER quality control and protein degradation, were measured. Overall, we demonstrated that PAO is a key factor for triggering of UPR in plants.

CS17.81 ROLE OF PROTEASOMES IN NON-SPECIFIC IMMUNE RESPONSE OF MARINE ANNELIDS

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Marine invertebrates represent the unique model for the molecular mechanisms of adaptation research. Polychaetes (annelids, Annelida) compose significant part of marine bottom ecosystems. Combination of annelids' anatomical features provides high level of adaptive plasticity and enables development of an effective immune (defensive) system. Free cell elements of the coelomic fluid, coelomocytes, constitute the main component of immune system in annelids. *Arenicola marina*, marine lugworm, is common species of cold-water seas intertidal zone. We investigated the specific features of proteasomes and chaperones functioning in *A. marina* coelomocytes during the experimental inflammation. To induce the inflammation, we injected the coelomic cavity of adult worms with bacterial cell wall lipopolysaccharide (LPS), dissolved in fresh sea water (FSW), in concentration about 50 ng/ml. The FSW was used in control experiments as well. 1 hour after LPS injection the content of proteasome subunits (including structural α and proteolytic β5 types) significantly increases, as well as the proteasomes' chymotrypsin-like activity (CLA). Also a new inducible form of Hsp70 chaperone appears after LPS injection. Proteasome complexes of annelid coelomocytes in normal conditions are characterised by relatively low amount of 26S proteasome form. This fact indicates the specialisation of annelid proteasome system on non-ubiquitinated proteins hydrolysis. The infection leads to full dissociation of macromolecular regulatory particles PA700 and PA200. The functional shift of coelomocytes proteasome system in conditions of considerable stress leads to implementation of proteolysis only on "pure" 20S proteasomes. This work was partially supported by 16-04-00454-a RFBR grant.

CS17.82 UBIQUITINATION IN WHEAT DEFENCE AGAINST SEPTORIA FUNGUS

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Wheat is a major food crop for much of the world, and with an ever-increasing population there is a rising demand to produce more food in a smaller area. Zymopseptoria tritici is a devastating foliar pathogen of wheat, which can lead to a 20% reduction in yield.

Plants have had to evolve a multitude of different defence mechanisms due to their sessile nature. Protein modifications, such as ubiquitination, have been shown to be central to plant defence.

In this study, Virus Induced Gene Silencing (VIGS) was used to investigate Triticum aestivum E2 ubiquitin conjugating (TaU) enzymes. The main focus of this study is TaU4, the E2 function of which has been proven through ubiquitin charging assays and the active site cysteine identified. The possible function of TaU4 in the Septoria-wheat interaction was investigated after silencing TaU4 in wheat and then infecting with Septoria. TaU4 silenced wheat leaves showed a delay in the onset of Septoria infection symptoms and had reduced pycnidia and spore counts when compared to the vector only control. It was concluded that TaU4 acts as a negative regulator of defence in wheat against Septoria fungal infection.

CS17.83 AN OMICS APPROACH TO INVESTIGATE THE EFFECT OF CLIMATE CHANGE IN *EUCALYPTUS*

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The *Eucalyptus* genus is composed of long-lived trees that produce a plethora of specialized metabolites to interact with their environment and to combat biotic and abiotic stress. Very little is known about how specialised metabolism is regulated in response to climatic changes. Therefore, this research project aims to examine the phenomic, metabolomic and proteomic changes in *Eucalyptus* leaves in response to enhanced temperature and atmospheric CO_2 concentration. *Eucalyptus grandis* and *E. cladocalyx* seedlings were grown in growth chambers subjected to elevated atmospheric CO_2 concentration (550 ppm), increased temperature (30 ºC) and a combination of both factors for eight weeks. Photosynthesis, biomass and volatile profile were assessed at the end of the growth period. No significant response to treatments was detected in total biomass or nitrogen content, and we hypothesize that the extra carbon is allocated to specialised metabolites, including volatile organic compounds (VOCs). We identified 64 biogenic VOCs emitted from leaves. Chemometrics analysis revealed changes in the volatile emission profile in response to treatment and key molecular markers were identified, such as isoprene, *p*-cymene, and linalool. High temperature is the most prominent factor, resulting in the increase of isoprene emission in *E. cladocalyx* by 2 fold. Proteomics analysis is currently being undertaken to examine the relationship between specialised metabolites, photosynthesis and carbon assimilation pathways. Changes in *Eucalyptus* chemical and physical parameters may affect the downstream food chain and feeding habits of herbivores, such as the iconic koala.

CS17.84 SELF-PROTECTION OF ARABIDOPSIS CYTOSOLIC MALATE DEHYDROGENASE AGAINST OXIDATIVE STRESS

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Plant malate dehydrogenase (MDH) isoforms are found in different cell compartments and function in key metabolic pathways. It is well known that the chloroplastic NADP-dependent MDH activity is strictly redox regulated and controlled by light. However, redox-dependence of other NAD-dependent MDH isoforms have been less studied. Here, we show by in vitro biochemical characterisation that the major cytosolic MDH isoform (cytMDH1) is sensitive to H_2O_2 through sulfur oxidation of cysteines and methionines. CytMDH1 oxidation affects the kinetics, secondary structure, and thermodynamic stability of cytMDH1. Moreover, mass-spectrometry analyses and comparison of crystal structures between the reduced and H_2O_2-treated cytMDH1 further show that a Trx-reversible homodimerization of cytMDH1 through Cys330 disulfide formation protects the protein from overoxidation. Consistently, we found that cytosolic thioredoxins interact specifically with cytosolic MDH in a yeast two-hybrid system. Importantly, we also show that cytosolic and chloroplastic, but not mitochondrial NAD-MDH activities are sensitive to H_2O_2 stress in Arabidopsis. NAD-MDH activities are decreased both in a catalase2 (cat2) mutant and a NADP thioredoxin reductase mutant (ntra ntrb), emphasizing the importance of the thioredoxin reducing system to protect MDH from oxidation in vivo. We propose that the redox switch of MDH activity contributes to adapt the cell metabolism to environmental constraints.

CS17.85 IDENTIFYING AND CHARACTERISING SUBSTRATES OF THE IMMUNE KINASE BIK1

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Pathogen-associated molecular patterns (PAMPs) are recognised by plant cell-surface pattern recognition receptors (PRRs), leading to pattern-triggered immunity (PTI). The cytoplasmic kinase BIK1 is an immediate substrate for many activated PRR complexes, and regulates multiple cellular outputs, wherein it acts as a convergent executor of immune signalling. Despite this central role, the protein substrates of BIK1 are poorly characterised. Previous work identified the NADPH oxidase RBOHD as one such substrate, directly linking BIK1 activation to apoplastic reactive oxygen species (ROS) production, a hallmark of PTI responses. Further work has identified 22 additional putative BIK1 substrates, which include several plasma membrane-localised transporter and/or signalling proteins. We are investigating the roles of these putative BIK1 substrates in immune signalling. Results from this work will further elucidate the hitherto unresolved molecular components linking ligand-induced PRR activation to downstream signalling outputs associated with plant immunity.

CS17.86 THE siRNA SUPPRESSOR RTL1 IS REDOX-REGULATED THROUGH GLUTATHIONYLATION OF A CONSERVED CYSTEINE IN THE DOUBLE-STRANDED-RNA-BINDING DOMAIN

MONDAY 11 DECEMBER 2017

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RNase III enzymes cleave double stranded (ds)RNA. This is an essential step for regulating the processing of mRNA, rRNA, snoRNA and other small RNAs, including siRNA and miRNA. *Arabidopsis thaliana* encodes nine RNase III: four DICER-LIKE (DCL) and five RNASE THREE LIKE (RTL). To better understand the molecular functions of RNase III in plants we developed a biochemical assay using RTL1 as a model. We show that RTL1 does not degrade dsRNA randomly, but recognizes specific duplex sequences to direct accurate cleavage. Furthermore, we demonstrate that RNase III and dsRNA binding domains (dsRBD) are both required for dsRNA cleavage. Interestingly, the four DCL and the three RTL that carry dsRBD share a conserved cysteine (C230 in *Arabidopsis* RTL1) in their dsRBD. C230 is essential for RTL1 and DCL1 activities and is subjected to post-transcriptional modification. Indeed, under oxidizing conditions, glutathionylation of C230 inhibits RTL1 cleavage activity in a reversible manner involving glutaredoxins. We conclude that the redox state of the dsRBD ensures a fine-tune regulation of dsRNA processing by plant RNase III.

CS17.87 IMPROVEMENT OF PLANT WATER USE EFFICIENCY VIA MODIFICATION OF STOMATAL SIGNALLING PATHWAYS

MONDAY 11 DECEMBER 2017

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Increasing atmospheric CO_2 concentrations are affecting plant growth and behaviour around the globe. Stomata control gas exchange between plants and their environment, and by balancing CO_2 uptake for photosynthesis and water loss via transpiration, they ensure plant survival and growth. These pores in the leaf epidermis are formed of specialised guard cells, and open and close in response to changes in environmental conditions through changes in intracellular ion concentrations. The plant stress hormone abscisic acid (ABA) and elevated CO_2 concentration are key triggers of stomatal closure. ABA triggers a signalling cascade, where protein phosphatases and kinases control the activation of ion channels leading to stomatal closure. The same post-translational protein modifications also contribute to CO_2-induced stomatal closure. However, recently a CO_2 -specific pathway was identified where, independently of ABA signalling, mitogen-activated protein kinases control the activation of guard cell anion channels in response to elevated CO_2 concentration. *Arabidopsis thaliana* mutants for the central phosphatases and kinases involved in causing alterations in stomatal movements have been isolated. We are crossing these mutations into plants with high stomatal density to create multiple mutant plants which have high numbers of ABA-hypersensitive, but CO_2 -insensitive stomata. These plants should have high photosynthetic levels at future elevated CO_2 concentrations if water is not limiting, but will be able to respond efficiently to drought conditions by stomatal closure and water preservation. As stomatal signalling pathways are highly conserved, we propose that a similar approach could be used to improve crop water use efficiency in the future.

CS17.88 CHARACTERISATION OF THE N-TERMINAL ACETYLATION BRANCH OF THE N-END RULE PATHWAY OF PROTEIN DEGRADATION IN *ARABIDOPSIS THALIANA*

MONDAY 11 DECEMBER 2017

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Regulating a diverse range of essential processes in eukaryotes, the N-end rule pathway is a highly conserved division of the ubiquitin proteasome system that controls protein stabilities within cells dependent on their N-terminal (Nt) identity. Recent studies have identified a novel branch of the pathway that specifically degrades N-terminally acetylated proteins - the Ac/N-end rule pathway. In yeast and mammals, this pathway plays a key role in regulating protein-complex homeostasis, peptide quality control and signal transduction. At present we do not know if the pathway exists in plants.

Degradation via the Ac/N-end rule involves two key steps: (1) Nt-acetylation by Nt-acetyltransferases (NATs), and (2) recognition of Nt-acetylated proteins and subsequent polyubiquitination by specific E3 ubiquitin ligases (Ac/N-recognins, to date NOT4 and DOA10/TEB4). Several NATs have been previously

characterised in plants, and we have identified putative homologues of both NOT4 and DOA10 in the *Arabidopsis thaliana* genome. In contrast to animals and yeast, *Arabidopsis* encodes for multiple copies of these E3s, suggesting potential divergence of function. Here we present our data on the functional characterisation of the plant Ac/N-recognins, and identification of their putative substrates in *Arabidopsis* using a combination of yeast and plant molecular genetics and protein biochemistry.

CS17.89 DYNAMIC INTERPLAY BETWEEN PHOSPHORYLATION AND UBIQUITINATION DURING PLANT RECEPTOR KINASE-MEDIATED IMMUNITY

MONDAY 11 DECEMBER 2017

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The first layer of plant immunity is mediated by the recognition of conserved microbial features known as pathogen-associated molecular patterns (PAMPs) by plasma membrane-localized pattern recognition receptors (PRRs). It leads to PAMP-triggered immunity (PTI) that restricts the multiplication of most potential pathogens. An immediate downstream substrate of activated PRR complexes is the cytoplasmic kinase BIK1, which positively regulates PTI signalling triggered by several PAMPs. However, the BIK1 downstream targets are mostly unknown. Here, we identified a new BIK1 phosphorylation substrate (BPS1), which is an E3 ubiquitin ligase. BPS1 interacts with BIK1 *in vivo* and is phosphorylated by BIK1 *in vitro*. Moreover, it associates with FLS2 and BAK1 upon flg22 treatment. The preliminary genetic data suggest that BPS1 is a positive regulator of plant immunity. Based on this, our main working hypothesis suggests that BPS1 either polyubiquitinates negative regulator(s) of PTI leading to its subsequent degradation by proteasome or activates positive regulator(s) of PTI through different type of ubiquitination mark. Now, we are looking for potential BPS1 substrates and the latest findings will be presented during the meeting. Via characterization of BPS1 function in PTI, we aim to improve our understanding of how activated PRR complexes regulate downstream immune signalling, which is a very important question in innate immunity, in both plants and mammals.

CS17.90 IDENTIFICATION OF BARLEY EXTA-HAUSTORIAL MEMBRANE PROTEINS IN THE HAUSTORIA OF THE OBLIGATE BIOTROPHIC FUNGAL PATHOGEN, *BLUMERIA GRAMINISS F.SP HORDEI*

MONDAY 11 DECEMBER 2017

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Diseases attributed to fungal pathogens are major contributors to reduction in crop yield, including *Blumeria graminis*, the causal agent of barley powdery mildew. This fungus is an obligate biotrophic pathogen producing haustorial feeding structures, which are required for infection and are located within the host epidermis,. Over 500 secreted *Blumeria* effector candidates (BECs) have been predicted, many of which were identified as haustoria specific proteins. Eight BECs were shown to be essential for infection. However there is sparse information about the plant derived extra-haustorial membrane (EHM) which surrounds the haustorial structure.

Haustoria and their associated EHM structures were isolated to characterise their specific proteomes using a differential approach. Proteins were extracted and precipitated in TCA-acetone. Tryptic peptides were analysed by nLC-nESI/MSMS.

A total of 185 barley proteins were found exclusively to the EHM in the enriched haustoria fraction, being absent in the infected epidermis sample. Included within these were pathogenesis related proteins, such as PR5, a known interactor with BEC1054,

as well as other PR proteins, peroxidases, kinases, leucine-rich repeat (LRR) containing proteins, and hormone and cell wall biosynthesis proteins. Many of these are likely to be involved in protein post-translational modifications, reactive oxygen species production, and other plant-pathogen responses.

We predict that some of the identified EHM specific proteins are putative key players of the host-biotroph interaction, where they may be acting as susceptibility factors, or playing a role in resistance.

CS17.91 PHOSPHORYLATION-MEDIATED STRESS SIGNALLING AND REDOX REGULATION

MONDAY 11 DECEMBER 2017

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Unfavourable environmental conditions and pathogen infections limit plant growth and development and thus reduce fitness. Plants have evolved complex cellular and physiological mechanisms to prevent damage and ensure growth under stress conditions. These responses are controlled by various stress-type specific but also common and interacting signalling pathways. Protein phosphorylation and reactive oxygen species (ROS) constitute two integral components of cellular stress signalling. In our work, we identified the *Arabidopsis* GSK3/Shaggy-like protein kinase ASKα as a positive regulator of both abiotic and biotic stress responses. Salt stress and pathogen infection enhance ASKα activity, which in turn phosphorylates the cytosolic glucose 6-phosphate dehydrogenase isoform G6PD6. G6PD is the key enzyme of the oxidative pentose phosphate pathway providing reducing equivalents in the form of NADPH. Stress-activated ASKα phosphorylates G6PD6 on the conserved threonine site Thr-467, thereby enhancing its enzymatic activity. Remarkably, while under salt stress conditions, enhanced G6PD activity contributes to the removal of excess levels of ROS via the ascorbate/glutathione cycle and thus stress tolerance, upon pathogen recognition G6PD activity is necessary for ROS production by NADPH oxidases and a successful defence response. Overall, our results reveal a novel mechanism of G6PD adaptive regulation. We provide evidence that ASKα and G6PD6 constitute a signalling module that links protein phosphorylation cascades to metabolic adjustment under both abiotic and biotic stress conditions.

CS17.92 IDENTIFICATION OF *NO GAMETOPHORES 1 (PPNOG1)*, A NOVEL REGULATOR OF THREE-DIMENSIONAL GROWTH IN *PHYSCOMITRELLA PATENS*

MONDAY 11 DECEMBER 2017

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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 first 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, which encodes a ubiquitin-associated protein. *PpNOG1* overexpression restored 3D growth to the mutant confirming correct gene identification.

CS17.93 PROTEASOME-ASSOCIATED HECT-TYPE UBIQUITIN LIGASE ACTIVITY IS REQUIRED FOR PLANT IMMUNITY

MONDAY 11 DECEMBER 2017

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In response to infection transcription factors play a central role in the immune response through extensive transcriptional reprogramming. In plants, the pathogen-induced immune hormone salicylic acid (SA) activates the transcriptional coactivator NPR1, resulting in activation of immune response genes. Ongoing stimulation of gene expression requires continuous instability of NPR1 at target gene promoters through its ubiquitin-dependent proteasomal degradation. However, the factors involved in regulation of NPR1 transcriptional activity are not fully understood. Here, we show that three members of the HECT domain containing family of E3 ligases, UPL1, UPL3 and UPL5 are required for NPR1-dependent gene activation. Disruption of these UPL ligases renders plants insensitive to SA and susceptible to infection. Disruption of UPL3 resulted in an overall reduction in protein ubiquitination following SA treatment. RNA-seq analysis demonstrated an almost complete loss of SA-and NPR1-induced immune responsive genes. Taken together, our study shows that the E3 ligase activities of UPL1, UPL3 and UPL5 play central roles in regulating NPR1-dependent immune responses in plants.

CS17.94 INSECTICIDAL FUSION PROTEINS: HOW DOES THE ORIENTATION OF THE TOXIN TO THE CARRIER AFFECT TOXICITY TO INSECTS?

MONDAY 11 DECEMBER 2017

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There are increasing demands being placed on food production to feed the growing population. Insecticidal control is a vital component to increase food supply, however there is increasing resistance developing in pest species. Additionally, effective insecticides are being removed from the market due to toxicity to the environment and pollinator species. A new fusion protein has been developed that utilises Snowdrop lectins *(Galanthus nivalisagglutinin;* GNA), which when fed orally is transported across the gut epithelium to the haemolymph of insects, however this carrier has little insecticidal toxicity. The toxicity can be increased by fusion to the spider-venom peptide ω-hexatoxin-Hv1a (Hv1a) from the Funnel web spider *(Hadronyche versuta)*. This peptide specifically targets insect's voltage-gated calcium channels in the central nervous system however, when fed on its own orally, has little toxic effect on *Mamestra brassicae* (cabbage moth) larvae and the aphid species *Acyrthosiphon pisum* (Pea), *Sitobion avenae* (Cereal) and *Myzus persicae* (Peach-potato). The fusion protein GNA-Hv1a, when orally delivered, is transported to the insect's haemolymph carrying the toxin to the site of action, reducing the survival of the insect pests. This technology provides a novel target in pest control and is non-harmful to the environment and non-target organisms. My work determined if the orientation of the toxin to the carrier affected the insecticidal activity of the fusion proteins. This involved expressing the fusion proteins in yeast *(Pichia pastoris)* as an expression host, purifying the protein, analysing the protein stability and carrying out bioassays on the aforementioned pest species.

CS17.95 PHOSPHOPROTEOMIC APPROACH TO SURVEY MOLECULAR COMPONENTS OF PLANT IMMUNE SYSTEM

MONDAY 11 DECEMBER 2017

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During the last several decades, extensive analyses revealed that plants utilize a two-branched immune system for defence against pathogens. In the first branch, transmembrane pattern recognition receptors (PRR) are used to recognise and respond to slowly evolving pathogen-associated molecular patterns (PAMP). In the second branch, either a direct or an indirect recognition of the pathogen through disease-resistance (R) proteins is used for response to pathogen virulence factors. While extensive genetic screens successfully identified a number of PRRs and components which affect abundance and maturation of PRRs, signal transduction mechanisms that lead to defence responses is thus far limited. This partly stems from limitations of forward genetics caused by lethality and/or genetic redundancy. Accordingly, we have been taking phosphoproteomic approach to understand the basic framework of the PRR-mediated immune system. Our recent findings based on phosphoproteomics in Arabidopsis and studies of an emerging model plant *Marchantia polymorpha* will be presented.

CS17.96 N-TERMINAL PEPTIDE ENRICHMENT AS A TOOL FOR STUDYING TARGETED PROTEIN DEGRADATION PATHWAYS AND PROTEOLYTIC EVENTS

MONDAY 11 DECEMBER 2017

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Proteolytic degradation is important for the bulk removal of damaged or unwanted proteins but specific proteolytic cleavage represents a pervasive and irreversible post-translational modification that can alter the fate and function of a given protein. One example of this is the Arg/N-end rule pathway of targeted protein degradation, which links the half-life of a protein to the identity of its amino (N-) terminal residue. Proteins become substrates for the pathway following proteolytic cleavage that may be followed by enzymatic modification including oxidation and arginylation to produce novel N-termini. These new N-termini can act as signals (degrons) for recognition by specific E3 ligases and subsequent degradation via the 26S proteasome. We will discuss the pros and cons of N-terminal peptide enrichment by Terminal Amine Isotope Labelling of Substrates (TAILS) as a means to identify potential substrates and downstream targets of the Arabidopsis N-end rule pathway. New insight into control of storage reserve mobilisation by the Arg/-N-end rule will be presented. The utility of TAILS for proteome annotation, analysis of N-terminal post-translational modifications and protease profiling will also be discussed.

CS17.97 INVESTIGATING THE DEGRADATION OF *ARABIDOPSIS* RIN4 FRAGMENTS AFTER CLEAVAGE BY THE *PSEUDOMONAS SYRINGAE* EFFECTOR AvrRpt2

MONDAY 11 DECEMBER 2017

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Plants rely on a complex immune system in order to defend themselves against invading pathogens. The *Arabidopsis thaliana* protein RPM1-INTERACTING PROTEIN 4 (RIN4) is a negative regulator of plant immunity that plays a role in both Pathogen-Associated Molecular Pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). RIN4 is targeted and modified by several bacterial effectors, including the *Pseudomonas syringae* protease effector AvrRpt2. Cleavage of RIN4 by AvrRpt2 results in the generation of three RIN4 fragments and resistance protein RPS2-mediated ETI. Notably, two of the RIN4 fragments that are released after AvrRpt2 cleavage bear so-called destabilising N-terminal residues, which could act as degradation signals and target the RIN4 fragments for destruction through the ubiquitin-dependent N-end rule pathway of protein degradation. In addition to RIN4, AvrRpt2 cleaves several other *Arabidopsis* proteins from the NOI-domain-containing protein family. In many cases, cleavage of these proteins by AvrRpt2 results in peptides that bear N-terminal destabilising residues. In this study, we sought to determine if protein fragments resulting from AvrRpt2 cleavage are degraded through the N-end rule pathway. Finding answers to this question could be key to our understanding of the immune responses that occur downstream cleavage of RIN4 and related proteins.

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