Membrane Transport across Polarized Epithelia

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Polarized epithelial cells line diverse surfaces throughout the body forming selective barriers between the external environment and the internal milieu. To cross these epithelial barriers, large solutes and other cargoes must undergo transcytosis, an endocytic pathway unique to polarized cell types, and significant for the development of cell polarity, uptake of viral and bacterial pathogens, transepithelial signaling, and immunoglobulin transport. Here, we review recent advances in our knowledge of the transcytotic pathway for proteins and lipids. We also discuss briefly the promise of harnessing the molecules that undergo transcytosis as vehicles for clinical applications in drug delivery.

Epithelial cells form delicate but highly effective single-cell-thick barriers that define the lumen of many tissues, including all secretory organs and mucosal surfaces. Endothelial cells form analogous single-cell-thick barriers that define blood vessels and capillaries. Both cell types function to separate and affect vastly different physiologic compartments. To achieve these functions, epithelial and endothelial cells must establish and maintain two structurally and functionally different apical and basolateral cell membranes, each interfacing with and affecting only one compartment. Such cell polarity is required to physiologically shape the different environments, in part by driving the vectorial transport of small and large solutes between them. In the case of large solutes, such as the immunoglobulins, albumin, and some signaling molecules, transport can occur only by moving these solutes through the cell via a

transcellular endocytic process termed transcytosis (Rojas and Apodaca 2002; Rath et al. 2014; Azizi et al. 2015; Tanigaki et al. 2016). Here, we address new developments in our understanding of transcytosis, the process of endosome trafficking unique to polarized cell types that connects one cell surface with the other. The reader is also referred to the most recent and comprehensive reviews of transcytosis (Rojas and Apodaca 2002; Tuma and Hubbard 2003) and endosome trafficking in polarized cell types (Ang and Folsch 2012; Apodaca et al. 2012; Bay et al. 2015; Folsch 2015).

SPECIALIZATIONS OF ENDOSOMES UNIQUE TO POLARIZED EPITHELIAL CELLS

The epithelial and endothelial cellular polarity required for barrier function and vectorial transport of peptide and protein solutes is

Editor: Keith E. Mostov

Additional Perspectives on Cell Polarity available at www.cshperspectives.org

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Cite this article as *Cold Spring Harb Perspect Biol* 2017;9:a027912

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achieved in large part by membrane trafficking. Fundamentally, it is membrane trafficking (vesicular transport) that accounts for cell polarity itself (Bryant et al. 2014), by enabling the sorting and delivery of specific membrane proteins and lipids to the appropriate cell surfaces of polarized cells and keeping them there (Mellman 1996; Folsch et al. 2009; Ang and Folsch 2012). Many adaptations of membrane trafficking are required to achieve cell polarity, and the general rules for vesicular transport that define endosome dynamics in nonpolarized cells do not always apply to polarized cell types. Some trafficking proteins, such as Rab11, function differently in polarized cells, and others are uniquely expressed such as AP1 μ B (Folsch et al. 1999; Wang et al. 2000b). The transcytotic pathway is a particularly important pathway to understand as the trafficking of membranes and cargoes by this process intersects with all the specialized endosomal compartments adapted by epithelial and endothelial cells to accommodate the polarized cell phenotype (Rojas and Apodaca 2002; Tuma and Hubbard 2003; Ang and Folsch 2012; Apodaca et al. 2012; Bryant et al. 2014; Rodriguez-Boulán and Macara 2014; Bay et al. 2015; Folsch 2015).

Polarized epithelial cells have adapted at least two major specializations of their endosomal compartments. One specialization is the establishment of distinct populations of apical and basolateral early sorting endosomes that receive membranes internalized from only one cell surface and that do not directly interact (Bomsel et al. 1989; Parton et al. 1989; Bomsel et al. 1990; Sheff et al. 2002). The different apical and basolateral sorting endosomes can rapidly recycle internalized components back to the cell surface where endocytosis originated, thus helping to maintain the specialized identity of the apical and basolateral membranes, or they can selectively deliver membrane and cargo to various intracellular compartments shared between them. These shared compartments include the late endosome and lysosome, the Golgi network, the endoplasmic reticulum, and the common/apical recycling endosome. Currently, neither the apical nor basolateral sorting endosomes are thought to sort and deliver cargo

directly to the contralateral plasma membrane (basolateral or apical, respectively) to mediate transcytosis (Huber et al. 2000). This is a function of the common endosome (Apodaca et al. 1994; Brown et al. 2000; Mostov et al. 2000).

The common/apical recycling endosome is the other major adaptation of the endosomal network unique to polarized epithelial cells (Parton et al. 1989; Hughson and Hopkins 1990; Apodaca et al. 1994; Barroso and Sztul 1994; Odorizzi et al. 1996; Brown et al. 2000; Mostov et al. 2000). It receives cargo from both apical and basolateral early sorting endosomes and can sort them back to the cell surface where endocytosis originated (recycling), or to the opposite cell surface to enable transcytosis. Although each of the organelles downstream of the early sorting endosomes of polarized epithelia (Golgi, common recycling endosome, lysosome) can transport membrane and cargo to either cell surface (Mellman 1996), the transcytotic pathway appears to originate from the common/apical endosomes. It does not intersect with the retrograde (Golgi and ER) or late endosome/lysosomal pathways (Hoppe et al. 1985; Apodaca et al. 1994; Saslow et al. 2013). The basic model for endosome structure and function in polarized (and nonpolarized) cells is shown in Figure 1. To date, many of the features originally proposed in 1994 remain relevant (Apodaca et al. 1994).

Early Apical and Basolateral Sorting Endosomes

The different early sorting endosomes are located immediately adjacent to the apical or basolateral plasma membrane. They are the first identifiable endocytic compartments after endocytosis, and they are typified by association with Rab5, EEA1, Rab4, Rab11, and transferrin receptor (TfnR) (Goldenring et al. 1996; Sheff et al. 1999; Sonnichsen et al. 2000; Hoekstra et al. 2004; Thompson et al. 2007). Both the apical and basolateral early sorting endosomes receive membrane and cargo from all forms of endocytosis, including clathrin- and non-clathrin-mediated processes (Mayor et al. 2014). Endocytosis by caveolae may predominate in the transcytotic

Figure 1. Endosome compartments and pathways. Diagram of pathways across endosomal compartments in (A) polarized epithelial, and (B) nonpolarized cells. Arrows represent transport from one organelle to the next. The apical and basolateral domains are indicated in green and red, respectively.

pathway of endothelial cells (Simionescu and Simionescu 1991; Simionescu et al. 2002; Preston et al. 2014). The early apical and basolateral sorting endosomes mediate recycling. They can also selectively sort membrane and cargo to the lysosome via a process of endosome maturation; or they can sort cargo to the common recycling/apical endosome (Hughson and Hopkins 1990). Sorting to destinations other than the late endosome requires the process of membrane budding and tubulation (Bonifacino and Rojas 2006). Delivery of endocytic cargo to the common/apical recycling endosome appears to be necessary for transcytosis for at least some membrane proteins, such as the immunoglobulin receptors (Apodaca et al. 1994; Tzaban et al. 2009). There are still many features of the early apical and basolateral sorting endosomes that remain unknown. How, for example, do they differ struc-

turally or mechanistically, and how do they discern between themselves and apical and basolateral membranes.

Common/Apical Recycling Endosomes

The common/apical recycling endosome is a separate and stable long-lived structure (Marsh et al. 1995) located in the apical perinuclear region of the cell (Hughson and Hopkins 1990; Apodaca et al. 1994) and dependent on the microtubule cytoskeleton (Bomsel et al. 1990; Breitfield et al. 1990; Hunziker et al. 1990; Apodaca et al. 1994, 2012; Casanova et al. 1999; Hoekstra et al. 2004; Maxfield and McGraw 2004; van Ijzendoorn 2006). It receives membrane and cargo from the different apical and basolateral early sorting endosomes (Hughson and Hopkins 1990; Apodaca et al. 1994; Barroso and Sztul 1994) and also from

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the biosynthetic pathway for membrane proteins (Lock and Stow 2005). Like the early sorting endosomes, the common/apical recycling endosome can sort and release cargo to other destinations (Apodaca et al. 1994; Marsh et al. 1995; Tzaban et al. 2009). In this case, it serves as a key site for polarized protein sorting and can selectively traffic membrane and cargo to either cell surface. This was first evidenced by studies on sorting of the polymeric immunoglobulin receptor (pIgR) and transferrin receptor (TfnR). These membrane proteins are internalized from basolateral membranes of polarized epithelia and localize in the common endosome together. However, pIgR is efficiently sorted apically and TfnR basolaterally (Breitfeld et al. 1989; Apodaca et al. 1994; Barroso and Sztul 1994; Gibson et al. 1998). Fluid phase cargo, which typifies the late endosome and lysosomal pathway, also enters the early sorting endosomes, but sorts to the lysosome and does not enter the common endosome. Thus, sorting to the late endosome/lysosome originates primarily from the early sorting endosomes (Apodaca et al. 1994). Established sorting destinations for membrane traffic out of the common/apical endosome of polarized cells include recycling back to the plasma membrane and by transcytosis to the contralateral membrane surface (Apodaca et al. 1994; Tzaban et al. 2009; Goldenring 2015). The mechanics for such selective and polarized sorting of cargo between recycling and transcytosis pathways likely overlaps with the machinery found in the *trans*-Golgi network (TGN) for the selective and polarized sorting of membrane proteins in the secretory pathway for membrane and secreted proteins of polarized cell types (Gan et al. 2002; Gravotta et al. 2007; Bay et al. 2015).

There is evidence for an additional endocytic compartment located immediately adjacent to the apical membrane, distal to the common endosome in the pathway for delivery of cargo to the apical cell surface, and termed the apical recycling endosome (ARE) (Apodaca et al. 1994; Casanova et al. 1999; van Ijzendoorn and Hoekstra 1999; Leung et al. 2000; Wang et al. 2000a,b; Lapierre et al. 2001; Lapierre and Goldenring 2005). The ARE appears to be

dedicated for apically directed recycling and transcytotic cargoes only. The physiologic significance of such a strict apically recycling compartment is emphasized by its ostensive role in regulating cell function, for example, fluid and ion transport across enterocytes (Forte et al. 1990; Casanova et al. 1999; Wang et al. 2000b; Tajika et al. 2004; Swiatecka-Urban et al. 2007); and in the biogenesis and maintenance of the apical membrane of intestinal and liver cells (Wakabayashi et al. 2005; Muller et al. 2008). Other lines of evidence, however, implicate the ARE and its functions as a sorting domain of the common recycling endosome, and not as a separate endocytic compartment (Sheff et al. 1999; Wang et al. 2000a; van Ijzendoorn 2006; Folsch 2008, 2015; Tanos and Rodriguez-Boulant 2008; Weisz and Rodriguez-Boulant 2009; Bay et al. 2015).

THE TRANSCYTOTIC PATHWAY

Molecular Mechanisms of Transcytosis

A number of proteins and genes have been implicated in the process of transcytosis, and common elements of the machinery for membrane transport in this pathway have begun to emerge. Here, we summarize the genes recently implicated in the pathway (Table 1).

First, the important roles of clathrin-mediated endocytosis (Hyman et al. 2006; Gravotta et al. 2007; Gonzalez and Rodriguez-Boulant 2009), microtubules (Jaulin et al. 2007; Perez Bay et al. 2013; Yui et al. 2013), myosin/actin (Tzaban et al. 2009), and the small GTPase ARF-6 (Shultz et al. 2006) were further implicated in the process. In MDCK cells (Shmuel et al. 2006), the ARF-6 exchange factor EFA6 was found to regulate apical-to-basolateral transcytosis of IgA (Shultz et al. 2006), possibly acting at the early sorting apical endosome. Knockdown of the Arp2/3 complex in the mouse small intestine caused defective post-endocytic vesicular trafficking and impaired transcytosis of IgG (Zhou et al. 2015). Silencing of the human inverted formin 2 (IFN-2) in HepG2 cells resulted in decreased apical sorting and transcytosis of GPI-APs (Madrid et al.

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Table 1. Genes and processes involved in transcytosis (2006–2016)

Family	Factors	Function	References
Clathrin adaptors	<i>AP-1B</i>	Sorting in the recycling and biosynthetic routes	Gravotta et al. 2007; reviewed in Gonzalez and Rodriguez-Boulan 2009
Nonclathrin	<i>Naked-2</i>	Cargo (TGF- α) recognition and targeting	Li et al. 2007
Actin	<i>EFA6</i>	ARF-6 GTPase-activating protein (GAP)/early endosome recruitment, regulation of IgA transcytosis	Shultz et al. 2006
	<i>INF-2</i> (human inverted formin 2)	Formation of actin filaments, regulation of apical transcytosis	Hyman et al. 2006; Madrid et al. 2010
	<i>Myosin Vb</i>	Common/apical recycling endosome	Swiatecka-Urban et al. 2007; Tzaban et al. 2009; Roland et al. 2011
Microtubules	Kinesin KIF16B	Tfn and pIgR transcytosis is microtubule dependent Common/apical recycling endosome	Perez Bay et al. 2013
Rab GTPases	<i>Rab11a</i>	Recycling endosome	Roland et al. 2011; Xu et al. 2011; Perez Bay et al. 2013; reviewed in Lapierre et al. 2012
	<i>Rab11a</i> family interacting protein	Rab11 effector, phosphorylation regulates tight junctions and transcytosis of Tfn	Ducharme et al. 2007, 2011; Lapierre et al. 2012
	<i>TBC1D9B</i>	Rab11a GAP, regulates IgA basolateral to apical transcytosis	Gallo et al. 2014
	<i>Rab35</i>	Docking of vesicles at apical plasma membrane and transcytosis	Mrozowska and Fukuda 2016
	<i>Rab25</i>	Transcytosis (common/apical recycling endosome)	Tzaban et al. 2009
SNAREs	Syntaxin-2	Interaction with sumyolated Rab17	Striz and Tuma 2016
	<i>VAMP-7</i> (<i>Ti-VAMP</i>)	Required for sorting of apical proteins and transcytosis	Pocard et al. 2007
	<i>VAMP-8</i>	Transcytosis to the apical membrane	Pocard et al. 2007
Retromer	Phosphoinositide-3 kinase	Controls retromer's role in pIgR–pIgA transcytosis	Verges et al. 2007
		Sorting of B-secretase in MDCK cells, TGF- β trafficking and localization to basolateral membrane	Cuartero et al. 2012; role of retromer reviewed in Yin et al. 2013; Verges 2016
Lipid rafts	<i>MAL</i> (myelin and lymphocyte protein)	Raft stabilizer, induces large clusters containing apical proteins	de Marco et al. 2002, 2006; Marazuela et al. 2004; Magal et al. 2009; In and Tuma 2010; Ramnarayanan and Tuma 2011
	Oligomerization	Increases GPI-APs affinity to DRMs and transcytosis to apical membrane	Galmes et al. 2013

Continued

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Table 1. *Continued*

Family	Factors	Function	References
Other	Galectin-4	Acts as an apical sorting signal and mediates TfR transcytosis	Perez Bay et al. 2014
	Ceramide	Transcytosis of oxLDL across the endothelial cell barrier	Li et al. 2014
	<i>Mfsd2</i>	Required for functional BBB; major transporter for DHA (omega-3 fatty acid) uptake into brain	Ben-Zvi et al. 2014; Nguyen et al. 2014; Guemez-Gamboa et al. 2015; Wang et al. 2016
	<i>Godzilla</i>	E3-ubiquitin ligase; Wingless signaling	Yamazaki et al. 2016.

2010). Cell polarity and apical transcytosis was also confirmed to be strongly dependent on specific microtubule motors (Jaulin et al. 2007; Perez Bay et al. 2013). These results strengthen the evidence for a central role of the actin and microtubule cytoskeletons in sorting cargo through the transcytotic pathway, as true for other endocytic pathways.

Other genes, some already known to be involved in membrane transport and others not, have newly been implicated in transcytosis. These include members of the SNARE fusion machinery—the v-SNAREs VAMP-7 and VAMP-8—which were found required for polarized sorting and transcytosis of transmembrane proteins and GPI-APs in FRT and Caco2 cells (Pocard et al. 2007). Another study showed that the plasma membrane-associated SNARE syntaxin-2 and its association with sumoylated Rab17 provides selectivity for syntaxin binding necessary for fusion of transcytotic vesicles at the apical surface (Striz and Tuma 2016). A role for retromer in polarized transport (Verges 2016) and in pIgR-pIgA transcytosis was also confirmed (Verges et al. 2004, 2007). Rab GTPase family members Rab 17, Rab 25, Rab 11, Rab 35, Rab 27 (Tzaban et al. 2009; Xu et al. 2011; Yasuda et al. 2012; Mrozowska and Fukuda 2016; Striz and Tuma 2016) and Rab 11-associated FIP family proteins (Ducharme et al. 2007, 2011; Su et al. 2010; Lapierre et al. 2012) were found required for epithelial cell polarization, vesicular transport to the apical plasma membrane, and transcytosis of IgA-pIgR, IgG-FcRn, and Tf-TfR. The *Drosophila* E3-ligase

Godzilla was discovered to affect Wnt signaling by affecting transepithelial transport of the molecule in the wing imaginal discs (Yamazaki et al. 2016). This gene product has not been previously implicated in membrane trafficking, and how Godzilla or any E3 ligase operates to affect transcytosis remains to be elucidated.

Evidence has also suggested that *N*-glycans and *O*-glycans act as luminal apical sorting signals in transcytosis, perhaps enabled by binding to the galectins such as galectin-3 (Honig et al. 2015) and galectin-4 (Perez Bay et al. 2014). Raft clustering, implicated in the biogenesis of apical membrane domains (Schuck and Simons 2004), may also contribute to transcytosis of GPI-APs (Galmes et al. 2013). The raft-associated protein MAL was found essential for enabling transcytosis of both pIgR and GPI-APs (de Marco et al. 2002, 2006).

Finally, in the context of endothelial cells, the major facilitator superfamily domain-containing protein 2A (*Mfsd2a*) was shown to cause a general down-regulation of transcytosis in brain endothelial cells. *Mfsd2a* is a Na⁺-dependent phospholipid transporter. How it operates as a master regulator of transcytosis remains unknown. Nevertheless, the diminished transcytotic pathway caused by expression of this gene in this cell type may in part functionally define the blood–brain barrier (Ben-Zvi et al. 2014).

Overall, a unified model explaining how sorting occurs in the apically and basolaterally directed transcytotic pathways has yet to emerge. Multiple mechanisms adapted to different cargoes and cell types will likely be found.

Transcytosis of the Immunoglobulins

Transcytosis for various cargoes (Table 2) has been documented in polarized epithelial and endothelial cells, neurons, and osteoclasts. The pathway is probably best studied for the immunoglobulin receptors, especially for IgA/IgM and IgG, which are transported by binding the polymeric immunoglobulin receptor pIgR (Apodaca et al. 1994; Rojas and Apodaca 2002) and the Fc-trafficking receptor FcRn (Baker et al. 2009; Pyzik et al. 2015), respectively (Table 2). In the case of transepithelial IgA transport, pIgR binds dIgA on the basolateral membrane and carries it sequentially into the early basolateral sorting endosome, the common/apical recycling endosome, and finally to the apical cell surface where the extracellular domain of the receptor is cleaved still bound to IgA for release into the lumen as secretory IgA (sIgA). Many of the regulatory components and structural elements of the pIgR required for

this pathway have been well reviewed (Rojas and Apodaca 2002; Apodaca et al. 2012). In contrast to pIgR, FcRn traffics immunoglobulin G (IgG) in both directions across polarized epithelial cells. Like pIgR, FcRn also traffics IgG through the common/apical recycling endosome (CRE), after which the actin motor myosin Vb and the GTPase Rab25 regulate a sorting step that specifies transcytosis. But these molecules do not affect the recycling of FcRn, suggesting their involvement in a sorting step specific to the transcytotic pathway (Tzaban et al. 2009). Another regulatory component of the CRE, Rab11a was found to be needed for transcytosis of pIgR (Bryant et al. 2010), but Rab11 was dispensable for transcytosis of FcRn (Tzaban et al. 2009). Thus, the two receptors likely do not use identical machinery for polarized sorting. In the case of FcRn, it appears that the cell can sort the receptor from the endosome to the cell surface via distinct recycling and transcytosis pathways. This model for separate transcyto-

Table 2. Cargoes undergoing transcytosis in polarized epithelia

Cargo	References
Immunoglobulins	
IgA/pIgR	Apodaca et al. 1994; Mostov 1994; Verges et al. 2004; Moon et al. 2014
IgG/ FcRn	Claypool et al. 2002; Li et al. 2011
IgE	Palaniyandi et al. 2015
EGF	Kozu et al. 1997; Maratos-Flier et al. 1987; Brandli et al. 1991
Endogenous cargoes	
Transferrin	Fishman et al. 1987; Roberts et al. 1993; Skarlatos et al. 1995; Broadwell et al. 1996; Descamps et al. 1996; Burdo et al. 2003; Du et al. 2013; Manich et al. 2013; Wiley et al. 2013;
Insulin	Azizi et al. 2015
DARC	Lee et al. 2003; Pruenster et al. 2009; Novitzky-Basso and Rot 2012; Minten et al. 2014
SorLA	Klinger et al. 2016
LDL	Candela et al. 2008; Bian et al. 2014; Li et al. 2014; Zhang et al. 2014
PI-CALM	Zhao et al. 2015
A1AT	Lockett et al. 2014
HTLV-1	Martin-Latil et al. 2012
Viral and bacterial pathogens	
P97	Tang et al. 2007
Botulinum toxin	Maksymowych and Simpson 1998; Ahsan et al. 2005; Miyashita et al. 2014
Listeria monocytogenes	Nikitas et al. 2011
Epstein–Barr virus	Tugizov et al. 2013
HIV	Dohgu et al. 2011; Gupta et al. 2013; Kinlock et al. 2014
Cholera toxin	Lencer et al. 1995; Saslowsky et al. 2013
Shiga toxin	Malyukova et al. 2008, 2009; Lukyanenko et al. 2011

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sis and recycling pathways is consistent with results of a targeted siRNA screen we recently completed in cultured MDCK epithelial cells for genes involved in transcytosis of FcRn (B Nelms and WI Lencer, unpubl.).

Other Proteins and Cargoes Undergoing Transcytosis

One of the important physiologic functions of transcytosis is to mediate transepithelial and transendothelial signal transduction. Insulin signaling, for example, requires transcytosis of the peptide hormone across tight endothelial barriers of adipose and muscle tissues, and this appears to require clathrin-dependent, not caveole-dependent, endocytosis (Azizi et al. 2015). Similarly, the Duffy antigen receptor for chemokines (DARC) acts as a trafficking chaperone to promote the transendothelial transport of inflammatory chemokines, including growth-related oncogene- α /CXC chemokine ligand 1 (GRO- α /CXCL1). This has been shown to be involved in regulating leukocyte migration and the inflammatory response (Lee et al. 2003; Pruenster et al. 2009; Novitzky-Basso and Rot 2012; Minten et al. 2014). The Vps10 family member SorLA, a transmembrane sorting receptor for several ligands including lipoprotein lipase and amyloid precursor protein, was recently found to cross MDCK cells by transcytosis from basolateral to apical membranes. The pathway intersects with the common endosome as measured by colocalization with TfR and may explain how lipoprotein lipase is secreted into breast milk (Klinger et al. 2016).

In some cases, transcytosis is implicated in the pathogenesis of human disease. Low-density lipoprotein (LDL) transcytosis across endothelial cells, for example, was recently linked to atherosclerosis and shown to be stimulated by C-reactive protein (Candela et al. 2008; Bian et al. 2014; Li et al. 2014; Zhang et al. 2014). Dietary DNA-fragments, even up to a few hundred base pairs, appear able to cross the intestinal barrier via vesicular transport to enter the bloodstream where they may function to regulate the immune system (Johannessen et al. 2013). Transcytosis of phosphatidylinositol-

binding clathrin assembly protein (PI-CALM) across the BBB has a role in brain homeostasis and is implicated in A β precursor protein (APP) clearance during Alzheimer's disease (Zhao et al. 2015). The glycoprotein serine protease inhibitor Alpha 1 antitrypsin (A1AT) modulates inflammatory responses in the lung endothelium. A recent report showed in vitro that A1AT is able to cross the lung endothelial barrier for delivery to adjacent epithelial cells from the circulation (Lockett et al. 2014).

Some microbes exploit transcytosis to cause disease (Bomsel 1997; Couesnon et al. 2008). The human T-cell leukemia virus type 1 (HTLV-1), the causative agent of adult T-cell leukemia/lymphoma, is one example of a pathogen that co-opts transcytosis to cross tight epithelial barriers and infect subepithelial dendritic cells (Martin-Latil et al. 2012). In another example, transcytosis of Botulinum toxin across T84-cell monolayers was recently visualized in vitro, confirming previous studies showing botulinum toxin transport across intestinal tissues (Maksymowych and Simpson 1998; Ahsan et al. 2005; Miyashita et al. 2014). *Listeria monocytogenes* undergoes transcytosis across the intestinal barrier on specific targeting of goblet cell E-cadherin (Nikitas et al. 2011). Epstein-Barr virus (EBV) appears to cross oral epithelial cells bidirectionally (Tugizov et al. 2013), and HIV transport across polarized epithelial cells may depend on IgG and trafficking by FcRn (Dohgu et al. 2011; Gupta et al. 2013; Kinlock et al. 2014). *Candida albicans* co-opts the transcytotic pathway of M cells as a portal of entry across the intestinal barrier (Albac et al. 2016). Human melanotransferrin (MTf, also named P97), a transferrin homolog undergoes basolateral to apical transcytosis and directs adeno-associated virus (AAV) transcytosis across the blood-brain barrier (Tang et al. 2007).

Clinical Applications

Transcytosis of membrane proteins may be harnessed for mucosal and transendothelial delivery of therapeutic peptides and proteins (Spiekermann et al. 2002; Li et al. 2011; Georgieva et al. 2014). Such transport of small molecule

drugs and larger biologics across polarized mucosal barriers for oral bioavailability is a major hurdle in drug development, and governed in part by the compartmentalized pathway and complex machinery involved in transcytosis (Fan et al. 2016). With the emerging fields of antibody-based therapeutics and oligonucleotide-based gene editing showing great promise, the failure of many biologic drug candidates to reach affected tissues underscores the importance of developing new technologies for drug delivery and for studies that further our understanding of the mechanisms responsible for transcellular transport (Juliano 2016; Parakh et al. 2016; Rabideau and Pentelute 2016).

Typically, large macromolecules either cannot enter the cell efficiently, or are internalized into endosomal compartments destined for lysosomal degradation (Pardridge 2015). Several approaches for drug delivery target endogenous protein receptors located on the apical membranes that enter the transcytotic pathways, such as TfR, FcRn, and insulin receptor (Lajoie and Shusta 2015; Martins et al. 2016). Here, the major application is to cross the endothelial microvasculature of the blood–brain barrier, with promising examples found with delivery of antibodies for A β amyloid (Niewoehner et al. 2014), and for delivering various lysosomal replacement enzymes (Pardridge 2015). For oral delivery via absorption through the intestinal epithelium, however, additional hurdles greatly impede drug delivery, such as the acidic gastric environment, an array of degradative enzymes, and the intestinal mucus layer. Recent advances in nanoparticle technologies can address some of these problems and engage the endocytic machinery via membrane receptors to cross enterocytes or M cells into the lamina propria (Yu et al. 2016). Overall, in spite of these challenges, it appears the transcytotic pathway of membrane proteins may be successfully exploited to deliver therapeutic molecules across polarized cell barriers.

Lipid Sorting in Polarized Epithelial Cells and Transcytosis

Several viral and bacterial toxins exploit membrane glycosphingolipids to breach epithelial

barriers by transcytosis (Lencer et al. 1995; Malyukova et al. 2008). Cholera toxin, for example, is found to traffic from apical to basolateral membranes of intestinal epithelial cells in vitro, and this depends on toxin binding via its B-subunit to its glycosphingolipid receptor GM1 (Lencer et al. 1995). The ability of cholera toxin to breach epithelial barriers by transcytosis may explain, in part, its well-known effects on mucosal vaccines (Stratmann 2015). The B-subunit of the toxin has the potential to bind up to five GM1 lipids at once, effectively cross-linking them in the plane of the membrane. We recently found that the structure of the ceramide domain of GM1 plays a decisive role in dictating the sorting and trafficking routes of the lipid in both polarized and nonpolarized cell types (Chinnapen et al. 2012; Saslowsky et al. 2013). In nonpolarized epithelial cells, GM1 species with short or unsaturated fatty acids traffic from the sorting endosome to recycling and late endosomes, the TGN, and to the ER, whereas long saturated chain GM1 were mainly found in late endosomes (Chinnapen et al. 2012). On cross-linking these lipids by binding the cholera toxin B-subunit, GM1 trafficking to the ER, and paradoxically also to the lysosome, is enhanced, possibly caused by association with lipid raft microdomains. In polarized cells, GM1 species containing short or unsaturated fatty acid chains traffic across the cell by transcytosis in the absence of toxin binding (Saslowsky et al. 2013). Transcytosis of GM1 does not require trafficking retrograde through the TGN (Saslowsky et al. 2013).

Experimental evidence has similarly implicated lipid sorting in the transcellular transport of Shiga Toxin-1 (Stx1) (Malyukova et al. 2008, 2009; Lukyanenko et al. 2011). Here, Stx1 binds to the globotriasylceramide Gb3 located on the apical outer leaflet of the membrane of host cells, and it is endocytosed by actin- and Src-dependent macropinocytosis. In this case, the B-subunit of Stx can cluster up to 15 Gb3 lipids greatly enhancing its endocytic and trafficking properties and specificities (Karve and Weiss 2014). When *Escherichia coli* O157/H7 strains that secrete Shiga toxins are allowed to colonize intestinal epithelial cells, shiga toxin is

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transported to basolateral membranes presumably via transcytosis of Gb3 lipids (Malyukova et al. 2008; Lukyanenko et al. 2011). Ceramide lipids have also been shown to affect the transcytotic pathway in endothelial cells for transport of oxidated low-density lipoprotein and albumin across the endothelial barrier in vitro and in vivo (Li et al. 2014; Kuebler et al. 2016).

How the cell recognizes and sorts the different ceramide domains of the different sphingolipid species remains unknown. The two major ideas in the field, that of curvature-dependent sorting by molecular shape and/or by association with membrane microdomains of lipid and protein mixtures connected to the membrane cytoskeleton (lipid rafts), or lack thereof, are plausible (Heinrich et al. 2010; Callan-Jones et al. 2011; Diaz-Rohrer et al. 2014). The former hypothesis is a biophysical sorting process, wherein the overall structural topology of the lipid dictates whether they can enter into sharp bends and turns in the early endosomal membrane that are needed to form sorting tubules to traffic to other cellular destinations; in effect, escaping the late endosome/lysosome pathway. The latter sorting mechanism is protein and lipid dependent, in which lateral heterogeneity in the membrane creates nanometer-sized domains of high order, and association with these domains is believed to lead to key signaling events, and connect to the endocytosis and sorting machinery. Glycolipid sorting could also be driven by lectin binding, such as that proposed for the galectins, and in clathrin-independent endocytosis of GM1 (Fajka-Boja et al. 2008; Lakshminarayan et al. 2014). None of these hypotheses are mutually exclusive, and it is believed that some processes may work within each other.

Clinical Applications

As for membrane proteins, lipid sorting pathways may also be harnessed for the clinical application of drug delivery via transepithelial or transendothelial transport. Our laboratory is currently developing an approach to deliver therapeutic peptides or proteins across a mucosal barrier that takes advantage of glycosphin-

golipid trafficking (te Welscher et al. 2014). Using short and unsaturated fatty acid containing GM1 as a trafficking platform, the cargo is covalently attached via the oligosaccharide head group. By fusion of nonnatural species of GM1 to glucagon-like peptide (GLP1), trans-epithelial transport was observed in vitro and in vivo. Subsequent studies on the structure of the ceramide chain show this approach to be robust (unpubl.). Another approach that co-opts endogenous glycosphingolipid trafficking uses a novel peptide that binds GM1 (Stojanov et al. 2012). When injected systemically, this peptide breeches the endothelial blood–brain barrier by transcytosis. In principle, both protein- and lipid-dependent trafficking across tight epithelial and endothelial barriers show promise for diverse clinical applications.

ACKNOWLEDGMENTS

We acknowledge the following funding sources: DK048106, DK0484424, and the Harvard Digestive Diseases Center DK034854. We also thank members of the Lencer laboratory for careful reading of the manuscript and for many helpful comments.

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Cold Spring Harb Perspect Biol 2017; doi: 10.1101/cshperspect.a027912 originally published online February 17, 2017

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