

**Fab1p and AP-1 are required for trafficking of endogenously ubiquitinated cargoes to the vacuole lumen in *Saccharomyces cerevisiae*.**

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The synthesis of phosphatidylinositol (3,5)-bisphosphate (PtdIns(3,5) $P_2$ ) by Fab1p in *S. cerevisiae* is required for several cellular processes, including an as yet undefined step in the ubiquitin-dependant trafficking of some integral membrane proteins from the *trans*-Golgi network to the vacuole lumen. AP-1 is a heterotetrameric clathrin adaptor protein complex that binds cargo proteins and clathrin coats, and regulates bi-directional protein trafficking between the *trans*-Golgi network and the endocytic/secretory pathway. Like *fab1* cells, AP-1 complex component mutants have lost the ability to traffic ubiquitinated cargoes to the vacuole lumen – the first demonstration that AP-1 is required for this process. Critically, these deletion mutants are distinct from the well-characterised class E mutants, where cargo proteins stall at aberrant endosomes adjacent to the vacuole. Deletion mutants of AP-1 complex components are compromised in their ability to synthesize PtdIns(3,5) $P_2$ , indicating that AP-1 is required for correct *in vivo* activation of Fab1p. Furthermore, wild-type protein sorting can be restored in AP-1 mutants by overexpression of Fab1p, implying that the protein-sorting defect in these cells is as a result of disruption of PtdIns(3,5) $P_2$  synthesis. We show that Vac14p, an activator of Fab1p, is also required for another AP-1-dependent process: chitin-ring deposition in *chs6* cells. Our data imply that AP-1 is required for some Fab1p and PtdIns(3,5) $P_2$ -dependent processes. Currently, we are working on a genetic screen to identify novel components of the AP-1/PtdIns(3,5) $P_2$ -dependant step(s) in sorting of ubiquitinated cargo to the Multi-vesicular Body (MVB)/vacuole.