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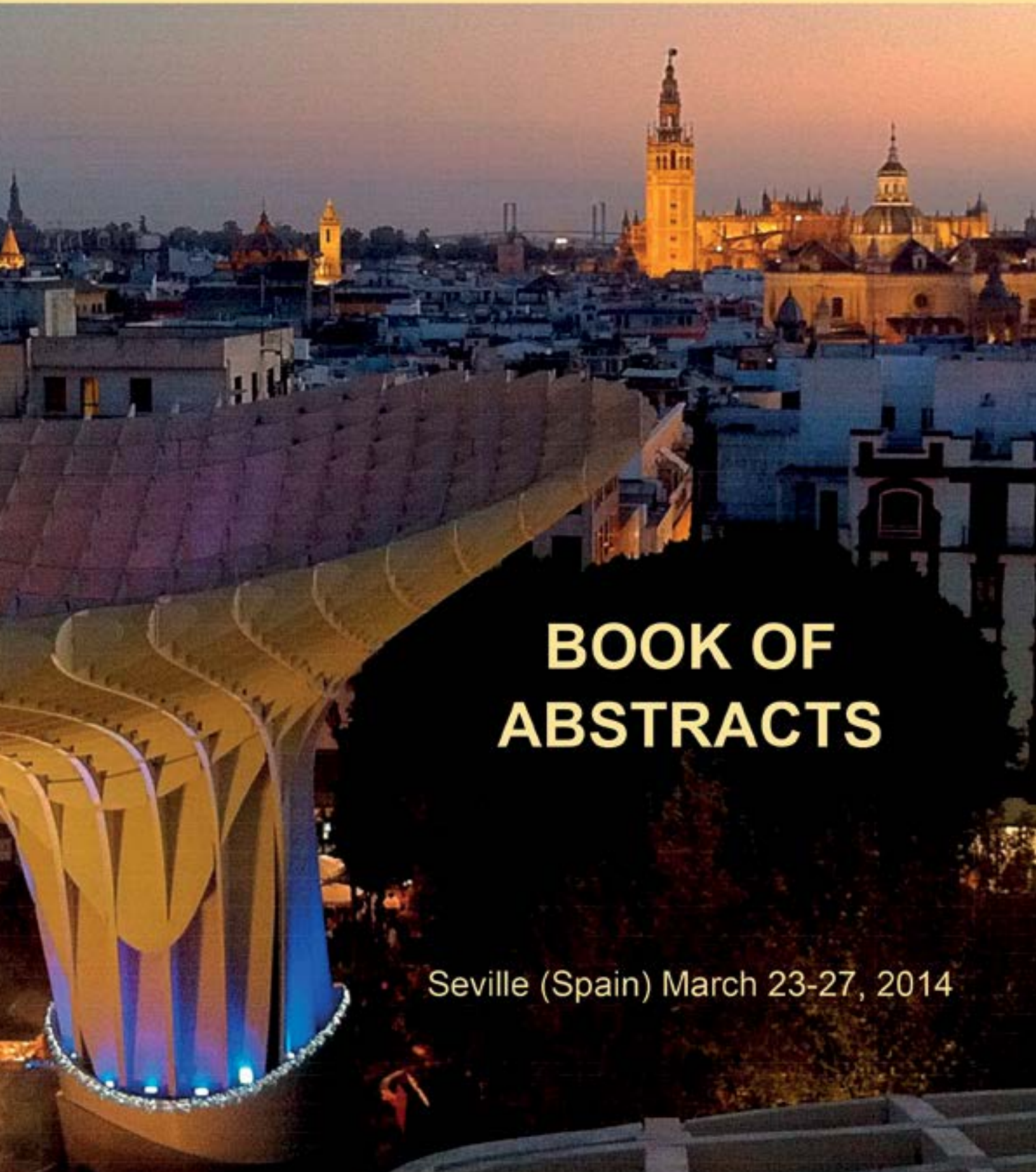

BOOK OF ABSTRACTS

Seville (Spain) March 23-27, 2014

CS7.7 INTERPLAY OF PHOSPHATASES AND KINASES: STRIPAK AND MAP KINASES REGULATE CELL DIFFERENTIATION IN SORDARIA MACROSPORA

INES TEICHERT[1], EVA STEFFENS[1], STEFFEN NORDZIEKE[1], NICOLE SCHNASS[1], THOMAS ZOBEL[2], BENJAMIN FRÄNZEL[1], CHRISTOPH KRISP[1], DIRK A. WOLTERS[1], ULRICH KÜCK[1]

[1] RUHR UNIVERSITY BOCHUM, GERMANY, [2] WESTFÄLISCHE WILHELMS-UNIVERSITÄT MÜNSTER, GERMANY

Phosphorylation and dephosphorylation are crucial for signal transduction and are carried out by kinases and phosphatases. Both play a role in fruiting body formation in the filamentous ascomycete Sordaria macrospora. This fungus has extensively been used as a model system for fungal cell differentiation, since developmental mutants are easily recognizable due to Sordaria`s homothallic lifestyle [1, 2]. We analyzed sterile mutants by complementation and next-generation sequencing and identified a number of proteins essential for fruiting body formation. Among these proteins were PRO22, PRO40 and PRO45 [3, 4]. Using affinity purification and MudPIT (multi-dimensional protein identification technology) mass spectrometry (AP-MS) with PRO22 and PRO40 as bait, we detected phosphatases and kinases as interaction partners. Strikingly, we identified the highly conserved striatin-interacting phosphatases and kinases (STRIPAK) complex as master regulator of fruiting body formation, containing PRO22, striatin homolog PRO11, SmMOB3, and protein phosphatase 2A subunits. We further characterized STRIPAK subunit PRO45, homologous to Neurospora crassa HAM4 and human sarcolemmal membrane-associated protein (SLMAP). Using AP-MS and co-immunoprecipitation, we confirmed that PRO45 is part of fungal STRIPAK. Super-resolution structured-illumination microscopy showed PRO45 localization to the nuclear envelope and to mitochondria. Our studies now focus on the significance of PRO45 localization to different compartments. Experimental evidence suggests interaction of STRIPAK with the cell wall integrity (CWI) MAP kinase cascade via developmental protein PRO40, a homolog of N. crassa SOFT. PRO40 binds to protein kinase C, MAPKKK MIK1, and MAPKK MEK1 via unstructured and WW domain-containing regions. We generated shared interaction networks of PRO40 and MEK1 and integrated data from recent transcriptomics analyses [5]. Our results strengthen our hypothesis that STRIPAK and the CWI pathway are interconnected and reveal a number of candidate proteins for future studies. Due to the evolutionary conservation of the protein complexes under investigation, our data are significant for invertebrate and vetebrate systems.

[1] Kück et al. 2009 The Mycota XV: 17-39 // [2] Engh et al. 2010 J Cell Biol 89:864-872 // [3] Bloemendal et al. 2012 Mol Microbiol 84:310-323 // [4] Engh et al. 2007 Eukaryot Cell 6:831-843 // [5] Teichert et al. 2012 BMC Genomics 13:511

CS7.8 CISTERNAL MATURATION WITHIN THE ASPERGILLUS NIDULANS GOLGI VISUALIZED IN VIVO

ARETI PANTAZOPOULOU[1], MARIO PINAR[1], MIGUEL HERNÁNDEZ-GONZÁLEZ[1], HERB N ARST[2], MIGUEL ANGEL PEÑALVA[1]

[1] CENTRO DE INVEST BIOLOG CSIC, SPAIN, [2] IMPERIAL COLLEGE LONDON, UK

115 years after Camillo Golgi´s description of the homonymous organelle, the mechanism by which proteins and lipids traffic in the secretory pathway, undergoing ordered modifications before being distributed to their target organelles, remains elusive and debated. Over the last few years, we have established that Golgi cisternae of the filamentous fungus Aspergillus nidulans are not stacked and are thus optically resolvable, as opposed to the mammalian Golgi, which is organized in stacks of sub-resolution cisternal distance. In hyphal cells, Golgi cisternae display polarized distribution towards the growing apex; however, the late Golgi is absent from a ≈3μm region immediately below the apex, where secretion predominates. This region is populated by both microtubules and the actin mesh emerging from the Spitzenkörper, where secretory membranes accumulate, awaiting fusion with the plasma membrane. According to the cisternal maturation model for cargo transport, acute impairment of traffic in the ER-Golgi interface would lead to rapid disorganization of both the early and the late Golgi cisternae, while the vesicular transport model anticipates that stable Golgi cisternae would not be affected under these conditions, at least not promptly. We have constructed appropriate conditional mutants and, using in vivo fluorescence microscopy, we observed that a reversible block in the ER-Golgi traffic results in the reversible disorganization of both the early and late Golgi cisternae within minutes, as predicted by the cisternal maturation model. Indeed, we have found that Golgi cisternae in growing hyphae are transient entities. By employing multidimensional microscopy, we are able to directly observe cisternal maturation; that is the de novo formation of an early Golgi compartment and its subsequent enrichment in a late Golgi marker with concomitant loss of the early Golgi marker, until the formation of a late Golgi compartment. In turn, the late Golgi eventually diminishes.

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