

DEVELOPMENT AT A GLANCE

The retromer complex in development and disease

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ABSTRACT

The retromer complex is a multimeric protein complex involved in recycling proteins from endosomes to the *trans*-Golgi network or plasma membrane. It thus regulates the abundance and subcellular distribution of its cargo within cells. Studies using model organisms show that the retromer complex is involved in specific developmental processes. Moreover, a number of recent studies implicate aberrant retromer function in photoreceptor degeneration, Alzheimer's disease and Parkinson's disease. Here, and in the accompanying poster, we provide an overview of the molecular and cellular mechanisms of retromer-mediated protein trafficking, highlighting key examples of retromer function *in vivo*.

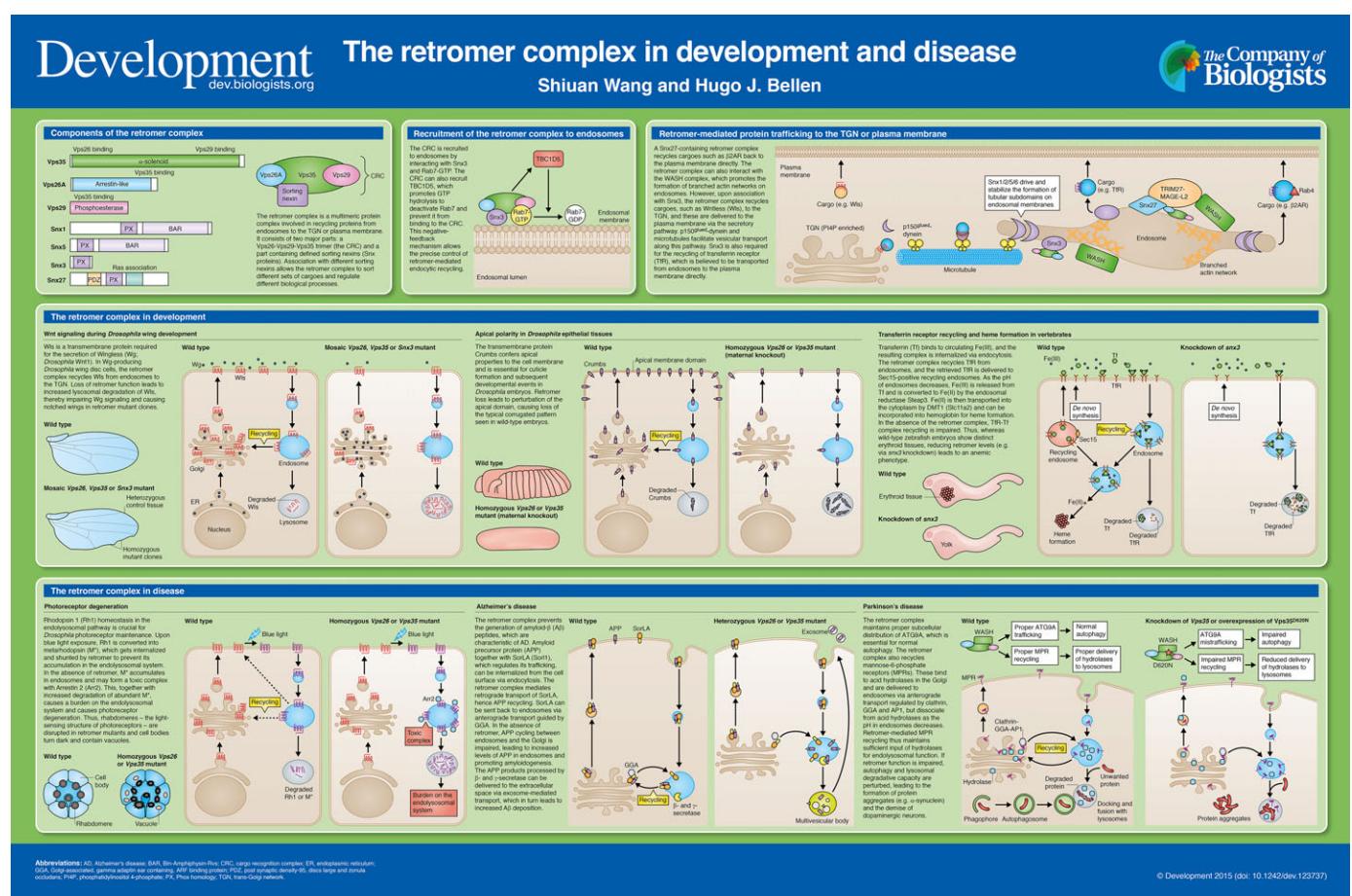
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Introduction

The retromer complex, a conserved protein complex originally identified in yeast, plays a crucial role in recycling endocytosed proteins from endosomes to the *trans*-Golgi network (TGN) or plasma membrane. Recent studies show that the retromer complex regulates the subcellular localization, and prevents the lysosomal degradation, of a select number of proteins, including signaling receptors, ion channels and small molecular transporters (Seaman and Williams, 2002; Steinberg et al., 2013; Temkin et al., 2011). Retromer function has therefore been implicated in various developmental contexts, including wing development in *Drosophila* (Belenkaya et al., 2008; Franch-Marro et al., 2008; Port et al., 2008), epithelial tissue polarity (de Vreede et al., 2014; Pocha et al., 2011; Zhou et al., 2011) and heme formation (Chen et al., 2013). In addition, the retromer complex is essential for the maintenance of neurons (Wang et al., 2014), and impaired retromer function has been implicated in



human neurodegenerative diseases, including Alzheimer's disease (Muhammad et al., 2008; Small et al., 2005; Wen et al., 2011) and Parkinson's disease (Vilariño-Güell et al., 2011; Zimprich et al., 2011). In this article, we summarize how the retromer complex functions and interacts with other molecules to mediate protein endocytic recycling. We further discuss several key *in vivo* functions for the retromer complex.

Components of the retromer complex

The retromer complex contains two major parts: a Vps26-Vps29-Vps35 trimer [termed the cargo recognition complex (CRC)] and a part containing defined sorting nexin (Snx) proteins (Burd and Cullen, 2014). Vps26, Vps29 and Vps35 form a stable tertiary structure both in yeast (Seaman et al., 1998) and mammalian cells (Haft et al., 2000), and loss of any of the components can abolish retromer function (Arighi et al., 2004; Seaman, 2004). Hence, the CRC is regarded as the core of the retromer complex.

Sorting nexins have been identified across species. Currently, there are 33 known sorting nexins in mammals (ten in yeast), of which ten are associated with the retromer complex (Gallon and Cullen, 2015). Vps5 and Vps17, which are the yeast homologs of Snx1/2 and Snx5/6, were initially found to be essential retromer components as they tightly associate with the CRC (Seaman et al., 1998). However, subsequent studies in mammalian cells reported that Snx3 is also involved in retromer function, showing stronger CRC-binding ability than Snx5 (Harterink et al., 2011). This suggests that the interaction between the CRC and sorting nexins varies in different species to regulate different biological processes. More recently, Snx27 was shown to regulate the trafficking of retromer cargoes from endosomes back to the plasma membrane rather than the TGN in mammalian cells (Temkin et al., 2011).

The selective transport of retromer cargoes

In vertebrates, the retromer complex regulates the transport of selective cargoes via different routes (Burd and Cullen, 2014). During this process, various factors are involved in first recruiting the CRC components of the complex to endosomes. For example, in HeLa cells, both SNX3 and RAB7 interact with the CRC directly and are required for the recruitment of the retromer complex to endosomal membranes (Harterink et al., 2011; Rojas et al., 2008; Seaman et al., 2009; Vardarajan et al., 2012). Localization of the retromer complex to endosomes can also be negatively regulated, for example by TBC1D5, a Tre2-Bub2-Cdc16 (TBC) family Rab GTPase-activating protein that decreases Rab7 activity (Seaman et al., 2009).

The association of the CRC with Snx proteins then determines cargo selection and the trafficking route, i.e. to the TGN or the plasma membrane. For example, upon association with Snx3, the retromer complex can sort specific cargoes, such as the seven transmembrane protein Wntless (Wls), which acts as a Wnt receptor, and the cation-independent mannose-6-phosphate receptor (CI-MPR), into the TGN pathway (Arighi et al., 2004; Harterink et al., 2011; Seaman, 2004). During this process, clathrin, clathrin-interacting proteins such as RME-8, and the WASH (Wiskott-Aldrich syndrome protein and SCAR homolog) complex, which promotes the formation of branched actin networks on endosomes, are involved in the formation, scission or stabilization of endosomal tubules (Gomez and Billadeau, 2009; Freeman et al., 2014; Popoff et al., 2009; Shi et al., 2009). Subsequently, cargo-containing vesicles associate with the p150^{glued}-dynein complex and are transported to the TGN along microtubule tracks (Wassmer et al., 2009). When retromer-coated vesicles arrive at the TGN, molecular tethers such as the Golgi-associated retrograde protein (GARP)

complex may be responsible for docking these vesicles (Perez-Victoria et al., 2008). The presence of phosphatidylinositol 4-phosphate (PI4P), a Golgi-enriched phosphoinositide, then facilitates the release of cargoes from transport vesicles to the TGN (Niu et al., 2013).

The retromer complex can also mediate protein transport from endosomes directly back to the plasma membrane. A crucial component involved in this pathway is Snx27, which binds to defined cargoes such as β 2-adrenergic receptor (β 2AR) (Temkin et al., 2011). Snx27 can also directly interact with the WASH complex to regulate Snx1- or Snx5-dependent protein trafficking (Temkin et al., 2011). The activity of the WASH complex can be regulated by the E3 RING ubiquitin ligase TRIM27 and its enhancer melanoma antigen-L2 (MAGE-L2) (Hao et al., 2013). Eventually, retromer-coated vesicles are delivered to the plasma membrane in a Rab4-dependent manner (Temkin et al., 2011). It should be noted that Snx3 can also regulate protein transport from endosomes to the plasma membrane. A recent report documented that Snx3 and Vps35 recycle the transferrin receptor (TfR) from endosomes to plasma membrane directly (Chen et al., 2013). Since Snx3 is crucial for the recruitment of the CRC, it is possible that Snx3 sorts numerous cargoes at endosomes and promotes the trafficking of different sets of cargoes to different destinations.

Functions of the retromer complex in development and neuronal maintenance

Although more than 150 retromer cargoes have been identified *in vitro* (Burd and Cullen, 2014), current studies suggest that the retromer complex regulates very specific cargoes and hence developmental processes *in vivo*. Below, we outline several examples of how the retromer complex is involved in tissue development, maintenance and function.

Retromer-mediated control of Wnt signaling during *Drosophila* wing development

The main ligand in the Wnt signaling pathway in *Drosophila* is Wingless (Wg), which is secreted from signal-sending cells and binds to Frizzled (Fz) receptors in signal-receiving cells (Port and Basler, 2010). The transmembrane protein Wls is required for Wg secretion; it binds to Wg in the Golgi and escorts Wg to the cell surface via vesicular transport (Bänziger et al., 2006; Bartscherer et al., 2006). Accordingly, the loss of Wls in wing imaginal discs leads to the intracellular accumulation of Wg in the signal-sending cell and is accompanied by the loss of Wg signaling (Bänziger et al., 2006; Bartscherer et al., 2006). The link between Wg signaling and the retromer complex became obvious when loss of retromer function was found to lead to Wg signaling defects (Coudreuse et al., 2006). Similar to loss of *wls*, loss of *Vps26* or *Vps35* leads to impaired Wg secretion from signal-sending cells and to reduced Wls abundance, indicating that the retromer complex is involved in the recycling of Wls (Belenkaya et al., 2008; Franch-Marro et al., 2008; Port et al., 2008; Yang et al., 2008).

Despite this role of the retromer complex in Wg signaling during wing development, very few other Wg signaling-dependent developmental events require retromer function. For example, unlike the loss of *wls* and *wg*, the loss of *Vps26* or *Vps35* does not cause oversized eyes (Bartscherer et al., 2006; Wang et al., 2014), suggesting that Wg signaling during eye development is not dependent on *Vps35* or *Vps26*. In addition, the retromer complex seems to be dispensable for the transduction or fine-tuning of other signaling pathways that are known to be regulated by vesicular trafficking. Indeed, no phenotypes consistent with defects in the

Hedgehog, Notch or BMP pathways during wing development have been observed following the knockdown of retromer components (Belenkaya et al., 2008; Franch-Marro et al., 2008; Port et al., 2008; Wang et al., 2014), suggesting that retromer-dependent trafficking in general does not play a crucial regulatory role in all developmental signaling pathways.

In summary, the current data in *Drosophila* only support a strong requirement for the retromer complex in Wg/Wnt signaling during wing development; signaling events in other tissues seem to occur normally in the absence of retromer function *in vivo*.

The retromer complex and apical polarity

The transmembrane protein Crumbs is essential for determining cell polarity. It confers apical identity to epithelial cells and delineates the boundary between the apical domain of the plasma membrane and adherens junctions (Tepass, 2012). Loss of *crumbs* leads to severe defects in embryogenesis and oogenesis in *Drosophila* (Tepass, 2012). Recent studies have linked the retromer complex to correct Crumbs function. Loss of *Vps35* disrupts the layering of the epithelial cells of the follicle epithelium during both oogenesis and embryogenesis (Pocha et al., 2011; Zhou et al., 2011). In the latter case, this is accompanied by the loss of apical markers such as Crumbs and Patj. However, overexpression of Crumbs rescues both the cuticle loss and polarity defects in *Vps35* mutant embryos (Zhou et al., 2011), suggesting that the retromer complex is required to recycle Crumbs and prevent its degradation in lysosomes. More recently, it was shown that knockdown of Scribble, and its associated proteins that regulate the basolateral identity of epithelial cells, impairs retromer-dependent sorting of Crumbs in wing imaginal discs (de Vree et al., 2014). Hence, the retromer complex may coordinate various polarity cues and sort proper cargoes to establish correct domains on the plasma membrane.

Retromer-dependent control of heme formation

Iron is an essential co-factor for numerous cellular and physiological processes, including the transport of oxygen by heme within erythrocytes. Iron homeostasis is crucial for heme production in erythrocytes, and impaired homeostasis leads to diseases such as anemia (De Domenico et al., 2008). Plasma iron levels are regulated by plasma transferrins (Tfs), which bind to iron and subsequently to cell-surface TfRs (De Domenico et al., 2008). The iron-Tf-TfR complexes are then endocytosed through clathrin-coated pits and iron is released from the complex in the acidic environment of endosomes (De Domenico et al., 2008).

A recent study has implicated an *Snx3*-containing retromer complex in this process. During erythroid maturation in vertebrates, *Snx3* is highly expressed, and decreasing its expression strongly reduces the total hemoglobin content in mouse fetal liver cells, suggesting that the abundance of *Snx3* is essential for heme production (Chen et al., 2013). Furthermore, TfRs physically bind to *Snx3* and *Vps35*, and loss of *Snx3* causes a reduction in TfRs (Chen et al., 2013), suggesting that an *Snx3*-containing retromer complex is required for the recycling of TfRs.

The retromer complex and the maintenance of photoreceptors

Rhodopsin is the light sensor in photoreceptors, and studies in *Drosophila* have shown that prolonged light exposure induces the internalization of rhodopsin into photoreceptor cells via endocytosis (Wang and Montell, 2007). Much of the endocytosed rhodopsin is degraded in lysosomes. However, recent studies indicate that a significant portion of internalized rhodopsin can be recycled via the retromer complex (Wang et al., 2014). In the absence of *Vps26* or

Vps35, rhodopsin aberrantly accumulates in endosomes and overwhelms the endolysosomal system, and it also forms a toxic complex with Arrestin 2 (Alloway et al., 2000), giving rise to the severe photoreceptor degeneration observed in retromer mutants upon light exposure (Wang et al., 2014).

Interestingly, increasing the function of the retromer complex can restore pathologies caused by impaired rhodopsin homeostasis in the endolysosomal system in several contexts. For example, dysfunction of the adapter protein AP- μ 3, which regulates the delivery of proteins to endolysosomal compartments (Dell'Angelica, 2009), or of phospholipase C (PLC), which regulates the activity of endocytosed rhodopsin (Wang and Montell, 2007), leads to the persistent presence of rhodopsin in endosomes, which in turn induces photoreceptor degeneration (Chinchore et al., 2009). Overexpressing *Vps26* or *Vps35*, however, alleviates photoreceptor degeneration in AP- μ 3 or PLC mutants (Wang et al., 2014). Hence, the retromer complex is able to actively maintain the function and integrity of photoreceptors.

The retromer complex and human disease

As highlighted above, the retromer complex plays a role in a number of physiological processes, and perturbations to retromer function can thus lead to pathologies, such as photoreceptor degeneration. Below we highlight further examples, notably those in neurons, of how retromer dysfunction can lead to disease.

Alzheimer's disease

Retromer dysfunction has recently been implicated in Alzheimer's disease (AD), which is characterized by excessive production of the amyloid β (A β) peptide (Alzheimer et al., 1995). In neurons, A β peptides are produced via the sequential proteolytic cleavage of amyloid precursor proteins (APPs) by β -secretase and γ -secretase (Small and Gandy, 2006). These proteases localize to endosomes; hence, the vesicular trafficking machinery may regulate A β production (Small and Gandy, 2006).

Consistent with a possible role of the retromer complex in AD, the levels of VPS35 and VPS26 were shown to be reduced in the brain of AD patients (Small et al., 2005). Subsequent studies showed that partial loss of *Vps26* or *Vps35* leads to increased cleavage of APP by β -secretase in mouse brains (Muhammad et al., 2008; Wen et al., 2011) due to the aberrant recycling of SorLA (SorL1), a protein essential for the endosome-Golgi trafficking of APP (Small and Gandy, 2006). These retromer-deficient mice also exhibit loss of neurons, defects in synaptic transmission and plasticity, and eventually show impaired cognition and memory (Muhammad et al., 2008; Wen et al., 2011).

Parkinson's disease

Abnormal retromer function has also been implicated in Parkinson's disease (PD), which is primarily a movement disorder that results from loss of dopamine-producing neurons in the substantia nigra of the brain. For example, an aspartate-to-asparagine (D620N)-causing mutation in *VPS35* has been identified as the cause of dominant late-onset PD in humans (Vilarino-Güell et al., 2011; Zimprich et al., 2011). Subsequent studies focused on the link between the retromer complex and PD and have been pursued in different model organisms. Decreased *Vps35* levels in *Drosophila* dopaminergic neurons were shown to lead to locomotor defects and reduced life span (Linhart et al., 2014; Macleod et al., 2013). In addition, ectopic expression of *Vps35*^{D620N} in the substantia nigra of rat brains was shown to cause a loss of dopaminergic neurons (Tsika et al., 2014). Further studies showed that the D620N mutation impairs the interaction between the retromer complex and the WASH complex,

which in turn perturbs normal trafficking of the autophagy protein ATG9A and autophagy function (McGough et al., 2014; Zavodszky et al., 2014). Hence, the data point to a direct role of the retromer complex in PD pathogenesis and suggest that the D620N alteration in *VPS35* acts as a dominant-negative mutation when overexpressed.

The retromer complex has also been shown to interact with other PD genes. Using primary rat neurons, a recent report documented that a glycine-to-serine (G2019S)-causing mutation in *LRRK2* (leucine-rich repeat kinase 2), a gene that has been implicated in dominant late-onset PD, leads to sorting defects in the endolysosomal system (Macleod et al., 2013). Interestingly, overexpression of *Vps35* restores the loss of dopaminergic neurons observed in fly brains that express the human *LRRK2*^{G2019S} mutant protein (Macleod et al., 2013) and suppresses their locomotor defects (Linhart et al., 2014), suggesting that elevated retromer activity can prevent neurodegeneration caused by *LRRK2* mutations.

Elevated levels of α -synuclein are a hallmark of PD in most sporadic late-onset cases (Bendor et al., 2013), and a link between α -synuclein and the retromer complex has recently been established (Miura et al., 2014; Zavodszky et al., 2014). Using HeLa cells, it was reported that cells expressing *Vps35*^{D620N} mutant protein exhibited autophagy defects and increased levels of autophagy (Zavodszky et al., 2014). Consistently, an independent study showed that reducing *Vps35* abundance affects the recycling of CI-MPR and impairs the activity of the lysosomal enzyme cathepsin D, which in turn causes aberrant α -synuclein accumulation in the endolysosomal compartments and enhances α -synuclein-induced locomotor defects in flies (Miura et al., 2014). These data indicate that the retromer complex is required to maintain endolysosomal function and prevent α -synuclein accumulation.

In summary, the retromer complex is required for key processes involved in protein degradation and interacts with PD genes that are implicated in endolysosomal trafficking or protein aggregation. Hence, the retromer complex appears to play a central role in regulating protein abundance and in preventing the accumulation of toxic or unwanted proteins that cause the demise of neurons.

Conclusions and perspectives

Work in recent years has revealed important functions for the retromer complex. Studies in yeast and cultured cells have uncovered dynamic interactions between core retromer components and various accessory proteins that allow the retromer complex to select different sets of cargoes. These cargoes are then delivered to their correct target compartments. Given its pivotal role in vesicular trafficking, the retromer complex has also been studied in various *in vivo* processes. Interestingly, many of the defects induced by deficient retromer function appear to be restricted to specific tissues during development, despite more than 100 retromer cargoes being identified *in vitro*. Hence, the dynamics of retromer-mediated protein trafficking *in vivo* might be much more specific than was anticipated. Systematic profiling of the interactions among retromer components, associated proteins and cargoes in various tissues using model organisms will be crucial to precisely elucidate retromer function in different developmental contexts.

Although the retromer complex regulates very specific signaling pathways in defined developmental processes, such as fly wing development, it seems to play a pervasive role in neurodegeneration. One possibility is that the retromer complex plays a major buffering role and helps maintain homeostasis. A reduction in this buffering capacity would thus partially perturb the homeostasis of numerous proteins. Although retromer mutant neurons may be able to cope

with impaired protein homeostasis for a short time, they might not be able to tolerate the stress (e.g. of having to continuously synthesize more cargoes and bear the increased load of proteins in the endolysosomal system) over a long period, and this might account for the premature aging observed in retromer mutant neurons. Further studies that systematically compare the abundance and distribution of proteins in neurons isolated from control and retromer mutant animals at different ages would be crucial to address this issue and to further understand the role of the retromer complex *in vivo*.

Competing interests

The authors declare no competing or financial interests.

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