COMMENTARY

Selective cargo recognition by vesicle tethering factors

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Received: July 16, 2024; Accepted: September 4, 2024; Published Online: October 16, 2024; https://doi.org/10.59717/j.xinn-life.2024.100094

Citation: Yong X. and Bonifacino J. S. (2024). Selective cargo recognition by vesicle tethering factors. The Innovation Life **2(4)**: 100094.

Vesicle trafficking is essential for the maintenance of cellular homeostasis by enabling the distribution of various intracellular components within eukaryotic cells. Disruptions in this process can lead to a range of diseases, including neurological, respiratory, immune, and metabolic disorders. The trafficking process involves several steps: (a) cargo recognition and selection by coat proteins at a donor membrane, (b) vesicle formation and scission, (c) vesicle transport along cytoskeletal tracks through association with motor proteins, (d) vesicle capture by tethering factors at an acceptor membrane, and finally, (e) vesicle fusion with the target membrane facilitated by SNARE proteins.[1]

It is widely accepted that selection of transmembrane cargoes is predominantly mediated by recognition of sequence motifs within the cytosolic tails of the cargoes by coat proteins at the donor membrane. This selection ensures that only specific proteins are transported. In theory, sequence-dependent cargo recognition could also occur at later stages of the transport process to further enhance the fidelity of protein trafficking. However, the existence of this additional recognition step was not proven until two recent studies by Deng et al.[2] and Cattin-Ortolá et al.[3] These authors report that tethering factors, such as the WDR11 complex and TBC1D23, interact directly with Super Acidic Cluster (SAC) and acidic-cluster-TLY motifs, respectively,

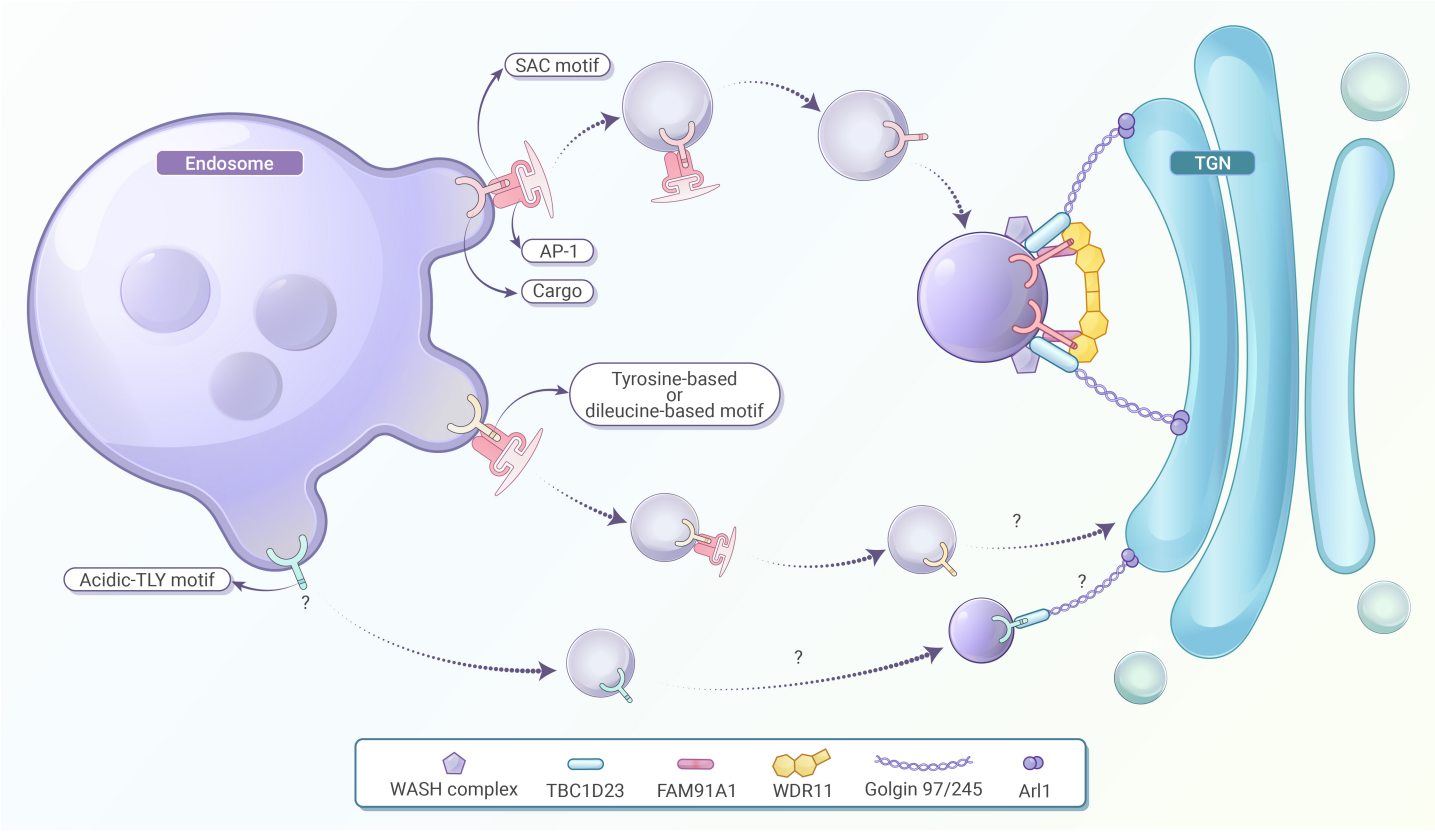

Figure 1. Selective cargo recognition and vesicle tethering at TGN The AP-1 complex on endosomes recognizes and transports cargo proteins containing SAC, tyrosine-based (YxxΦ), and dileucine-based ([D/E]xxxL[L/I]) motifs. Downstream of the AP-1 complex, the WDR11 complex specifically recognizes the SAC motif, facilitating the delivery of SAC-containing cargoes to the TGN. WDR11's recognition of the SAC motif within the cytosolic tail of cargo proteins is enhanced by its dimerization. FAM91A1, through its N-terminal domain, interacts with TBC1D23, which is localized to the TGN by binding to golgin 97/245. TBC1D23 itself recognizes cargoes with acidic-TLY motifs in their cytosolic tails, but critical data on TBC1D23's role in transporting these acidic-TLY cargoes are still lacking. At present, it is unclear if tyrosine-based and dileucine-based motifs are also recognized at the tethering step and if cargoes containing the acidic-TLY motif exit endosomes in a manner dependent on AP-1 or other retrograde transport machinery. Together, the WDR11-FAM91A1 complex and TBC1D23 promote the endosome-to-TGN trafficking of SAC- and acidic-TLY containing cargoes. TGN: *trans*-Golgi network. WASH (Wiskott-Aldrich syndrome protein and SCAR homolog), and Arl1 (ADP-ribosylation factor-like protein 1).

within the cytosolic tails of transmembrane proteins, thus contributing to their selective delivery from endosomes to the *trans*-Golgi network (TGN) (Figure 1). Together, these studies suggest that direct recognition of sequence motifs also occurs at later steps of vesicle trafficking.

The WDR11 complex consists of WDR11 and FAM91A1 subunits in eukaryotes (referred to as the WDR11-FAM91A1 complex hereafter), and a third C17orf75 subunit in vertebrates. The WDR11 complex directly interacts via FAM91A1 with TBC1D23 and mediates endosome-to-TGN trafficking of many cargoes, including the cation-independent mannose 6-phosphate receptor (CI-MPR). Of note, loss-of-function mutations in TBC1D23 or WDR11 give rise to pontocerebellar hypoplasia (PCH) or PCH-like disease.[4]

WDR11 harbors two WD40 domains at its N-terminus and an α-solenoid domain at its C-terminus. Both WD40 domains of WDR11 interact directly with the C-terminal domain of FAM91A1. Additionally, WDR11 dimerizes

through intermolecular binding between its α-solenoid domains, leading to the formation of a stable tetrameric complex, FAM91A1-WDR11-WDR11-FAM91A1. Notably, disruption of either the WDR11-FAM91A1 interaction or WDR11 dimerization impairs the endosome-to-TGN trafficking of multiple cargoes, such as the CI-MPR and KIAA0319L, indicating that the assembly of the WDR11-FAM91A1 complex is crucial for the proper trafficking of these cargoes.

Most importantly, using *in silico* predictions and biochemical and cellular assays, Deng et al. demonstrate that WDR11 directly recognizes the SAC motif in cargo proteins and that this interaction is critical for cargo trafficking[2] (Figure 1). The SAC motif consists of 6-10 acidic residues (E/D/S), flanked on each side by one or two bulky hydrophobic residues ($\Phi[D/E/S]_{6\text{-}10}\Phi\Phi$). Both the acidic residues and the flanking hydrophobic residues are important for the interaction with WDR11. A large set of proteins, in addition to CI-MPR and KIAA0319L, harbor the SAC motif, including ATG9A, a phospholipid scramblase essential for autophagy. Moreover, Deng et al. demonstrate that the SAC motif is crucial for intracellular trafficking of multiple proteins, such as the CI-MPR, ATG9A, KIAA0319L, and VAMP4.[2] Depletion of WDR11 in zebrafish leads to defects that mimic many features of human PCH. These defects can be rescued by wild-type WDR11, but not by a WDR11 mutant defective in binding to the SAC motif, highlighting the physiological importance of the WDR11-SAC interaction.

The clathrin-associated adaptor protein 1 complex (AP-1) recognizes various sorting signals, such as tyrosine-based and dileucine-based motifs[1], in addition to the SAC motif. It could be hypothesized that AP-1 packages proteins containing multiple motifs into vesicles, whereas WDR11 prioritizes the delivery of vesicles containing SAC-motif proteins among different cargoes. It is unclear whether tyrosine-based and dileucine-based motifs could also contribute to selective vesicle tethering through recognition by other proteins analogous to WDR11 (Figure 1).

TBC1D23 encompasses three structural domains: an N-terminal TBC domain, a rhodanese-like domain, and a C-terminal PH domain. The TBC and rhodanese-like domains jointly interact with TGN-localized golgin 97/245, and the PH domain binds to the WASH complex subunit FAM21.[5] Furthermore, a linker region between the rhodanese-like domain and the PH domain directly interacts with FAM91A1.[4] Cattin-Ortolá et al.[3] report that the C-terminal domain of TBC1D23 can bind to an acidic-TLY motif in cargoes. They further determine the structure of a complex between the C-terminal domain of TBC1D23 and an acidic-TLY motif-containing peptide, and suggest that TBC1D23 might be involved in selecting vesicles containing proteins with acidic-TLY motifs (Figure 1). However, it remains to be determined if these interactions are required for cargo transport to the TGN, as demonstrated for WDR11. More importantly, since both the WDR11 complex and TBC1D23 are involved in vesicle tethering and cargo recognition, their potential relationship in the transport of cargoes containing both SAC and acidic-TLY motifs, such as carboxypeptidase D (CPD), remains to be defined. Together, these two studies suggest that tethering factors may select specific vesicles for attachment to the TGN by interacting with certain proteins on the vesicles, providing a fresh insight into the mechanisms of vesicle trafficking and their functions in health and disease.

Like many groundbreaking studies, these two studies raise additional questions. First, vesicle tethers can be found in many subcellular compartments, and are involved in virtually all vesicle trafficking pathways. Do similar mechanisms exist in other vesicle trafficking pathways? Second, the domain organization of WDR11 closely resembles that of β′-COP in the COPI complex, which forms a triskelion-like structure through its N-terminal WD40 domain. Can the WDR11 complex further oligomerize through its C-terminal α-solenoid domains, similar to the COPI complex and other coat proteins? Last but not least, in addition to WDR11 and FAM91A1, the WDR11 complex in vertebrates also includes C17orf75. Although C17orf75 is not essential for the location and stability of WDR11 and FAM91A1, the structure and function of C17orf75 remain to be elucidated.

REFERENCES

1. Bonifacino, J.S. and Glick, B.S. (2004). The mechanisms of vesicle budding and fusion. Cell **116**: 153–166. DOI: 10.1016/s0092-8674(03)01079-1.
2. Deng, H., Jia, G., Li, P., et al. (2024). The WDR11 complex is a receptor for acidic-cluster-containing cargo proteins. Cell **187**: 4272–4288. DOI: 10.1016/j.cell.2024.06.024.
3. Cattin-Ortola, J., Kaufman, J.G.G., Gillingham, A.K., et al. (2024). Cargo selective vesicle tethering: The structural basis for binding of specific cargo proteins by the Golgi tether component TBC1D23. Sci. Adv. **10**: eadl0608. DOI: 10.1126/sciadv.adl0608.
4. Zhao, L., Deng, H., Yang, Q., et al. (2023). FAM91A1-TBC1D23 complex structure reveals human genetic variations susceptible for PCH. Proc. Natl. Acad. Sci. USA **120**: e2309910120. DOI: 10.1073/pnas.2309910120.
5. Shin, J.J.H., Gillingham, A.K., Begum, F., et al. (2017). TBC1D23 is a bridging factor for endosomal vesicle capture by golgins at the trans-Golgi. Nat. Cell Biol. **19**: 1424–1432. DOI: 10.1038/ncb3627.

FUNDING AND ACKNOWLEDGMENTS

Work in the authors' laboratories is supported by the Natural Science Foundation of China (NSFC, #32200559), China Postdoctoral Science Foundation (#2021M702364), "From 0 to 1" Innovation Research Project by Sichuan University (#2023SCUH0022), Sichuan Science and Technology Program (#2024NSFSC1291) to X.Y., and the Intramural Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD. Project ZIA HD001607 to J.S.B.). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

X.Y. and J.S.B. conceived and wrote this paper. All authors read and approved the article.

DECLARATION OF INTERESTS

The authors declare no competing interests.