Single-particle analysis of the HOPS complex

The 660-kDa multisubunit tethering complex HOPS is a major organizer of membrane tethering and fusion within the endo-lysosomal system of all eukaryotes. The misregulation or mutation of human HOPS can lead to developmental defects, neurodegenerative diseases, and cancer. HOPS interacts with SNARE proteins, Rab GTPases, specific lipids and vesicle coats. To understand the molecular mechanisms underpinning HOPS function, we want to determine its structure and characterize its interactions with other key components of the membrane trafficking machinery.

Previous negative-stain EM studies of the Saccharomyces cerevisiae HOPS complex yielded mutually inconsistent low-resolution structures (Fig. 1). We have therefore chosen the HOPS complex from the thermotolerant fungus *Chaetomium thermophilum* for single-particle cryo-EM, complemented with X-ray crystallographic analysis of individual subunits or domains thereof, with the goal of determining a structure at atomic or near-atomic resolution. The optimization of the protein sample preparation is ongoing. Currently, we can purify large amounts of individual subunits and subcomplexes of HOPS after expression in *E. coli*, and small amounts of the fully assembled complex after expression in insect cells (Fig. 2). Recently, we started analyzing subcomplexes of HOPS with negative-stain and cryo-EM (Fig. 3).

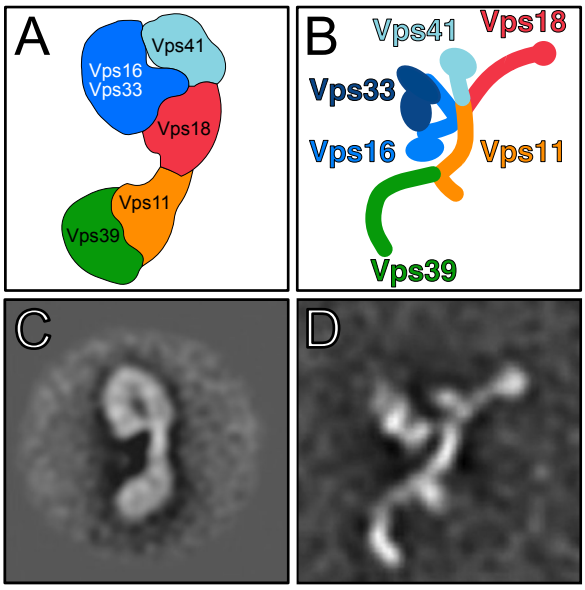

Fig. 1 Proposed architecture of the HOPS complex. (A,B) Schematic drawings of the proposed subunit organization of HOPS and (C,D) selected class averages derived from negative-stain EM (modified from [1,2], respectively). (A,C) show samples that were cross-linked with glutaraldehyde.

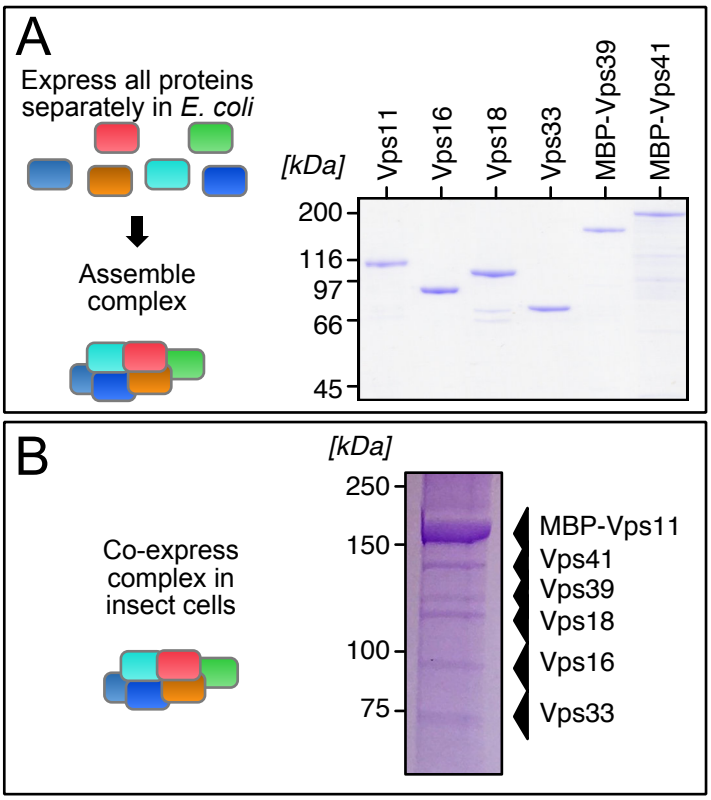

Fig. 2 Purification strategies for the HOPS complex. (A) HOPS subunits can be purified after recombinant expression in *E. coli* and assembled into a complex (B) or the entire HOPS complex can be purified after recombinant co-expression in insect cells.

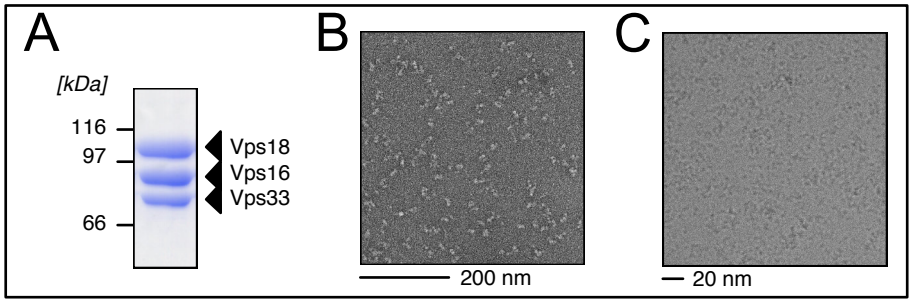

Fig. 3 Initial EM studies of a subcomplex of HOPS. (A) A complex containing three HOPS subunits was purified after recombinant expression in *E. coli*. (B) The complex was visualized by negative-staining with uranyl acetate and (C) cryo-EM. Due to aggregation, potential conformational heterogeneity and complex dissociation as well as low contrast, the identification of individual particles in the cryo-EM micrographs is challenging.

References

[1] Bröcker C et al. (2012) Proc Natl Acad Sci U S A, 109(6):1991-1996.

[2] Chou HT et al. (2016) Nat Struct Mol Biol, 23:761-763.