

**P003** Ambient pH signalling protease PalB associates with ESCRT-III via Vps24

**O. Rodríguez-Galán<sup>1</sup>, A. Galindo<sup>1</sup>, A. Hervás-Aguilar, H.N. Arst<sup>2</sup>, Miguel A. Peñalva<sup>1</sup>**

(1) *Centro de Investigaciones Biológicas, Madrid*, and (2) *Imperial College, London*

Regulation of gene expression by ambient pH in the filamentous ascomycete *Aspergillus nidulans* involves the transcription factor PacC, which undergoes two-step proteolytic activation in response to ambient alkaline pH. The first proteolytic step and the only one which is ambient pH-regulated is almost certainly catalysed by the calpain-like cysteine protease PalB, one of the six dedicated proteins involved in pH signalling. These proteins are organized into two complexes: a 'plasma membrane complex' involving the 7-TMD receptor PalH, the 3-TMD protein PalI and the PalF arrestin and an 'endosomal membrane complex' involving the Vps32-interactor PalA and PacC, which is recruited to this complex via PalA. PalC, which localises to cortical punctate structures in a PalH/alkaline pH-dependent manner and contains a Bro1-like Vps32-binding domain, is a candidate to liaise between these two complexes. PalB is partially associated with membrane fractions. PalB specifically binds Vps24, as determined by 2H and GST pull-down assays. This interaction is mediated by the N-terminal PalB MIT domain and the C-terminal MIM motif in Vps24. Deletion of the MIT domain impairs but does not prevent PacC proteolysis and partially relocalises membrane-associated PalB to the cytosol. Attachment of Vps24 to MIT domain-deleted PalB restores membrane association and, to a significant extent, PalB function. We conclude that PalB is a component of the ESCRT-III-associated endosomal pH signalling complex.