PS7.1

The Pals wink at the ESCRT: pH signalling in the plasma membrane

Miguel A. Peñalva [1] A.M. Calcagno-Pizarelli [2] Herb N. Arst [2] Jr. ,Antonio Galindo [1]

[1]*Department of Molecular Medicine; Centro de Investigaciones Biológicas CSIC, Ramiro de Maeztu 9, Madrid 28040, Spain* [2]*Section of Microbiology, Imperial College London, Flowers Building, Armstrong Road, London SW7 2AZ, UK*

The fungal *pal*/*RIM* signalling pathway regulates gene expression in response to environmental pH. In *Aspergillus nidulans* it involves six dedicated proteins, PalA, PalB, PalC, PalF, PalH and PalI, which mediate the proteolytic activation of the transcription factor PacC. In addition, it requires several components of the ESCRT (endosomal sorting complex required for transport) complexes, which mediate multivesicular body biogenesis at endosomes. This fact suggested that pH signalling proteins might assemble on endosomal platforms. Amongst Pal proteins are the plasma membrane receptor PalH and its coupled arrestin, PalF. PalF becomes ubiquitylated in an alkaline pH- and PalH-dependent manner; three other Pal proteins are ESCRT-III associates, and thus they were considered potentially endosomal. These are the Vps32-interactors PalA and PalC and the Vps24-interactor calpain-like PalB. Therefore previous models speculated that intracellular traffic would mediate the connection between plasma membrane- and endosomal membrane-associated complexes.

We studied by *in vivo* microscopy the subcellular localization at which signalling takes place after activating the pathway by shifting ambient pH to alkalinity. Rather than localising to endosomes, Vps32 interactors PalA and PalC oscillate at the plasma membrane, transiently co-localising to alkaline pH-induced cortical structures in a PalH-dependent manner. Notably, the assembly of this cortical structures is Vps23 (ESCRT-I)- and Vps32 (ESCRT-III)-dependent but Vps27 (ESCRT-0)-independent. These cortical structures are dramatically more stable under conditions leading to Vps4 deficiency, indicating that their half-life depends on ESCRT-III disassembly. Pull-down studies demonstrated that Vps23 interacts strongly with the PalF arrestin. Notably Vps23 co-immunoprecipitates exclusively ubiquitylated PalF forms from extracts. Endogenously tagged Vps23-GFP is also recruited to cortical structures, in addition to endosomes, in a PalF- and alkaline pH-dependent manner. These Vps23-GFP structures become particularly obvious in *vps27Δ* cells where the conspicuous endosomal localisation of Vps23 is prevented. Dual-channel time-lapse epifluorescence microscopy showed that PalC arrives to cortical complexes before PalA. As PalC recruitment is PalA-independent and PalA recruitment is PalC-dependent but PalB-independent, these data complete the participation order of Pal proteins in the pathway. Importantly, they strongly support a model in which pH signalling takes place in ESCRT-containing, plasma membrane-associated, rather than endosome-associated, signalling complexes.

PS7.2

pH control of infectious growth in *Fusarium oxysporum* involves reprogramming of MAPK signalling cascades

Antonio Di Pietro, Manuel S. Lopez-Berges, David Segorbe, Elena Perez-Nadales, David Turra
Universidad de Cordoba

In plant pathogenic fungi, contact with the host triggers a developmental and metabolic transition towards infectious growth. We are interested in the environmental and host-derived stimuli and cellular pathways that regulate infectious growth in *Fusarium oxysporum*, a soilborne pathogen causing vascular wilt disease on a wide range of plant species and opportunistic infections in immunocompromised humans. One of the key players in plant pathogenicity is Fmk1, a conserved mitogen-activated protein kinase (MAPK) that is essential for infection-related processes such as chemotropism, root adhesion, penetration and invasive growth. Most Fmk1-dependent virulence functions require the homeodomain transcription factor Ste12, and are repressed in the presence of the preferred nitrogen source ammonium through a mechanism that requires the transporter MepB and the bZIP factor MeaB. Repression of invasive growth by ammonium also occurs in *Magnaporthe oryzae*and *Fusarium graminearum*, suggesting that this mechanism is conserved in biologically divergent plant pathogens. Recent data suggest that ammonium repression is mediated by a shift in extracellular pH, which results in rapid changes in the phosphorylation pattern of different MAPKs. Thus, ambient pH controls invasive growth of *F. oxysporum* by reprogramming the activation status of cellular MAPK signalling cascades.

PS7.3

Subcellular Localization of the *Neurospora crassa* MAP Kinase MAK-2 Influence its Activity and Function During Cell-Cell Signalling

Julia Illgen, Timo Schuerg, Ulrike Brandt, Andre Fleissner
Technische Universität Braunschweig, Institut für Genetik, Spielmannstraße 7, 38106 Braunschweig, Germany

Germinating vegetative spores of the filamentous fungus *Neurospora crassa* mutually attract and grow towards each other and eventually fuse. Earlier studies identified the SO protein and the MAP kinase MAK-2 as essential players for this chemotropic interaction. Both proteins show dynamic recruitment to the tips of opposing germlings and concentrate at the fusion point once the two cells get into physical contact (Fleissner et al, 2009). Our analysis of the subcellular localization of the upstream kinases of MAK-2 suggest similar dynamics for the MAPKK STE-7. Colocalization experiments were performed using heterokaryons expressing *ste-7-gfp* and *mak-2-cherry* constructs. Analysis of the subcellular localization of the MAPKKK NRC-1 indicate a concentration of the kinase at the fusion point after the fusion cells established physical contact.

To test if the dynamic localization of MAK-2 is essential for chemotropic growth, we artificially tethered it permanently to the plasma membrane via a farnesyl anchor. Expression of the respective constructs does not rescue the Δ*mak-2* defects and results in a dominant phenotype in the wild-type background. Western-Blot analysis revealed that the membrane bound form of MAK-2-GFP is hyperactivated. Together these results indicate that the recruitment of MAK-2 to the plasma membrane promotes activation of this kinase and that the dynamic localization of the MAP kinase is essential for cell signalling.

PS7.4

Functional characterization of G-protein-coupled receptors in the cereal pathogen *Fusarium graminearum*

Van Thuat Nguyen, Jörg Bormann, Wilhelm Schäfer
Biozentrum Klein-Flottbek, Universität Hamburg

G-protein-coupled receptors (GPCRs) are a large family of transmembrane proteins that perceive external signals and communicate them to an intracellular heterotrimeric G-protein-signaling cascade. Thus, GPCRs play a pivotal role in the adaptation of entities to environmental stresses. During establishment of a compatible interaction, pathogenic fungi have to deal with numerous, rapid changes in regard to stresses and nutrition. Here, we present a comprehensive functional characterization of nine GPCRs of the cereal pathogen *F. graminearum*, the causal agent of Fusarium head blight (FHB) of small grain cereals. Single deletion mutants of five putative cAMP receptor-like GPCRs (cAMP-GPCR), two putative nitrogen sensors (NS), one pheromone receptor (PR) and one putative carbon sensor (CS) GPCR were generated. Subsequently, the mutants were characterized in regard to vegetative growth on different media (e.g. different C- and N-sources, various stresses), different conditions (temperature, pH), sexual and vegetative reproduction, cAMP production, lipase acitivity, virulence towards wheat, and deoxynivalenol production. To our surprise, deletion of one cAMP-GPCR (FG7716) and both NS (FG8496 and FG5579) does not provoke any obvious phenotype. Deletion mutants of the cAMP-GPCR FG1861 showed a higher stress tolerance towards oxidative, fungicide and temperature stresses and were reduced in virulence compared to wild type. The latter also applies for deletion mutants of the cAMP-GPCRs FG3023 and FG5239 and the PR FG2655. These four mutants were also reduced in DON-production, which might explain the reduced virulence towards wheat. The CS FG5006 was drastically reduced in the intracellular cAMP level indicating that this GPCR acts upstream of the adenylatcyclase.

PS7.5

Specific Structural Features Of Sterols Affect Cell-Cell Signalling And Fusion In *Neurospora crassa*

Martin Weichert[1] Ewald Priegnitz[1] Raphael Brandt[1] Thorben Nawrath[2] Stefan Schulz[2] André Fleißner[1]
[1] *Institut für Genetik, Technische Universität Braunschweig, Spielmannstraße 7, D-38106 Braunschweig*
[2] *Institut für Organische Chemie, Technische Universität Braunschweig, Hagenring 30, D-38106 Braunschweig*

In the early stages of colony formation in *Neurospora crassa*, germinating spores mutually attract each other, establish physical contact, and finally fuse. Communication between germlings requires the coordinated dynamic recruitment of the MAP kinase MAK-2 and the cytoplasmic protein SO to the tips of interacting cells. Subsequent plasma membrane fusion is facilitated by the transmembrane protein PRM1.

Here, we report that mutants affected in the biosynthesis of ergosterol, a major constituent of the fungal cell membrane, show distinct defects during germling fusion. Deletion of *erg-2*, which encodes an enzyme mediating the last step in the pathway, strongly impairs both tropic interactions and cell fusion. Interestingly, both MAK-2 and SO mislocalize at the tips of interacting Δ*erg-2* germlings. In contrast, the absence of ERG-10a and ERG-10b, two enzymes with redundant function that act upstream of ERG-2, does not affect cell-to-cell communication. However, Δ*erg-10a* Δ*erg-10b* germling pairs show Δ*Prm1*-like deficiencies in plasma membrane merger.

By relating the sterol composition and fusion competence of several *erg* mutants, we find that not the absence of ergosterol, but the accumulation of sterol intermediates specifically impairs distinct steps of germling fusion. While the presence of two double bonds in the sterol side chain provokes Δ*erg-2*-like deficiencies, the absence of a double bond in the sterol ring system causes Δ*Prm1*-like defects.

These data suggest that specific structural features of sterols differentially affect membrane properties and functions, such as the membrane recruitment of proteins, the assembly of signalling complexes, and plasma membrane fusion.

PS7.6

Structural and Functional Comparison of Pyrrolnitrin- and Iprodione-induced Modifications in the Class III histidine-kinase Bos1 of *Botrytis cinerea*

Sabine Fillinger[1] Sakhr Ajouz[2] Philippe Nicot[2]Pierre Leroux[1] Marc Bardin[2]
[1] INRA UR 1290, BIOGER CPP, BP01, F-78850 Thiverval-Grignon, France [2.] INRA, UR407, Plant Pathology Unit, Domaine St Maurice, F-84140 Montfavet, France

Dicarboximides and phenylpyrroles are commonly used fungicides against plant pathogenic ascomycetes. Although their effect on fungal osmosensing systems has been shown in many studies, their modes-of-action still remain unclear. Laboratory- or field-mutants of fungi resistant to either or both fungicide categories generally harbour point mutations in the sensor histidine kinase of the osmotic signal transduction cascade. We compared the mechanisms of resistance to the dicarboximide iprodione and to pyrrolnitrin, a structural analogue of phenylpyrroles, in *Botrytis cinerea*. Pyrrolnitrin-induced mutants and iprodione-induced mutants of *B*. *cinerea* were produced *in vitro*. For the pyrrolnitrin-induced mutants, high level of resistance to pyrrolnitrin was associated with a high level of resistance to iprodione. For the iprodione-induced mutants, the high level of resistance to iprodione generated variable levels of resistance to pyrrolnitrin and phenylpyrroles. All selected mutants showed hypersensitivity to high osmolarity and regardless of their resistance levels to phenylpyrroles, they showed strongly reduced fitness parameters (sporulation, mycelial growth, aggressiveness on plants) compared to the parental phenotypes. The sequences of the osmosensing class III histidine kinase encoding gene *bos1* showed different mutations in both types of mutants. All of them affected the HAMP-domains of the histidine-kinase showing that each of the six HAMP domains is important for signal-transduction. Structure modelling of the HAMP domains revealed that the replacements of hydrophobic residues within the HAMP domains generally affected their helical structure, probably abolishing signal transduction. The mutation of residues E529, T581, or E692 – without consequences on HAMP structure – highlighted their involvement in signal transduction.

PS7.7

Transcriptomic and molecular analysis of germination and plant infection of Botrytis cinerea

Michaela Leroch, Matthias Hahn
University of Kaiserslautern

Germination of *Botrytis cinerea* conidia can be induced by several stimuli. Carbon source-induced germination is dependent on cAMP-dependent signaling, whereas for germination of conidia on hydrophobic surfaces without nutrients, an intact Ste11-Ste7-Bmp1 MAP kinase cascade is required. We have performed transcriptome studies to follow gene expression changes during germination and differentiation of *Botrytis cinerea* wild type conidia and deletion mutants strains. The results showed that in general the greatest changes of gene expression occur between 0 and 1 hour (before germ tube emergence). The genes that were specifically upregulated during germination (1-4 h.p.i.), were found to be enriched in genes encoding secreted proteins, indicating a strong secretory activity during the early stages of development. In contrast in the *bmp1* MAP kinase mutant, which is essential for germination on a hydrophobic surface and host penetration, an upregulation of many of these genes was not observed. As a putative sensor protein Msb2, was identified. Δ*msb2* mutants showed normal germination on hydrophobic surfaces but no appressoria formation and impaired primary lesion formation. Comparison of the transcript profiles of *msb2* and *bmp1* deletion mutants supports a regulatory link of both signal transduction components. Our further research focuses on the contribution of Msb2 in the Bmp1 dependent signaling cascade by phosphorylation studies and yeast-2-hybrid experiments.