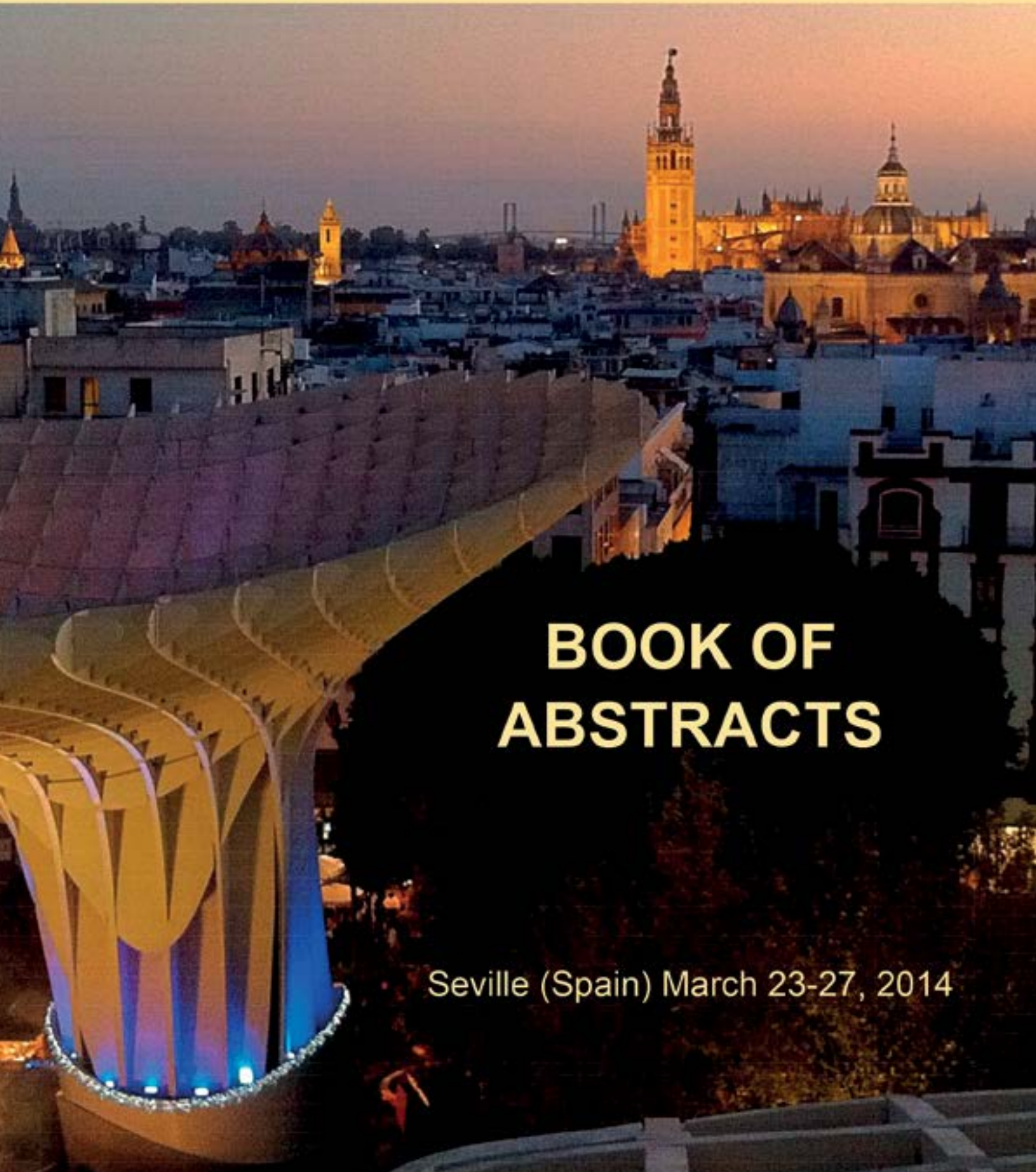
ECFG12
Seville 2014

12th European Conference on Fungal Genetics



BOOK OF ABSTRACTS

Seville (Spain) March 23-27, 2014

083 AUTOPHAGY CONTROLS NUCLEAR DYNAMICS DURING VEGETATIVE HYPHAL GROWTH AND FUSION OF FUSARIUM OXYSPORUM

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In the fungal pathogen Fusarium oxysporum, vegetative hyphal fusion triggers a series of nuclear events including mitosis in the invading hypha, nuclear migration into the receptor hypha and subsequent degradation of the resident nucleus. Here we examined the role of autophagy in fusion-induced nuclear degradation. A search of the F. oxysporum genome database for autophagy pathway (Atg) components identified putative orthologues of 16 core ATG genes in yeast, including the ubiquitin-like protein ATG8 which is required for the formation of autophagosomal membranes. F. oxysporum Δatg8 mutants were generated in a strain harbouring H1::ChFP-labelled nuclei to facilitate analysis of nuclear dynamics. The Δatg8 mutants failed to develop autophagic compartments in contrast to the wild type strain, suggesting that ATG8 is required for autophagy in F. oxysporum. The Δatg8 strains displayed reduced rates of hyphal growth and fusion, and were significantly attenuated in virulence on tomato plants and on the non-vertebrate animal host Galleria mellonella. Whereas wild type hyphae were almost exclusively composed of uninucleated cells, the hyphae of the Δatg8 mutant contained a significant fraction of cells with two or more nuclei. The increase in the number of nuclei per cell was particularly evident after hyphal fusion events between Δatg8 hyphae, or between hyphae of the Δatg8 and wild type strains. Furthermore, time-lapse microscopy analyses revealed abnormal mitotic patterns during vegetative growth in the Δatg8 mutants. Our results suggest that autophagy mediates nuclear degradation after vegetative hyphal fusion, and may function as a general mechanism to control the number of nuclei per cell in F. oxysporum.

084 AUTOPHAGY IN ASPERGILLUS NIDULANS. THE ER AS A POSSIBLE SOURCE OF MEMBRANES FOR AUTOPHAGY

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The genetic model Aspergillus nidulans, whose multinucleated hyphal cells are notably larger than those of Saccharomyces cerevisiae, is ideally suited for in vivo microscopy and intracellular trafficking studies. Therefore, we have exploited these advantages to investigate autophagy. A protein playing a central role in autophagy in yeast is the ubiquitin-like Atg8, which localizes to the phagophore assembly site (PAS). This location depends on the conjugation of phosphatidylethanolamine (PE) to this molecule, mediated by a set of proteins, including Atg4 cysteine protease and E1-like Atg7. This protein modification is necessary to anchor Atg8 to membranes and plays a key role in autophagosome biogenesis. In A. nidulans the localization of Atg8 to the PAS is independent of PE conjugation to the protein, since it is located in this structure in the absence of Atg4 and Atg7. Under nitrogen starvation conditions, GFP-Atg8 containing pre-autophagosomal puncta give rise to cup-shaped phagophores and circular (0.9-µm diameter) autophagosomes that disappear in the proximity of the vacuoles after their shape becomes irregular and their GFP-Atg8 fluorescence decays. Autophagy does not require endosomal maturation or ESCRTs, as autophagosomes fuse with the vacuole in a RabS (RAB7) / HOPS dependent manner. Also, does not require Golgi or post-Golgi traffic since mutations affecting known Golgi resident proteins, or mutations in proteins involved in the post-Golgi trafficking to the plasma membrane or endosomes do not affect the formation of autophagosomes and their fusion with the vacuole. By using a ts mutation in rabO, we have seen that autophagy it is dependent on this Rab protein. RabO (RAB1) localizes to phagophores and autophagosomes. Additionally TRAPPIII-specific factor Trs85 localizes to the PAS. The critical role of RabO (Rab1) in autophagy, combined with the fact that the traffic through the Golgi is not required for this process, suggest that the ER could be a potential source of autophagic membranes. In fact we have detected the presence of omegasome-like structures, similar to those described in mammalian cells, associated with fungal autophagosomes.

Pinar M. et al. Autophagy. 2013 Jul; Volume 9, Issue 7: 1024 -43.

Pinar M. et al. Molecular Microbiology. 2013 Jul;89:228-48.

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