From endosomes to the *trans*-Golgi network

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Key words

Endosome, *trans*-Golgi network, retrograde membrane trafficking, retromer

Abbreviations

AD, Alzheimer disease; AP1, adaptor protein 1; APP, amyloid precursor protein; BAR, Bin/Amphiphysin/Rvs; CD-M6PR, cation-dependent mannose-6-phosphate receptor; CI-M6PR, cation-independent mannose-6-phosphate receptor; COG, conserved oligomeric Golgi complex; GRIP, Golgin97-RanBP-Imh1p-p230 *trans* Golgi protein; CTxB, Cholera toxin B fragment; EE, early endosome; EHD1, eps15 homology domain 1; EpsinR, Epsin related protein; ER, endoplasmic reticulum; FKBP, FK506 binding protein; FRB, FKBP rapamycin binding protein; GARP/VFT, Golgi associated retrograde protein complex/Vps fifty three; GGA, Golgi localized, γ-ear containing, ARF interacting protein; LDL, low-density lipoprotein; LE, late endosome; MTOC, microtubule organizing center; NSF, *N*-ethylmaleimide-sensitive factor; OCRL1, oculocerebrorenal syndrome of Lowe protein 1; PACS1, phosphofurin acidic cluster sorting protein 1; PE, Pseudomonas exotoxin; PI(3)P, phosphotidylinositol 3-phosphate; PI(3,5)P_2, phosphotidylinositol 3,5-bisphosphate; PI(4)P, phosphotidylinositol 4-phosphate; PI(4,5)P_2, phosphotidylinositol 4,5-bisphosphate; PM, plasma membrane; RE, recycling endosome; SNAP, soluble NSF attachment protein; SNARE, soluble NSF attachment protein receptor; SNX, sorting nexin; STxB, Shiga toxin B fragment; Syn, Syntaxin; TGN, *trans*-Golgi network; WASH, Wiskott-Aldrich syndrome protein and SCAR homolog.

Abstract

The retrograde trafficking from endosomes to the *trans*-Golgi network (TGN) is one of the major endocytic pathways to divert proteins and lipids away from lysosomal degradation. Retrograde transported cargos enter the TGN via two itineraries from either the early endosome/recycling endosome or the late endosome and involve various machinery components such as retromer, sorting nexins, clathrin, small GTPases, tethering factors and SNAREs. Recently, the pathway has been recognized for its role in signal transduction, physiology and pathogenesis of human diseases.

Contents

In recent years, the endosome-to-TGN trafficking has been recognized as one of the major retrograde pathways and received significant attention. This review focuses on our general understanding of this retrograde trafficking pathway in mammalian cells. Additional details and discussions can be found in several excellent reviews published recently [1-4].

1 Introduction of the endosome-to-TGN trafficking

1.1 The pathway to recycle the secretory machinery

In the secretory or biosynthetic pathway, cargos targeted to the endoplasmic reticulum (ER) sequentially pass through Golgi cisternae to the *trans*-Golgi network (TGN). The TGN is a hub of the membrane trafficking network of a mammalian cell. By default, secretory cargos are constitutively delivered to the plasma membrane (PM) for exocytosis. Alternatively, cargos with sorting signals could diverge from this default route to reach endosomes via the TGN-to-endosome pathway. The reverse pathway, the endosome-to-TGN trafficking, serves as an essential pathway to recycle the secretory machinery components, such as cargo adaptors or receptors. For example, lysosomal hydrolases are luminal soluble enzymes that are synthesized in the ER. Newly synthesized hydrolases acquire mannose-6-phosphate (M6P) modification once they are delivered to the Golgi apparatus. M6P acts as a unique sorting signal that is recognized by the M6P receptor (M6PR) at the TGN. When lysosomal hydrolases reach the TGN, the association between M6PR and M6P results in lysosomal hydrolases being packed into transport carriers destined for lysosomes via endosomes (the TGN-to-endosome pathway). Once delivered to endosomes, the acidic environment of endosomal lumen releases hydrolases from the M6PR. As endosomes mature to become lysosomes, hydrolases accumulate and become activated in lysosomes. Complimentary to this TGN-to-endosome pathway, the endosome-to-TGN trafficking retrieves M6PR back to the TGN for further rounds of loading of hydrolases [5](Fig. 1). Failure in the retrieval of the M6PR results in the secretion of lysosomal hydrolases into the extracellular space instead of the lumen of lysosomes, leading to aberrant lysosomal functions.

1.2 The pathway to diverge from lysosomal degradation

In the endocytic pathway, cargos on the PM are internalized to the early endosome (EE) or the sorting endosome, which, by default, gradually matures to become the late endosome (LE). The LE eventually becomes a lysosome by fusing to existing lysosomes, resulting in the degradation of cargos in the lysosome lumen. Cargos can be selectively salvaged from the degradation fate by two major recycling pathways: 1) the endosome-to-PM pathway, including the one via the recycling endosome (RE), and 2) the endosome-to-TGN pathway, including those from the EE, the RE and the LE (Fig. 1). Because of the central position of the TGN, cargos recycled via the endosome-to-TGN pathway could further access a variety of organelles of secretory and endocytic pathways. The endosome-to-TGN pathway can be hijacked by exogenous pathogens for targeting to their destined organelles. For example, Shiga toxin, Cholera toxin and Ricin evade lysosomal degradation after endocytosis by the endosome-to-TGN pathway before reaching the ER for its cytotoxic effects (see section 3.2) [6]. Inhibition of retrograde trafficking pathways provides an effective therapeutic strategy to combat the infection of these toxins. For example, the treatment with the inhibitor of the retrograde trafficking, manganese [7], which is

specific to Shiga toxin, or the small molecular compound Retro-1 or 2 [8], protects cultured cells or animals from toxic effects of these toxins.

2 Two endocytic pathways leading to the TGN

The EE/RE-to-TGN and the LE-to-TGN pathways have been proposed as two major endocytic itineraries leading to the TGN. They were originally defined and represented by Tac-TGN38 and Tac-furin [9, 10] (Fig. 1), which are the fusion of two type I transmembrane proteins, the extracellular domain of interleukin 2 receptor α subunit (Tac) and the cytosolic domain of rat TGN38 or furin. Shortly after endocytosis, both Tac-TGN38 and Tac–furin first reach the EE. However, their subsequent itineraries differ. A significant amount of Tac-TGN38 enters the RE or the endocytic recycling compartment. The RE comprises of a cluster of membrane tubules localized around the microtubule organizing center (MTOC) and the Golgi complex. In contrast, the EE is mainly peripherally distributed. From the RE, a fraction of Tac-TGN38 recycles back to the PM while the rest enters the TGN. Although the significance of the RE in the endosome-to-TGN trafficking of TGN38 is challenged by recent findings [11], it is agreed upon that TGN38 reaches the TGN without passing through the LE. Beside TGN38 (or TGN46 in human), the cation-independent (CI)-M6PR [12, 13] and Shiga toxin B fragment (STxB) [11, 13, 14] have also been well documented to utilize similar trafficking itineraries and often serve as markers for the EE/RE-TGN pathway. The endocytic itinerary of Tac-furin is different from Tac-TGN38. Tac-furin remains in the same compartment when the EE matures to become the LE, from which it enters the TGN via the LE-to-TGN pathway, bypassing the RE [10, 15].

Endocytic trafficking involves dynamic and continuous maturation of endosomal compartments. The EE, the RE and the LE represent endosomal compartments of different maturation stages and thus clearly defined boundaries among them do not exist. Hence, it is possible that the retrograde trafficking of a cargo to the TGN takes place continuously during the EE to LE maturation, with some cargos predominantly sorted at the EE while others at the LE. Supporting this view, it has been documented that a certain amount of furin and CI-M6PR could also be sorted to the TGN from the EE and the LE, respectively [15-17] (also see section 5.1.4).

3 Cargos utilizing the endosome-to-TGN trafficking

Although the TGN-to-PM trafficking is rapid and generally considered to be by default, most PM membrane cargos do not take retrograde routes back to the TGN as revealed by monitoring the re-sialylation (a TGN specific enzymatic reaction) of de-sialylated surface membrane proteins [18]. This finding indicates that the endosome-to-TGN pathways could be restricted to privileged cargos. On the other hand, almost all yeast membrane proteins residing on the late Golgi (equivalent to the TGN in mammalian cells) cycle between endosomes and the TGN [19-21], suggesting there could be a vast range of cargos utilizing the endosome-to-TGN trafficking pathway in mammals. The repertoire of mammalian cargos transiting this pathway is expanding rapidly. A list of cargos discussed in this review, which is by no means exhaustive, is included in the Table 1. Both proteins, including membrane and soluble proteins, and lipids could undergo the endosome-to-TGN trafficking.

3.1 Membrane proteins

Membrane proteins transiting this pathway include soluble NSF attachment protein receptors (SNAREs) such as GS15 [22] and Vamp4 [23], transporters such as GLUT4 [24], enzymes such as endoprotease furin (see section 2) and carboxypeptidase D [25], Vps10 domain family cargo receptors such as the cation-dependent (CD)-or CI-M6PR (see section 1.1), sortilin [26] and SorLA/SorL1 [27], GPI-anchored proteins such as CD59 and GPI-GFP [28] and others with undefined cellular functions such as TGN38/46 (see section 2), GPP130 [29] and GPP73 [29].

3.2 Soluble proteins

Known soluble proteins that utilize the endosome-to-TGN pathway are exogenous bacterial and plant toxins, such as Shiga toxin, Cholera toxin, Pseudomonas exotoxin (PE) and Ricin [6], which take advantage of this retrograde pathway to avoid lysosomal degradation. These toxins comprise of two types of fragments—A and B. Shiga toxin and Cholera toxin are AB_5 type toxins, consisting of one A fragment and a homopentameric B fragment. PE and Ricin are AB type toxins with one A and B fragment. The non-toxic B fragment binds to the cell surface receptor and transports the toxic A fragment via the endosome-to-TGN pathway to the TGN. From the TGN and via the B fragment, the toxin can be further transported to the ER lumen (another retrograde pathway), where the A fragment retrotranslocates to the cytosol to execute its cytotoxic effect.

The cell surface receptors for STxB and Cholera toxin B fragment (CTxB) have been identified as glycosphingolipid globoside/Gb3 [30] and GM1 [31], respectively, which presumably reside in lipid microdomains and direct the endocytosis and intracellular trafficking itineraries of B fragments [28, 32]. While both STxB and CTxB take the EE-to-TGN route [6, 33], their trafficking mechanisms are different. At the EE, STxB, but not CTxB, associates with another receptor, transmembrane protein GPP130, which targets STxB to the Golgi complex [7]. Furthermore, the endosome-to-TGN trafficking of STxB, but not CTxB, is inhibited by manganese ion which induces the degradation of GPP130. Under such treatment, STxB is eventually degraded in the lysosome. The B fragment of Ricin binds to cell surface glycoproteins or glycolipids containing galactose [6]. The receptor for PE has been reported to be low-density lipoprotein receptor related protein [34]. While Ricin adopts the EE-to-TGN route [35, 36], PE could enter the TGN from either the EE or the LE [37].

Two small molecular compounds, Retro-1 and 2, have been shown to specifically inhibit the endosome-to-TGN trafficking of exogenous toxins, including Shiga toxin, Cholera toxin and Ricin, but not endogenous cargos such as CI-M6PR and TGN46 [8]. Although it is unclear how the two compounds work, the finding highlights mechanistic differences between these soluble toxins and membrane cargos in retrograde trafficking.

3.3 Lipids

The trafficking of STxB and CTxB indirectly demonstrates that endogenous glycosphingolipids such as globoside and GM1, when highly clustered by the homopentameric B fragment, are able to traffic in the retrograde direction to the TGN. Furthermore, in normal cells, fluorescence conjugated sphingolipids, such as lactosylceramide, glucosylceramide, sphingomyelin and globoside (STxB receptor), were also found to target to the Golgi complex via the endosome-to-

TGN trafficking [38-41]. Using fluorescence conjugation, glucosylceramide and sphingomyelin have been shown to follow the EE/RE-to-TGN in neuronal cells [39], while lactosylceramide have been implicated in the LE-to-TGN pathway [38]. The retrograde trafficking of these non-toxin clustered sphingolipids suggested that certain endogenous lipids could constitutively cycle between endosomes and the TGN. The Golgi trafficking of certain sphingolipids could be blocked by a high cellular cholesterol level. In sphingolipid storage disease cells, the high intracellular cholesterol accumulated in endosomes perturbs the endosome-to-TGN trafficking of lactosylceramide and GM1 (as probed by CTxB) [38, 42]. Such a trafficking block could be released by cholesterol depletion or the overexpression of Rab7 or 9 [38], two Rab GTPases involved in the endosome-to-TGN pathway (see section 5.1.1 and 5.1.4). Although it is still unclear how the overexpression of Rab7 or 9 works here, it would be interesting to test the possible role of the retrograde trafficking in the pathogenesis of sphingolipid storage diseases.

4 Methodology

Two types of methods have been used to assay the endosome-to-TGN trafficking in mammalian cells. The biochemical type of assays take advantage of unique enzymatic reactions of the TGN, such as sialylation of glycans and tyrosine sulfation, catalyzed by TGN localized enzymes — sialyltransferase [43] and tyrosyl protein sulfotransferase [22, 44, 45], respectively. In tyrosine sulfation based assay, an artificial tyrosine sulfation site is engineered within the luminal region of cargos, such as M6PR, STxB or Ricin, and the retrograde transport is measured as the incorporation of S^{35}-sulphate into these proteins by autoradiography [22, 44, 45].

The imaging based assays, which are widely used, determine the endosomal and the TGN distributions of reporters via high resolution microscopy. The commonly used reporters are type I transmembrane fusion proteins consisting of luminal and transmembrane domain of CD8A or Tac and the cytosolic domain of M6PR, furin or TGN38 [9, 10, 46]. The reporters could also be recombinant bacterial toxins, such as STxB and CTxB [14, 47, 48]. The endocytic trafficking of these reporters could be followed under conditions in which a potential trafficking component is knocked-down or over-expressed. The endosome-to-TGN trafficking is qualitatively or quantitatively measured by the amount of reporter colocalizing with the TGN markers. A caveat is that the very close localization of the reporter to a TGN marker could be an unreliable evidence for trafficking to the TGN lumen due to limited resolution of light microscopy, which is ~250 nm. Hence, it could be hard to unambiguously resolve if a cargo is at the RE or the TGN since both are tightly adjacent to each other at the peri-nuclear or MTOC region of a mammalian cell. On the other hand, a distinct but adjacent localization from a TGN marker does not clearly indicate that the reporter is outside the TGN lumen due to the presence of morphologically different domains of the TGN [49]. Therefore, the field currently needs an image based assay utilizing a TGN specific enzyme reaction to unambiguously detect the arrival of cargos in the TGN lumen.

Since anterograde and retrograde trafficking are tightly coupled, the disruption of trafficking in one direction would eventually compromise the trafficking in the other direction. Therefore, sometimes it is difficult to pinpoint the involvement of a protein in anterograde or retrograde pathway by slow disruption approaches such as siRNA mediated knockdown or mutant over-

expression. Acute disruption approaches, such as administration of small molecule inhibitors or microinjection of the neutralizing antibody, would provide a more direct effect on the endosome-to-TGN trafficking. The “knocksideways” method could provide a general acute disruption approach [50]. In this technique, the endogenous protein of interest is knocked-down. Cells are subsequently introduced with the FKBP (FK506 binding protein)-tagged protein of interest (siRNA resistant) and FRB (FKBP rapamycin binding protein)-tagged mitochondrial targeting signal. Upon the addition of small molecule rapamycin, the FKBP-tagged protein of interest rapidly mislocalizes to mitochondria and thus is effectively depleted at its functional sites by the formation of very tight FKBP/FRB/rapamycin complex. The “knocksideways” method has provided a key evidence that supports the direct role of AP1 in the endosome-to-TGN trafficking [50, 51].

5 Molecular mechanism

Many endosomal or Golgi proteins have been implicated in the endosome-to-TGN trafficking. However, the detailed cellular studies of many of them are still lacking. Therefore, it is still hard to combine all proteins as a picture within the frame of our knowledge. This review attempts to integrate those that we know the best.

In the endosome-to-TGN trafficking, membrane carriers, such as vesicles or tubules, emerge from endosomes, travel along cytoskeleton tracks, tether at and then fuse with the TGN membrane. Similar to other membrane trafficking routes, the machinery of the endosome-to-TGN trafficking is highly conserved from yeast to human and involves small GTPases, coat proteins, tethering factors and SNAREs (Fig. 2).

5.1 Formation of transport carriers from endosomes

5.1.1 Retromer—the central sorting machinery of retrograde trafficking

Retromer is a hetero-pentameric complex whose structure and function are evolutionary conserved from yeast to human [52, 53]. The membrane localization of retromer is mediated by its association with SNXs (sorting nexins) and Rab7. Therefore, retromer mainly localizes to the transitional endosome from the EE to the LE, which is positive for both SNXs and Rab7 [53]. Retromer has two sub-complexes: the cargo selection trimer consisting of Vps26, Vps29 and Vps35 and the membrane association SNX dimer. The cargo selection trimer directly binds to sorting motifs in the cytosolic domains of an expanding list of membrane cargos, such as those belonging to Vps10 domain receptor family. The trimer can associate with either a heterodimer of BAR (Bin/Amphiphysin/Rvs) domain containing SNX-BARs, such as the heterodimer between SNX1/2 and SNX5/6/32, or a possible homodimer of SNX3 to form two types of retromers—SNX-BAR-retromer and SNX3-retromer [52]. The SNX family proteins comprise a PX domain that binds phosphoinositides, such as PI(3)P and PI(3,5)P_2. In addition to the PX domain, SNX-BARs contain a BAR domain that is able to drive, sense and stabilize membrane curvature [54]. SNX-BAR-retromer is able to sort cargos into high curvature membrane tubules or vesicles. It is essential for most known cargos transiting from the endosome-to-TGN, especially the EE/RE-to-TGN, such as STxB, TGN38, M6PRs and SorLA [52]. The trafficking of furin in the LE-to-TGN pathway does not require retromer, suggesting the cargo selectivity of retromer [46]. The best

known SNX3-retromer specific cargo is Wntless [55, 56] (see section 6). However, without the assistance of a BAR domain, it is unclear how SNX3 facilitates retromer in generating transport carriers.

A model has emerged to explain the molecular role of SNX-BAR-retromer in the endosome-to-TGN trafficking. After endocytosis, membrane cargos enter the EE, which is characterized by the enrichment of PI(3)P. The SNX-BAR dimer binds PI(3)P and its BAR domains bend the EE membrane to form membrane tubules or vesicles, where the SNX-BAR dimer subsequently partitions and coats. At this stage, the EE develops a vacuolar domain destined for lysosomal degradation and multiple tubular domains for the retrieval and recycling of membrane cargos. The cargo selection trimer recruits membrane cargos from the vacuolar domain to the retromer coated tubular domain. The SNX-BAR dimer associates with EHD1 (eps15 homology domain 1) which, through its ATPase activity, could aid in the scission of the tubule for the biogenesis of transport carriers [57, 58]. Retromer can recruit accessory regulatory proteins such as WASH (Wiskott-Aldrich syndrome protein and SCAR homolog) and dynein motor complex to connect membrane tubules to cytoskeleton. WASH complex probably provides the force for tubule formation and scission by actin polymerization [59], while dynein-mediated minus end transport along microtubule probably brings the retromer decorated carriers to the TGN at MTOC [60, 61]. The TGN-enriched phosphoinositide, PI(4)P, disassembles the association between dynein motor and retromer/cargo complex. Consequently, the membrane carriers loaded with cargos are specifically targeted and delivered to the TGN [61]. Cargo proteins left at the vacuolar domain of the endosome end up in lysosomes via the LE for degradation. In addition to its role in the endosome-to-TGN pathway, retromer also participates in the trafficking of membrane cargos from the EE to the PM or the RE by associating with other members of SNX family [54].

5.1.2 Clathrin and adaptors

Coat proteins are important machinery for membrane deformation and cargo selection in vesicular trafficking. Clathrin coats localize to post-Golgi secretory organelles and are essential for the endosome-to-TGN trafficking [62]. Adaptor proteins are required for connecting polymerized clathrin coat to cargos and membrane. There are two types of clathrin adaptor proteins—monomeric and tetrameric. The heterotetrameric clathrin adaptor protein 1 (AP1) has long been proposed to function in the endosome-to-TGN pathway to retrieve membrane cargos, including SNAREs, M6PRs, sortilin and furin [51, 63-65]. Within endosomes, AP1 could directly sort membrane cargos by interacting with YXXΦ and [DE]XXXL[LI] motifs in their cytosolic domains [5]. AP1 can also indirectly sort cargos by interacting with phosphofurin acidic cluster sorting protein 1 (PACS1), a soluble protein that can bind acidic cluster sorting motifs of cargos such as M6PRs and furin [66]. EpsinR (Epsin related protein) is a monomeric clathrin adaptor protein found in the Golgi complex and endosomes [67, 68]. EpsinR binds both AP1 and clathrin [67] and, similar to AP1, its disruption inhibits the retrograde trafficking of STxB to the TGN [62]. The endosome-to-TGN trafficking mediated by EpsinR seems to prefer SNARE Vti1b as a cargo, instead of the related SNARE Vti1a and conventional membrane cargos, such as M6PRs and furin [63]. The recruitment of AP1 and EpsinR to endosomal membrane requires the phosphoinositide PI(4)P [68, 69], which could be produced from the PM derived $PI(4,5)P_2$ by the oculocerebrorenal syndrome of Lowe protein 1 (OCRL1) [70], an inositol polyphosphate 5-

phosphatase. AP1, EpsinR, PACS1 and OCRL1 could work synergistically in cargo sorting and the budding of clathrin-coated vesicles at the EE.

5.1.3 The relationship between clathrin and retromer

Although both clathrin and retromer are key components in the retrograde trafficking, it is obscure if and how they work together or independently [71-73]. AP1 has been observed on the tubular/vesicular/bud profiles on the EE membrane [74-76], suggesting that clathrin-coated transport carriers could form in the vacuolar or tubular domain of the EE. The finding that SNX1 interacts with not only Hrs, a clathrin coat polymerizing and ESCRT-0 protein, but also clathrin coat disassembling factor Rme-8/Hsc70 argues an alternative model where clathrin and retromer could function sequentially [72, 77, 78]. With the aid of Hrs, clathrin and its accessory proteins could form a flat clathrin coat to recruit cargos. The subsequent arrival of retromer and Rme-8 could depolymerize clathrin coat and transfer cargos to tubular domains.

5.1.4 The roles of Rab9 and its effectors at the LE

The LE localized small GTPase Rab9 is essential for the retrograde trafficking of furin and M6PRs from the LE to the TGN [15-17]. M6PRs are probably incompletely sorted by retromer and clathrin/AP1 machinery and are left behind as the endosomal compartment matures to become the LE. At the LE, Rab9 recruits its effectors—p40 and TIP47 to membrane [79, 80]. The adaptor protein TIP47 subsequently recognizes the cytosolic domain of M6PR and, together with Rab9 and p40, it packs M6PRs into Rab9 positive transport vesicles, probably non-clathrin coated, destined for the TGN [81].

5.2 Tethering and fusion at the TGN

5.2.1 Tethering factors

Upon the arrival of transport carriers, tethering factors residing on the TGN membrane loosely capture carriers at a long distance before the subsequent SNARE pairing and membrane docking/fusion. The short range interaction between the v-SNARE on the membrane carrier and the t-SNARE complex on the TGN membrane is greatly facilitated by this long range tethering process. A tethering factor could be either a homodimeric or oligomeric complex. Golgins are Golgi localized homodimeric tethering factors with extensive coiled-coil regions [82]. They are proposed to adopt a long rod-like structure with one end anchoring on the Golgi membrane and the other end binding to transport carriers [82]. GM130, p115 and GMAP-210 are *cis*-Golgi Golgins that are proposed to tether the ER-to-Golgi anterograde transport carriers [83, 84]. On the TGN membrane, the most studied tethering factors for the endosome-derived carriers are GRIP (Golgin97-RanBP-Imh1p-p230 *trans* Golgi) domain Golgins, including Golgin97, Golgin245, GCC88 and GCC185 [82]. For GRIP Golgins, the GRIP domains at their C-termini specifically interact with ARF family small GTPase—Arl1 [85]. By Arl1-GRIP interaction, active Arl1 recruits GRIP domain Golgins to the TGN membrane. STxB based transport assays showed that both Arl1 and Golgin97 are essential for the endosome-to-TGN transport and Golgin97 was found to be required at a step before the t-SNARE Syn16 (Syntaxin16), suggesting a tethering function of Golgin97 [48]. Other GRIP Golgins, including Golgin245,

GCC88 and GCC185, were also found to participate in the retrograde trafficking [86-90]. Among them, GCC185 seems to specifically tether transport carriers from the LE as its depletion resulted in the accumulation of Rab9 positive vesicles [15, 87] (see section 5.1.4).

Together with yeast data, an ARF family GTPase signaling cascade was proposed to regulate the retrograde trafficking. In this signaling pathway, active ARFRP1, another ARF family small GTPase, activates Arl1, which in turn recruits GRIP domain Golgins to tether retrograde carriers from endosomes [91-93]. Another TGN Golgin relevant to retrograde trafficking is TMF/ARA160 [94]. The small GTPase Rab6 seems to recruit TMF to the Golgi membrane by specifically binding to its C-terminus [95].

Oligomeric tethering factors are present in most vesicular trafficking pathways [96, 97]. Studies in yeast established that COG (conserved oligomeric Golgi complex) and GARP/VFT (Golgi associated retrograde protein complex/Vps fifty three) participate in the endosome to the late Golgi (TGN) trafficking as tethering factors [98-100]. Mammalian orthologous complexes could have the same conserved functions. Two features emerged for COG and GARP complexes. First, their disruption results in the accumulation of non-tethered transport vesicles containing retrograde cargos [101-103]; second, independent of their tethering function, they regulate the assembly of SNARE complexes on the TGN membrane, such as those containing GS28 or Syn16 (see section 5.2.2) [101, 104].

5.2.2 SNAREs

A SNARE is usually a C-terminus tail-anchored membrane protein with one or two SNARE motifs in its cytosolic domain [105]. In the SNARE hypothesis, a v-SNARE (v is for vesicle) on the transport vesicle specifically interacts with its cognate t-SNARE (t is for target) on the target membrane. A t-SNARE is usually a complex consisting of three SNARE motifs contributed by three Syn or Syn-like SNARE motifs, while a v-SNARE usually has one SNARE motif. During the v-t SNARE pairing, the formation of a very stable four-helix bundle brings the two membrane sheets to such a close proximity that fusion would occur. The v-t SNARE bundle has to be pried open and hence regenerated by NSF (*N*-ethylmaleimide-sensitive factor) ATPase and α-soluble NSF attachment protein (α-SNAP) at the expense of cellular energy.

In mammalian cells, there are about a dozen SNAREs residing in the Golgi complex. A systematic screening of these SNAREs identified four v-t SNARE complexes involved in the endosome-to-TGN trafficking—Vamp3/Syn6/Syn16/Vti1a, Vamp4/Syn6/Syn16/Vti1a, GS15/Syn5/GS28/Ykt6 and Vamp3/Syn10/Syn16/Vti1a [22, 106-108]. The SNARE regulators such as NSF and α-SNAP are also essential for the endosome-to-TGN trafficking [44, 106]. Among SNARE complexes involved, Vamp3, Vamp4 and GS15 are v-SNAREs residing on the membrane carriers originated from endosomes, and the rest are t-SNARE hetero-trimers localized to the TGN. The involvement of various SNARE complexes probably reflects multiple trafficking carriers transporting different cargos from distinct endosomal domains. For example, Vamp3/Syn10/Syn16/Vti1a is specifically required for retrograde trafficking of M6PRs [108] from the LE to the TGN while the two SNARE complexes containing Syn16 are required for STxB, CTxB and TGN38/46 from the EE/RE to the TGN [106].

6 Retrograde trafficking in cellular signaling and neurodegenerative diseases

Recent advancement in this field revealed important roles of retrograde trafficking in cellular signaling and neurodegenerative diseases through its core machinery – retromer. The Wnt family secreted morphogens are essential for the proper patterning of various tissues in metazoans [109]. The secretion of Wnt depends on its interaction with Wntless, a multi-transmembrane protein cycling among the PM, the endosome and the Golgi [110]. After biosynthesis of Wnt in the ER, Wnt enters the secretory pathway and is transported to the Golgi complex. Wntless associates with Wnt at the Golgi and targets it to the PM, where Wnt is exocytosed to extracellular space, while Wntless is subsequently endocytosed from the PM to the EE. Work from multiple labs showed that the retromer-mediated endosome-to-TGN trafficking plays an essential role in the continuous secretion of Wnt in worms and vertebrates [111-115]. Via the interaction between Vps35 and Wntless cytosolic domain, retromer retrieves Wntless away from the lysosomal degradative pathway and delivers it to the Golgi for further rounds of Wnt targeting and secretion. Interestingly, SNX3-retromer, instead of SNX-BAR-retromer, is required for the recycling [55, 56]. Disrupting the SNX3-retromer complex causes the lysosomal degradation of Wntless and the impaired secretion of Wnt morphogen. Similarly to its role in Wnt signaling, retromer was also found to regulate apical-basal polarity signaling in epithelial cells by recycling transmembrane protein Crumbs to the TGN from lysosomal degradation pathway[116, 117]. Retromer is also able to terminate the production of cAMP by diverting activated parathyroid hormone receptor from the EE to the Golgi [118].

The pathogenesis of neurodegenerative Alzheimer's disease (AD) is contributed by the accumulation of Aβ peptide, which is generated by the sequential cleavage of amyloid precursor protein (APP) by β and γ-secretase (amyloidogenic cleavage). Alternatively, APP is first cleaved within the Aβ peptide by α-secretase and therefore the production of Aβ peptide is inhibited (non-amyloidogenic cleavage). APP, β- and γ-secretase are transmembrane proteins that cycle among post-Golgi organelles, including the PM, the endosome and the TGN [119, 120]. As the amyloidogenic processing of APP is initiated by β-secretase, the subcellular location of APP and β-secretase can determine the production of Aβ peptide. β-secretase has been reported to localize to the EE/RE [121, 122] and the TGN [119]. Although a pool of APP is known to reside on the PM [123], whether the endocytosed APP undergoes lysosomal degradation [122] or the endosome-to-TGN trafficking [124] is in debate recently. Therefore, two possible Aβ peptide biogenesis sites have been proposed — the EE and the TGN based on different models of APP intracellular itineraries. However, it seems clear that altering the trafficking of APP and β-secretase could change the production of Aβ peptide. The trafficking of APP has been found to be regulated by SorLA, a member of Vps10 domain receptor family, and retromer [125]. SorLA cycles between the TGN and the endosome and binds and transports APP by interacting with the retromer subunit Vps26. In cultured cells or animal models, disruption of retromer [124, 126-129] or SorLA [130] has been reported to affect the production of Aβ peptide. Supporting their roles in AD, both retromer and SorLA have been implicated in the late-onset AD [128, 130]. Interestingly, mutations of Vps35, a key subunit in the cargo selection trimer of retromer, were also found in the late-onset Parkinson's disease [131, 132], which is characterized by the formation of protein aggregates called Lewy's bodies. The manifestation of the disease could be due to the disrupted trafficking of CI-M6PR and its cargo cathepsin D (a lysosomal hydrolase),

leading to compromised degradation and thus aggregation of α-synuclein — a major component of Lewy's body [133].

7 Conclusions and future directions

The Endosome-to-TGN retrograde trafficking has been recognized as an important route to retrieve cargos from the endo-lysosome degradation pathway. Core machinery components involved in this trafficking pathway have been identified. Especially, the sorting, packing and/or targeting of many cargos converge on the retromer protein complex on the endosomal membrane. However, we don't have a coherent and complete molecular picture on how they work together in vivo, which requires extensive future work. Novel assays for probing endosome-to-TGN trafficking are needed to sort out the large amount of data available in the literature.

Roles of this pathway in the regulation of signal transduction and neurodegenerative diseases are beginning to emerge and we expect more would be revealed. A general theme involving retromer has emerged. After endocytosis, signaling receptors are retrieved or diverted to the TGN by retromer via the retrograde trafficking, thus depleting their endosomal pools or avoiding their degradation in lysosomes. Since the retrograde trafficking plays such important roles in signaling, it could be regulated by certain cellular signals, which remain unknown currently. Research along this line would greatly help us to understand the molecular pathogenesis of certain human diseases.

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Figure captions

Figure 1 Various post-Golgi trafficking pathways. The two endosome-to-TGN retrograde trafficking pathways, the EE/RE-to-TGN and the LE-to-TGN, are highlighted using thick green lines. Some cargos detailed in this review are listed in boxes. PM, plasma membrane; EE, early endosome; RE recycling endosome; LE, late endosome; TGN, *trans*-Golgi network; ER, endoplasmic reticulum.

Figure 2 Major machinery components involved in endosome-to-TGN trafficking. Machinery for transport carrier formation on the EE/RE: clathrin and accessory proteins, retromer and accessory proteins, cytoskeletons/motors, Hrs and Rme-8; and on the LE: Rab9 and accessory proteins. Machinery on the TGN to receive transport carriers: small GTPases, tethering factors and SNAREs. EE, early endosome; RE recycling endosome; LE, late endosome; TGN, *trans*-Golgi network; MTOC, microtubule organizing center.

Table 1 List of cargos discussed in the review which utilize the endosome-to-TGN trafficking pathway.

cargo	pathway	reference
	membrane cargos	
GS15	EE/RE-to-TGN	[22]
Vamp4	EE/RE-to-TGN	[23]
GLUT4	EE/RE-to-TGN	[24]
CI- or CD-M6PR	EE/RE-to-TGN	[12, 13]
TGN38/46	EE/RE-to-TGN	[9]
furin	LE-to-TGN	[10, 15]
GPP130	EE-to-TGN	[29]
GPP73	EE-to-TGN	[29]
SorLA/SorL1	EE-to-TGN	[27]
Wntless	EE-to-TGN	[110]
Crumbs	EE-to-TGN	[116, 117]
parathyroid hormone receptor	EE-to-TGN	[118]
carboxypeptidase D		[25]
sortilin		[26]
CD59		[28]
GPI-GFP		[28]
APP		[124]
β-secretase		[119]
	soluble proteins	
Shiga toxin	EE/RE-to-TGN	[11, 13, 14]
Cholera toxin	EE-to-TGN	[33]
Ricin	EE-to-TGN	[35, 36]
Pseudomonas exotoxin (PE)	EE-to-TGN and LE-to-TGN	[37]
	lipids	
globoside	EE/RE-to-TGN	[11, 13, 14, 40]
GM1	EE-to-TGN	[33]
glucosylceramide	EE-to-TGN	[39]
lactosylceramide	LE-to-TGN	[38, 40]
sphingomyelin	EE-to-TGN	[39-41]

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