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WILEY Traffic

PERSPECTIVE

Retromer and the cation-independent mannose 6-phosphate receptor—Time for a trial separation?

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The retromer cargo-selective complex (CSC) comprising Vps35, Vps29 and Vps26 mediates the endosome-to-Golgi retrieval of the cation-independent mannose 6-phosphate receptor (CIMPR). Or does it? Recently published data have questioned the validity of this long-established theory. Here, the evidence for and against a role for the retromer CSC in CIMPR endosome-to-Golgi retrieval is examined in the light of the new data that the SNX-BAR dimer is actually responsible for CIMPR retrieval.

KEYWORDS
cargo-selection, CIMPR, endosome-to-Golgi retrieval, Retromer, SNX-BAR, sorting motif

1 | INTRODUCTION

Since it was first characterized nearly 20 years ago there has been a panoply of published reports detailing the functioning of the retromer complex in trafficking a varied array of membrane proteins from endosomes to either the Golgi complex or the cell surface.[1,2] One of the most widely accepted tenets of retromer function is that, in mammalian cells, the retromer complex mediates the endosome-to-Golgi retrieval of the cation-independent mannose 6-phosphate receptor (CIMPR) through the association of the cytoplasmic tail of the CIMPR with the cargo-selective complex (CSC) of retromer comprising the Vps35, Vps29 and Vps26 proteins. Now though, this has been thrown into doubt after studies from Cullen and Steinberg report that the retromer CSC does not associate with the tail of the CIMPR and is not required for the endosome-to-Golgi retrieval of the CIMPR. Rather, the SNX-BAR dimer, comprising SNX1 or SNX2 with either SNX5 or SNX6 associate with the CIMPR to mediate its retrieval.[3,4]

2 | SOME HISTORY

How and why was the retromer CSC believed to mediate the endosome-to-Golgi retrieval of the CIMPR for so long if that theory is wrong? The first reports that the retromer CSC was required to sort the CIMPR for retrieval to the Golgi came from studies independently conducted in the labs of Bonifacino and myself.[5,6] But those studies followed on from the initial work in yeast which favoured a direct association of the retromer CSC with cargo proteins, such as Vps10p.[7,8] As Vps10p in yeast performs the same task as the CIMPR in mammalian cells, it seemed natural that the retromer CSC would associate with the tail of the CIMPR and mediate its endosome-to-Golgi retrieval. Indeed the study from the Bonifacino lab reported a direct interaction between Vps35 and the CIMPR tail. Both studies reported that the CIMPR requires the function of the retromer CSC to be retrieved from endosomes to the Golgi.[5,6] Loss of retromer CSC function through knockdown or genetic knockout results in mislocalization of the CIMPR to endosomes and its subsequent degradation in lysosomes. We also reported that mistrafficking of the CIMPR results in a failure to deliver lysosomal hydrolases such as Cathepsin D to the lysosome—a predictable outcome if the retrieval of the CIMPR is impaired.

3 | IMPORTANT ADVANCES

Thus, early on in the studies of the retromer complex in mammals, it was established that the retromer CSC associates with the tail of the CIMPR to mediate its endosome-to-Golgi retrieval. The motif in the CIMPR tail that is required for retromer-mediated endosome-to-Golgi retrieval was identified through a tried-and-tested approach employing CD8 reporters and mutagenesis of the CD8-CIMPR construct. That study identified a sequence comprising Trp-Leu-Met (WLM) in

the CIMPR tail as necessary for its retrieval.[9] Interestingly, the reports from the Cullen and Steinberg labs reveal that the SNX-BAR dimer associates with the CIMPR tail via the WLM motif.[3,4]

Over time, the functioning of retromer-mediated endosome-to-Golgi retrieval has become better understood. A major advance by the Cullen laboratory was the identification of SNX5 and SNX6 as retromer components and the demonstration that these two SNX-BAR proteins could associate with dynein by binding to dynactin.[10,11] Subsequently, it was recognized that mammalian retromer, unlike the complex in yeast, is not a stable heteropentamer but a much looser association of the retromer CSC with the SNX-BAR dimer.[12] Thus, the retromer CSC requires the Rab7a and Snx3 proteins for its membrane association, whereas the SNX-BAR dimer can associate with endosomes through the phox-homology (PX) domains that bind to phosphotidyl inositol 3-phosphate.[13–16] The mammalian retromer CSC, unlike the yeast CSC, associates with a collection of accessory proteins, for example, the WASP and Scar Homolog (WASH) complex that is recruited to endosomes through an interaction between the Vps35 protein and the Fam21 subunit of the WASH complex.[17–20] The patho-physiological importance of the retromer-WASH complex interaction is underscored by the fact that a Parkinson's disease (PD)-causing mutation in Vps35 impairs the binding of the WASH complex to the retromer CSC resulting in less endosomally localized WASH complex leading to trafficking defects.[21–23]

4 | OUT WITH THE OLD, IN WITH THE NEW?

The recent reports from the Cullen and Steinberg labs have suggested that the role of the retromer CSC in sorting the CIMPR for retrieval to the Golgi is questionable because they could not show any mislocalization of the CIMPR when components of the retromer CSC are silenced by RNAi or deleted by targeted knockout. They cite the studies from my lab and that of Bonifacino[5,6] as the sources for the evidence that the retromer CSC is necessary for the retrieval of the CIMPR but of course there are other published studies that agree with those early papers. There are the data from Bulankina et al that was part of their investigation into the function of the TIP47 protein[24] and the study of Hao et al confirmed the requirement for the retromer CSC to mediate the endosome-to-Golgi retrieval of the CIMPR in their analysis of WASH complex regulating machinery.[25]

Could the differences in effects on CIMPR trafficking boil down to different assays—what is the best way to measure the endosome-to-Golgi retrieval of the CIMPR? There is no definitive answer to that question and different labs have employed an array of techniques that are centred on the use of microscopy to visualize the CIMPR. The assay used by Hao et al[25] measures the dispersal of the CIMPR to peripheral structures and has been used previously in retromer-focussed studies.[26] It was suggested however that the CIMPR dispersal assay employed by Hao et al may be insufficiently robust to properly determine the effect of loss of retromer CSC function on the localization of the CIMPR.[4] Curiously, one of the reports to show that the retromer CSC is required for CIMPR retrieval was when the role of SNX5 and SNX6 in CIMPR retrieval was identified using a CIMPR dispersal assay.[10] On that occasion knockdowns of Vps35, Vps29 or Vps26 all impaired CIMPR retrieval in a manner similar to a SNX5 or SNX6 knockdown. My own lab generally favours the use of automated microscopy as a means of measuring endosome-to-Golgi retrieval to avoid potential bias introduced at the time of imaging. Using such an assay, we have shown that loss of Snx3, Rab7a, Vps26a, Vps35 or SNX1 function all cause a significant endosome-to-Golgi retrieval defect of a CD8-CIMPR reporter protein.[17,27] Another way to measure CIMPR retrieval to the TGN is the application of immunofluorescence with Pearson's correlation—the technique employed in the paper by Kvainickas et al, where they report no role for the retromer CSC in CIMPR retrieval.[4] In a surprising volte-face, the study by Kvainickas et al stands in stark contrast with a previous report where it was shown that the PD-causing Vps35 mutant was equivalent to a Vps35 null with respect to impairing CIMPR retrieval to the TGN using immunofluorescence and Pearson's correlation to measure retrieval.[22]

5 | CONCLUDING REMARKS

The finding that the SNX-BAR dimer can associate with the tail of the CIMPR is undoubtedly a significant advance in the understanding of how the endosome-to-Golgi retrieval of the CIMPR occurs. But does the observation that the SNX-BAR dimer can bind the CIMPR tail negate the role of the retromer CSC? There is sufficient evidence, accumulated by labs operating independently (including studies authored by Cullen and Steinberg[10,22]), that the retromer CSC is required for the retrieval of the CIMPR to argue that it has a major role—possibly by directly associating with the CIMPR tail.[5,6,9,10,14,17,22,24,25,28] What then is the truth? Without further experimentation the answer to that question will have to wait. I suspect however that the "truth" will end up being a version of both models where the tail of the CIMPR can associate with the retromer CSC, possibly to ensure it is concentrated, albeit briefly, in endosomal microdomains that may require the presence of WASH-complex generated F-actin. Following the concentration of the CIMPR in the endosomal membrane, the SNX-BAR proteins may bind and direct the CIMPR into a tubule for retrieval to the Golgi. Proteins such as RME-8 that can associate with both the SNX-BAR dimer and the WASH complex[22,29–31] may coordinate the transfer of cargo such as the CIMPR from the retromer CSC to the SNX-BAR dimer. Both sets of proteins then will have a direct role in the CIMPR retrieval but the relative importance of the retromer CSC and SNX-BAR dimer may vary from cell-to-cell and tissue-to-tissue according to the primary function of the cell(s) in question.

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