Insights into the Structure and Regulation of Endosomal Cargo Transporters

Dr. Daniel G. S. Capelluto, Associate Professor
Protein Signaling Domains Laboratory
Department of Biological Sciences, Fralin Life Sciences Institute
And Center for Soft Matter and Biological Physics
Virginia Tech, Blacksburg, VA

The cargo transporter TOM1, an ESCRT-0 component, facilitates the transport of endosomal ubiquitinated proteins destined for lysosomal degradation. To do so, TOM1 forms a complex with TOLLIP, through recognition of the cargo ubiquitin moieties. However, the mechanism of TOM1-TOLLIP complex formation as well as their regulation remain unknown. Using a range of biophysical, cellular, and computational tools, we demonstrate that TOM1 binds TOLLIP through a coupled folding and binding mechanism. This association precludes binding of TOLLIP to endosomal phosphatidylinositol 3-phosphate, increasing the commitment of TOLLIP for cargo trafficking. We also identified an adjacent DXXLL motif-containing region to the TOM1 VHS domain that markedly enhanced the affinity for ubiquitin, resembling the strength of the contiguous GAT domain, and that it can be modulated by phosphorylation. TOM1 is an endosomal phosphatidylinositol 5-phosphate (PtdIns5P) effector under Shigella flexneri infection. With the use of NMR spectroscopy and liposome co-sedimentation assays, we pinpointed a consensus PtdIns5P-binding motif in the VHS domain. We show that PtdIns5P binding by TOM1 is pH-dependent, similarly observed in its binding partner TOLLIP. Under acidic conditions, TOM1 retained its complex formation with TOLLIP, but lost its ability to bind ubiquitin. S. flexneri infection inhibits pH-dependent endosomal maturation, leading to reduced protein degradation. We propose a model wherein pumping of H+ to the cytosolic side of endosomes contributes to the accumulation of TOM1, and possibly TOLLIP, at these sites, thereby promoting PtdIns5P- and pH-dependent signaling, facilitating bacterial survival.

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