

**P001** Evolution of the endocytic ubiquitylation system  
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Uptake and lysosomal targeting of ubiquitylated endocytic cargo is mediated in part by the ESCRT complex, E3 ubiquitin ligases and by several proteins bearing ubiquitin-interaction motifs (UIMs), a system well conserved between animals and fungi. We used comparative genomics and a combination of sequence analysis and phylogenetics to reconstruct the evolution of the ESCRT system together with analysis of the E3 ligases associated with endocytosis, and the epsin family. We find that ESCRT factors are also well conserved across the eukaryotic lineage, with ESCRT complexes I, II, III and III-associated almost completely retained, and CHMP7 more widely distributed than previously suspected, all indicating an early origin. Two groups of homologous proteins (Vps20/32/60 and Vps2/24/46) populate the modern ESCRT III and III-associated complexes and suggest a concerted model of ESCRT III and III-associated evolution. ESCRT 0 however, which functions in recognition of ubiquitylated cargo, is restricted to the Opisthokonta. Additionally, we find a complete absence of E3 ligases related to Rsp5 or c-Cbl, suggesting a novel ligase mediates attachment of ubiquitin to cell surface components. Finally, we demonstrate that the combined presence of an ENTH domain and a UIM, as in epsin, is restricted to the Opisthokonts, while epsinR, which lacks the UIM, is widely retained and hence ancestral. These data highlight both broad conservation and a complex ancient endocytic system but also distinctive lineage-specific features differences between higher eukaryotes and the majority of taxa.