PS6.1

ROS signal transduction and cell differentiation in filamentous fungi

Fernando Lara-Rojas, Olivia Sánchez, Jesús Aguirre
Instituto de Fisiología Celular, Universidad Nacional Autónoma de México

A phosphorelay system coupled to a MAP kinase module is involved in sensing and processing environmental signals in Fungi. In *Aspergillus nidulans*, response regulator (RR) SskA transmits oxidative stress signals to the stress MAPK (SAPK) SakA, which in turns physically interacts with ATF/CREB transcription factor AtfA in the nucleus. This defines a general stress-signalling pathway, which plays differential roles in oxidative stress responses during growth and development. AtfA is needed for the expression of several genes, the conidial accumulation of SakA and the viability of conidia. Furthermore, SakA is active (phosphorylated) in asexual spores, remaining phosphorylated in dormant conidia and becoming dephosphorylated during germination. SakA phosphorylation in spores depends on certain (SskA) but not other (SrrA and NikA) components of the phosphorelay system. Constitutive phosphorylation of SakA prevents both, germ tube formation and nuclear division. Similarly, *Neurospora crassa* SakA orthologue OS-2 is phosphorylated in intact conidia and gets dephosphorylated during germination. We propose that SAPK phosphorylation is a conserved mechanism to regulate transitions between non-growing (spore) and growing (mycelia) states. The *Aspergilli* contain a second SAPK called MpkC. Although *mpkC* mutants are not sensitive to oxidative or osmotic stress, they produce more spores that the wild type strain, suggesting that SakA and MpkC regulate processes related to the production and germination of spores. In addition, to the SakA pathway, RR SrrA and the AP-1 trancription factor NapA are differentially involved in ROS signalling and cell differentiation.

PS6.2

The NADPH Oxidase Complexes in *Botrytis cinerea*

Ulrike Siegmund, Sabine Giesbert, Paul Tudzynski
WWU Münster

Reactive oxygen species (ROS) act as messenger molecules for intercellular signaling or play a role during defense mechanisms against pathogens. One good example is the oxidative burst, within which plants rapidly produce large amounts of ROS as the first defense reaction towards pathogen attacks. NADPH oxidases (Nox) are the most common enzymatic system to produce ROS. Nox are enzymes, which transport electrons through biological membranes and therewith reduce oxygen to superoxide. In fungi they are shown to be involved in differentiation processes and pathogenicity and are therewith in our focus to gain insights into plant - fungi interactions.

Two NADPH oxidases (BcNoxA and BcNoxB) as well as their putative regulator (BcNoxR) were previously identified in the phytopathogenic fungus *B. cinerea* [1]. Besides their involvement in pathogenicity and sclerotia production, deletion studies have revealed that BcNoxA and BcNoxR are also involved in hyphal germling fusions[2].

Preliminary analysis indicate a localization of the catalytical subunits BcNoxA and BcNoxB to the ER and partly to the plasma membrane of hyphae.

Nox are multi-enzyme complexes, whose regulatory process and the participating proteins are well described in mammals. Though, in fungi not all components have been identified, yet. For *B. cinerea* interaction studies with potential candidates identified the regulatory subunit BcNoxR, the small GTPase Rac, the GEF BcCdc24, the scaffold protein BcBem1 and the PAK BcCla4 as interacting proteins within the BcNox complex.

[1] Segmueller N. et al., (2008) *Mol Plant Microbe Interact* **21:** 808-808-819.
[2] Roca M.G. et al., (2011) *Fungal Biology* (in press)

PS6.3

Identifying targets of NADPH oxidase-mediated redox signalling in *Fusarium graminearum* using proteomics approaches.

Manisha Joshi, Rajagopal Subramaniam, Christof Rampitsch
Agriculture and Agrifood Canada

Regulated production of reactive oxygen species by NADPH oxidases (NoxA, NoxB) in *Fusarium graminearum* is essential for the establishment of Fusarium head blight in wheat: the knock-out mutant *FgrΔNoxAB* is non-pathogenic, although it produces normal levels of the mycotoxin (and virulence factor) deoxynivalenol. Nox A and B produce O_2^- and thence H_2O_2 during infection, creating a reducing environment in which susceptible cysteine-cysteine disulphide bonds or other Cys-S-R, can be reduced to native Cys-SH. This can profoundly affect the activity of targeted proteins. Two strategies were used to identify targeted proteins: 1) 2-D electrophoresis, using monobromo-bimane to label reduced Cys residues and 2) an affinity-enrichment strategy based upon biotinylation of targeted cys residues, i.e. those where reduction occurs in WT but not in *FgrΔNoxAB*, under mycotoxin-inducing conditions *in vitro*. We identified 13 potentially targeted proteins by 2-DE and 29 by affinity enrichment, with Cys-S-R in *FgrΔNoxAB* and Cys-SH in WT. One of these proteins, FG10089 – homologous to a putative sporulation specific (SPS2) protein – has been knocked out, and *FgrΔ10089* presents the same phenotype as *FgrΔNoxAB*: non-pathogenic on wheat, but with WT levels of deoxynivalenol. FG10089 is therefore a possible downstream target of NoxAB-mediated redox signalling. Quantification of all Cys-peptides of interest by LC-MRM/MS and conversion of targeted Cys to Ser are underway, with the aim of further understanding the role of redox regulation in *F. graminearum* pathogenesis.

PS6.4

Production and Epidemiological Importance of Photodynamic Toxins Produced by the Necrotrophic Fungus *Ramularia collo-cygni*

Michael Hess[1] Marika Nyman[1] Neil Havis[2] James Fountaine[2] Harald Schempp[1]
[1] Technische Universität München [2] Scottish Agricultural College

Leaf spotting is a common symptom in barley observed in particular since the late 1980s. The phenomenon is frequently associated with a physiologic reaction of the host plant to abiotic stresses like changes in temperature and radiation involving Reactive Oxygen Species (ROS). The frequent occurrence in past years seems related to climate change. At the same time the fungus *Ramularia collo-cygni* was identified as the causal agent of the Ramularia leaf spot disease. A particularity of the fungus is the production of photodynamic toxins, anthraquinone derivates named Rubellins. After activation through light Rubellins produce ROS, which were shown to be able to cause symptom development through chlorophyll bleaching and lipid peroxidation leading to cell wall degradation and the formation of necrotic spots. It was hypothesized that Rubellins play as virulence factor a role in the host parasite interaction. This is further supported by the correlation of symptom development and epidemics with the breakdown of the antioxidative system during plant senescence.

The testing of archive samples with PCR methods have shown that the fungus was present on seed long before becoming a major disease in barley and the detection during the growth season has discovered a high latency and the opportunity of seed transfer.

The current investigations quantify Rubellins through HPLC and compare it to the occurrence of symptoms and DNA content of *Ramularia collo-cygni* in the plant to find out where and when Rubellins are produced and to further elucidate the role of ROS and abiotic stresses in epidemics.

PS6.5

ROS Damage Defence Mechanisms in *Podospora anserina*

Andrea Hamann, Sandra Zintel, Heinz Dieter Osiewacz
J.W. Goethe-University Frankfurt, Germany

Reactive oxygen species (ROS) like superoxide or hydrogen peroxide are central components of the 'free radical theory of aging'. One major source for ROS in biological systems is their emergence as by-product during electron transport processes. Fortunately, various pathways to deal with ROS-induced damage have evolved. A first protective mechanism is the prevention of damage by effective ROS scavenging. One central ROS scavenging enzyme is the superoxide dismutase (SOD) converting superoxide into hydrogen peroxide. A number of studies demonstrate a clear impact of this enzyme family on lifespan. However, in the fungal aging model *Podospora anserina* the deletion of all three *Sod* genes hardly affects lifespan. This surprising dispensability of SODs in *P. anserina* is even more astonishing if considering that lack of PaSOD1, the cytosolic Cu/ZnSOD as well as of PaSOD3, the mitochondrial MnSOD results in paraquat sensitivity. We provide data suggesting a 'hormesis' effect of low superoxide doses on lifespan.

While low ROS doses seem to be beneficial, long-term or higher stress needs efficient degradation/recycling of damaged components. We therefore recently started to investigate another protective mechanism, selective autophagy, which degrades and recycles damaged components. The selectivity of this process is determined by the cargo receptor interacting with ATG8, an ubiquitin-like protein required for autophagosome formation. In filamentous fungi, there is only very little information available about cargo receptors. Thus, we used a two-hybrid screen with PaATG8 as bait to identify potential cargo receptors. Here, we provide first data on their characterisation.

PS6.6

Mitophagy is linked to the general stress response pathway in *Saccharomyces cerevisiae*

Andreas S. Reichert

Mitochondriale Biologie, Zentrum für Molekulare Medizin, Goethe Universität Frankfurt am Main, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany Mitochondrial Biology, Frankfurt Institute for Molecular Life Sciences, Max-von-Laue-Str. 15, 60438 Frankfurt am Main, Germany

Mitochondria form a dynamic reticular network that is maintained by balance fusion and fission events. Impairment of this dynamic behavior is associated with a number of neuropathies such as optic atrophy and Parkinson's disease. In dysfunctional mitochondria the fusion machinery become inactivated and, as fission is maintained, such mitochondria become spatially separated from the intact network. By that mechanism dysfunctional mitochondria have been proposed to be targeted for selective degradation, by mitophagy, providing a quality control system for mitochondria. In yeast conflicting results concerning the role of mitochondrial dynamics in mitophagy were reported. We investigated the effects of altering mitochondrial fission and fusion on mitophagy using biochemical as well as fluorescence based assays. Rapamycin induced mitophagy depended on Atg11, Atg20, and Atg24 confirming that a selective type of autophagy was induced. Fragmentation of mitochondria or inhibition of oxidative phosphorylation was not sufficient to trigger mitophagy. Neither expression of dominant-negative variants of Dnm1, nor deletion of the fission factors Dnm1, Fis1, Mdv1, or Caf4 impaired mitophagy. Instead, we found that reduced mitophagy initially observed in a Δ*fis1* mutant was not due to the absence of Fis1 but rather due to a secondary mutation in *WHI2*, encoding a factor reported to function in the general stress response and the Ras/PKA signaling pathway. We propose that mitochondrial fission is not a prerequisite for the selective degradation of mitochondria in yeast and that mitophagy is linked to the general stress response and the Ras/PKA signaling pathway. Future studies will address how Whi2 is mechanistically linked to mitophagy.

PS6.7

***Botrytis*-plant interaction: interplay of cell death**

Neta Shlezinger, Yonathan Gur, Amir Sharon

Tel Aviv University, Department of Molecular Biology and Ecology of Plants, Tel Aviv 79978, Israel

Botrytis cinerea is used as a model system to study the pathogenicity of necrotrophic fungi. As inferred by the term "necrotrophic", such fungi first kill the tissue and then obtain nutrients from the dead plant cells. Hence, the most critical step in necrotrophic development is fast killing of the host cells. Previous studies have shown that *B. cinerea* promotes apoptotic cell death in infected plants, thereby promoting lesion spreading. How the fungus survives the first encounter with living plant tissue remained unclear. Here we report on the characterization of apoptotic cell death in *Botrytis cinerea* during plant infection and on the role of an anti-apoptotic response in disease establishment.

Useing an automatic search protocol we mapped the fungal homologues of mammalian apoptotic proteins and domains. Among all known apoptotic domains, only BIR domain was found in fungi. We isolated the BIR-containing protein from *B. cinerea* and determined its role in apoptosis and pathogenicity. Knockout or over expression strains of *BcBIR1* revealed that BcBir1 has antiapoptotic activity. We found that the fungus undergoes massive programmed cell death during early stages of infection, but then fully recovers upon transition to second phase of infection. Further studies using the fungal mutants in combination with mutant *Arabidopsis* lines showed that virulence was fully correlated with ability of the fungus to cope with plant-induced PCD. Time lapse analysis showed that the reduced pathogenicity of the *Bcbir1* mutant was due to slow development in the initial infection phase, whereas there was no change in development of the mutant during the second infection phase. A model of the infection process is proposed in which the anti-apoptotic machinery is only necessary during the initial infection phase, during which the fungus is in direct contact with living plant cells.

PS6.8

Farnesol-induced cell death in the filamentous fungus Aspergillus nidulans

Gustavo Goldman
Universidade de São Paulo

FOH (farnesol), a non-sterol isoprenoid produced by dephosphorylation of farnesyl pyrophosphate, has been shown to inhibit proliferation and induce apoptosis. We have been using Aspergillus nidulans and FOH as a model system and cell death stimulus, respectively, aiming to understand by which means filamentous fungi are driven towards cell death. Previously, we demonstrated that the A. nidulans calC2 mutation in protein kinase C pkcA was able to confer tolerance to FOH. We demonstrate that pkcA overexpression during FOH exposure causes increased cell death. FOH is also able to activate several markers of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR). Our results suggest an intense cross-talk between PkcA and the UPR during FOH-induced cell death. Furthermore, the overexpression of pkcA increases both mRNA accumulation and metacaspases activity, and there is a genetic interaction between PkcA and the caspase-like protein CasA. Mutant analyses imply that MAP kinases are involved in the signal transduction in response to the effects caused by FOH.

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Monday 2 April

Parallel session 7: Sensing and responding

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.