

**P007** K63-linked polyubiquitylation of the yeast Gap1 permease is required for its sorting into the multivesicular body pathway

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Covalent attachment of the small protein ubiquitin to membrane cargoes constitutes a sorting signal at different steps in the endocytic pathway. Although several plasma membrane proteins are modified with K63-linked polyubiquitin chains, the influence of this modification as opposed to linkage of a single ubiquitin to multiple lysines remains unclear. The general amino acid permease Gap1 is one of the protein cargoes of *S. cerevisiae* used to investigate the role of ubiquitin in trafficking of membrane proteins. Upon addition of a favoured nitrogen source, Gap1 undergoes K63-linked polyubiquitylation on two residues (K19 and K16). We show here that the Gap1 protein with both K9 and K16 accessible only for monoubiquitylation is internalized but fails to be correctly sorted into the multivesicular body (MVB) pathway. In contrast, a Gap1 mutant presenting only one lysine accessible for polyubiquitylation enters the MVB pathway with good efficiency, whereas this sorting step is defective if K63-polyubiquitin chain formation is prevented. Finally, the Gap1 permease that fails to reach the vacuolar lumen because of defective polyubiquitylation tends to be recycled to the cell surface. Our findings suggest that K63-polyubiquitylation is critical for cargo handling by the yeast MVB machinery, and thereby plays a previously underestimated role in vacuolar/lysosomal targeting of membrane proteins.