



12th European Conference on Fungal Genetics

A nighttime photograph of Seville, Spain, showing the illuminated Giralda tower and the Seville Cathedral in the background. In the foreground, a large, illuminated, abstract sculpture with a curved, ribbed structure is visible, lit with blue and yellow lights.

BOOK OF ABSTRACTS

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RAPID DISORGANIZATION OF THE GOLGI APPARATUS BY BLOCKING THE EXIT OF COPII TRAFFIC FROM THE ENDOPLASMIC RETICULUM

MIGUEL HERNÁNDEZ GONZÁLEZ, MIGUEL ÁNGEL PEÑALVA, ARETI PANTAZOPOULOU

CIB-CSIC, SPAIN

The mechanistic bases of the biogenesis and maintenance of the cisternae of the Golgi apparatus are still subject to debate. Proteins and lipids synthesized at the endoplasmic reticulum (ER) are sent to the Golgi apparatus into vesicles. The formation of these vesicles is a crucial step of the secretory pathway involved in Golgi function and maintenance. Coat protein complex II (COPII) is a set of highly conserved proteins that mediates the biogenesis of those membrane vesicles. Sar1 is a p21 GTPase that triggers and regulates the assembly of COPII. sarA, the *Aspergillus nidulans* sar1 orthologue, is essential. We generated a saturated library of mutant alleles by random PCR combined with gene replacement and selected a collection of temperature-sensitive alleles that are useful to specifically block traffic from the ER to the Golgi apparatus. Following a temperature shift-up, we found that Sec23, a subunit of COPII, shifts its localization from the ER to the cytosol, suggesting that COPII vesicles do not form. In addition, an acute and rapid disorganization of the Golgi apparatus occurs: RerA (Rer1), a Golgi resident protein that cycles between Golgi and ER to retrieve proteins to the ER, abnormally labels ER membranes, indicating that its steady-state equilibrium has changed, and the late Golgi marker PHosbp becomes cytosolic. Our data seem incompatible with the stable cisternae model, supporting instead cisternal maturation.

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REGULATION OF SNARES IN THE ENDOVACUOLAR SYSTEM OF ASPERGILLUS NIDULANSMANUEL SÁNCHEZ LÓPEZ-BERGES⁽¹⁾, HERBERT N. ARST, JR⁽²⁾, MIGUEL ÁNGEL PEÑALVA SOTO⁽¹⁾⁽¹⁾ CIB/CSIC, SPAIN, ⁽²⁾ IMPERIAL COLLEGE, UK

Aspergillus nidulans presents many advantages for the study of the traffic within the endocytic pathway. Probably, the main one is the ease to differentiate by fluorescent microscopy highly motile early endosomes (EEs) from late endosomes (LEs), larger and relatively static, and from vacuoles, spherical, static and with an optically visible luminal space. Previous work in our lab defined Rab5 and Rab7 domains in EEs and vacuoles, respectively, and also provided evidence of maturation of the former into the latter [1,2,3]. Maturation of EEs into LEs is essential but, once EEs has matured, homotypic fusion of LEs/vacuoles is not vital. In the present work we address the study of endosomal maturation focusing in the regulation of syntaxins, one kind of t-SNAREs, in the endovacuolar system. Pep12 is the sole syntaxin of *A. nidulans* in this context, which lacks a Vam3 homolog. Vps45 and Vps33 are SM proteins that positively regulate syntaxins. Vps45 binds tightly to the late-Golgi/endosomal syntaxin Tlg2 but surprisingly, inactivation of Tlg2 gave rise to no growth alterations while a vps45 null mutant is markedly affected. These data suggest that Vps45 must be regulating an additional syntaxin and we hypothesize that Pep12 is this second target. In fact, there is evidence of functional [4] and, debatably, physical [5,6] interaction between both proteins in yeast and deletion of *A. nidulans* pep12 recapitulates the vps45Δ phenotype. We have proved experimentally that rabbit polyclonal antiserum against Pep12 is able to specifically immunoprecipitate Vps33 and Vps45 but not Sly1, the SM of the early-Golgi syntaxin Sed5, while Tlg2 antiserum exclusively and strongly immunoprecipitate Vps45. We believe that Pep12 must be regulated at three different levels by two SM proteins: Vps33, in the EEs CORVET and LEs/vacuole HOPS contexts, and Vps45, in the Golgi-to-endosomes traffic. Additionally, we are identifying all the SNARE complexes formed in the endovacuolar system to establish a physical map of interactions. Even though much more effort is required to understand the source of all interactions our results indicate that Pep12 is regulated by two different SM proteins along the endovacuolar system.

1. Abenza et al., 2009. *Traffic* 10:57-75 // 2. Abenza et al., 2010. *Mol Biol Cell* 21:2756-693. Zhang et al., 2011. *J Cell Biol* 193:1245-55 // 4. Webb et al., 1997. *Genetics* 147:467-785. Burd et al., 1997. *Mol Biol Cell* 8:1089-104 // 6. Dulubova et al., 2002. *EMBO J* 21:3620-31



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