Analysis of the Retromer complex-WASH complex interaction illuminates new avenues to explore in Parkinson disease

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The retromer complex mediates endosomal protein sorting by concentrating membrane proteins (cargo) into nascent tubules formed through the action of sorting nexin (SNX) proteins. The WASH complex is recruited to endosomes by binding to the VPS35 subunit of retromer and facilitates cargo protein sorting by promoting formation of endosomally-localized F-actin. The VPS35 protein is mutated in Parkinson disease (PD) and a recent report has revealed that the PD-causing mutation impairs the association of retromer with the WASH complex leading to perturbed endosomal protein sorting. Another important player in endosomal protein sorting is the DNAJC13/RME-8 protein, which associates with SNX1 and has also recently been linked to PD. An additional recent report has now shown that RME-8 also interacts with the WASH complex thus establishing retromer and WASH complex-mediated endosomal protein sorting as a key pathway linked to the pathology of PD and providing new avenues to explore in the search for insights into the disease mechanism.

The retromer complex—a vital component of endosomal sorting machinery

Within eukaryotic cells, endosomes are key sorting stations that receive material (proteins and lipids) from both the biosynthetic and the endocytic pathways. A membrane protein arriving at an endosome can be sorted for delivery to one of three destinations: the cell surface, the Golgi, and the lysosome. The retromer complex is a conserved multimeric protein complex that is required to sort proteins into both the endosome-to-Golgi, and endosome-to-cell surface pathway. The role of cargo selection within retromer is performed by a trimer of the VPS35, VPS26, VPS29 proteins where binding to cargo is known to be mediated by both VPS35 and VPS26.[1] The other key activity of retromer is membrane bending to form tubules into which cargo is sorted. Tubules are formed by the action of the sorting nexin (SNX) dimer component of retromer, which associates loosely with the trimeric cargo-selective complex (CSC). The SNX dimer binds to phosphatidyl inositol 3-phosphate (PtdIns 3P) in the membrane and is able to generate tubules through an intrinsic self-assembly activity and the action of C-terminal Bin-Amphiphysin-Rvs (BAR) domains that adopt a curved banana-shaped conformation to drive tubulation.[2] The SNX1 protein is a key element of retromer-mediated endosomal protein sorting and can generate membrane tubules both in vivo and in vitro.[3]

In addition to mediating sorting of membrane proteins into nascent tubular domains for delivery to the Golgi or the cell surface, the retromer CSC also functions as a hub for recruiting a host of accessory proteins to endosomes.[4] A key retromer accessory is the WASH complex—a pentameric protein complex that mediates production of filamentous (F-) actin patches on endosomes to facilitate the partitioning of membrane proteins into discrete regions of the endosome so that sorting into distinct pathways is accomplished. Loss of WASH complex function results in an accumulation of endosomal tubules indicating
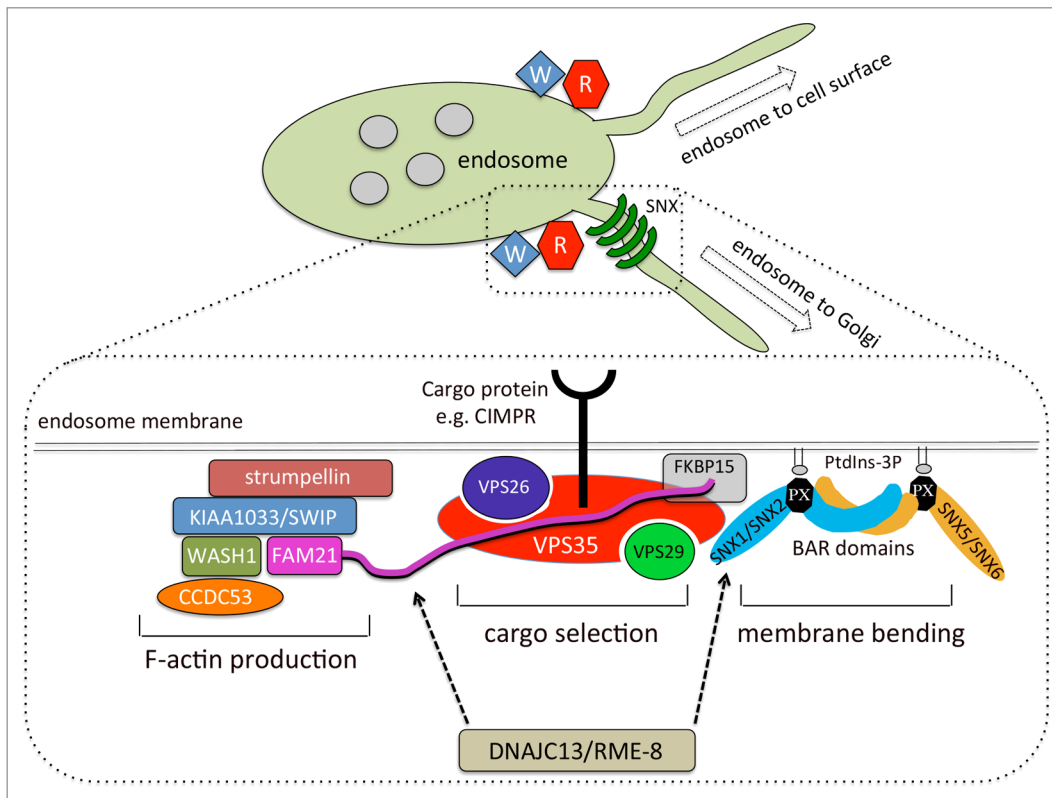
Figure 1. Schematic diagram of the retromer and WASH complexes. The retromer cargo-selective complex (CSC) associates with endosomal membranes to sort cargo proteins (e.g. the cation-independent mannose 6-phosphate receptor—CIMPR) into tubules formed by the sorting nexin (SNX) dimer. The retromer CSC also recruits the WASH complex that mediates F-actin production through VPS35 binding to the extended tail of the FAM21 protein (shown in pink). The FKBP15 protein binds to both VPS35 and FAM21. The RME-8 protein has recently been shown to associate with the WASH complex through binding to FAM21 and regulates the kinetics of SNX dimer association-dissociation with the membrane.

that the WASH complex contributes to the scission of tubules, possibly through an association with Dynamin-2.[5] The recruitment of the WASH complex to endosomes occurs through the interaction of the VPS35 protein with the extended "tail" domain of the FAM21 protein of the WASH complex.[6-8] Along with binding to the WASH complex, VPS35 also interacts with a protein called FKBP15 (also known as FKBP133 and WAFL). Thus a tripartite complex comprising the retromer CSC, the WASH complex, and FKBP15 is formed. The precise role of FKBP15 has yet to be determined but it may function to modulate actin dynamics at the endosome.[4,6,9] The components of the retromer and WASH complexes are shown schematically in **Figure 1**.

RME-8, an accessory of the WASH complex and SNX dimer

The sorting nexin dimer, including the SNX1 protein, also associates with accessory factors; one of which is

a protein called RME-8—a member of the DNAJ family of proteins that are implicated in regulating the assembly and disassembly of macromolecular complexes via an interaction with chaperone proteins.[10] In a recent publication, it has been shown that RME-8 also associates with the WASH complex through binding to the tail of the FAM21 protein.[11] When RME-8 expression was abolished using RNAi, the morphology of endosomes was profoundly altered becoming a highly branched network of membrane tubules that contained many membrane proteins that require retromer and the WASH complex for their proper localization including the iron transporter protein SLC11a2 (also known as DMT1-II) and the α5β1 integrin complex. Proteins that comprise the retromer CSC, i.e., VPS26 and VPS35, also became extensively localized to tubules, as did the SNX1 protein.[11,12] Endosomal tubulation

observed after loss of RME-8 is shown in **Figure 2**.

It was hypothesised that the increase in membrane tubules observed after loss of RME-8 function may be due, at least in part, to reduced kinetics of SNX1 association and dissociation with the membrane. This was investigated using Fluorescence Recovery After Photobleaching (FRAP) experiments that confirmed that the kinetics of SNX1 association with the membrane were altered so that the recovery of GFP-SNX1 fluorescence was markedly slower after loss of RME-8 expression. The precise role of RME-8 in modulating the activity of the WASH complex has yet to be elucidated but it is possible that RME-8 functions to regulate the localization of the WASH complex so that the WASH complex is primarily localized to vesicular endosomes and not tubules as the function of the WASH complex to promote F-actin production is required on

vesicular endosomes to facilitate sorting of membrane proteins. Consistent with this hypothesis, the FAM21 protein and a GFP-tagged WASH1 protein become localized to endosomal tubules following siRNA-mediated silencing of RME-8.[11]

Parkinson disease-causing mutations to VPS35 and RME-8

Interestingly, the *DNAJC13* gene that encodes RME-8 has recently been shown to be mutated in an inherited form of Parkinson disease (PD).[13] It is not currently known whether the PD-causing mutation affects the association of RME-8 with either the WASH complex or SNX1 but this may be a fruitful area to investigate. The identification of DNAJC13/RME-8 as a player in PD cements the importance of endosomal protein sorting in the pathogenesis of PD and follows the identification of *VPS35* as a PD gene.[14-16] For VPS35, the PD-causing mutation is a substitution of Aspartate for Asparagine at residue 620 (D620N). This mutation has now been shown to impair the association of the WASH complex with the retromer CSC resulting in reduced WASH complex recruitment to the endosome.[17] Thus the FAM21 protein of the WASH complex interacts with two proteins—VPS35 and RME-8—both of which are mutated in PD. This suggests that WASH complex function may be especially important in the mechanisms that underlie PD. In fact, WASH complex function is already associated with another neurodegenerative movement disorder, namely hereditary spastic paraplegia (HSP), as the strumpellin component of the WASH complex is encoded by the *KIAA0196* gene at the SPG8 locus—a locus associated with HSP.[18] Currently it is not known why mutations in strumpellin cause HSP whilst mutations to WASH complex-associated proteins result in PD.

Curiously, although the retromer-WASH complex association is impaired by the VPS35 PD mutation, the most pronounced effect of the VPS35 D620N mutation is on the association with FKBP15, a protein whose role in endosomal protein sorting remains poorly understood.[17] One role of the FKBP15 protein may be to facilitate or stabilize
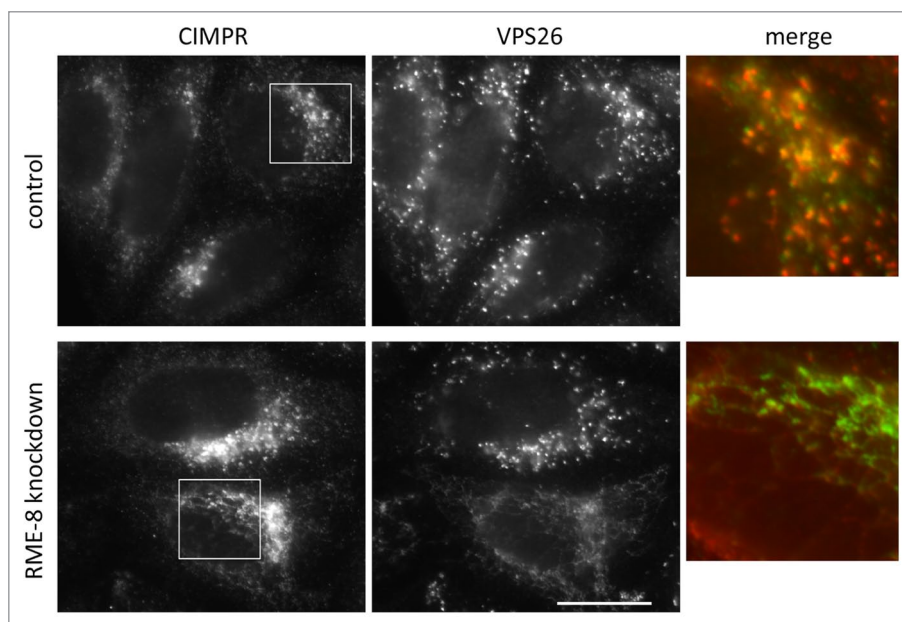

Figure 2. Loss of RME-8 function leads to extensive tubulation of endosomes. The expression of RME-8 was silenced using RNAi. After fixation and labeling with antibodies, cells were imaged using an epifluorescence microscope. The knockdown of RME-8 leads to an accumulation of endosomal tubules that are positive for retromer proteins and cargo such as the CIMPR. Scale bar = 20 μm.

the retromer-WASH complex association as increased FKBP15 expression can enable retromer to co-immunoprecipitate the WASH complex under less favourable conditions. Indeed when FKBP15 is overexpressed, in a subset of highly expressing cells, it forms cytoplasmic aggregates that contain both the WASH complex and RME-8 (see **Fig. 3**) indicating that FKBP15 forms a strong association with the WASH complex and can exert dominant negative effects on WASH-complex associated proteins such as RME-8. The FKBP15 protein is linked to inflammatory bowel disease but it is currently not known what role FKBP15 plays in this disease.[19]

Among the PD-causing genes, mutations in *LRRK2* are a frequent form of inherited PD.[20] It has recently been reported that overexpression of wild-type VPS35 could rescue lysosomal defects caused by LRRK2 mutants but the VPS35 D620N mutant does not rescue suggesting that retromer function is possibly downstream of LRRK2 activity.[21] Interestingly, it has been reported that the loss of LRRK2 function can also cause inflammatory bowel disease although it is mechanistically unclear

how this occurs.[20,22] As both LRRK2 and FKBP15 have been linked to inflammatory bowel disease and both are players in PD (FKBP15 through the VPS35 D620N mutant) it is tempting to speculate that a common mechanism or pathway involving FKBP15 and LRRK2 underlies both pathologies.

Conclusions

The retromer and WASH complexes play key roles in endosomal protein sorting and are emerging as cellular machinery that is especially important in many neurological diseases, including PD. The convergence of genetic studies identifying disease-causing mutations with the functional studies of endosomal protein sorting represents a significant advance in understanding the molecular basis of diseases such as PD, but there is much left to learn about how the various proteins operate in endosomal protein sorting and what consequences arise when these processes are perturbed. The next few years offer the potential for further advances that will illuminate both the pathology of PD and also the basic mechanisms of endosomal protein sorting.
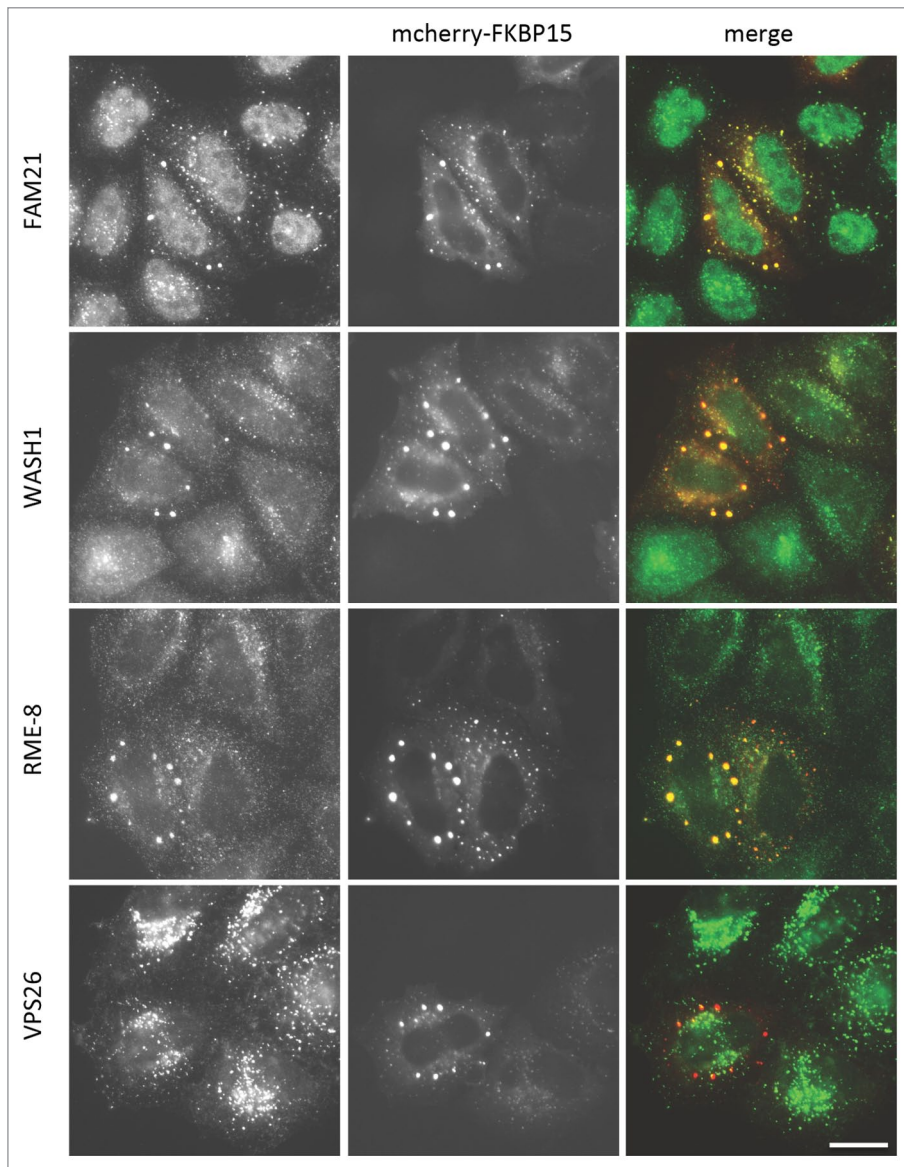
Figure 3. FKBP15 is important for WASH complex localization. Cells were transiently transfected with mcherry-tagged FKBP15. In the highly expressing cells FKBP15 forms bright cytoplasmic aggregates that are also positive for WASH complex proteins and RME-8. The retromer protein VPS26 remains associated with endosomes however. Scale bar = 20 μm.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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