Lipid-Mediated Endocytosis

Helge Ewers and Ari Helenius

Cold Spring Harb Perspect Biol 2011;3:a004721 originally published online May 16, 2011

Lipid-Mediated Endocytosis

Helge Ewers[1] and Ari Helenius[2]

[1]Laboratorium für Physikalische Chemie, ETH Zurich, 8093 Zurich, Switzerland

[2]Institute of Biochemistry, ETH Zurich, 8093 Zurich, Switzerland

Correspondence: ari.helenius@bc.biol.ethz.ch

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Receptor-mediated endocytosis is used by a number of viruses and toxins to gain entry into cells. Some have evolved to use specific lipids in the plasma membrane as their receptors. They include bacterial toxins such as Shiga and Cholera toxin and viruses such as mouse polyoma virus and simian virus 40. Through multivalent binding to glycosphingolipids, they induce lipid clustering and changes in membrane properties. Internalization occurs by unusual endocytic mechanisms involving lipid rafts, induction of membrane curvature, *trans*-bilayer coupling, and activation of signaling pathways. Once delivered to early endosomes, they follow diverse intracellular routes to the lumen of the ER, from which they penetrate into the cytosol. The role of the lipid receptors is central in these well-studied processes.

Endocytosis is a general term for the internalization of particles, solutes, fluid, and membrane components by invagination of the plasma membrane (PM) and internalization of membrane vesicles. It plays a central role in the life of eukaryotic cells by mediating important aspects in the communication between the cell and the outside world, and by serving as a sensitive regulator of cell dynamics and homeostasis (Collinet et al. 2010). The endocytosed cargo includes nutrients, receptor-ligand complexes, lipids, antigens, membrane proteins, toxins, and pathogens, to name a few.

In this article, we limit our discussion to a special form of endocytosis; the internalization of viruses and bacterial toxins that use neutral and acidic glycosphingolipids (SP05/SP06) as receptors for endocytic uptake. They exploit these receptors and the endocytic machinery of the cell for infection and intoxication. The endocytic processes and intracellular trafficking routes differ in many respects from those used by most other cargo.

In a typical animal cell, several different mechanisms of endocytosis operate side by side. The most important for the present discussion are the clathrin-mediated and various lipid raft-dependent pathways because they are the main pathways taken by lipid-bound ligands. The clathrin-mediated pathway is continuously ongoing, whereas the others are ligand-induced and involve signaling pathways that engage tyrosine kinases and other cytosolic signaling factors (Sandvig et al. 2008; Mercer et al. 2010).

Editor: Kai Simons

Additional Perspectives on The Biology of Lipids available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a004721
Cite this article as *Cold Spring Harb Perspect Biol* 2011;3:a004721

GLYCOSPHINGOLIPIDS

Glycosphingolipids are synthesized in the Golgi apparatus and enriched in the outer bilayer leaflet of the plasma membrane (PM). They have bulky hydrophilic head groups composed of an oligosaccharide with up to seven monosaccharide units. Gangliosides (SP0601) constitute a subgroup of acidic glycosphingolipids (SP06) with one or more terminal sialic acids. The total ganglioside content of the plasma membrane (PM) can reach up to 1%–2%, with individual gangliosides in the range of 0.01%–0.1% (Levis et al. 1976; Ortegren et al. 2004; Sonnino et al. 2006). The glycosphingolipids are enriched in the apical domain of polarized cells (Crespo et al. 2008), and they are especially abundant in neurons (Sonnino et al. 2006).

The larger the oligosaccharide, the more likely gangliosides are to cluster in membrane regions of positive curvature (Sonnino et al. 2007). The preference for membrane curvature may be the reason, why gangliosides can be detected in the neck region of caveolae (Parton 1994). Exogenously added gangliosides enter caveolae (Fra et al. 1995; Pitto et al. 2000), and are endocytosed through these (Choudhury et al. 2006), but this association is transient and not observed under steady state conditions (Chigorno et al. 2000).

Because of the high content of long, saturated alkyl chains and the propensity to associate with other sphingolipids and cholesterol, glycosphingolipids are thought to promote the formation of lipid domains with reduced fluidity and tight packing (Tsamaloukas et al. 2006; Sonnino et al. 2007). This process has been studied extensively in artificial membranes that have a lipid composition similar to that of the PM (McConnell and Vrljic 2003; Feigenson 2007; Blanchette et al. 2008).

LIGANDS THAT BIND TO GLYCOSPHINGOLIPIDS: BACTERIAL TOXINS AND VIRUSES

Endocytosed ligands known to bind to glycosphingolipids include certain bacterial toxins, viruses, lectins, and antibodies (Schengrund 2003). Unlike other toxins and viruses that use cell surface proteins as their receptors, those that bind to glycosphingolipids are special because they are usually transported to the endoplasmic reticulum (ER) from where they penetrate into the cytosol. Once in the cytosol, the bacterial toxins and plant lectins manifest their toxicity by interfering with critical cell functions such as protein translation and regulation of membrane traffic (Sandvig et al. 2010). The viruses take over the cellular machinery for their own replication. After entering the ER, the best studied of the viruses—the mouse polyoma virus (mPy) and simian virus 40 (SV40)—continue their voyage to the nucleus to replicate.

Tables 1 and 2 list some of the pathogenic ligands in question and some of their properties. In the case of Cholera (CTX) and Shiga

Table 1. Toxins that use glycosphingolipids as cell surface receptors

Bacterial toxins		
Cholera toxin *Vibrio cholera*	A_1B_5	GM1; Heyningen 1974
Shiga toxin *Shigella dysenteriae*	$A_1A_2B_5$	Gb3 (CD77); Lindberg et al. 1987
Shiga-like toxins (SLT1 and SLT 2) *Escherichia coli*	$A_1A_2B_5$	Gb3 (CD77)
Heat labile toxin 1 *E. coli*	A_1B_5	GM1; Lencer and Saslowsky 2005
Heat labile toxin IIb *E. coli*	A_1B_5	GD1a
Tetanus neurotoxin TeNT *Clostridium tetani*	GT1b, GD1b, GQ1b, etc.	Brunger and Rummel 2009
Botulinium toxin BoNT *Clostridium botulinum*		GD1a, GT1b, etc.
Plant toxins		
Ricin	AB	Protein and lipid-linked galactosides; Sandvig and van Deurs 2000
Abrin	AB	Same

Table 2. Viruses that use glycosphingolipids as cell surface receptors

Nonenveloped viruses	Organization	Specificity
Simian virus 40	VP1 pentamers	GM1
Murine polyomavirus	VP1 pentamers	GD1a, GT1b
BK virus	Likely VP1 pentamers	GD1b, GT1b
Merkel cell polyomavirus	Likely VP1 pentamers	GT1b
Murine norovirus	VP1?	GD1a

(STX) toxins, the glycosphingolipids GM1 (Heyningen 1974) and globotriasylceramide, Gb3 (Lindberg et al. 1987), respectively, are likely to be the main receptors needed for endocytosis. In the case of the clostridium neurotoxins, TeNT and BoNT, polysialic gangliosides serve as attachment receptors. They concentrate the toxins on the cell surface, and promote subsequent binding to specific protein receptors such as synaptotagmin expressed in presynaptic membranes (Montecucco et al. 1996; Brunger and Rummel 2009).

Most of the toxins in Table 1 contain two subunits (A and B). These are usually derived from a common precursor protein through cleavage by target cell proteases. The A-subunits are the actual toxins or effectomers delivered to the cytosol. The B-subunits are so called haptomers responsible for binding to the carbohydrate moiety of GSLs and internalization. The B-subunits are often five in number, and organized as a flat ring with a single A-subunit on one side and the glycan binding sites on the other (Fig. 2). The Clostridial neurotoxins differ in having a single (i.e., nonpentameric) binding subunit, but they have additional coreceptor binding sites (Binz and Rummel 2009; Brunger and Rummel 2009).

Although many animal viruses bind to carbohydrate moieties of cell surface glycoconjugates, only few interact with glycosphingolipids and use them as receptors (Table 2). For the polyoma virus family, glycosphingolipids are likely to constitute primary receptors, but a role for additional coreceptors cannot be excluded. Polyomaviruses are nonenveloped DNA viruses that replicate in the nucleus. Several members of the family are recognized as human pathogens.

The viral capsids are icosahedral with 72 VP1 protein pentamers serving as basic structural units (Liddington et al. 1991; Stehle et al. 1994). The VP1 pentamers carry five glycosphingolipid binding sites as shown in Figure 1. There is no sequence homology between VP1 and the bacterial toxins, but the overall pentameric structures are remarkably similar compared to the equally pentameric, membrane-binding B-subunits of STX and CTX, so called AB5 toxins (Fig. 1). Especially striking is the similar organization of binding sites always spaced about 3 nm from each other. Noroviruses are RNA viruses and replicate in the cytosol, but little is known about their entry mechanisms.

INITIAL BINDING

Binding of viruses and toxins to glycosphingolipids is highly specific. In gangliosides, the sialic acids play a central role in defining the specificity. In the case of SV40, it is interesting to note in this context that a single atom in the carbohydrate moiety can make a difference. Thus N-acetyl sialic acid in human GM1 provides less efficient binding to cells and less efficient infection than the N-glycolyl group present in simian GM1 (Campanero-Rhodes et al. 2007).

The crystal structures available for the toxins and viruses in association with their receptors show that 2–3 of the saccharides in the glycosphingolipid contact the protein subunit directly (Merritt et al. 1994; Stehle et al. 1994; Neu et al. 2008, 2009). Although the affinity of glycosphingolipid binding is often relatively low, the avidity of toxin and virus binding to membrane bilayers and cells is high because of the multivalency of binding (Mammen et al. 1998). The avidity of CTX to GM1-containing supported membrane bilayers is, for example, three-orders of magnitude higher than its affinity to the carbohydrate moiety of GM1 in solution (Schon and Freire 1989; Kuziemko et al. 1996; Turnbull et al. 2004). A mutant cholera

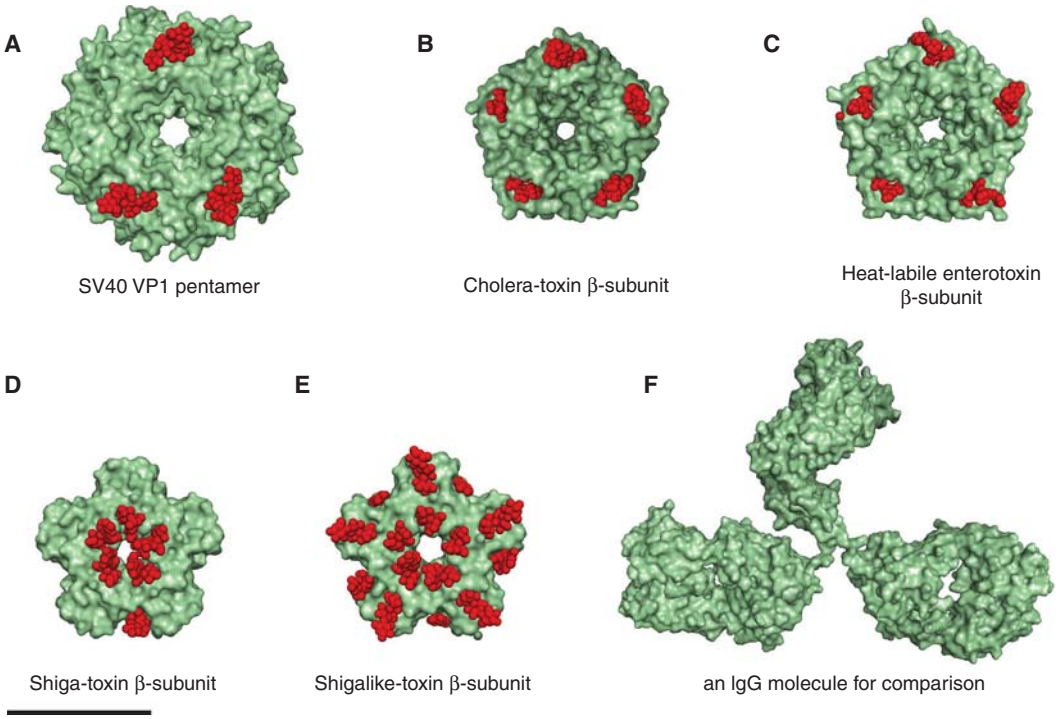

Figure 1. Organization of binding sites in ganglioside ligands. (*A*) SV40 coat protein VP1 pentamer cocrystallized with GM1 pentasaccharide, three of five binding sites are occupied in the structure. PDB-file: 3bwr. (*B*) Pentameric cholera toxin β-subunit cocrystallized with GM1 pentasaccharide, PDB file: 1ct1. (*C*) Pentameric *Escherichia coli* heat-labile enterotoxin β-subunit cocrystallized with nitrophenyl-galactoside, PDB file: 1pzi. (*D*) Pentameric Shiga toxin β-subunit cocrystallized with GB3 trisaccharide, six of up to 15 reported binding sites are occupied. PDB file: 1cqf. (*E*) Pentameric *Escherichia coli* Shigalike toxin β-subunit cocrystallized with a GB3 analog, PDB file: 1bos. (*F*) Bivalent antibody (*Mus musculus* IgG2a), PDB file: 1igt. The structures are shown to scale. In some crystals, not all binding sites are occupied by ligands. (Scale bar, 5 nm.)

toxin with a reduced number of functional binding sites shows reduced affinity for cell membranes, and it is endocytosed less efficiently (Wolf et al. 2008). The individual binding sites of SV40 for the carbohydrates in GM1 have a K_d in the millimolar range as estimated in ligand-soaked crystals (Neu et al. 2008), but binding of SV40 virions to GM1 containing membranes is virtually irreversible (H Ewers and A Helenius, unpubl.).

ASSOCIATION OF GSLs AND LIGANDS WITH LIPID DOMAINS

Individual gangliosides diffuse in the plasma membrane of cells with a diffusion constant (D) in the range of a few μm^2/sec. Interaction with cholesterol leads to short-lived confinement in the nanometer range (Spiegel et al. 1984; Eggeling et al. 2009; Sahl et al. 2010). The diffusion of GM1-bound CTX is more than an order of magnitude slower than that of free GM1 in supported membrane bilayers, plasma membrane vesicles, and cellular membranes (Spiegel et al. 1984; Kenworthy et al. 2004; Burns et al. 2005; Lingwood et al. 2008; Eggeling et al. 2009). The lateral mobility of GSL-bound polyomaviridae is even lower (Ewers et al. 2007; Kukura et al. 2009).

Why the diffusion of lipid-bound ligands is reduced to such an extent is not clear, especially because the binding of antibody to lipids does not result in a similar reduction (Lee et al. 1991). Interestingly, the binding of cholera

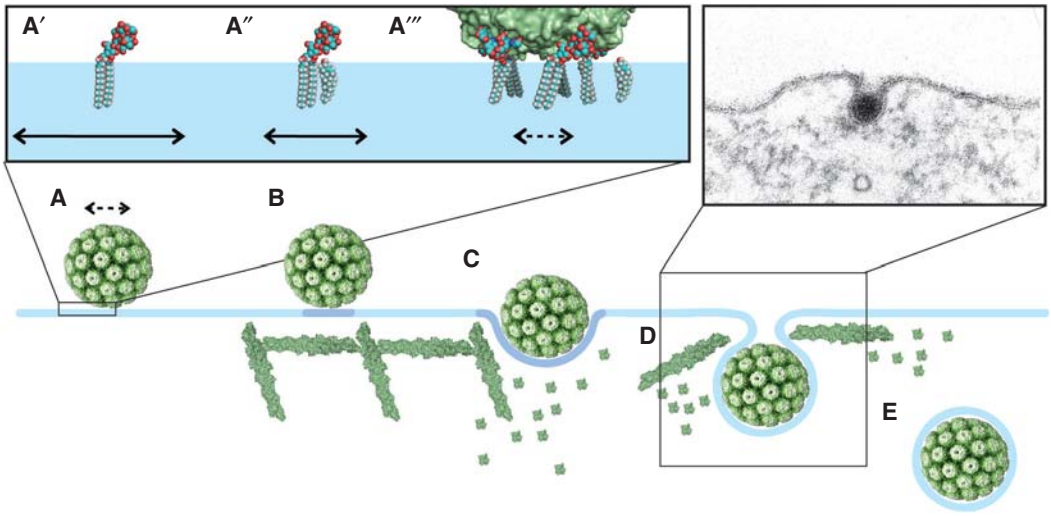

Figure 2. First steps in SV40 endocytosis. (*A*) Binding to GM1 and lateral motion in the plane of the plasma membrane, (*B*) formation of a membrane microdomain "lipid raft" and actin-dependent immobilization, (*C*) invagination of the plasma membrane, (*D*) recruitment of dynamin-independent scission machinery (actin?) and tyrosine kinase-dependent internalization, and (*E*) intracellular transport. (*Insets*) (*Left*) GM1 mobility in membranes. Individual GM1 molecules undergo fast diffusion in cholesterol-free membranes (*A′*), but are transiently confined in nanoscopic domains in the presence of cholesterol (*A″*). When SV40 binds to GM1, diffusion speed is greatly reduced (*A‴*) and the SV40-GM1-complex likely recruits cholesterol to form a membrane microdomain or "lipid raft." (*Right*) Individual SV40 virions bound to cells seem to imprint their shape onto the plasma membrane and are found in small, tight-fitting invaginations. Image of SV40 courtesy of Jürgen Kartenbeck. Structures used for this figure: SV40: 1sva; SV40 pentamer: (Campanero-Rhodes et al. 2007); actin:1j6z, 3b63.

toxin to GM1 strongly reduces the diffusion of other lipid molecules in the same membrane (Forstner et al. 2006). The binding interface between CTX and SV40 pentamers and the membrane is quite tight, with the protein surface contacting the top bilayer leaflet (Campanero-Rhodes et al. 2007; Neu et al. 2008). This may be important for the inclusion into membrane microdomains or for *trans*-bilayer coupling.

Single particle tracking has shown that when incoming mPy viruses bind to the PM of cells, they first undergo random lateral movement for 5–15 sec with a diffusion constant of $\sim$0.01 μm^2/sec (Ewers et al. 2005). The permanent or transient loss of mobility after the initial phase of free diffusion depends on the actin cytoskeleton. In the presence of inhibitors of actin polymerization, the lateral diffusion continues without confinement. The reason for the confinement could be *trans*-membrane interactions with cortical actin. Virus-receptor complexes could, because of their size, also be confined by a "picket fence" of *trans*-membrane molecules that are anchored to the actin meshwork and act as obstacles to lateral diffusion (Kusumi et al. 2005). Alternatively, the confinement could be explained by the virus-induced invagination of the plasma membrane described below. Although the viruses do not enter coated pits, a minority population is trapped in caveolae in cells that express caveolin-1.

In supported membrane bilayers, the viruses are highly mobile, no confinement is observed (Ewers et al. 2005; Kukura et al. 2009). However, an exception can be observed when the bilayer contains high amounts of GM1 (1 mol %). Instead of undergoing lateral motion, SV40 virions wobble back and forth with a step size of a few nanometers, likely by oscillating between

adjacent VP1 pentamers (Kukura et al. 2009). How virions become immobilized in such a way is not clear. A binding-induced membrane deformation or local patches of gangliosides interacting across the bilayer with the substrate may be involved.

The clustering of glycosphingolipids induced by the viruses and the toxins has a general effect on membrane lipids and their organization. Association of CTX with GM1 in the PM, for example, increases membrane order (Gaus et al. 2006). It also leads to lateral segregation of CTX-containing liquid-ordered membrane domains from more liquid-disordered membrane both in PM-blebs (Lingwood et al. 2008) and in artificial membranes (Hammond et al. 2005). Binding of CTX also increases the melting temperature (Forstner et al. 2006) and viscosity (Yamazaki et al. 2005) of supported membrane bilayers. Furthermore, after binding to the PM of cells, CTX, STX, and SV40 are found to associate with detergent-resistant membranes (DRM) (Falguieres et al. 2001; Wolf et al. 2002; Damm et al. 2005). Both intact SV40 particles and isolated VP1 pentamers cluster when bound to GM1 in artificial membranes (Ewers et al. 2010). If such membranes have liquid-ordered and liquid-disordered phases, they accumulate in the liquid-ordered membrane (Ewers et al. 2010).

For many of these phenomena, the detailed structure of the GM1 is important. Only GM1 molecules with long alkyl chains support partitioning of virus with the liquid-ordered phase in vitro, and thus promote local enrichment of virion-receptor complexes (Ewers et al. 2010). Studies using GM1 supplementation into mutant cells that do not contain glycosphingolipids of their own have, moreover, shown that although GM1 supports SV40 binding independently of its hydrocarbon chains, only those with long and preferably saturated alkyl chains promote association with the liquid-ordered phase (Ewers et al. 2010). Unlike GM1 with short alkyl chains, they also allow endocytosis and infection.

These findings are consistent with the view that clustering of glycosphingolipids with long aliphatic chains by multivalent ligands in the presence of cholesterol can drive the nucleation of membrane domains (i.e., so-called lipid rafts [Hancock 2006; Lingwood and Simons 2010]). The spacing of glycosphingolipid binding sites may be important given that the ~30 Å distance is virtually identical in the polyomaviruses and the pentamer-containing toxins. In antibodies, the two binding sites are arranged flexible to one another and antibodies to GM1 indeed fail to induce lateral segregation of membranes (Ewers et al. 2010).

INDUCTION OF CURVATURE

Electron microscopy studies show that compared with other viruses, the association of mPy and SV40 with the plasma membrane is unique in that the space between the surface of the virus and the membrane is almost nonexistent (Hummeler et al. 1970; MacKay 1976; Maul et al. 1978). The reason for this is no doubt that the receptors are lipids, and the binding sites in the virus are not located on spikes or extensions. In addition, these viruses often occur in invaginations in which the membrane contour gives the impression that the viruses are "budding" into the cell (Fig. 2). The endocytic vesicles formed from these inward "buds" are tight-fitting, with the virus particle occupying all of the inner volume.

We have recently shown that SV40 actively induces membrane curvature. This is most dramatically illustrated by the effects observed when the pinching-off of SV40-containing vesicles from surface buds is inhibited by ATP depletion of the cell. The small invaginations containing single virions grow into elongated, PM-attached, virus-filled membrane tubules extending far into the cytoplasm (Ewers et al. 2010). Under ATP-depletion conditions, STX, CTX, and SV40 VP1 pentamers also induce similar tubules indicating that pentameric glycosphingolipid binders are sufficient to induce the curvature (Römer et al. 2007; Ewers et al. 2010).

Tubule formation by these ligands can also readily be observed in giant unilamellar vesicles (GUVs) containing glycosphingolipid receptors. After addition of SV40 or any of

the pentameric ligands, long inward-directed tubules are formed emanating from the limiting membrane. Both in cells and in GUVs the same rules seem to apply; for SV40 and VP1 pentamers to form clusters and tubules the GM1 must have long aliphatic chains.

How do virions and AB5 toxins manage to overcome membrane tension and induce such dramatic membrane curvature? Substantial forces are required for the deformation of membranes into tubes and vesicles. In receptor-mediated endocytosis, the intracellular domains of *trans*-membrane proteins provide sites for coatomers to bind to and to deform membrane into vesicles. In the case of lipid-mediated endocytosis, the interactions leading to curvature must occur on the external surface of the plasma membrane. How is force generated under these circumstances?

In the case of virions, the binding energy between VP1 and GM1 is likely to play an important role. The virus has 360 GM1 binding sites. They are arranged 30 Å apart in a pentamer, and the pentamers are evenly distributed 9–10 nm apart over the surface of the 50 nm diameter particle. The virus can "wrap" itself in membrane by zipper-like association of GM1 molecules to an increasing number of binding sites. Membrane invagination is a consequence of the spatial organization of the binding sites on a spherical surface. If abscission does not take place—as is the case in energy-depleted cells and GUVs—a second virion can use the existing invagination and thus induce the formation of a tubule. Additional virions will allow the emerging structure to grow, resulting in the formation of long, virus-filled tubules.

In addition to the energy provided by the multivalent binding of GM1 to the virus surface, it is possible that the local accumulation of gangliosides under viruses or AB5 toxins generates a lipid domain with a composition distinct from the bulk membrane. This in turn will induce line tension within the membrane, which may lead to membrane invagination. Such deformation helps to reduce the length of the phase boundary, and thus the energy of line tension (Baumgart et al. 2003). Once the membrane is deformed, the induction of curvature by the bound virus and AB5 toxin can further enhance phase separation, which may act as a feedback loop (Parthasarathy et al. 2006).

The physical mechanisms may not be identical for virions and toxins (Ewers et al. 2010). Although for virions the binding energy is important because of the intrinsic curvature of the particle, the line tension is likely to be the dominant driving force for AB5 toxins (Ewers et al. 2010). This may explain why the induction of curvature is faster for the virus than for the toxins, and viral particles can overcome higher membrane tension.

SCISSION OF ENDOCYTIC VESICLES

In artificial membranes composed of domain-forming lipid mixtures, line tension can lead to fission of vesicles from invaginations when the system is cooled rapidly (Baumgart et al. 2003; Allain et al. 2004; Roux et al. 2005; Rozovsky et al. 2005). When energy-depleted cells with STX-induced tubules are cooled, STX becomes internalized as well (Römer et al. 2010) suggesting a role for line tension in cellular membrane fission. Under normal growth conditions however, AB5 toxins and virions can deform cellular membranes without the need of cellular energy consumption, but the scission reaction needed to generate an endocytic vesicle requires the participation of cellular factors and energy in the form of ATP or GTP.

Liu et al. describe a general model for eukaryotic endocytosis in which lipid-binding proteins and coatomers protect the forming vesicle and the neck of the invagination respectively from phosphatases that digest phosphatidylinositol-4,5-bisphosphate (PIP2) specifically at the ingression between these domains (Liu et al. 2009). Spatially constricted removal of PIP2 may in turn lead to an interfacial force or line tension sufficient for fission. Line tension-mediated fission could thus be a common principle of membrane scission events. At the same time, the requirement of organized parallel threads of interacting molecules during

this process ensures tight control of this important cellular process.

That the scission of STX and SV40 containing tubules in cells depends on the presence of cholesterol in the plasma membrane argues for a role of line tension as well (Römer et al. 2007; Ewers et al. 2010). Nevertheless, cholesterol and ganglioside clustering alone are not sufficient for fission, as STX, CTX, or SV40-containing tubules are stable in cholesterol-containing GUVs. Some cellular factors and energy are required to induce fission. In the case of STX tubules, the polymerization of actin is sufficient to induce fission in GUVs (Römer et al. 2010), but in cells the situation likely is more complex. What factors are involved in the scission of vesicles in lipid-mediated endocytosis as described here is unclear.

The scission of vesicles in cells is often catalyzed by dynamin, a GTPase that binds to the highly curved membrane in the stalk region of the forming vesicle (Bashkirov et al. 2008). However, there are endocytic mechanisms that work independently of dynamin. The endocytosis of SV40 seems to belong to these. Whereas STX and CTX can be internalized by multiple pathways, the tubular pathway described here is dynamin-independent as well.

Studies on SV40 entry indicate that scission is the result of a complex series of processes that depend on *trans*-bilayer coupling and activation of tyrosine and other kinases. Given that the virus interacts with receptors only in the outer lipid leaflet of the plasma membrane, one must assume that a *trans*-membrane signal is generated by virus binding. Whether the coupling involves the lipids alone or also proteins on the inside surface of the PM remains to be determined. A fraction of virions enters caveolar invaginations that are rich in signaling molecules at the plasma membrane (Anderson et al. 1996, 1998; Stang et al. 1997), and the addition of SV40 particles activates caveolar trafficking (Tagawa et al. 2005). However, while shRNA against Caveolin-1 reduces infection significantly in HeLa cells (Pelkmans et al. 2005), Caveolin-1 deficient cells are readily infected and internalize SV40 at an even faster rate than wt cells (Damm et al. 2005). In many cell types, the caveolin-independent route is likely to represent the main route of endocytosis leading to infection. Coupling may be induced simply by the membrane curvature resulting from viral binding, as highly curved membranes attract and increase the activity of many important molecules in membrane traffic (Bigay et al. 2003; Yoshida et al. 2004; McMahon and Gallop 2005; Frolov and Zimmerberg 2008; Lundmark et al. 2008). However, it is well documented that tyrosine- and ser/thr kinases are activated, and phosphatase inhibitors elevate vesicle formation (Richards et al. 2002). In the case of STX and CTX, binding to the plasma membrane can activate Src-family kinases (SFKs) that co-purify in detergent resistant membrane fractions (Katagiri et al. 1999) and regulate endocytosis (Lauvrak et al. 2006).

TRANSPORT AND SORTING IN THE ENDOCYTIC PATHWAY

Although lipid-bound ligands are internalized by several, possibly parallel endocytic mechanisms, they all seem to deliver their cargo to the endosome network. The main compartments in this complex and interconnected system are shown in Figure 3. Early endosomes (EEs) serve as the portal of entry from the plasma membrane, and constitute the main sorting compartment. Late endosomes (LEs) provide a unidirectional feeder function to lysosomes. They fuse with these to form endolysosomes, in which the endocytic cargo is degraded. In addition, many cell types have perinuclear recycling endosomes (REs) that mediate recycling of membrane components and fluid to the plasma membrane. Endosomes are connected with each other by vesicle traffic and direct fusion, and with the TGN and the Golgi complex by bidirectional membrane traffic. There is also trafficking from lysosomes to the ER directly, but it is not clear to what extent such traffic is vesicular.

The sorting of cargo from EE to LEs depends on a complex maturation process whereby the vacuolar parts of EEs are converted to LEs. The conversion involves a gradual drop in pH, formation of intralumenal vesicles, Rab

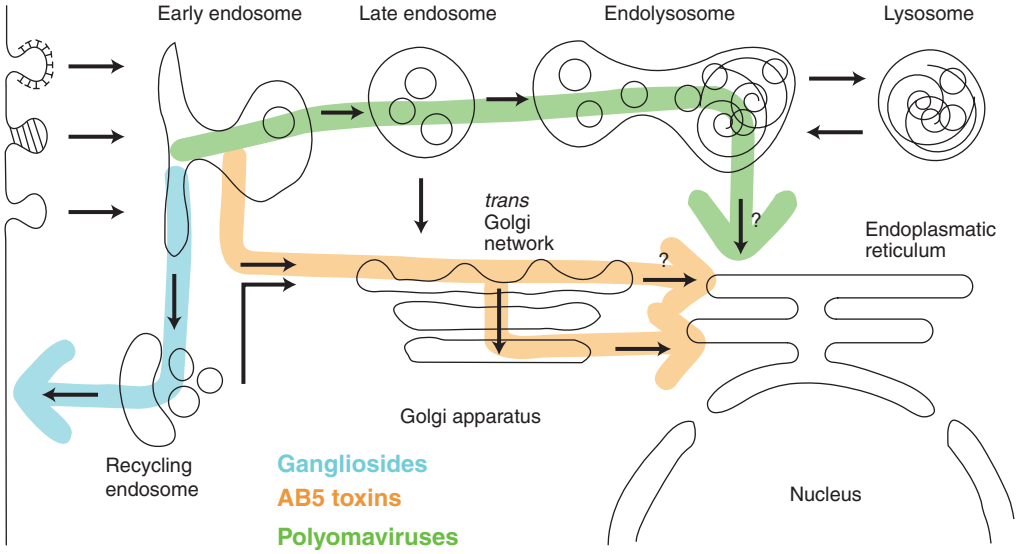

Figure 3. Trafficking in the endocytic pathway. The main organelles are EEs, LEs, REs, and lysosomes. EEs are complex organelles with tubular and vacuolar domains. The vacuolar domains dissociate with their contents and undergo microtubule-mediated, dynein-dependent movement to the perinuclear region. They mature to LEs, which can fuse with each other and eventually with lysosomes generating endolysosomes. At the level of EEs, LEs, and REs, the endocytic pathway is connected to the TGN and the Golgi complex by vesicle transport and possibly with the ER at the level of endolysosomes. The main trafficking pathways are emphasized for mono- or paucivalent gangliosides (blue), AB5 toxins (red), and polyomaviruses (green).

switching, microtubule-mediated movement, phosphatidylinositide conversion, and acquisition of acid hydrolases.

SORTING IN ENDOSOMES

The lipid-binding toxins, STX and CTX, are both transported via EEs and the TGN to the lumen of the ER. A detailed discussion of the literature describing these phenomenona can be found in recent reviews (Chinnapen et al. 2007; Johannes and Romer 2010; Sandvig et al. 2010). STX reaches peripheral EEs 5 min, perinuclear EEs in 10–20 min, TGN and Golgi complex in 30–45 min, and the ER in 180 min or longer (McKenzie et al. 2009). Cholesterol is essential for trafficking, and the STX remains associated with detergent resistant membranes (Falguieres et al. 2001).

As recently discussed (Sandvig et al. 2009; Johannes and Romer 2010), the EE to Golgi transport step requires a spectrum of cellular factors some of which are activated by the toxin and its association with the cell (Mallard et al. 1998). The retromer complex and its subunits (SNX1 and 2, VPS), known to promote EE to Golgi transport of cellular proteins (Rojas et al. 2008), seem to play an important role. However, transport also depends on activation of signaling pathways involving at least two kinases (p38a and protein kinase C δ) and remodeling of the EE compartment through the association of arrestins and p38a with each other and with EEs. In addition, there is evidence that further components such as clathrin, dynamin-2, epsinR, and syntaxins 6 and 16 play a role. The dependence on actin and microtubules shows that the cytoskeleton is also important (Valderrama et al. 2001; Luna et al. 2002; Duran et al. 2003). A small fraction (5%–10%) of the STX has been shown to escape already at the endosomal level (McKenzie et al. 2009).

The retrograde transport from the Golgi complex to the ER is the slowest step in the pathway. Unlike cholera toxin, STX does not have a

carboxy-terminal KDEL sequence. Both COPI-dependent and independent pathways to the ER have been proposed (Girod et al. 1999).

The transport of CTX to the ER has many similarities with STX (Schapiro et al. 1998; Lencer and Saslowsky 2005). CTX is transported via the TGN to the ER. Although it has a carboxy-terminal KDEL sequence that allows binding to the retrograde transport receptor, the KDEL receptor, it is apparent that the use of this receptor is not essential for intoxication by CTX. The toxin has five binding sites for GM1 and its internalization and transport is dependent on multivalent binding to these lipids. It has been suggested that it induces or uses a lipid-dependent pathway from the PM to the ER (Chinnapen et al. 2007).

INTRACELLULAR TRAFFICKING OF GLS BOUND VIRUSES

After internalization by small monopinocytic vesicles, the various polyomaviruses are also delivered to the ER. However, this is preceded by slow passage through endocytic compartments (Liebl et al. 2006; Qian et al. 2009; Engel et al. 2011). The first station in the entry of mPy and SV40 seems to be the EE followed by either the RE or LE and endolysosomes. Eventually, from these endocytic organelles, the viruses move by poorly defined mechanisms to the ER (Kartenbeck et al. 1989; Qian et al. 2009). For mPy and SV40, the best characterized of the polyoma viruses, the transport is slow. It takes 4–10 h before the viruses reach the ER, and passes into the cytosol and nucleus (Schelhaas et al. 2007). Passage through the Golgi complex is unlikely in this case but cannot be ruled out.

PERSPECTIVES

The interaction of toxins and viruses with their target cells provides powerful systems to study animal cells. The incoming pathogens make use of numerous cellular mechanisms and pathways that are otherwise difficult to study. Intoxication and infection provide easily quantifiable biological read-outs for the successful outcome of the complex entry process.

In the case of the toxins and viruses discussed in this review, the breadth of existing data is already impressive. It ranges from detailed structural information on receptor/ligand complexes, to genetic analysis, in vitro systems, signal transduction, and intracellular membrane trafficking. The dependence of these particular ligands on lipids and the detailed biophysical properties of bilayers make them useful as tools in the study of lipids and membranes for which there are few powerful biological model systems. In particular, when combined with imaging techniques, they allow studies addressing dynamic aspects of membranes in live cells. To single out one aspect that deserves further study, the valency of binding of these ligands to gangliosides has important functional consequences. The multivalency is apparently the underlying principle that triggers lipid domain formation, membrane invagination, *trans*-bilayer coupling, signaling, and intracellular routing.

First of all, CTX, SV40 pentamers, and SV40 particles coalesce in clusters on GUVs, what antibodies to GM1 do not. Furthermore, CTX, SV40 pentamers, and SV40 induce tubules in GUVs whereas cell and antibodies to GM1 do not. This discrepancy leads to dramatic differences in intracellular trafficking. Although antibody-bound gangliosides are recycled to the PM, cholera-toxin clustered GM1 is routed from perinuclear endosomes via the Golgi apparatus to the ER (Crespo et al. 2008). On the other hand, SV40 virions, binding to the same receptor as CTX or the antibody, seem to bypass the Golgi on the way to the ER (Fig. 3). Interestingly, when quantum dots are functionalized either with GD1a antibodies or cholera toxin, they are not recycled like ganglioside antibodies or transported to the Golgi apparatus like cholera toxin, but sorted to the ER, suggesting that the three-dimensional organization of binding site on colloids may play a role in intracellular sorting (Tekle et al. 2008; Qian et al. 2009). A similar clustering dependent defect of trafficking to the Golgi has been observed for ricin (van Deurs et al. 1986).

As the important role of lipids in the mechanics of endocytosis is gaining more deserved attention (Sens et al. 2008; Frolov and Zimmerberg 2010; Liu et al. 2010), the well-understood systems described here will allow the investigation of important questions in membrane biology. What is the minimum requirement for membrane domain formation after binding? What is the composition of binding-induced microdomains? How is *trans*-bilayer coupling achieved in the absence of *trans*-membrane proteins? What confers identity to the lipid-ligand containing vesicles pinching off the plasma membrane and how do they acquire fusion machinery? How are cellular lipid environments tuned for the formation of different microdomains?

ACKNOWLEDGMENTS

We discussed here a wide and complex field and unfortunately could not cite all relevant literature. For convenience of the reader new to this field, we cited several reviews on different aspects, in which more detailed references can be found. We apologize to our colleagues whose work was not cited. A.H. is supported by grants from the Swiss National Research Fund, the Swiss SystemsX.ch initiative, grant LipidX-2008/011LipidX, the National Institutes of Health, US, by An ERC advanced grant, and by ETH Zurich. H.E. is supported by the NCCR Neural Plasticity and Repair.

REFERENCES

Allain JM, Storm C, Roux A, Ben Amar M, Joanny JF. 2004. Fission of a multiphase membrane tube. *Phys Rev Lett* **93:** 158104.

Anderson HA, Chen Y, Norkin LC. 1996. Bound simian virus 40 translocates to caveolin-enriched membrane domains, and its entry is inhibited by drugs that selectively disrupt caveolae. *Mol Biol Cell* **7:** 1825–1834.

Anderson HA, Chen Y, Norkin LC. 1998. MHC class I molecules are enriched in caveolae but do not enter with simian virus 40. *J Gen Virol* **79:** 1469–1477.

Bashkirov PV, Akimov SA, Evseev AI, Schmid SL, Zimmerberg J, Frolov VA. 2008. GTPase cycle of dynamin is coupled to membrane squeeze and release, leading to spontaneous fission. *Cell* **135:** 1276–1286.

Baumgart T, Hess ST, Webb WW. 2003. Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension. *Nature* **425:** 821–824.

Bigay J, Gounon P, Robineau S, Antonny B. 2003. Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature. *Nature* **426:** 563–566.

Binz T, Rummel A. 2009. Cell entry strategy of clostridial neurotoxins. *J Neurochem* **109:** 1584–1595.

Blanchette CD, Lin WC, Orme CA, Ratto TV, Longo ML. 2008. Domain nucleation rates and interfacial line tensions in supported bilayers of ternary mixtures containing galactosylceramide. *Biophys J* **94:** 2691–2697.

Brunger AT, Rummel A. 2009. Receptor and substrate interactions of clostridial neurotoxins. *Toxicon* **54:** 550–560.

Burns AR, Frankel DJ, Buranda T. 2005. Local mobility in lipid domains of supported bilayers characterized by atomic force microscopy and fluorescence correlation spectroscopy. *Biophys J* **89:** 1081–1093.

Campanero-Rhodes MA, Smith A, Chai W, Sonnino S, Mauri L, Childs RA, Zhang Y, Ewers H, Helenius A, Imberty A, et al. 2007. *N*-glycolyl GM1 ganglioside as a receptor for simian virus 40. *J Virol* **81:** 12846–12858.

Chigorno V, Palestini P, Sciannamblo M, Dolo V, Pavan A, Tettamanti G, Sonnino S. 2000. Evidence that ganglioside enriched domains are distinct from caveolae in MDCK II and human fibroblast cells in culture. *Eur J Biochem* **267:** 4187–4197.

Chinnapen DJ-F, Chinnapen H, Saslowsky D, Lencer WI. 2007. Rafting with cholera toxin: Endocytosis and trafficking from plasma membrane to ER. *FEMS Microbiol Lett* **266:** 129–137.

Choudhury A, Marks DL, Proctor KM, Gould GW, Pagano RE. 2006. Regulation of caveolar endocytosis by syntaxin 6-dependent delivery of membrane components to the cell surface. *Nat Cell Biol* **8:** 317–328.

Collinet C, Stoter M, Bradshaw CR, Samusik N, Rink JC, Kenski D, Habermann B, Buchholz F, Henschel R, Mueller MS, et al. 2010. Systems survey of endocytosis by multiparametric image analysis. *Nature* **464:** 243–249.

Crespo PM, von Muhlinen N, Iglesias-Bartolome R, Daniotti JL. 2008. Complex gangliosides are apically sorted in polarized MDCK cells and internalized by clathrin-independent endocytosis. *Febs J* **275:** 6043–6056.

Damm EM, Pelkmans L, Kartenbeck J, Mezzacasa A, Kurzchalia T, Helenius A. 2005. Clathrin- and caveolin-1-independent endocytosis: Entry of simian virus 40 into cells devoid of caveolae. *J Cell Biol* **168:** 477–488.

Duran JM, Valderrama F, Castel S, Magdalena J, Tomas M, Hosoya H, Renau-Piqueras J, Malhotra V, Egea G. 2003. Myosin motors and not actin comets are mediators of the actin-based Golgi-to-endoplasmic reticulum protein transport. *Mol Biol Cell* **14:** 445–459.

Eggeling C, Ringemann C, Medda R, Schwarzmann G, Sandhoff K, Polyakova S, Belov VN, Hein B, von Middendorf C, Schönle A, et al. 2009. Direct observation of the nanoscale dynamics of membrane lipids in a living cell. *Nature* **457:** 1159–1162.

Engel S, Heger T, Mancini R, Herzog F, Kartenbeck J, Hayer A, Helenius A. 2011. Role of endosomes in simian virus 40 entry and infection. *J Virol* **85:** 4198–4211.

Ewers H, Jacobsen V, Klotzsch E, Smith AE, Helenius A, Sandoghdar V. 2007. Label-free optical detection and tracking of single virions bound to their receptors in supported membrane bilayers. *Nano Lett* **7:** 2263–2266.

Ewers H, Römer W, Smith AE, Bacia K, Dmitrieff S, Chai W, Mancini R, Kartenbeck J, Chambon V, Berland L, et al. 2010. GM1 structure determines SV40-induced membrane invagination and infection. *Nat Cell Biol* **12:** 11–12.

Ewers H, Smith AE, Sbalzarini IF, Lilie H, Koumoutsakos P, Helenius A. 2005. Single-particle tracking of murine polyoma virus-like particles on live cells and artificial membranes. *Proc Natl Acad Sci* **102:** 15110–15115.

Falguieres T, Mallard F, Baron C, Hanau D, Lingwood C, Goud B, Salamero J, Johannes L. 2001. Targeting of Shiga toxin B-subunit to retrograde transport route in association with detergent-resistant membranes. *Mol Biol Cell* **12:** 2453–2468.

Feigenson GW. 2007. Phase boundaries and biological membranes. *Annu Rev Biophys Biomol Struct* **36:** 63–77.

Forstner MB, Yee CK, Parikh AN, Groves JT. 2006. Lipid lateral mobility and membrane phase structure modulation by protein binding. *J Am Chem Soc* **128:** 15221–15227.

Fra AM, Masserini M, Palestini P, Sonnino S, Simons K. 1995. A photo-reactive derivative of ganglioside GM1 specifically cross-links VIP21-caveolin on the cell surface. *FEBS Lett* **375:** 11–14.

Frolov VA, Zimmerberg J. 2008. Flexible scaffolding made of rigid BARs. *Cell* **132:** 727–729.

Frolov VA, Zimmerberg J. 2010. Cooperative elastic stresses, the hydrophobic effect, and lipid tilt in membrane remodeling. *FEBS Lett* **584:** 1824–1829.

Gaus K, Le Lay S, Balasubramanian N, Schwartz MA. 2006. Integrin-mediated adhesion regulates membrane order. *J Cell Biol* **174:** 725–734.

Girod A, Storrie B, Simpson JC, Johannes L, Goud B, Roberts LM, Lord JM, Nilsson T, Pepperkok R. 1999. Evidence for a COP-I-independent transport route from the Golgi complex to the endoplasmic reticulum. *Nat Cell Biol* **1:** 423–430.

Hammond AT, Heberle FA, Baumgart T, Holowka D, Baird B, Feigenson GW. 2005. Crosslinking a lipid raft component triggers liquid ordered–liquid disordered phase separation in model plasma membranes. *Proc Natl Acad Sci* **102:** 6320–6325.

Hancock JF. 2006. Lipid rafts: Contentious only from simplistic standpoints. *Nat Rev Mol Cell Biol* **7:** 456–462.

Heyningen SV. 1974. Cholera toxin: Interaction of subunits with ganglioside GM1. *Science* **183:** 656–657.

Hummeler K, Tomassini N, Sokol F. 1970. Morphological aspects of the uptake of simian virus 40 by permissive cells. *J Virol* **6:** 87–93.

Johannes L, Romer W. 2010. Shiga toxins—From cell biology to biomedical applications. *Nat Rev Microbiol* **8:** 105–116.

Kartenbeck J, Stukenbrok H, Helenius A. 1989. Endocytosis of simian virus 40 into the endoplasmic reticulum. *J Cell Biol* **109:** 2721–2729.

Katagiri YU, Mori T, Nakajima H, Katagiri C, Taguchi T, Takeda T, Kiyokawa N, Fujimoto J. 1999. Activation of Src family kinase Yes induced by Shiga toxin binding to globotriaosyl ceramide (Gb3/CD77) in low density, detergent-insoluble microdomains. *J Biol Chem* **274:** 35278–35282.

Kenworthy AK, Nichols BJ, Remmert CL, Hendrix GM, Kumar M, Zimmerberg J, Lippincott-Schwartz J. 2004. Dynamics of putative raft-associated proteins at the cell surface. *J Cell Biol* **165:** 735–746.

Kukura P, Ewers H, Müller C, Renn A, Helenius A, Sandoghdar V. 2009. High-speed nanoscopic tracking of the position and orientation of a single virus. *Nat Methods* **6:** 923–927.

Kusumi A, Nakada C, Ritchie K, Murase K, Suzuki K, Murakoshi H, Kasai RS, Kondo J, Fujiwara T. 2005. Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: High-speed single-molecule tracking of membrane molecules. *Annu Rev Biophys Biomol Struct* **34:** 351–378.

Kuziemko GM, Stroh M, Stevens RC. 1996. Cholera toxin binding affinity and specificity for gangliosides determined by surface plasmon resonance. *Biochemistry* **35:** 6375–6384.

Lauvrak SU, Walchli S, Iversen TG, Slagsvold HH, Torgersen ML, Spilsberg B, Sandvig K. 2006. Shiga toxin regulates its entry in a Syk-dependent manner. *Mol Biol Cell* **17:** 1096–1109.

Lee GM, Ishihara A, Jacobson KA. 1991. Direct observation of Brownian motion of lipids in a membrane. *Proc Natl Acad Sci* **88:** 6274–6278.

Lencer WI, Saslowsky D. 2005. Raft trafficking of AB5 subunit bacterial toxins. *Biochim Biophys Acta* **1746:** 314–321.

Levis GM, Evangelatos GP, Crumpton MJ. 1976. Lipid composition of lymphocyte plasma membrane from pig mesenteric lymph node. *Biochem J* **156:** 103–110.

Liddington RC, Yan Y, Moulai J, Sahli R, Benjamin TL, Harrison SC. 1991. Structure of simian virus 40 at 3.8-A resolution. *Nature* **354:** 278–284.

Liebl D, Difato F, Hornikova L, Mannova P, Stokrova J, Forstova J. 2006. Mouse polyomavirus enters early endosomes, requires their acidic pH for productive infection, and meets transferrin cargo in Rab11-positive endosomes. *J Virol* **80:** 4610–4622.

Lindberg AA, Brown JE, Stromberg N, Westling-Ryd M, Schultz JE, Karlsson KA. 1987. Identification of the carbohydrate receptor for Shiga toxin produced by Shigella dysenteriae type 1. *J Biol Chem* **262:** 1779–1785.

Lingwood D, Ries J, Schwille P, Simons K. 2008. Plasma membranes are poised for activation of raft phase coalescence at physiological temperature. *Proc Natl Acad Sci* **105:** 10005–10010.

Lingwood D, Simons K. 2010. Lipid rafts as a membrane-organizing principle. *Science* **327:** 46–50.

Liu J, Sun Y, Drubin DG, Oster GF. 2009. The mechanochemistry of endocytosis. *PLoS Biol* **7:** e1000204.

Liu J, Sun Y, Oster GF, Drubin DG. 2010. Mechanochemical crosstalk during endocytic vesicle formation. *Curr Opin Cell Biol* **22:** 36–43.

Luna A, Matas OB, Martinez-Menarguez JA, Mato E, Duran JM, Ballesta J, Way M, Egea G. 2002. Regulation of protein transport from the Golgi complex to the

endoplasmic reticulum by CDC42 and N-WASP. *Mol Biol Cell* **13:** 866–879.

Lundmark R, Doherty GJ, Vallis Y, Peter BJ, McMahon HT. 2008. Arf family GTP loading is activated by, and generates, positive membrane curvature. *Biochem J* **414:** 189–194.

MacKay RL, Consigli RA. 1976. Early events in polyoma virus infection: Attachment, penetration, and nuclear entry. *J Virol* **19:** 620–636.

Mallard F, Antony C, Tenza D, Salamero J, Goud B, Johannes L. 1998. Direct pathway from early/recycling endosomes to the Golgi apparatus revealed through the study of Shiga toxin B-fragment transport. *J Cell Biol* **143:** 973–990.

Mammen M, Choi SK, Whitesides GM. 1998. Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors. *Angew Chem Int Ed Engl* **37:** 2755–2794.

Maul GG, Rovera G, Vorbrodt A, Abramczuk J. 1978. Membrane fusion as a mechanism of Simian Virus 40 entry into different cellular compartments. *J Virol* **28:** 936–944.

McConnell HM, Vrljic M. 2003. Liquid–liquid immiscibility in membranes. *Annu Rev Biophys Biomol Struct* **32:** 469–492.

McKenzie J, Johannes L, Taguchi T, Sheff D. 2009. Passage through the Golgi is necessary for Shiga toxin B subunit to reach the endoplasmic reticulum. *FEBS J* **276:** 1581–1595.

McMahon HT, Gallop JL. 2005. Membrane curvature and mechanisms of dynamic cell membrane remodelling. *Nature* **438:** 590–596.

Mercer J, Schelhaas M, Helenius A. 2010. Virus entry by endocytosis. *Annu Rev Biochem* **79:** 803–833.

Merritt EA, Sarfaty S, van den Akker F, L'Hoir C, Martial JA, Hol WG. 1994. Crystal structure of cholera toxin B-pentamer bound to receptor GM1 pentasaccharide. *Protein Sci* **3:** 166–175.

Montecucco C, Schiavo G, Rossetto O. 1996. The mechanism of action of tetanus and botulinum neurotoxins. *Arch Toxicol Suppl* **18:** 342–354.

Neu U, Stehle T, Atwood WJ. 2009. The Polyomaviridae: Contributions of virus structure to our understanding of virus receptors and infectious entry. *Virology* **384:** 389–399.

Neu U, Woellner K, Gauglitz G, Stehle T. 2008. Structural basis of GM1 ganglioside recognition by simian virus 40. *Proc Natl Acad Sci* **105:** 5219–5224.

Ortegren U, Karlsson M, Blazic N, Blomqvist M, Nystrom FH, Gustavsson J, Fredman P, Stralfors P. 2004. Lipids and glycosphingolipids in caveolae and surrounding plasma membrane of primary rat adipocytes. *Eur J Biochem* **271:** 2028–2036.

Parthasarathy R, Yu CH, Groves JT. 2006. Curvature-modulated phase separation in lipid bilayer membranes. *Langmuir* **22:** 5095–5099.

Parton RG. 1994. Ultrastructural localization of gangliosides; GM1 is concentrated in caveolae. *J Histochem Cytochem* **42:** 155–166.

Pelkmans L, Fava E, Grabner H, Hannus M, Habermann B, Krausz E, Zerial M. 2005. Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. *Nature* **436:** 78–86.

Pitto M, Brunner J, Ferraretto A, Ravasi D, Palestini P, Masserini M. 2000. Use of a photoactivable GM1 ganglioside analogue to assess lipid distribution in caveolae bilayer. *Glycoconj J* **17:** 215–222.

Qian M, Cai D, Verhey KJ, Tsai B. 2009. A lipid receptor sorts polyomavirus from the endolysosome to the endoplasmic reticulum to cause infection. *PLoS Pathog* **5:** e1000465.

Richards AA, Stang E, Pepperkok R, Parton RG. 2002. Inhibitors of COP-mediated transport and cholera toxin action inhibit simian virus 40 infection. *Mol Biol Cell* **13:** 1750–1764.

Rojas R, van Vlijmen T, Mardones GA, Prabhu Y, Rojas AL, Mohammed S, Heck AJ, Raposo G, van der Sluijs P, Bonifacino JS. 2008. Regulation of retromer recruitment to endosomes by sequential action of Rab5 and Rab7. *J Cell Biol* **183:** 513–526.

Römer W, Berland L, Chambon V, Gaus K, Windschiegl B, Tenza D, Aly MRE, Fraisier V, Florent J-C, Perrais D, et al. 2007. Shiga toxin induces tubular membrane invaginations for its uptake into cells. *Nature* **450:** 670–675.

Römer W, Pontani L-L, Sorre B, Rentero C, Berland L, Chambon V, Lamaze C, Bassereau P, Sykes C, Gaus K, et al. 2010. Actin dynamics drive membrane reorganization and scission in clathrin-independent endocytosis. *Cell* **140:** 540–553.

Roux A, Cuvelier D, Nassoy P, Prost J, Bassereau P, Goud B. 2005. Role of curvature and phase transition in lipid sorting and fission of membrane tubules. *EMBO J* **24:** 1537–1545.

Rozovsky S, Kaizuka Y, Groves JT. 2005. Formation and spatio-temporal evolution of periodic structures in lipid bilayers. *J Am Chem Soc* **127:** 36–37.

Sahl SJ, Leutenegger M, Hilbert M, Hell SW, Eggeling C. 2010. Fast molecular tracking maps nanoscale dynamics of plasma membrane lipids. *Proc Natl Acad Sci* **107:** 6829–6834.

Sandvig K, van Deurs B. 2000. Entry of ricin and Shiga toxin into cells: Molecular mechanisms and medical perspectives. *EMBO J* **19:** 5943–5950.

Sandvig K, Bergan J, Dyve AB, Skotland T, Torgersen ML. 2009. Endocytosis and retrograde transport of Shiga toxin. *Toxicon* **56:** 1181–1185.

Sandvig K, Torgersen ML, Engedal N, Skotland T, Iversen T-G. 2010. Protein toxins from plants and bacteria: Probes for intracellular transport and tools in medicine. *FEBS Lett* **584:** 2626–2634.

Sandvig K, Torgersen ML, Raa HA, van Deurs B. 2008. Clathrin-independent endocytosis: From nonexisting to an extreme degree of complexity. *Histochem Cell Biol* **129:** 267–276.

Schapiro FB, Lingwood C, Furuya W, Grinstein S. 1998. pH-independent retrograde targeting of glycolipids to the Golgi complex. *Am J Physiol* **274:** C319–332.

Schelhaas M, Malmstrom J, Pelkmans L, Haugstetter J, Ellgaard L, Grunewald K, Helenius A. 2007. Simian Virus 40 depends on ER protein folding and quality control factors for entry into host cells. *Cell* **131:** 516–529.

Schengrund CL. 2003. "Multivalent" saccharides: Development of new approaches for inhibiting the effects of glycosphingolipid-binding pathogens. *Biochem Pharmacol* **65:** 699–707.

Schon A, Freire E. 1989. Thermodynamics of intersubunit interactions in cholera toxin upon binding to the oligosaccharide portion of its cell surface receptor, ganglioside GM1. *Biochemistry* **28:** 5019–5024.

Sens P, Johannes L, Bassereau P. 2008. Biophysical approaches to protein-induced membrane deformations in trafficking. *Curr Opin Cell Biol* **20:** 476–482.

Sonnino S, Mauri L, Chigorno V, Prinetti A. 2007. Gangliosides as components of lipid membrane domains. *Glycobiology* **17:** 1R–13R.

Sonnino S, Prinetti A, Mauri L, Chigorno V, Tettamanti G. 2006. Dynamic and structural properties of sphingolipids as driving forces for the formation of membrane domains. *Chem Rev* **106:** 2111–2125.

Spiegel S, Schlessinger J, Fishman PH. 1984. Incorporation of fluorescent gangliosides into human fibroblasts: Mobility, fate, and interaction with fibronectin. *J Cell Biol* **99:** 699–704.

Stang E, Kartenbeck J, Parton RG. 1997. Major histocompatibility complex class I molecules mediate association of SV40 with caveolae. *Mol Biol Cell* **8:** 47–57.

Stehle T, Yan Y, Benjamin TL, Harrison SC. 1994. Structure of murine polyomavirus complexed with an oligosaccharide receptor fragment. *Nature* **369:** 160–163.

Tagawa A, Mezzacasa A, Hayer A, Longatti A, Pelkmans L, Helenius A. 2005. Assembly and trafficking of caveolar domains in the cell: Caveolae as stable, cargo-triggered, vesicular transporters. *J Cell Biol* **170:** 769–779.

Tekle C, Deurs Bv, Sandvig K, Iversen T-G. 2008. Cellular trafficking of quantum dot-ligand bioconjugates and their induction of changes in normal routing of unconjugated ligands. *Nano Lett* **8:** 1858–1865.

Tsamaloukas A, Szadkowska H, Heerklotz H. 2006. Thermodynamic comparison of the interactions of cholesterol with unsaturated phospholipid and sphingomyelins. *Biophys J* **90:** 4479–4487.

Turnbull WB, Precious BL, Homans SW. 2004. Dissecting the cholera toxin-ganglioside GM1 interaction by isothermal titration calorimetry. *J Am Chem Soc* **126:** 1047–1054.

Valderrama F, Duran JM, Babia T, Barth H, Renau-Piqueras J, Egea G. 2001. Actin microfilaments facilitate the retrograde transport from the Golgi complex to the endoplasmic reticulum in mammalian cells. *Traffic* **2:** 717–726.

van Deurs B, Tonnessen TI, Petersen OW, Sandvig K, Olsnes S. 1986. Routing of internalized ricin and ricin conjugates to the Golgi complex. *J Cell Biol* **102:** 37–47.

Wolf AA, Fujinaga Y, Lencer WI. 2002. Uncoupling of the cholera toxin-G(M1) ganglioside receptor complex from endocytosis, retrograde Golgi trafficking, and downstream signal transduction by depletion of membrane cholesterol. *J Biol Chem* **277:** 16249–16256.

Wolf AA, Jobling MG, Saslowsky DE, Kern E, Drake KR, Kenworthy AK, Holmes RK, Lencer WI. 2008. Attenuated endocytosis and toxicity of a mutant cholera toxin with decreased ability to cluster ganglioside GM1 molecules. *Infect Immun* **76:** 1476–1484.

Yamazaki V, Sirenko O, Schafer RJ, Groves JT. 2005. Lipid mobility and molecular binding in fluid lipid membranes. *J Am Chem Soc* **127:** 2826–2827.

Yoshida Y, Kinuta M, Abe T, Liang S, Araki K, Cremona O, Di Paolo G, Moriyama Y, Yasuda T, De Camilli P, et al. 2004. The stimulatory action of amphiphysin on dynamin function is dependent on lipid bilayer curvature. *EMBO J* **23:** 3483–3491.