

P026 Investigating the role of phosphatidylinositol 3,5-bis-phosphate and AP-1 in multivesicular body protein sorting.

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We are investigating the role of AP-1 and Fab1p in the sorting of ubiquitinated cargoes at the multivesicular body (MVB) and vacuole in yeast. Previously we have shown that both AP-1, a heterotetrameric clathrin binding complex and PtdIns(3,5) P_2 (the product of Fab1p) are required for sorting the cargo carboxypeptidase S (CPS) at the MVB where it is recognised and ubiquitinated by ESCRT machinery. In AP-1 deletion mutants CPS sorting is disrupted and CPS is sorted to the outer vacuole membrane, however, correct sorting is restored by the covalent fusion of ubiquitin to the protein. Similarly, the over-expression of Fab1p in these cells restores CPS sorting into the vacuole lumen suggesting that both Fab1p and ubiquitination are required for this process. Our current work is focused on exploring mechanisms behind these sorting events. To do this we will identify and characterise other proteins involved in the sorting of CPS and subject them to a variety of phenotypic assays that will hopefully reveal insights into this interesting and complex sorting route for proteins.