THE EMBO JOURNAL

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The EMBO Journal (2011) 30, 2988–2989 | © 2011 European Molecular Biology Organization | All Rights Reserved 0261-4189/11
www.embojournal.org

Signalling gets sorted by retromer

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The EMBO Journal (2011) **30,** 2988–2989. doi:10.1038/emboj.2011.232

Wnt proteins are secreted glycoproteins required for intercellular communication and important for development and tissue homoeostasis. Wnts are secreted through association to the sorting receptor Wntless, which promotes transport of Wnt from the trans-Golgi network (TGN) to the plasma membrane. Harterink *et al* (2011) and Silhankova *et al* (2010) have investigated the intracellular recycling of the Wnt-sorting receptor and identified a novel retromer-dependent traffic route from early endosomes to the TGN that is dependent on the sorting nexin SNX3 and is regulated by PI3P levels on early endosomes.

Wnt signalling is an important process in the development of metazoa. The secretion of wnt molecules requires the presence of an escort protein/sorting receptor called Wntless (wl). After delivery of wnt at the plasma membrane, the escort protein is endocytosed and recycled from endosomal structures to the trans-Golgi network (TGN), where wl can be again loaded with wnt and be exported back to the cell surface. Retrograde transport from endosomes to the TGN requires the action of the retromer complex, which consists of members of the sorting nexin family and Vps26, Vps29 and Vps35. It is thought that cargo recognition occurs through the trimer consisting of Vps26–Vps29–Vps35, while membrane deformation would be achieved by the BAR domain containing sorting nexins. Retromer-dependent transport to the trans-Golgi is thought to mainly occur from endosomal compartments. These membranes are enriched in either PI3P (early) or PI3,5P_2 (late), while the trans-Golgi is rich in PI4P. The conversion of phosphoinositide species occurs through phospho-lipid kinases and phosphatases. Proteins that have to be retrieved from endosomes to the TGN must be recognized by the retromer complex, included into phospholipids that may have to change their phosphoinositide identity to be compatible with TGN lipid composition.

Two recent publications (Silhankova *et al*, 2010; Harterink *et al*, 2011) studied the requirement for wnt secretion and wl receptor recycling in *D. melanogaster* and *C. elegans*. The elegant studies revealed that only a part of the retromer complex components, namely SNX-3, had a role in endosome–TGN transport, while the well-established BAR domain containing SNX1/2 and SNX5/6 was dispensable for this process (Harterink *et al*, 2011). These findings open the possibility that from numerous parts of the endosomal system retrieval processes could be initiated and that depending on the cargo to be retrieved, different subcomplexes of retromer would be involved in the retrieval processes. Interestingly, SNX3 has a PX domain with a preferential binding to PI3P, which is essential for SNX3's association with endosomes (Xu *et al*, 2001). In co-IP experiments Harterink demonstrated that SNX3 and SNX5/6 are in mutually exclusive retromer subcomplexes and hence are implicated in carrier formation containing distinct cargo subpopulations. These results are supported by previous findings in yeast: Strochlic *et al* (2008) reported the SNX3-dependent retrieval of iron transporters in a sort of tug-of-war with the ESCRT complex at common endosomal compartments, while

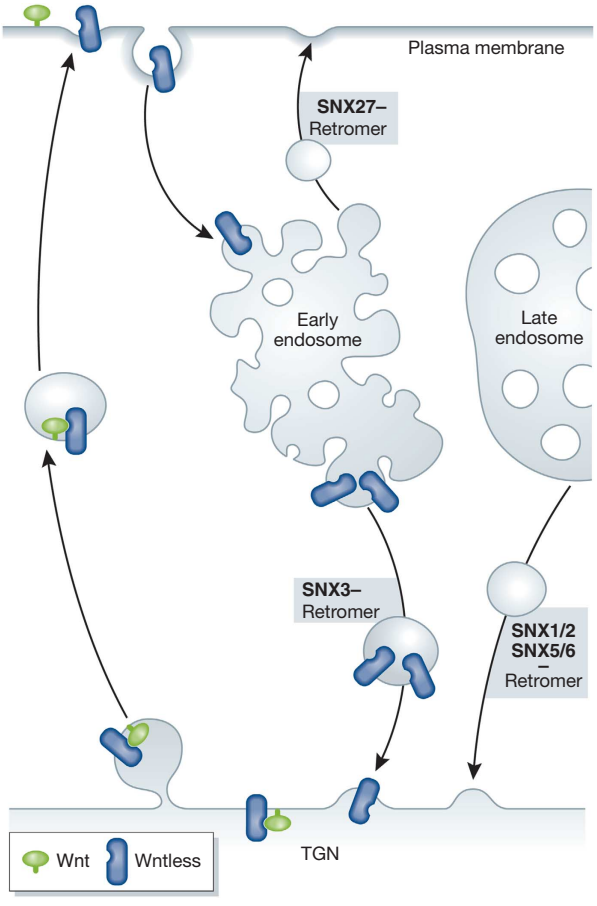

Figure 1 Schematic depiction of different retromer-dependent transport steps. Emphasis on the role of retromer in Wnt and Wntless trafficking is given.

in the initial retromer complex identification Vps10 was retrieved from late endosomes to the TGN (Seaman *et al*, 1998). This retromer complex contained homologues of the mammalian SNX1/2 and SNX5/6. Finally, Temkin *et al* (2011) showed very recently that β2-adrenergic receptor uses SNX27 in conjunction with the retromer cargo recognition subcomplex and Rab4 to recycle from early endosomes to the plasma membrane. Collectively, these data suggest that different retromer complexes drive sorting away form the degradative pathway at different steps along the endocytic route (Figure 1).

How could such differential sorting be achieved? The cargo recognition subcomplex of retromer seems to be always the same. Hence, the SNX subcomplex must at least in part provide the specificity. SNX proteins contain PX domains that bind phosphoinositide, but not all of them bind to all phosphoinositide subspecies with the same affinity. For example, the PX domain of SNX3 has a high affinity for PI3P, and SNX3 is recruited to early endosomes depending on the PI3P levels and induce specific retrieval of wl from early endosomes. Interestingly, Silhankova *et al* (2010) identified two members of the myotubularin family of phosphoinositide phosphatases, MTM-6 and MTM-9 as being required for proper wnt signalling in worms and flies. These two MTMs are supposed to form a heterodimer and act on PI3P. Mutants in *mtm-6* increased the association of SNX3 with early endosomes; yet, efficient recycling of wl was inhibited and wl was degraded in the lysosome.

How could the loss from endosomes and the increased association with endosomes lead both to the degradation of wl? As early endosomes mature to late endosomes, a number of processes take place that need to be coordinated in at least some way: (1) the pH of the endosomes will gradually drop, (2) protein recycling to the plasma membrane and to the TGN is initiated, (3) initiation of the formation of intraluminal vesicles in which most endocytosed receptors end up that cannot enter recycling pathways, (4) in some systems, the endosomes will move during maturation from the cell surface to the cell centre and (5) the phosphoinositide composition of endosomes will change. At the beginning of the maturation process, PI3P levels will increase through the action of the Rab5 effector Vps34 and when the switch of early-to-late endosomes is about to happen, PI3P levels will decrease through the phosphorylation of PI3P to PI3,5P and dephosphorylation of PI3P (Spang, 2009; Poteryaev *et al*, 2010). Thus, in the absence of PI3P phosphatases, this concerted action will be perturbed. The PI3P levels will raise too quickly and perhaps endosome maturation occurs much faster. As a consequence, sorting of proteins at the stage of recycling to the TGN or being included into intraluminal vesicles may not occur and as default wl would enter the degradation pathway. An alternative, but not mutually exclusive, possibility would be that due to the high PI3P levels in *mtm-6* mutants, SNX3 is locked on endosomes and cannot drive carrier formation.

Thus, a picture seems to emerge in which a number of different escape routes exist that can prevent degradation of proteins, and in particular receptors, in lysosomes. The entry into these escape routes could be regulated through feedback mechanisms. The increasing variety of recycling pathways indicates the benefits of different recycling and probably plasma membrane expression kinetics in signal transduction processes.

Conflict of interest

The author declares that she has no conflict of interest.

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