European Journal of Cell Biology 89 (2010) 379–393

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Identification of different itineraries and retromer components for endosome-to-Golgi transport of TGN38 and Shiga toxin

Zi Zhao Lieu, Paul A. Gleeson *

The Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, Victoria 3010, Australia

ARTICLE INFO

Article history:
Received 28 August 2009
Received in revised form
22 October 2009
Accepted 29 October 2009

Keywords:
Retrograde transport
Golgi
trans-Golgi network
Shiga toxin
TGN38

ABSTRACT

The retrograde transport pathways from early/recycling endosomes are critical for recycling a range of endogenous cargo, as well as internalisation of bacterial and plant toxins. We have previously shown that the retrograde transport of the two model cargos, TGN38 and Shiga toxin, differs in the requirement for TGN golgins; transport of TGN38 requires the TGN golgin GCC88 whereas that of Shiga toxin requires GCC185. Here we have further defined the retrograde transport requirements of these two cargos. Tracking the transport of these cargos demonstrated that the bulk of Shiga toxin is transported from early endosomes to recycling endosomes en route to the TGN whereas the bulk of TGN38 is transported from early endosomes to the TGN with only low levels detected in recycling endosomes. In cells depleted of the TGN t-SNARE syntaxin 16, TGN38 accumulated predominantly in early endosomes whereas Shiga toxin accumulated in Rab11-positive recycling endosomes, suggesting distinct routes for each cargo. Retrograde transport of Shiga toxin and TGN38 requires retromer, however, whereas sorting nexin 1 (SNX1) is specifically required for transport of Shiga toxin, sorting nexin 2 (SNX2) is required for the transport of TGN38. Overall, our data have identified different itineraries for the retrograde transport of Shiga toxin and TGN38 and distinct retromer components that regulate the transport of these cargos.

Introduction

Retrograde transport from the endosomal system to the trans-Golgi network (TGN) is important for the recycling of endogenous proteins including the sorting receptors mannose-6-phosphate receptor (M6P-R), sortilin and wntless, transmembrane peptidases such as furin, SNAREs, and ion and glucose transporters (Ghosh et al., 1998; Lewis et al., 2000; Ghosh et al., 2003; Shewan et al., 2003; Sandvig and van Deurs, 2005; Bonifacino and Rojas, 2006; Johannes and Popoff, 2008). In addition, bacterial and plant toxins, such as Shiga toxin, cholera toxin, pertussis toxin and ricin, are internalised by endocytosis and then use the retrograde transport pathway to mediate cytotoxicity (Sandvig and van Deurs, 2000; Utskarpen et al., 2006; Plaut and Carbonetti, 2008). By analysing the trafficking of individual cargos several retrograde transport pathways from the endosomal compartments to the TGN have been identified (Sannerud et al., 2003; Bonifacino and Rojas, 2006; Johannes and Popoff, 2008). These transport pathways include routes from the early/recycling endosomes to the TGN as well trafficking from late endosomes to the TGN. A diverse range of factors have been identified which regulate these retrograde transport pathways (reviewed in (Bonifacino and Rojas, 2006; Johannes and Popoff, 2008)).

The transport routes between early/recycling endosomes and the TGN are predicted to involve the budding of membrane-enclosed transport carriers from endosomes and the subsequent fusion with the TGN. A number of components of the molecular machinery involved in these transport steps have been identified. Of particular importance, the retromer complex has been shown to mediate the retrograde transport of a number of cargos from the early endosome (Bujny et al., 2007; Bonifacino and Hurley, 2008; Franch-Marro et al., 2008; Port et al., 2008). Retromer was first identified as important for the retrograde transport of cation-independent mannose 6-phosphate receptor (CI-M6P-R) (Arighi et al., 2004; Seaman, 2004), and more recently also shown to regulate retrograde transport of other cargos such as wntless, Shiga toxin, and polymeric immunoglobulin receptors (Verges et al., 2004; Popoff et al., 2007; Belenkaya et al., 2008; Franch-Marro et al., 2008; Port et al., 2008; Yang et al., 2008). Retromer comprises two sub-complexes: a cargo recognition trimer of Vps26-Vps35-Vps29 and a sorting nexin (SNX) dimer that contains PX and Bar domains that sense membrane curvature

Abreviations: TGN, Trans-Golgi network; STx-B, Shiga toxin B subunit; M6P-R, Mannose-6-phosphate receptor; CI-M6P-R, Cation-independent mannose-6-phosphate receptor; SiRNA, Small interfering RNA; SNX1, Sorting nexin 1; SNX2, Sorting nexin 2; GFP, Green fluorescent protein; PCC, Pearson's correlation coefficient.

* Corresponding author. Tel.: +61 3 8344 2354; fax: +61 3 9348 1428.
E-mail address: pgleeson@unimelb.edu.au (P.A. Gleeson).

0171-9335/$ - see front matter
doi:10.1016/j.ejcb.2009.10.021

and in some cases can bind lipid headgroups and promote membrane curvature (Seaman, 2005; Rojas et al., 2007). Retromer plays a critical role not only in the sorting of cargos but also the generation of transport intermediates (Popoff et al., 2007; Rojas et al., 2007; Bonifacino and Hurley, 2008; Cullen, 2008; Wassmer et al., 2009). SNX1, SNX2 , SNX5 and SNX6 have been shown to be important components of retromer (Carlton et al., 2004; Rojas et al., 2007; Cullen, 2008), and recent studies have identified multiple forms of retromer which contain specific combinations of the four sorting nexins (Wassmer et al., 2009). In addition to retromer, clathrin (Saint-Pol et al., 2004; Bujny et al., 2007; Popoff et al., 2007; Utskarpen et al., 2007) and clathrin adaptors, such as epsinR, have also been demonstrated to play a role in trafficking of cargo from the early endosome (Mallard et al., 1998; Saint-Pol et al., 2004). Studies from a number of laboratories have shown that the machinery involved in the docking and fusion of retrograde transport carriers with the TGN include tethering factors, small GTPases and SNAREs (Sannerud et al., 2003; Bonifacino and Rojas, 2006; Johannes and Popoff, 2008), although the link between the individual components and the precise identity of the transport pathway(s) is not always clear.

TGN38 and Shiga toxin are two model cargos used to study retrograde transport in mammalian cells (Sannerud et al., 2003). TGN38 is a transmembrane protein whereas Shiga toxin is a soluble toxin that binds to the glycosphingolipid Gb3 on the luminal leaflet of the plasma membrane. These cargos are transported to the TGN via early/recycling endosomes and are independent of the Rab9-late endosome-to-TGN pathway (Mallard et al., 1998, 2002; Reddy et al., 2006). Both TGN38 and Shiga toxin are internalised into transferrin receptor-positive compartments, indicating that TGN38 and Shiga toxin utilise a retrograde transport pathway from the early endosome or the recycling endosome (Mallard et al., 1998, 2002). A number of common components have also been identified for endosome-to-TGN transport of Shiga toxin and TGN38, for example epsinR, syntaxin 16, and the mammalian Golgi-associated retrograde protein (GARP) complex (Saint-Pol et al., 2004; Popoff et al., 2007; Perez-Victoria et al., 2008). Based on these findings TGN38 and Shiga toxin have been considered to be transported to the TGN by the same retrograde transport pathway, however, there have been few studies to directly compare the trafficking routes of these two cargos.

Our previous studies have focused on the role of a family of TGN golgins in the regulation of membrane transport. There are four human TGN golgins, namely p230/golgin-245, golgin-97, GCC185 and GCC88 (Kooy et al., 1992; Fritzler et al., 1995; Erlich et al., 1996; Gleeson et al., 1996; Griffith et al., 1997; Luke et al., 2003a). TGN golgins are peripheral membrane proteins that are recruited to the TGN by a targeting sequence located at the C-terminus, called the GRIP domain (Barr, 1999; Kjer-Nielsen et al., 1999a; Munro and Nichols, 1999). Each of the TGN golgins appears to have independent functions (Gleeson et al., 2004; Derby and Gleeson, 2007). In particular, different TGN golgins regulate the retrograde transport of TGN38 and Shiga toxin (Derby et al., 2007; Lieu et al., 2007). The TGN golgin, GCC88, is essential for efficient retrograde transport of TGN38 from endosomes (Lieu et al., 2007), whereas efficient transport of Shiga toxin is dependent on the golgin GCC185 (Derby et al., 2007). RNAi silencing of GCC88 resulted in the accumulation of TGN38 in early endosomes while RNAi silencing of GCC185 resulted in accumulation of Shiga toxin in recycling endosomes (Derby et al., 2007; Lieu et al., 2007). The accumulation of TGN38 and Shiga toxin in different endocytic compartments after silencing TGN golgins indicates that these two cargos may utilise different transport pathways. Here we have further compared the itinerary, and the retromer components required, for the retrograde transport of the two cargos, TGN38 and Shiga toxin. Our findings demonstrate major differences in the retrograde transport of these two cargos.

Materials and methods

Plasmids, antibodies and reagents

TGN38-CFP (Keller et al., 2001) encodes a C-terminal fusion protein with the fluorescent protein. pIRES-TGN38 encodes an untagged version of TGN38 as described (Lieu et al., 2007). GFP-Rab11 and GFP-Rab7(Q67L) are N-terminal fusions with GFP, as described (Zhang et al., 2004). Myc-SNX2 encodes a C-terminal fusion protein with myc epitope tag as described (Kerr et al., 2006) and was obtained from Dr Rohan Teasdale, University of Queensland. Human autoantibodies to p230 (Kooy et al., 1992) and early endosome-associated protein 1 (EEA1) (Mu et al., 1995) are as described. The 9E10 mouse monoclonal antibody specific for the myc epitope has been described (Evan et al., 1985). Mouse monoclonal antibodies to rat TGN38 were from Transduction Labs (Lexington, USA). Mouse monoclonal antibodies to SNX1 and SNX2 were purchased from BD Biosciences (North Ryde, NSW, Australia). Mouse monoclonal anti-α-tubulin was obtained from GE Healthcare (Rydalmere, NSW, Australia). 13C4 mouse monoclonal antibodies to Shiga toxin B fragment B (STx-B) were purified as previously described (Johannes et al., 1997). Rabbit polyclonal antibodies to rat TGN38 were from Sigma Aldrich (Lismore, Australia), syntaxin 16 from Synaptic Systems, (Germany) and Vps26 from Abcam (Cambridge, UK). Rabbit polyclonal antibodies to syntaxin 16 (Mallard et al., 2002) were obtained from Wanjin Hong (Institute of Molecular Cell Biology, Singapore) and to human syntaxin 10 were obtained from Dr Rohan Teasdale, University of Queensland. Rabbit polyclonal antibodies to human GCC88 and GCC185 were previously described (Luke et al., 2003b; Derby et al., 2007). Rabbit polyclonal anti-human GMAP-210 has been previously described (Infante et al., 1999), a kind gift from Michel Bornens (Curie Institute, Paris). Rabbit polyclonal antibodies to Rab11 has been previously described (Wilcke et al., 2000) and was provided by Bruno Goud (Curie Institute, Paris). Goat polyclonal antibody to human Vps35 was purchased from Abcam (Cambridge, UK). Purification of Shiga toxin B fragment was carried out as previously described (Johannes et al., 1997). Cy3 conjugated Shiga toxin B fragment (STx-B) (Mallard et al., 1998) was a kind gift from Bruno Goud (Curie Institute, Paris).

Secondary antibodies used for immunofluorescence were sheep anti-rabbit Ig-FITC (fluorescein isothiocyanate) and sheep anti-human Ig-FITC (from Silenus laboratories, Melbourne Australia) while goat anti-rabbit IgG-Alexa Fluor™ 568 nm, goat anti-mouse IgG-Alexa Fluor™ 568 nm, goat anti-rabbit IgG-Alexa Fluor™ 488 nm, goat anti-mouse IgG-Alexa Fluor™ 488 nm, goat anti-mouse IgG-Alexa Fluor™ 647 nm, goat anti-rabbit IgG-Alexa Fluor™ 647 nm, goat anti-human Alexa Fluor™ 647 nm and goat anti-human Alexa Fluor™ 594 nm were from Molecular Probes (Invitrogen, Carlsbad, California, USA). Horse-radish peroxidase-conjugated rabbit anti-goat Ig, horse-radish peroxidase-conjugated sheep anti-rabbit Ig and anti-mouse Ig were from DAKO Corporation (Carpinteria, CA, USA).

Cell culture and transient transfections

HeLa cells were maintained as semi-confluent monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) foetal calf serum (FCS), 2 mM L-glutamine, 100 units/μl