Published in final edited form as:

Annu Rev Cell Dev Biol. 2021 October 06; 37: 257–283. doi:10.1146/annurev-cellbio-120219-054401.

Glycocalyx Curving the Membrane: Forces Emerging from the Cell Exterior

Joe Chin-Hun Kuo[1], **Matthew J. Paszek**[1,2,3]

[1]Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, New York 14853, USA

[2]Field of Biomedical Engineering and Field of Biophysics, Cornell University, Ithaca, New York 14853, USA

[3]Kavli Institute at Cornell for Nanoscale Science, Ithaca, New York 14853, USA

Abstract

Morphological transitions are typically attributed to the actions of proteins and lipids. Largely overlooked in membrane shape regulation is the glycocalyx, a pericellular membrane coat that resides on all cells in the human body. Comprised of complex sugar polymers known as glycans as well as glycosylated lipids and proteins, the glycocalyx is ideally positioned to impart forces on the plasma membrane. Large, unstructured polysaccharides and glycoproteins in the glycocalyx can generate crowding pressures strong enough to induce membrane curvature. Stress may also originate from glycan chains that convey curvature preference on asymmetrically distributed lipids, which are exploited by binding factors and infectious agents to induce morphological changes. Through such forces, the glycocalyx can have profound effects on the biogenesis of functional cell surface structures as well as the secretion of extracellular vesicles. In this review, we discuss recent evidence and examples of these mechanisms in normal health and disease.

Keywords

glycocalyx; membrane morphology; cell shape; cancer; microvesicle; mucin

INTRODUCTION

Almost all cells in the human body carry a pericellular coat comprised of a polymer meshwork known as the glycocalyx (Reily et al. 2019). Along with polysaccharides anchored on the cell surface, the glycocalyx consists of proteins and lipids carrying covalent sugar chains called glycans. A key feature of the glycocalyx is its adaptability. Cells can dynamically change the composition and architecture of the glycocalyx to modulate their behavior. The mechanisms through which glycocalyx assembly can be reprogrammed are multifaceted (Pinho & Reis 2015). These include the regulation of glycan-related transcripts,

jck267@cornell.edu .

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

tuning of the secretory system flux, and rewiring cellular metabolism, which generate the necessary building blocks for glycan biosynthesis. As such, the glycocalyx structure is intimately coupled with cellular states, and its restructuring likely accompanies all major physiological changes, including specification and transformation (Pinho & Reis 2015, Stowell et al. 2015). An active search is now underway to uncover how specific glycocalyx assemblies support the complex processes of multicellular life.

Of the many possibilities, recent work has suggested a powerful role for the glycocalyx in sculpting the plasma membrane architecture. Due to the cylindrical shape of most phospholipids, the plasma membrane prefers a planar geometry (Frolov et al. 2011). Deforming the membrane into curved forms comes with considerable energy costs that are associated with the resistance to both membrane bending and, to a lesser extent, membrane stretching (Stachowiak et al. 2013). Tremendous efforts in past decades have identified mechanisms that physically contribute the energy needed for membrane bending. These include spontaneous curvature by asymmetric lipid composition as well as unbalanced steric pressure exerted by membrane-associated proteins across the lipid bilayer (Stachowiak et al. 2013). As we review here, more recent theoretical and experimental analyses have suggested that enthalpic and entropic interactions within the glycocalyx can also contribute substantially to the overall balance of forces that defines membrane shape. These investigations have been aided by the development of modern tools to edit and visualize the glycocalyx composition and structure (see sidebar titled New Techniques Used to Study the Glycocalyx), which have otherwise remained inaccessible to conventional biochemical methods.

Curved membrane projections control the cellular exchange of chemical factors and information with the microenvironment (Anvarian et al. 2019, Colombo et al. 2014, Orbach & Su 2020, Sauvanet et al. 2015). A new appreciation for the glycocalyx's role in regulating the full repertoire of functional membrane projections is beginning to emerge. Microvilli, cilia, stereocilia, filopodia, and neurites all possess glycocalyx constituents that have been implicated in generating membrane curvature (Table 1). Multiple biopolymer classes found within the glycocalyx can trigger the outward budding and release of small, spherical vesicles from the plasma membrane (Rilla et al. 2013, Shurer et al. 2019). Intercellular membrane bridges, such as tunneling nanotubes, are common on tumor cell types that construct a particularly dense glycocalyx (Barnes et al. 2018, Kim et al. 2020, Osswald et al. 2015). Although in many cases the relationship between altered glycocalyx states and changes in functional membrane architecture remain correlative, the emerging tools of glycoscience are poised to finally reveal cause-and-effect relationships.

In this review, we focus on membrane curvature arising from exoplasmic forces generated by the glycocalyx and discuss its significance in normal health and disease. The ubiquity of curvature-generating elements within the glycocalyx suggests that the glycocalyx may have a role in the biogenesis of most, if not all, functional projections of the plasma membrane.

GLYCOCALYX BIOPOLYMERS ARE CURVATURE-GENERATING MACHINES

The mammalian cell glycocalyx has abundant polymeric elements and is often enriched in one or more specialized types of biopolymers, including glycosaminoglycans (GAGs) as well as sugar and protein copolymers called mucins (Reily et al. 2019). Historically known for barrier and molecular sieve functions, these long-chain and disordered glycopolymers are increasingly recognized as machinery for generating membrane curvature (Figure 1). The regulation of cell morphology may thus be a general function of glycocalyx biopolymers that are present on a diverse set of membrane structures (Table 1).

At a sufficiently high amount of membrane coverage, theoretical calculations and modeling suggest steric pressures arising from macromolecular crowding can overcome the energy cost of membrane bending (Busch et al. 2015, Nawrocki et al. 2019, Sens & Turner 2004). When macromolecules are densely crowded on a surface, they have less space to explore through diffusive movements and conformational changes such as the wiggling motions of unstructured polymers. The reduced entropy of the system forms a thermodynamic basis for increased pressure on the membrane. Stated simply, membrane bending is energetically favorable, because it creates more space for the macromolecules to rearrange.

Model membranes indeed buckle outward to form bud, tubule, and pearl morphologies when sufficiently dense synthetic polymers and structured proteins are crowded on the outer membrane surface (Decher et al. 1989; Stachowiak et al. 2010, 2012). Consistent with molecular crowding, this phenomenon shows dependency on membrane coverage regardless of how proteins are attached to the membrane (Stachowiak et al. 2012). Compared to folded proteins, intrinsically disordered proteins and polymers are more effective crowding agents, as they tend to occupy greater volumes. Consequently, membrane bending is enhanced for disordered macromolecules compared to structured proteins of similar molecular weight and surface density (Busch et al. 2015).

Evidence implicating the crowding of glycocalyx biopolymers in morphological changes of the plasma membrane has accumulated over the past two decades. Early studies (such as Scholl et al. 1999) reported that the mucin-type protein podoplanin could induce filopodia formation in keratinocytes. Later, the large mucin podocalyxin was found to dramatically increase the induction of microvilli-like structures in cell models (Nielsen et al. 2007). Importantly, these effects did not require the podocalyxin cytoplasmic tail, which can mediate intracellular signaling. This finding suggested that the induction of membrane protrusions may be linked to the biophysical actions of the mucin ectodomain. Early evidence also implicated GAG polymers and GAG-presenting syndecans in membrane shape regulation (Berndt et al. 2004, Granés et al. 1999). Studying the assembly of hyaluronan [also called hyaluronic acid (HA)] polymers, Kultti et al. (2006) and Rilla et al. (2013) further demonstrated that the expression of HA synthases (HASs), enzymes that catalyze HA biosynthesis, induced the extension of microvilli-like projections (Koistinen et al. 2015). In these studies (such as Kultti et al. 2006), the effects of HA on morphological transitions did not appear to occur through biochemical signaling, leading to the conclusion that the crowding of HA molecules in the glycocalyx might generate forces sufficient for membrane deformation.

Genetically encoded systems for controlling the size, chemistry, and density of mucin biopolymers in the glycocalyx are advancing our understanding of glycocalyx polymers as curvature-generating machines. Structurally, mucins are defined by their central polypeptide backbones and the dense grafting of serine- and threonine-linked *O*-glycans (Hattrup & Gendler 2008). These features afford many advantages for glycocalyx editing (H. Pan et al. 2019, Shurer et al. 2017). First, mucin length, backbone chemistry, and the distribution of potential glycosylation sites are precisely encoded in the DNA Second, powerful genetic tools and data resources for manipulating mucin-type *O*-glycans have come online (Narimatsu et al. 2018, 2019; Zhu et al. 2021). Shifts in *O*-glycan extension or charge (i.e., sialylation) can be readily programmed through genetic manipulation of the governing glycosyltransferases.

Recent studies (Shurer et al. 2019) taking advantage of these genetic systems strongly support the concept that molecular crowding is a general principle by which glycocalyx polymers generate forces to curve the membrane. Indeed, mucins with broadly varying structural attributes and glycosylation patterns can induce morphological transitions of the plasma membrane, if expressed at sufficient density. However, the precise structure and surface levels of the mucin polymers can dictate the types of membrane protrusions generated. According to Shurer et al. (2019), low densities of mucin biopolymers on the plasma membrane favored blebbing, whereas high densities favored thin microvilli-like tubules and very high densities triggered pearled membrane structures, likely arising through induced membrane instabilities.

These membrane features are likely to occur in coordination with cytoskeletal dynamics and other intracellular processes. Tubular protrusions induced by glycocalyx polymers typically have an actin core (Kultti et al. 2006, Nielsen et al. 2007, Shurer et al. 2019). While certain glycocalyx compositions may lower the barrier for protrusion, the successful extension of fingerlike protrusions likely requires the polymerization of cytoskeletal filaments. Likewise, while certain polymer compositions may make blebbing more favorable, membrane expansion still requires cytosolic pressures generated by actomyosin contractility. Theoretical calculations indicate that modest cytosolic pressures can provide sufficient energy to maintain blebs, but with increasing mucin densities the pressure required for such maintenance goes above the physiological range (Shurer et al. 2019). Instead, the high curvature induced by crowded mucins is expected to decrease the required force for tubular extensions to a level comparable to the polymerization force of a single actin polymer. At very high mucin crowding, tubular membrane projections are expected to collapse into pearled structures upon depolymerization of the supporting cytoskeletal core. The regulation of cytoskeletal organization and dynamics by mucin cytoplasmic tails has been documented (Gipson et al. 2014). However, our understanding of the collaboration between glycocalyx polymers and the cytoskeleton in controlling membrane architecture remains limited.

The potential for specific polymer attributes to favor the distinct types of membrane projection is intriguing, given the enormous structural diversity of native mucins (Hattrup & Gendler 2008). The largest membrane-tethered mucin, MUC16, carries >22,000 amino acids, reaching >2.5 MDa in molecular weight (MW), and extends to a length of 1.5 μm when fully stretched on the cell surface. More modest mucin polymers implicated

in membrane protrusions include podoplanin (MW ~45 kDA), podocalyxin (MW ~165 kDa), and MUC1 (MW ~450 kDa). Steric and electrostatic repulsions between glycan side chains on the polypeptide backbone can profoundly impact the physical properties and conformations of these glycopolymers (Kramer et al. 2015). The stochastic nature of glycan assembly contributes to structural diversity in a combinatorial manner for mucins with many glycosylation sites. Differentiation, transformation, and other changes in cell states that alter glycosylation patterns are expected to shift the mean structural attributes of mucins in the glycocalyx and, thus, membrane projections (Stowell et al. 2015). For example, the disruption of glycan side chain extension or sialylation can alter the frequency of tubular membrane protrusions (Shurer et al. 2019). The field of biophysical glycoscience is in its infancy and the full biophysical implications of the glycome on membrane shape regulation await clarification.

The cost of membrane bending by the glycocalyx is ultimately paid for by the consumption of cellular nutrients and the energy put into polypeptide and glycan biosyntheses. Branching from glycolysis, the hexosamine biosynthetic pathway (HBP) diverts glucose metabolism for the construction of sugar nucleotides that serve as building blocks for glycan and GAG assembly (Reily et al. 2019). Under normal cellular conditions, 2–5% of glucose uptake is consumed by the HBP (Bouche et al. 2004). The possibility that biopolymer synthesis could serve as a conduit through which metabolic programming and membrane architecture are connected is provocative. For example, enhanced nutrient flux through the HBP in tumor cells displaying glycolytic phenotypes may partly explain the thick glycocalyx and tubulated membrane morphologies that many cancer cells exhibit (Kolata 1975, Pinho & Reis 2015, Ying et al. 2012), although such possibilities must be tested directly. In addition to the demands associated with biosynthesis, cellular energy must be consumed to insert newly synthesized biopolymers into the crowded glycocalyx. Overall, connecting cellular energetics with membrane regulation by glycocalyx polymers is a largely unexplored and exciting avenue for future study.

ESTIMATION OF FORCES GENERATED BY GLYCOCALYX POLYMERS

The macromolecular crowding pressures in the glycocalyx have not been directly measured. However, unstructured glycopolymers such as mucin and hyaluronan are anchored to the plasma membrane in such a way that long polymer loops or chains extend from the cell surface (Hattrup & Gendler 2008, Lee et al. 1993). This ensemble resembles a polymer brush, whose physical behavior can be captured with well-validated theories. The total free energy per area and two-dimensional (2D) crowding pressure in the plane of the membrane for a theoretical glycocalyx brush increases in a highly nonlinear manner with biopolymer surface density (Gandhi et al. 2019). The free energy and 2D pressure vary roughly as a power of the surface density. An estimation of the free energy and 2D pressure for a theoretical MUC1 brush provides an illustrative case study (Figure 1). Well-known systems for membrane bending in endocytosis—clathrin and actin polymerization—generate an energetic driving force of approximately 0.1 $k_B T$/nm^2. On a flat segment of membrane, a mucin brush can generate a similar energetic driving force at a surface density of approximately 3,000 lb/μm^2. This mucin density is most likely met or exceeded on specialized cell types, such as polarized epithelia and enterocytes, as well as on mucin-

overexpressing tumor cells (see below) (Button et al. 2012, Shurer et al. 2019). At these densities, glycocalyx biopolymers are predicted to generate piconewton-level pressures on other macromolecules on the cell surface. For instance, a typical glycoprotein with a 2D circumference of 15 nm is expected to experience a force of about 1 pN in a glycocalyx brush with a mucin density of approximately 1,000 lb/μm^2 (Figure 1). While such estimates provide a useful general sense of forces, the exact crowding pressures in a glycocalyx depend on the precise physiochemical properties of its biopolymer constituents as well as the composition of other macromolecules in the glycocalyx. Direct measurement of forces in the glycocalyx has remained elusive in part due to the tiny, nanometer-scale thickness of the glycocalyx. New experimental approaches that can probe the physical properties of the glycocalyx are needed.

GLYCOCALYX BIOPOLYMERS DRIVE MEMBRANE VESICLE RELEASE

Extracellular vesicles (EVs) are important intercellular communicators due to their ability to transfer nucleic acids and protein cargos (Colombo et al. 2014). Glycocalyx polymers, including mucins, podocalyxin, and HA, decorate the surfaces of some EV subtypes (Table 1). Rather than being passive cargos, emerging evidence suggests that glycocalyx polymers may be important machinery for EV biogenesis. In particular, the expression of glycocalyx polymers can trigger the release of microvesicles, an important class of EVs that bud off directly from the plasma membrane. The precise mechanisms underlying microvesicle induction by the glycocalyx are not fully resolved, but several distinct possibilities have been described.

In one mechanism, microvesicle generation occurs through tip budding from tubular protrusions (Figure 2a). Overexpressing HAS3 to increase HA surface density can dramatically enhance microvesicle release that coincides with long membrane protrusions frequently displaying vesiculated tips (Rilla et al. 2013). Molecular myosin motors also accumulate at these vesiculated tips, suggesting their involvement in vesicle budding (Koistinen et al. 2015) (Figure 2b). Such mechanisms may be similar to processes that occur in vivo, where microvilli are stabilized by interprotrusion linkages and vesicle release from vesiculated tips is driven by myosin motors (McConnell et al. 2009).

In a second mechanism, glycocalyx polymers are proposed to trigger vesicle release through the induction of membrane instabilities. Studies on synthetic membranes (Decher et al. 1989, Kozlovsky & Kozlov 2003, Tsafrir et al. 2001) have demonstrated that unstructured polymers anchored to one side of the membrane can generate curvature to destabilize tubular structures into pearled morphologies that can spontaneously fissure at their narrow necks (Figure 2c). Crowded polymers in the glycocalyx may exert similar effects to release microvesicles from tubulated cell protrusions. Upon depolymerization of the actin core, membrane instabilities are triggered by the curvature-generating actions of densely crowded polymers in the glycocalyx, likely resulting in EV release from membrane pearls (Figure 2a) (Shurer et al. 2019). Indeed, treatments to disrupt the cytoskeleton can rapidly induce pearling instabilities and vesicle release.

The full significance of glycocalyx-induced vesicles must still be determined. It is unclear whether polymer structure or size drive distinct vesicle-budding mechanisms. It is also unclear whether glycopolymer-induced vesicles are similar to classical EVs in their molecular markers, cargo contents, and communication potencies (Jeppesen et al. 2019). Besides microvesicles, most cells secrete exosomes. Exosome biogenesis begins with the inward budding of endosomal membranes to form multivesicular bodies, which can subsequently fuse with the plasma membrane for vesicle release. Interestingly, exosomes are enriched with mucins (Kesimer et al. 2009, D. Pan et al. 2019). The molecular crowding of cargo molecules has been proposed to generate membrane deformation and drive budding during the biogenesis of multivesicular bodies (Adell et al. 2017). Whether steric pressure from crowded mucin polymers can drive these reverse budding events also needs further validation.

GLYCOLIPIDS BEND MEMBRANE OUTWARD

Glycolipids are amphipathic lipids carrying hydrophilic headgroups of sugar polymers attached to hydrophobic ceramide tails (Lingwood 2011). Gangliosides, the best-characterized class of glycolipids, accumulate on outward protrusions such as cilia, microvilli, and neurite tips and are particularly enriched in the brain, where cell shape plasticity is functionally important (Kaiser et al. 2020, Sipione et al. 2020). Studies have linked gangliosides to morphogenesis ranging from neurite outgrowth to immune and stem cell activation and differentiation (Sonnino et al. 2018). However, how glycolipids regulate membrane curvature remains unclear. Insights from model membranes and dynamic simulations suggest that glycolipids influence membrane organization and biophysical properties. For instance, gangliosides in model systems may cluster into distinct liquid-ordered regions that decrease bilayer bending rigidity and generate membrane curvature (Fricke & Dimova 2016, Patel et al. 2016). Besides ceramide tail composition, these properties have been attributed to the sugar polymers that glycolipids carry as their headgroups (Liu et al. 2019).

Glycolipid headgroups can be remarkably large compared to the major bilayer-forming lipids such as phosphatidylcholine (PC). For example, while a fully stretched PC headgroup is ~1 nm long, the headgroup on the well-documented ganglioside GM1 is a chain of five sugar monomers ~2.2 nm in length when fully stretched (McIntosh & Simon 1994). GM1 headgroups in model bilayers can extend ~1.2 nm above PC, suggesting a near fully extended glycan chain with a portion that intercalates between PC heads (McIntosh & Simon 1994). GM1 may also adopt an orientation that buries the glycan headgroup in the membrane (Jedlovszky et al. 2009). Whether the glycan chain is extended or embedded would have an impact on the overall molecular shape that a glycolipid adopts as well as on its steric and charge interactions with neighboring lipids (Frey & Lee 2013).

Large glycan heads may shape gangliosides into an inverted cone with a propensity for outward curvature (Hägerstrand et al. 2006, Pei & Chen 2003) (Figure 3a). In contrast, bilayer-forming lipids tend to have a cylindrical shape, as their headgroups and tails are similar in size (Frolov et al. 2011). Lipid shapes dictate the amount of stored elastic stresses in the membrane, which bends to minimize these stresses (Gruner 1985). In solution, the

cylindrical shape allows lipids such as PC to pack uniformly and form nonstressed flat bilayers. With an inverted conical shape, gangliosides instead favor membrane bending away from the bulky headgroups. Complex gangliosides, unlike smaller lipids, are predicted to have a strong preference for curvature due to their large molecular area (Kamal et al. 2009). In solution, gangliosides carrying a simple glycan headgroup such as GM3 can still form frustrated vesicles, but those with more complex sugar polymers such as GM1 spontaneously form micelles with high curvatures that typify inverted conical lipids (Sonnino et al. 2018). Mixed gangliosides thus form micelles with larger headgroups occupying regions of higher curvature. The volume of hydrated glycolipid headgroups is expected to increase with glycan chain complexity, which may influence their bulkiness and contribute to steric pressures on the membrane for bending (Sonnino et al. 2018).

Ganglioside enrichment on the exoplasmic lipid leaflet might be crucial for curvature generation. The asymmetric distribution of lipids across a bilayer is proposed to drive spontaneous curvature (Frolov et al. 2011). Each leaflet of a lipid bilayer can have a different lateral stress and bending moment, based on its composition and lipid density. Balancing this bending torque is thought to generate spontaneous curvature (Hossein & Deserno 2020). In theory, stresses imposed by gangliosides on one lipid leaflet may cause the whole bilayer to bend due to coupling (Sheetz & Singer 1974). Even small composition asymmetries of bulky glycolipids can induce membrane curvature in computer simulations (Dasgupta et al. 2018, Patel et al. 2016, Sreekumari & Lipowsky 2018). On model vesicles, asymmetrically distributed GM1 indeed generates spontaneous membrane tubulation and pearled morphologies (Dasgupta et al. 2018). Enriching GM1 in either the inner or outer lipid leaflet on model vesicles drives inward or outward tubulation, respectively (Bhatia et al. 2018). Note that a small amount of symmetrically or asymmetrically distributed GM1 in model vesicles has the opposite effect on membrane stiffness: Symmetry increases bilayer rigidity, while asymmetry softens the bilayer to facilitate deformation (Brocca et al. 2004).

How asymmetrically distributed glycolipids regulate cell shape remains ill defined, as membrane composition in living cells is extremely complex and difficult to manipulate. Simulations of complex plasma membranes also indicate that membrane bending tends to occur at the boundary of glycolipid clusters (Ingólfsson et al. 2017). On live cells, the application of exogenous gangliosides and the inhibition of glycolipid synthesis can stimulate or abolish membrane protrusions, respectively, implicating glycolipids in these processes (Lingwood 2011, Sonnino et al. 2018). Interestingly, GM1 sorting into erythrocyte protrusions and exovesicles is attributed to its intrinsic conical shape (Hägerstrand et al. 2006), although whether such protrusions are driven by GM1 enrichment is unclear.

Studies on neuronal cells further suggest that glycolipid-stimulated protrusions may involve carbohydrate-binding proteins known as lectins. For instance, GM1 binding to laminin-1 and galectin-1 stimulates neurite outgrowth (Wu et al. 2016). GD1a andGT1b gangliosides binding to myelin-associated glycoprotein and Nogo receptor NgR1 instead inhibits this process (Williams et al. 2008). This inhibition in turn is abolished by enzymes that convert GD1a and GT1b to GM1, postinjury, for axon regeneration (Kappagantula et al. 2014). Here, GD1a is proposed to act as a reserve pool for GM1 for neurite branching and outgrowth (Ledeen & Wu 2015). Interestingly, exogenous lectins and antibodies that

cross-link glycolipids may have opposing effects on protrusion regulation in neuronal cells (Ledeen & Wu 2015). Furthermore, the actin cytoskeleton may organize glycolipids on distinct curved structures, and actin polymerization for protrusion, in turn, has an interdependence on glycolipid presence (Kaiser et al. 2020). Together these studies emphasize the complex actions of glycolipids on live cells, where curvature generation is likely cell type and stage dependent and may require other factors to take advantage of the curvature preference of asymmetrically distributed glycolipids and their effects on membrane softening.

GLYCOLIPIDS CURVE MEMBRANE INWARD

Glycolipids are co-opted for bending plasma membranes into budding and tubular invaginations that serve as clathrin-independent carriers (CLICs) for endocytosis (Johannes et al. 2016) (Figure 3b). Actin- and ATP-mediated processes are redundant for invagination but promote membrane scission at later endocytic steps (Renard et al. 2015). For instance, ganglioside GM1 clustering by cholera toxin subunit B (CTxB) or polyomavirus SV40 VP1 capsids and globoside Gb3 clustering by Shiga toxin subunit B (STxB) can induce membrane invagination (Ewers et al. 2010, Römer et al. 2007). These lectins share convergently evolved pentameric structures that are similar even down to the 3-nm spacing between their carbohydrate-binding pockets and the angle at which these binding sites face the membrane, though the number of binding sites differ (Johannes et al. 2016).

Precise geometry of glycolipid clustering imposed by lectin structures may be essential for inward membrane bending. Antibody cross-linking of GM1, Gb3, or even monovalent CTxB bound to GM1 does not appear to initiate the necessary curvature (Ewers et al. 2010, Kabbani et al. 2020, Römer et al. 2007). Likewise, mutations that alter the geometry of glycolipid binding on CTxB and STxB abolish curvature induction (Kabbani et al. 2020, Römer et al. 2007). At least two GM1 glycolipids binding to one CTxB pentamer may be necessary for membrane bending (Kabbani et al. 2020). Interestingly, this minimum requirement is recapitulated by divalent synthetic lectins that tubulate membranes only if adjacent glycolipid-binding sites are close enough (Arnaud et al. 2014).

Large headgroups on glycolipids have a height mismatch with neighboring phospholipids (McIntosh & Simon 1994). Binding glycolipids in a defined geometry may indeed generate curvature that compensates for the lack of intrinsic curvatures in flat lectins such as STxB (Ling et al. 1998). In silico, Gb3 binding to the middle of an STxB pentamer is found to push down on the membrane, while Gb3 binding to its periphery exerts an upward pull (Pezeshkian et al. 2016). This convex curvature is also speculated to occur with GM1 binding to CTxB (Pezeshkian et al. 2017b). Otherwise, the Gb3-STxB cluster is shown to induce lipid compression and long-range lipid reorganization that may produce asymmetric bilayer stresses and favor inward membrane bending (Watkins et al. 2019). Membrane fluctuation–induced forces may in turn drive positively cooperated clustering of glycolipid-lectin complexes for tubule growth (Pezeshkian et al. 2017a). Although theoretical models predict that glycolipid-lectin interactions can provide sufficient energy for engulfing whole bacteria (Eierhoff et al. 2014), mechanisms to generate enough energy for curvature and invagination induced by small lectins and viruses need further elucidation.

Glycolipid interaction with endogenous lectins also generates CLIC invaginations. Galactose-binding lectin galectin-3 represents a well-studied example (Lakshminarayan et al. 2014). Unlike toxins and viruses, the initial recruitment of galectin-3 to the cell membrane is decoupled from membrane bending (Figure 3b). Monomeric galectin-3 is first recruited by its cargo glycoproteins before binding to glycolipids, through oligomerization, for inducing membrane invagination and cargo endocytosis. Galectin-8 may similarly sort cargo glycoproteins into CLICs for endocytosis (Renard et al. 2020). Interestingly, galectin-3 can form pentamers, although its significance in membrane deformation remains to be determined (Ahmad et al. 2004). Galectin-3 and STxB, which bind different glycolipids, localize to discrete tubule invaginations, suggesting the presence of distinct CLIC populations (Lakshminarayan et al. 2014). Furthermore, lectins that bind the same glycolipid can segregate into distinct CLIC tubules, which suggests the orientation of glycan headgroups may be important (Schubert et al. 2020). Altogether, glycolipid-lectin interactions may represent a common yet diverse approach for inward membrane deformation (Johannes et al. 2016).

How glycolipids regulate inward and outward bending on the same membrane is unclear. On erythrocytes, Gb3 binding to STxB instead induces the formation and release of outward-budding microvesicles that are important in bacterial infection and pathogenesis (Willysson et al. 2020). Uptake of these toxin-coated microvesicles again requires Gb3 on recipient cells (Johansson et al. 2020). Intriguingly, Gb3-STxB interaction on HeLa cells is reported to induce inward membrane bending for tubular invagination as well as outward membrane bending for microvesicle generation (Römer et al. 2007, Willysson et al. 2020). Both of these processes occur on minute timescales. Whether local enrichment of Gb3, differential Gb3 content on HeLa clones, distinct Gb3 species, or headgroup orientations determine the direction of membrane bending is an intriguing question (Schubert et al. 2020, Shin et al. 2009). In neuronal cells, ganglioside interaction with CTxB and galectin-3 can promote neurite outgrowth (Ledeen & Wu 2015). Neuronal cells also release microvesicles for intercellular communication (Budnik et al. 2016), though the importance of glycolipid-lectin interactions remains to be determined. Therefore, the context in which glycolipids and lectins interact could play a key role in determining the outcome of curvature generation, which requires further investigation.

CURVATURE SIGNALING

Signal coordination for glycocalyx-induced curvature generation is underdefined. On cell protrusions, glycopolymers and glycolipids often colocalize with cytoskeletal elements (Kaiser et al. 2020, Kultti et al. 2006, Shurer et al. 2019), possibly indirectly through cytosolic anchors such as ezrin that themselves are recruited by curvature sensors (Bennett et al. 2001, Koistinen et al. 2015, Nielsen et al. 2007, Tsai et al. 2018). On the basal membrane, glycopolymers such as dystroglycan may control protrusion length, branching, and orientation (Eyermann et al. 2012, Palmieri et al. 2017, Saito et al. 2003), and extracellular matrix proteins including laminins may organize glycolipids for curvature generation (Ledeen & Wu 2015). Protrusion initiation may require membrane-cytoskeletal detachment (Welf et al. 2020), which could be driven by pressures from the glycocalyx. Protrusion growth depends on cytoskeletal polymerization that results in membrane tension

(Sitarska & Diz-Muñoz 2020), which may in turn act as a long-range signal inhibitor for protrusions elsewhere (Houk et al. 2012). Self-amplifying signals that regulate the expression, density, and clustering of glycocalyx components are likely to enhance curvature. For example, autocrine maintenance of podocyte foot processes by cytoskeletal organization is achieved through the clustering of the glycopolymer syndecan and the glycolipid GM3 (Jin et al. 2012). Conversely, curvature may be discouraged by pathways that downregulate, disperse, and reduce the density of glycocalyx components, such as uterine epithelial flattening by removing MUC1-induced microvilli (Kelleher et al. 2016). These together point to the need for detailed investigations into how the glycocalyx, cytoskeleton, membrane tension, extracellular cues, and signaling events coordinate to organize cell morphology.

SIGNAL REGULATION BY GLYCOCALYX-MEDIATED CURVATURE

Glycocalyx-induced membrane curvature may profoundly impact signaling events emanating from the cell surface. Membrane geometry can influence the lateral organization and activities of transmembrane receptors and downstream effector proteins (Cheng & Smith 2019). For example, transmembrane receptors may experience thermodynamic sorting to match receptor shape and local curvature that is fine-tuned by ligand activation and receptor oligomerization (Needham et al. 2016, Rosholm et al. 2017). Downstream cytosolic effectors recruited by signaling receptors may have curvature-sensing motifs that further reinforce curvature and coordinate with the underlying cytoskeleton for signal propagation (Ebrahimkutty & Galic 2019). Moreover, membrane curvature can change receptor exposure to freely diffusing ligands and cytosolic effectors that generates a spatial gradient of activated receptors by simple reaction–diffusion kinetics (Schmick & Bastiaens 2014). Interestingly, cellular protrusions may enhance the accumulation of activated receptor complexes by simply increasing the membrane surface-to-cytosolic volume and, hence, the local density of membrane-bound complexes (Neves et al. 2008). These mechanisms are poorly understood in the context of glycocalyx-induced curvature and are worth further exploration, especially as receptor enrichment on membrane protrusions is potentially a general organization principle for cell signaling (Anvarian et al. 2019, Lange 2011, Orbach & Su 2020).

EXAMPLES OF GLYCOCALYX CELL PROTRUSIONS IN NORMAL HEALTH AND DISEASE

Podocalyxin on Podocyte Morphology

Podocyte foot processes represent a well-illustrated example of glycocalyx-regulated cell shape. Normal kidney function relies on well-spaced neighboring podocytes to extend interdigitated foot processes and form specialized intercellular filtration junctions known as slit diaphragms to prevent protein loss in urine production (Kopp et al. 2020). Podocalyxin is the major bottlebrush glycopolymer expressed on podocyte apical surfaces and foot processes above slit diaphragms (Kerjaschki et al. 1984). Podocalyxin directs the formation of foot processes and their presumptive microvilli precursors as well as slit diaphragms, as its homozygous deletion eliminates such structures and reduces podocyte spacing in

human organoid and mouse models to block urine production, causing renal failure and lethality (Doyonnas et al. 2001, Kim et al. 2017). This recapitulates the symptoms of human congenital podocalyxin deficiency (Kang et al. 2017). Mice heterozygous for podocalyxin remain healthy with subtly altered foot processes but become highly susceptible to proteinuria upon injury (Refaeli et al. 2020). This likely reflects a loss of heterozygosity and more closely mimics the onset of adult human diseases, such as those associated with environmental factors including obesity (Refaeli et al. 2019). In mice, ablating glycosylation enzymes to shorten *O*-glycan side chains on glycopolymers can flatten foot processes and induce slit diaphragm loss and proteinuria, producing conditions that resemble human nephrotic syndrome (Song et al. 2017, Stotter et al. 2020). This suggests that glycan-mediated steric stabilization is crucial for podocalyxin polymer conformation and function in podocyte morphology.

As podocalyxin carries glycans with negatively charged sialic acids, its effects on membrane morphology and cell spacing are attributed to charge repulsion (Kim et al. 2017). Indeed, animals deficient in sialylation or treated to remove charge or sialic acids develop altered foot processes and nephropathology (Galeano et al. 2007). However, electrostatic repulsion between glycans, which is often overlooked, may impact glycopolymer conformation and crowding for membrane curvature generation (Shurer et al. 2019) as well as steric hindrance that likely defines intercellular spacing (Komatsu et al. 1997, Nielsen et al. 2007). How these effects define podocyte morphology and spacing needs to be addressed. As sialic acid and its metabolic precursors are promising treatments for human kidney diseases (Huizing et al. 2019), the effect of sialylation on podocalyxin polymer conformation also warrants further investigation.

Other glycocalyx components also regulate podocyte morphology. Foot process flattening and simplified interdigitation are observed in dysregulation of GM3-mediated cell shape maintenance, antibody targeting glycolipid GD3 and dystroglycan dysregulation (Jin et al. 2012, Kojima et al. 2011, Simons et al. 2001). Foot processes are thought to compress the glomerular basement membrane to counteract capillary hydrostatic filtration pressure and prevent proteinuria (Butt et al. 2020). In turn, effacement compromises the maintenance of proper tension and permeability of the basement membrane by foot processes. Future studies need to address the role of glycocalyx components in these processes.

Microvilli can originate from the apical surfaces of healthy podocytes that are distinct from foot processes and can become dramatically numerous in diseased-induced transformation (Wrede et al. 2020). Podocyte apical microvilli contact protrusions from parietal epithelial cells and may mediate intercellular communication (Wrede et al. 2020). Note that podocalyxin induces microvilli in cell models, presumably due to biophysical crowding effects (Nielsen et al. 2007). Apical microvilli tip vesiculation on podocytes can shed podocalyxin-coated microvesicles, which are increased in kidney diseases (Hara et al. 2010). Interestingly, foot processes also project protrusions with bulbous tips, although it is unclear whether vesicles are released (Rice et al. 2013). These observations hint at the possible role of podocalyxin in microvesicle production that requires further verification. Finally, MUC1 is also expressed apically on developing podocytes as well as other kidney cell types that exhibit numerous protrusions (Al-bataineh et al. 2017, Rice et al. 2013). Familial mutations

in the ectodomain of MUC1 can cause kidney diseases and alter foot process morphology in humans (Kirby et al. 2013). The function of MUC1 in generating these protrusions in vivo needs further study (Al-bataineh et al. 2017).

Mucin-Coated Epithelia

Unlike podocalyxin, membrane regulation by canonical mucins has not been demonstrated in vivo. Membrane-tethered mucins participate in epithelial polarization and often enrich apical protrusions (Hattrup & Gendler 2008). For example, MUC1 adorns microvilli, while MUC4 and MUC20 decorate cilia on the airway epithelium (Kesimer et al. 2013). MUC17 is found almost exclusively on enterocytic microvilli, which suggests a functional association (Pelaseyed & Hansson 2020). In cell models, the overexpression of MUC1 and synthetic mucins induces membrane protrusions, while deleting MUC16 or MUC17 results in fewer microplicae or disorganized microvilli, respectively, suggesting mucins contribute to these structures (Gipson et al. 2014, Kim et al. 2014, Shurer et al. 2019). Microvesicle biogenesis also occurs from mucin-induced protrusions in vitro and possibly in vivo (McConnell et al. 2009, Shurer et al. 2019).

However, whether animals lacking membrane-tethered mucins show abnormal protrusions is not clear (Hattrup & Gendler 2008). Given the critical roles of protrusions in vivo, they are likely maintained by compensatory mechanisms (Sauvanet et al. 2015). Indeed, epithelial cells typically express multiple mucins and, in vivo, thin protrusions are further stabilized by glycoprotein bridges that may also carry unstructured mucin-like domains (Crawley et al. 2014, Hattrup & Gendler 2008). It remains to be seen whether deleting multiple mucins, perhaps concurrently with interprotrusion linkages, would ablate mucin-coated protrusions.

Insights into uterine receptivity may exemplify membrane regulation by mucins. Epithelial flattening that eliminates microvilli is essential to provide a smooth surface for embryo implantation (Murphy 2004). Temporal MUC1 downregulation mediates embryo attachment and dysregulated MUC1 expression coincides with failed microvilli flattening in disease models (Kelleher et al. 2016). This has profound implications, as, for example, heightened endometrial MUC1 expression is associated with human infertility and spontaneous abortion (Chauhan et al. 2015, Horne et al. 2005, Margarit et al. 2010). Downregulation of other membrane-tethered mucins such as MUC4 and MUC16 may also occur at the onset of receptivity for embryo implantation (Gipson et al. 2008, McNeer et al. 1998). Polymorphisms in mucin ectodomains, which are critical for mucin polymer size and conformation, also contribute to infertility (Chang et al. 2011, Horne et al. 2001). Interestingly, deleting or enzymatically removing MUC1 can improve embryo attachment (DeSouza et al. 2000). In addition to the steric hindrance suggested by early reports, future studies should consider mucin-induced protrusions with implications for improving infertility treatments.

Hyaluronan Coat

HA polymers form pericellular coats on diverse cell types that exhibit elaborate microvilli and cilia. The function of the HA coat is typically attributed to its mechanical and electrosteric properties (Salustri et al. 2019), but the structural biology of HA-coated

protrusions is often overlooked. In cell models, the overexpression of HASs, especially HAS3, dramatically thickens the pericellular coat and induces membrane protrusions (Kultti et al. 2006, Shurer et al. 2019). These protrusions contain basal actin and are vesiculated at their tips, which may bud off microvesicles in a myosindependent manner (Koistinen et al. 2015, Noble et al. 2020). Primary mesothelial cells undergoing epithelial-to-mesenchymal transition also upregulate HA synthesis in parallel with protrusion and microvesicle generation (Koistinen et al. 2017), suggesting that HA-induced curvature is involved in cell transformation. Membrane regulation by HA has not been demonstrated in vivo, but enzymatically digesting HA severely diminishes HA-enriched protrusions, such as those on synovial tissue explants (Shurer et al. 2019).

Tissues apically enriched in HA often display numerous protrusions and release EVs, some of which may originate from protrusion tips. Notable examples include synovium (Mustonen et al. 2016, Shikichi et al. 1999), cartilage (Cohen et al. 2003, Hale & Wuthier 1987), mesothelium (Koistinen et al. 2016), and alveolar epithelium (Lee et al. 2018, Ochs et al. 2020). HA-induced microvesicles may mediate intercellular communication (Arasu et al. 2020). Likewise, HA-coated protrusions may participate in cellular communications, which is currently underexplored. Examples may include osteocyte networking and mesothelium postsurgical adhesion (Burra et al. 2010, Fischer et al. 2020).

These mechanisms may be especially important for protrusion-based gap junctional communications in oocyte development. Interconnecting oocyte–somatic cell protrusions are likely HA enriched (Baena & Terasaki 2019, El-Hayek et al. 2018). Maturing oocytes upregulate HAS3 concurrently with the formation of elaborate microvilli (Makabe et al. 2006, Zhang et al. 2018). They also induce supporting granulosa cells to synthesize an HA-rich matrix that coincides with the formation of numerous protrusions, through the expression of HAS2 and binding factors that extend HA polymers including versican, tumor necrosis–stimulated gene-6, and pentraxin-3 (Chang et al. 2016, El-Hayek et al. 2018). Communication through microvesicles, possibly HA enriched, may also take place (Machtinger et al. 2016). In fact, HAS2 and factors that bind HA are potential competence markers for assisted reproduction (Uyar et al. 2013), but how HA-enriched protrusions and vesicles contribute to these processes remains unclear. Furthermore, oocyte microvilli mediate sperm fusion and may block polyspermy through vesicle release to shed fusion receptors (Bianchi et al. 2014). Therefore, in addition to the well-known properties of HA matrices, HA-enriched structures, which have been largely neglected, may be crucial to mammalian reproduction (Salustri et al. 2019). Future studies need to delineate HA-regulated membrane structures and their broad functions in normal physiology and disease.

Glycocalyx-Regulated Mitosis

Classical literature suggests, with cell cycle progression, that cells transform a relatively flat membrane with blebs and stubby protrusions into one that accumulates densely arrayed lengthy microvilli (Follett & Goldman 1970, Lundgren & Roos 1976). Although curvature by the glycocalyx is poorly defined in mitosis, such morphological changes closely resemble those observed with increasing glycopolymer densities (Shurer et al. 2019). Indeed, HA synthesis peaks at mitosis, which parallels the formation of elaborate microvilli on cultured

cells (Evanko et al. 1999, Tukaj et al. 2002). Displacing HA polymers or blocking their synthesis inhibits division before cell rounding (Evanko et al. 1999). Incorporating long synthetic mucin polymers or the MUC1 ectodomain also promotes G1 cell cycle progression in mammary epithelial and cancer cells (Woods et al. 2017). Conversely, *MUC1* or *MUC4* deletion causes G1 arrest in glioma and pancreatic cancer cells (Jonckheere et al. 2012, Kim et al. 2020). As cell cycle inhibitions are promising therapeutic interventions for diseases such as cancers (Mills et al. 2018), understanding membrane and signaling regulation by glycopolymers in these processes would be paramount.

Glycocalyx and Shape in Cancer Cells

Microvilli-like protrusions are classically associated with transformed and isolated cancer cells (Kolata 1975). This may correspond to the upregulation of glycolipids, mucins, and HA observed broadly in cancer with poor outcomes (Pinho & Reis 2015). Increased cell surface density of mucin and HA are likely to enhance protrusion generation through polymer crowding. For instance, MUC1 overexpression occurs across many cancer types, and human breast and cervical cancer cells sorted for high MUC1 surface levels have significantly more protrusions than do native cells (Shurer et al. 2019). Similarly, HA accumulates in multiple cancers, and cell surface HA synthesis is responsible for protrusion generation in esophageal cancer cells and correlates with protrusion growth and density in breast cancer cells (Kyykallio et al. 2020, Twarock et al. 2010). Glycopolymer accumulation, besides the upregulation of their gene expression, is likely mediated by reprogrammed cancer metabolism (Pinho & Reis 2015).

Glycopolymers may have altered conformations and physical properties in cancer cells due to changes in side chains along the polymer backbone (Stowell et al. 2015). Cancer mucins are typically grafted by an abnormally high density of shorter O-glycan side chains due to upregulated and mislocalized glycosylation enzymes. How altered glycan chain length and grafting density affect the properties of cancer mucins needs further investigation. Steric repulsion between densely arrayed glycan chains may stretch mucin polymers to increase their extension. For instance, the upregulation of the mucin *O*-glycosylation enzyme GALNT7 increases glycocalyx thickness upon oncogenic *KRAS* activation and correlates with poor survival in pancreatic ductal adenocarcinoma (Möckl et al. 2019). It remains to be seen whether aberrant glycosylation of cancer mucins changes the preferred types and frequencies of membrane protrusions. In addition, factors that assemble on glycocalyx polymers could increase their occupied hydrodynamic volume to achieve greater crowding coverage and enhance membrane bending. Indeed, factors such as aggrecan binding to extend a preexisting HA coat can induce protrusion formation in prostate cancer cells (Nijenhuis et al. 2012). Future studies should provide details linking metabolism, oncogenic activation, glycopolymer conformation, and density for their contributions to cancer cell morphology.

The functions of glycocalyx-induced membrane structures in cancer are underdefined. Microvilli on circulating tumor cells are classically postulated to interact with the endothelium for extravasation (Domagala & Koss 1978, Kramer & Nicolson 1979). Such membrane protrusions may be enriched by glycopolymers. CD34, a podocalyxin-related

glycopolymer, regulates microvilli formation and the rolling of human hematopoietic stem/progenitor and myelogenous leukemia cells (AbuSamra et al. 2017). MUC1, which is especially enriched on circulating tumor cells, may carry the necessary epitopes to engage endothelium for leukocyte-like rolling and firm adhesion before tissue extravasation (Geng et al. 2012, Paszek et al. 2014). Interestingly, podocalyxin promotes extravasation by inducing invasive foot processes in breast cancer cells attached to the endothelial monolayer (Fröse et al. 2018). Newly extravasated cells then extend protrusions for tumor outgrowth to develop macroscopic metastases (Shibue et al. 2012), though the involvement of the glycocalyx in this process is unclear. Membrane bridges between cancer cells are proposed for intercellular communication to propagate disease (Osswald et al. 2015). In this regard, MUC1 overexpression may enrich glioblastoma networking (Barnes et al. 2018, Kim et al. 2020, Osswald et al. 2015). Microvesicles are also important communicators in cancer (Xu et al. 2018). Cancer patient–derived microvesicles are MUC1 enriched, and cervical cancer cells sorted for high MUC1 expression also secrete significantly more microvesicles than do native cells (Shurer et al. 2019), although whether they carry messages for communication needs to be verified. Likewise, melanoma cells overexpressing HAS3 can secrete HA-coated microvesicles to stimulate the proliferation and epithelial-to-mesenchymal transition in normal target cells (Arasu et al. 2020). These observations highlight an urgent unmet need for unraveling the functions of glycocalyx-induced structures in cancer.

Glycolipids and Protrusion Biology

Glycolipid metabolic reprogramming, mediated by biosynthetic enzyme regulation, can switch glycolipid classes and transition simple to complex glycolipids to drive a broad range of cell differentiation and cell fate determination (Russo et al. 2018, Sipione et al. 2020). These metabolic programs parallel membrane protrusions that form structures such as neurites, stereocilia, microvilli, and cilia (Jennemann et al. 2012, Kaiser et al. 2020, Ledeen & Wu 2015). Genetic models have revealed glycolipid-regulated membrane morphologies in vivo. For instance, mice lacking the glycolipid initiation enzyme UGCG have normal congenital microvilli that later malform in postnatal intestines but not in the liver or kidney (Jennemann et al. 2012). Specific glycolipids may regulate distinct structures. Mice lacking the synthase for GM3, but not GM1, recapitulate the hearing impairment of human GM3 deficiency and show immature stereocilia replaced by abnormally giant structures in postnatal development (Yoshikawa et al. 2015). Conversely, GM3-only mice, devoid of complex gangliosides, suffer from lethal audiogenic seizures, though stereocilia abnormalities are not characterized (Kawai et al. 2001). In cultured cells, GM3 predominantly populates highly curved protrusion peaks, while GM1 resides mainly along protrusion slopes and valleys (Chen et al. 2008). Whether observations in mice reflect the ability of distinct glycolipids to generate curved membrane regions needs further validation, especially as GM3 supplementation may potentially improve hearing impairment and neurodevelopment in newborns (Wang et al. 2019).

Complex gangliosides accumulate at neurite tips in the developing brain and regulate protrusion branching and outgrowth in isolated neuronal cells (Ledeen & Wu 2015, Sipione et al. 2020). Molecular details are emerging, but how such glycolipids induce membrane bending is not well-defined. This is important, as glycolipid deficiencies due to

loss-of-function mutations can induce severe congenital neuropathy and altered ganglioside levels are common in neurodegenerative diseases (Sipione et al. 2020). Abnormal neuron morphology is widespread among animal models lacking complex gangliosides that display neuron degeneration, retarded brain development, reduced cognitive functions, and other symptoms that resemble human pathology (Allende & Proia 2014, Sipione et al. 2020). Remarkably, ganglioside administration can partially rescue these phenotypes and has shown promise in stimulating neuron repair and regeneration for treating nerve damage and neurodegenerative diseases in preclinical and clinical studies (Magistretti et al. 2019). Therefore, ganglioside-regulated neuronal cell morphologies warrant further elucidation.

CONCLUDING REMARKS

Cell morphogenesis is largely attributed to cytoskeletal dynamics, and molecular details are currently being unraveled. Often overlooked is that the glycocalyx also can generate pressures on the membrane through processes that are becoming better understood. Mechanisms are now emerging to assign biophysical functions to the glycocalyx, which has long been observed to coat diverse membrane structures. The significance of such protrusions and secreted vesicles in intercellular communication requires further elucidation. These studies are important, as a detailed understanding of glycocalyx-membrane regulation can direct potential therapies for conditions ranging from infertility to neurodegenerative diseases and cancer.

ACKNOWLEDGMENTS

This investigation was supported by National Institutes of Health New Innovator grant DP2 GM229133, National Cancer Institute grant U54 CA210184, and National Institute of General Medical Sciences grant R01 GM138692-02. We thank Ling-Ting Huang for assistance with the calculations in Figure 1.

LITERATURE CITED

AbuSamra DB, Aleisa FA, Al-Amoodi AS, Jalal Ahmed HM, Chin CJ, et al. 2017. Not just a marker: CD34 on human hematopoietic stem/progenitor cells dominates vascular selectin binding along with CD44. Blood Adv. 1(27):2799–816 [PubMed: 29296932]

Adell MAY, Migliano SM, Upadhyayula S, Bykov YS, Sprenger S, et al. 2017. Recruitment dynamics of ESCRT-III and Vps4 to endosomes and implications for reverse membrane budding. eLife 6:e31652 [PubMed: 29019322]

Ahmad N, Gabius HJ, Andre S, Kaltner H, Sabesan S, et al. 2004. Galectin-3 precipitates as a pentamer with synthetic multivalent carbohydrates and forms heterogeneous cross-linked complexes. J. Biol. Chem 279(12):10841–47 [PubMed: 14672941]

Ajo-Franklin CM, Ganesan PV, Boxer SG. 2005. Variable incidence angle fluorescence interference contrast microscopy for z-imaging single objects. Biophys. J 89(4):2759–69 [PubMed: 16085775]

Al-bataineh M, Sutton TA, Hughey RP 2017. Novel roles for mucin 1 in the kidney. Curr. Opin. Nephrol. Hypertens 26(5):384–91 [PubMed: 28622163]

Allende ML, Proia RL. 2014. Simplifying complexity: genetically resculpting glycosphingolipid synthesis pathways in mice to reveal function. Glycoconj. J 31(9):613–22 [PubMed: 25351657]

Anvarian Z, Mykytyn K, Mukhopadhyay S, Pedersen LB, Christensen ST. 2019. Cellular signalling by primary cilia in development, organ function and disease. Nat. Rev. Nephrol 15(4):199–219 [PubMed: 30733609]

Arasu UT, Deen AJ, Pasonen-Seppänen S, Heikkinen S, Lalowski M, et al. 2020. HAS3-induced extracellular vesicles from melanoma cells stimulate IHH mediated c-Myc upregulation via the

hedgehog signaling pathway in target cells. Cell Mol. Life Sci 77(20):4093–115 [PubMed: 31820036]

Arnaud J, Tröndle K, Claudinon J, Audfray A, Varrot A, et al. 2014. Membrane deformation by neolectins with engineered glycolipid binding sites. Angew. Chem 126(35):9421–24

Baena V, Terasaki M. 2019. Three-dimensional organization of transzonal projections and other cytoplasmic extensions in the mouse ovarian follicle. Sci. Rep 9:1262 [PubMed: 30718581]

Barnes JM, Kaushik S, Bainer RO, Sa JK, Woods EC, et al. 2018. A tension-mediated glycocalyx-integrin feedback loop promotes mesenchymal-like glioblastoma. Nat. Cell Biol 20(10):1203–14 [PubMed: 30202050]

Bennett R, Järvelä T, Engelhardt P, Kostamovaara L, Sparks P, et al. 2001. Mucin MUC1 is seen in cell surface protrusions together with ezrin in immunoelectron tomography and is concentrated at tips of filopodial protrusions in MCF-7 breast carcinoma cells. J. Histochem. Cytochem 49(1):67–77 [PubMed: 11118479]

Berndt C, Montañez E, Villena J, Fabre M, Vilaró S, Reina M. 2004. Influence of cytoplasmic deletions on the filopodia-inducing effect of syndecan-3. Cell Biol. Int 28(11):829–33 [PubMed: 15563406]

Bhatia T, Agudo-Canalejo J, Dimova R, Lipowsky R. 2018. Membrane nanotubes increase the robustness of giant vesicles. ACS Nano 12(5):4478–85 [PubMed: 29659246]

Bianchi E, Doe B, Goulding D, Wright GJ. 2014. Juno is the egg Izumo receptor and is essential for mammalian fertilization. Nature 508(7497):483–87 [PubMed: 24739963]

Blalock TD, Spurr-Michaud SJ, Tisdale AS, Heimer SR, Gilmore MS, et al. 2007. Functions of MUC16 in corneal epithelial cells. Invest. Ophthalmol. Vis. Sci 48(10):4509–18 [PubMed: 17898272]

Bouché C, Serdy S, Kahn CR, Goldfine AB. 2004. The cellular fate of glucose and its relevance in type 2 diabetes. Endocr. Rev 25(5):807–30 [PubMed: 15466941]

Braun D, Fromherz P 1997. Fluorescence interference-contrast microscopy of cell adhesion on oxidized silicon. Appl. Phys. A 65(4):341–48

Brocca P, Cantù L, Corti M, Del Favero E, Motta S. 2004. Shape fluctuations of large unilamellar lipid vesicles observed by laser light scattering: influence of the small-scale structure. Langmuir 20(6):2141–48 [PubMed: 15835663]

Budnik V, Ruiz-Cañada C, Wendler F. 2016. Extracellular vesicles round off communication in the nervous system. Nat. Rev. Neurosci 17(3):160–72 [PubMed: 26891626]

Burra S, Nicolella DP, Francis WL, Freitas CJ, Mueschke NJ, et al. 2010. Dendritic processes of osteocytes are mechanotransducers that induce the opening of hemichannels. PNAS 107(31):13648–53 [PubMed: 20643964]

Busch DJ, Houser JR, Hayden CC, Sherman MB, Lafer EM, Stachowiak JC. 2015. Intrinsically disordered proteins drive membrane curvature. Nat. Commun 6:7875 [PubMed: 26204806]

Butt L, Unnersjö-Jess D, Höhne M, Edwards A, Binz-Lotter J, et al. 2020. A molecular mechanism explaining albuminuria in kidney disease. Nat. Metab 2(5):461–74 [PubMed: 32694662]

Button B, Cai L-H, Ehre C, Kesimer M, Hill DB, et al. 2012. A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. Science 337(6097):937–41 [PubMed: 22923574]

Chang CY-Y, Chang H-W, Chen C-M, Lin C-Y, Chen C-P, et al. 2011. *MUC4* gene polymorphisms associate with endometriosis development and endometriosis-related infertility. BMC Med. 9:19 [PubMed: 21349170]

Chang H-M, Qiao J, Leung PCK. 2016. Oocyte–somatic cell interactions in the human ovary—novel role of bone morphogenetic proteins and growth differentiation factors. Hum. Reprod. Update 23(1):1–18 [PubMed: 27797914]

Chauhan M, Balakrishnan M, Chan R, Yallampalli C. 2015. Adrenomedullin 2 (ADM2) regulates mucin 1 at the maternal-fetal interface in human pregnancy. Biol. Reprod 93(6):1–8

Chen Y, Qin J, Chen ZW. 2008. Fluorescence-topographic NSOM directly visualizes peak-valley polarities of GM1/GM3 rafts in cell membrane fluctuations. J. Lipid Res 49(10):2268–75 [PubMed: 18603643]

Cheng X, Smith JC. 2019. Biological membrane organization and cellular signaling. Chem. Rev 119(9):5849–80 [PubMed: 30747526]

Cohen M, Klein E, Geiger B, Addadi L. 2003. Organization and adhesive properties of the hyaluronan pericellular coat of chondrocytes and epithelial cells. Biophys. J 85(3):1996–2005 [PubMed: 12944312]

Colom A, Derivery E, Soleimanpour S, Tomba C, Molin MD, et al. 2018. A fluorescent membrane tension probe. Nat. Chem 10(11):1118–25 [PubMed: 30150727]

Colombo M, Raposo G, Théry C. 2014. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu. Rev. Cell Dev. Biol 30:255–89 [PubMed: 25288114]

Crawley SW, Shifrin DA, Grega-Larson NE, McConnell RE, Benesh AE, et al. 2014. Intestinal brush border assembly driven by protocadherin-based intermicrovillar adhesion. Cell 157(2):433–46 [PubMed: 24725409]

Dasgupta R, Miettinen MS, Fricke N, Lipowsky R, Dimova R. 2018. The glycolipid GM1 reshapes asymmetric biomembranes and giant vesicles by curvature generation. PNAS 115(22):5756–61 [PubMed: 29760097]

Decher G, Kuchinka E, Ringsdorf H, Venzmer J, Bitter-Suermann D, Weisgerber C. 1989. Interaction of amphiphilic polymers with model membranes. Angew. Makromol. Chem 166(1):71–80

DeSouza MM, Surveyor GA, Price RE, Julian J, Kardon R, et al. 2000. MUC1/episialin: a critical barrier in the female reproductive tract. J. Reprod. Immunol 45(2):127–58

Domagala W, Koss LG. 1978. Configuration of surfaces of human cancer cells in effusions. Virchows Arch. B Cell Path 26:27–42

Doyonnas R, Kershaw DB, Duhme C, Merkens H, Chelliah S, et al. 2001. Anuria, omphalocele, and perinatal lethality in mice lacking the Cd34-related protein podocalyxin. J. Exp. Med 194(1):13–28 [PubMed: 11435469]

Ebrahimkutty MP, Galic M. 2019. Receptor-free signaling at curved cellular membranes. BioEssays 41(10):1900068

Eierhoff T, Bastian B, Thuenauer R, Madl J, Audfray A, et al. 2014. A lipid zipper triggers bacterial invasion. PNAS 111(35):12895–900 [PubMed: 25136128]

El-Hayek S, Yang Q, Abbassi L, FitzHarris G, Clarke HJ. 2018. Mammalian oocytes locally remodel follicular architecture to provide the foundation for germline-soma communication. Curr. Biol 28(7):1124–31.e3 [PubMed: 29576478]

Evanko SP, Angello JC, Wight TN. 1999. Formation of hyaluronan- and versican-rich pericellular matrix is required for proliferation and migration of vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol 19(4):1004–13 [PubMed: 10195929]

Ewers H, Römer W, Smith AE, Bacia K, Dmitrieff S, et al. 2010. GM1 structure determines SV40-induced membrane invagination and infection. Nat. Cell Biol 12:11–18 [PubMed: 20023649]

Eyermann C, Czaplinski K, Colognato H. 2012. Dystroglycan promotes filopodial formation and process branching in differentiating oligodendroglia. J. Neurochem 120(6):928–47 [PubMed: 22117643]

Fischer A, Koopmans T, Ramesh P, Christ S, Strunz M, et al. 2020. Post-surgical adhesions are triggered by calcium-dependent membrane bridges between mesothelial surfaces. Nat. Commun 11:3068 [PubMed: 32555155]

Follett EAC, Goldman RD. 1970. The occurrence of microvilli during spreading and growth of BHK21/C13 fibroblasts. Exp. Cell Res 59(1):124–36 [PubMed: 5448185]

Frey SL, Lee KYC. 2013. Number of sialic acid residues in ganglioside headgroup affects interactions with neighboring lipids. Biophys J. 105(6):1421–31 [PubMed: 24047994]

Fricke N, Dimova R. 2016. GM1 softens POPC membranes and induces the formation of micron-sized domains. Biophys. J 111(9):1935–45 [PubMed: 27806275]

Frolov VA, Shnyrova AV, Zimmerberg J. 2011. Lipid polymorphisms and membrane shape. Cold Spring Harb. Perspect. Biol 3(11):a004747 [PubMed: 21646378]

Frose J, Chen MB, Hebron KE, Reinhardt F, Hajal C, et al. 2018. Epithelial-mesenchymal transition induces podocalyxin to promote extravasation via ezrin signaling. Cell Rep. 24(4):962–72 [PubMed: 30044991]

Galeano B, Klootwijk R, Manoli I, Sun M, Ciccone C, et al. 2007. Mutation in the key enzyme of sialic acid biosynthesis causes severe glomerular proteinuria and is rescued by N-acetylmannosamine. J. Clin. Invest 117(6):1585–94 [PubMed: 17549255]

Gandhi JG, Koch DL, Paszek MJ. 2019. Equilibrium modeling of the mechanics and structure of the cancer glycocalyx. Biophys. J 116(4):694–708 [PubMed: 30736980]

Geng Y, Yeh K, Takatani T, King MR. 2012. Three to tango: MUC1 as a ligand for both E-selectin and ICAM-1 in the breast cancer metastatic cascade. Front. Oncol 2. 10.3389/fonc.2012.00076

Gipson IK, Blalock T, Tisdale A, Spurr-Michaud S, Allcorn S, et al. 2008. MUC16 is lost from the uterodome (pinopode) surface of the receptive human endometrium: in vitro evidence that MUC16 is a barrier to trophoblast adherence. Biol. Reprod 78(1):134–42 [PubMed: 17942799]

Gipson IK, Spurr-Michaud S, Tisdale A, Menon BB. 2014. Comparison of the transmembrane mucins MUC1 and MUC16 in epithelial barrier function. PLOS ONE 9(6):e100393 [PubMed: 24968021]

Godula K, Umbel ML, Rabuka D, Botyanszki Z, Bertozzi CR, Parthasarathy R. 2009. Control of the molecular orientation of membrane-anchored biomimetic glycopolymers. J. Am. Chem. Soc 131(29):10263–68 [PubMed: 19580278]

Granés F, Garciá R, Casaroli-Marano RP, Castel S, Rocamora N, et al. 1999. Syndecan-2 induces filopodia by active cdc42Hs. Exp. Cell Res 248(2):439–56 [PubMed: 10222136]

Gruner SM. 1985. Intrinsic curvature hypothesis for biomembrane lipid composition: a role for nonbilayer lipids. PNAS 82(11):3665–69 [PubMed: 3858841]

Hägerstrand H, Mrówczyñska L, Salzer U, Prohaska R, Michelsen KA, et al. 2006. Curvature-dependent lateral distribution of raft markers in the human erythrocyte membrane. Mol. Membr. Biol 23(3):277–88 [PubMed: 16785211]

Hale JE, Wuthier RE. 1987. The mechanism of matrix vesicle formation. Studies on the composition of chondrocyte microvilli and on the effects of microfilament-perturbing agents on cellular vesiculation. J. Biol. Chem 262(4):1916–25 [PubMed: 3543016]

Hara M, Yanagihara T, Hirayama Y, Ogasawara S, Kurosawa H, et al. 2010. Podocyte membrane vesicles in urine originate from tip vesiculation of podocyte microvilli. Hum. Pathol 41(9):1265–75 [PubMed: 20447677]

Hattrup CL, Gendler SJ. 2008. Structure and function of the cell surface (tethered) mucins. Annu. Rev. Physiol 70:431–57 [PubMed: 17850209]

Horne AW, Lalani E-N, Margara RA, Ryder TA, Mobberley MA, White JO. 2005. The expression pattern of MUC1 glycoforms and other biomarkers of endometrial receptivity in fertile and infertile women. Mol. Reprod. Dev 72(2):216–29 [PubMed: 15971251]

Horne AW, White JO, Margara RA, Williams R, Winston RM, Lalani E-N. 2001. MUC 1: a genetic susceptibility to infertility? Lancet 357(9265):1336–37 [PubMed: 11343742]

Hossein A, Deserno M. 2020. Spontaneous curvature, differential stress, and bending modulus of asymmetric lipid membranes. Biophys. J 118(3):624–42 [PubMed: 31954503]

Houk AR, Jilkine A, Mejean CO, Boltyanskiy R, Dufresne ER, et al. 2012. Membrane tension maintains cell polarity by confining signals to the leading edge during neutrophil migration. Cell 148(1–2):175–88 [PubMed: 22265410]

Houser JR, Hayden CC, Thirumalai D, Stachowiak JC. 2020. A Forster resonance energy transfer-based sensor of steric pressure on membrane surfaces. J. Am. Chem. Soc 142(49):20796–805 [PubMed: 33237768]

Huizing M, Yardeni T, Fuentes F, Malicdan MCV, Leoyklang P, et al. 2019. Rationale and design for a Phase 1 study of *N*-acetylmannosamine for primary glomerular diseases. Kidney Int. Rep 4(10):1454–62 [PubMed: 31701055]

Ingólfsson HI, Carpenter TS, Bhatia H, Bremer P-T, Marrink SJ, Lightstone FC. 2017. Computational lipidomics of the neuronal plasma membrane. Biophys. J 113(10):2271–80 [PubMed: 29113676]

Jedlovszky P, Sega M, Vallauri R. 2009. GM1 ganglioside embedded in a hydrated DOPC membrane: a molecular dynamics simulation study. J. Phys. Chem. B 113(14):4876–86 [PubMed: 19275209]

Jennemann R, Kaden S, Sandhoff R, Nordström V, Wang S, et al. 2012. Glycosphingolipids are essential for intestinal endocytic function. J. Biol. Chem 287(39):32598–616 [PubMed: 22851168]

Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, et al. 2019. Reassessment of exosome composition. Cell 177(2):428–45.e18 [PubMed: 30951670]

Jin J, Sison K, Li C, Tian R, Wnuk M, et al. 2012. Soluble FLT1 binds lipid microdomains in podocytes to control cell morphology and glomerular barrier function. Cell 151(2):384–99 [PubMed: 23063127]

Johannes L, Wunder C, Shafaq-Zadah M. 2016. Glycolipids and lectins in endocytic uptake processes. J. Mol. Biol 428(24, Part A):4792–818

Johansson K, Willysson A, Kristoffersson A-C, Tontanahal A, Gillet D, et al. 2020. Shiga toxin-bearing microvesicles exert a cytotoxic effect on recipient cells only when the cells express the toxin receptor. Front. Cell. Infect. Microbiol 10. 10.3389/fcimb.2020.00212

Jonckheere N, Skrypek N, Merlin J, Dessein AF, Dumont P, et al. 2012. The mucin MUC4 and its membrane partner ErbB2 regulate biological properties of human CAPAN-2 pancreatic cancer cells via different signalling pathways. PLOS ONE 7(2):e32232 [PubMed: 22393391]

Kabbani AM, Raghunathan K, Lencer WI, Kenworthy AK, Kelly CV. 2020. Structured clustering of the glycosphingolipid GM1 is required for membrane curvature induced by cholera toxin. PNAS 117(26):14978–86 [PubMed: 32554490]

Kaiser F, Huebecker M, Wachten D. 2020. Sphingolipids controlling ciliary and microvillar function. FEBS Lett. 594(22):3652–67 [PubMed: 32415987]

Kamal MM, Mills D, Grzybek M, Howard J. 2009. Measurement of the membrane curvature preference of phospholipids reveals only weak coupling between lipid shape and leaflet curvature. PNAS 106(52):22245–50 [PubMed: 20080790]

Kang HG, Lee M, Lee KB, Hughes M, Kwon BS, et al. 2017. Loss of podocalyxin causes a novel syndromic type of congenital nephrotic syndrome. Exp. Mol. Med 49(12):e414 [PubMed: 29244787]

Kappagantula S, Andrews MR, Cheah M, Abad-Rodriguez J, Dotti CG, Fawcett JW. 2014. Neu3 sialidase-mediated ganglioside conversion is necessary for axon regeneration and is blocked in CNS axons. J. Neurosci 34(7):2477–92 [PubMed: 24523539]

Kawai H, Allende ML, Wada R, Kono M, Sango K, et al. 2001. Mice expressing only monosialoganglioside GM3 exhibit lethal audiogenic seizures. J. Biol. Chem 276(10):6885–88 [PubMed: 11133999]

Kelleher AM, Burns GW, Behura S, Wu G, Spencer TE. 2016. Uterine glands impact uterine receptivity, luminal fluid homeostasis and blastocyst implantation. Sci. Rep 6:38078 [PubMed: 27905495]

Kerjaschki D, Sharkey DJ, Farquhar MG. 1984. Identification and characterization of podocalyxin–the major sialoprotein of the renal glomerular epithelial cell. J. Cell Biol 98(4):1591–96 [PubMed: 6371025]

Kesimer M, Ehre C, Burns KA, Davis CW, Sheehan JK, Pickles RJ. 2013. Molecular organization of the mucins and glycocalyx underlying mucus transport over mucosal surfaces of the airways. Mucosal Immunol. 6(2):379–92 [PubMed: 22929560]

Kesimer M, Scull M, Brighton B, DeMaria G, Burns K, et al. 2009. Characterization of exosome-like vesicles released from human tracheobronchial ciliated epithelium: a possible role in innate defense. FASEB J. 23(6):1858–68 [PubMed: 19190083]

Kim S, Seo Y, Chowdhury T, Yu HJ, Lee CE, et al. 2020. Inhibition of MUC1 exerts cell-cycle arrest and telomerase suppression in glioblastoma cells. Sci. Rep 10:18238 [PubMed: 33106534]

Kim SH, Chi M, Yi B, Kim SH, Oh S, et al. 2014. Three-dimensional intestinal villi epithelium enhances protection of human intestinal cells from bacterial infection by inducing mucin expression. Integr. Biol 6(12):1122–31

Kim YK, Refaeli I, Brooks CR, Jing P, Gulieva RE, et al. 2017. Gene-edited human kidney organoids reveal mechanisms of disease in podocyte development. Stem Cells 35(12):2366–78 [PubMed: 28905451]

Kirby A, Gnirke A, Jaffe DB, Barešová V, Pochet N, et al. 2013. Mutations causing medullary cystic kidney disease type 1 lie in a large VNTR in *MUC1* missed by massively parallel sequencing. Nat. Genet 45(3):299–303 [PubMed: 23396133]

Koistinen V, Härkönen K, Kärnä R, Arasu UT, Oikari S, Rilla K. 2017. EMT induced by EGF and wounding activates hyaluronan synthesis machinery and EV shedding in rat primary mesothelial cells. Matrix Biol. 63:38–54 [PubMed: 28043889]

Koistinen V, Jokela T, Oikari S, Kaärnä R, Tammi M, Rilla K. 2016. Hyaluronan-positive plasma membrane protrusions exist on mesothelial cells in vivo. Histochem. Cell Biol 145(5):531–44 [PubMed: 26821263]

Koistinen V, Kärnä R, Koistinen A, Arjonen A, Tammi M, Rilla K. 2015. Cell protrusions induced by hyaluronan synthase 3 (HAS3) resemble mesothelial microvilli and share cytoskeletal features of filopodia. Exp. Cell Res 337(2):179–91 [PubMed: 26162854]

Kojima K, Nosaka H, Kishimoto Y, Nishiyama Y, Fukuda S, et al. 2011. Defective glycosylation of α-dystroglycan contributes to podocyte flattening. Kidney Int. 79(3):311–16 [PubMed: 20944549]

Kolata GB. 1975. Microvilli: a major difference between normal and cancer cells? Science 188(4190):819–20 [PubMed: 17769885]

Komatsu M, Carraway CA, Fregien NL, Carraway KL. 1997. Reversible disruption of cell-matrix and cell-cell interactions by overexpression of sialomucin complex. J. Biol. Chem 272(52):33245–54 [PubMed: 9407114]

Kopp JB, Anders H-J, Susztak K, Podestà MA, Remuzzi G, et al. 2020. Podocytopathies. Nat. Rev. Dis. Primers 6:68 [PubMed: 32792490]

Kozlovsky Y, Kozlov MM. 2003. Membrane fission: model for intermediate structures. Biophys.J 85(1):85–96 [PubMed: 12829467]

Kramer JR, Onoa B, Bustamante C, Bertozzi CR. 2015. Chemically tunable mucin chimeras assembled on living cells. PNAS 112(41):12574–79 [PubMed: 26420872]

Kramer RH, Nicolson GL. 1979. Interactions of tumor cells with vascular endothelial cell monolayers: a model for metastatic invasion. PNAS 76(11):5704–8 [PubMed: 293673]

Kultti A, Rilla K, Tiihonen R, Spicer AP, Tammi RH, Tammi MI. 2006. Hyaluronan synthesis induces microvillus-like cell surface protrusions. J. Biol. Chem 281(23):15821–28 [PubMed: 16595683]

Kyykallio H, Oikari S, Bueno Álvez M, Gallardo Dodd CJ, Capra J, Rilla K. 2020. The density and length of filopodia associate with the activity of hyaluronan synthesis in tumor cells. Cancers 12(7):1908

Lakshminarayan R, Wunder C, Becken U, Howes MT, Benzing C, et al. 2014. Galectin-3 drives glycosphingolipid-dependent biogenesis of clathrin-independent carriers. Nat. Cell Biol 16(6):592–603

Lange K 2011. Fundamental role of microvilli in the main functions of differentiated cells: outline of an universal regulating and signaling system at the cell periphery. J. Cell. Physiol 226(4):896–927 [PubMed: 20607764]

Ledeen RW, Wu G. 2015. The multi-tasked life of GM1 ganglioside, a true factotum of nature. Trends Biochem. Sci 40(7):407–18 [PubMed: 26024958]

Lee GM, Johnstone B, Jacobson K, Caterson B. 1993. The dynamic structure of the pericellular matrix on living cells. J. Cell Biol 123(6):1899–907 [PubMed: 8276905]

Lee H, Zhang D, Laskin DL, Jin Y. 2018. Functional evidence of pulmonary extracellular vesicles in infectious and noninfectious lung inflammation. J. Immunol 201(5):1500–9 [PubMed: 29997122]

Ling H, Boodhoo A, Hazes B, Cummings MD, Armstrong GD, et al. 1998. Structure of the Shiga-like toxin I B-pentamer complexed with an analogue of its receptor Gb_3. Biochemistry 37(7):1777–88 [PubMed: 9485303]

Lingwood CA. 2011. Glycosphingolipid functions. Cold Spring Harb. Perspect. Biol 3(7):a004788 [PubMed: 21555406]

Liu Y, Barnoud J, Marrink SJ. 2019. Gangliosides destabilize lipid phase separation in multicomponent membranes. Biophys.J 117(7):1215–23 [PubMed: 31542224]

Lundgren E, Roos G. 1976. Cell surface changes in HeLa cells as an indication of cell cycle events. Cancer Res. 36(11,Part 1):4044–51 [PubMed: 61800]

Machtinger R, Laurent LC, Baccarelli AA. 2016. Extracellular vesicles: roles in gamete maturation, fertilization and embryo implantation. Hum. Reprod. Update 22(2):182–93 [PubMed: 26663221]

Magistretti PJ, Geisler FH, Schneider JS, Li PA, Fiumelli H, Sipione S. 2019. Gangliosides: treatment avenues in neurodegenerative disease. Front. Neurol 10. 10.3389/fneur.2019.00859

Makabe S, Naguro T, Stallone T. 2006. Oocyte-follicle cell interactions during ovarian follicle development, as seen by high resolution scanning and transmission electron microscopy in humans. Microsc. Res. Tech 69(6):436–49 [PubMed: 16718658]

Margarit L, Taylor A, Roberts MH, Hopkins L, Davies C, et al. 2010. MUC1 as a discriminator between endometrium from fertile and infertile patients with PCOS and endometriosis. J. Clin. Endocrinol. Metab 95(12):5320–29 [PubMed: 20826587]

McConnell RE, Higginbotham JN, Shifrin DA Jr., Tabb DL, Coffey RJ, Tyska MJ. 2009. The enterocyte microvillus is a vesicle-generating organelle. J. Cell Biol 185(7):1285–98 [PubMed: 19564407]

McIntosh TJ, Simon SA. 1994. Long- and short-range interactions between phospholipid/ganglioside GM1 bilayers. Biochemistry 33(34):10477–86 [PubMed: 8068686]

McNeer RR, Carraway CAC, Fregien NL, Carraway KL. 1998. Characterization of the expression and steroid hormone control of sialomucin complex in the rat uterus: implications for uterine receptivity. J. Cell. Physiol 176(1):110–19 [PubMed: 9618151]

Mills CC, Kolb E, Sampson VB. 2018. Development of chemotherapy with cell-cycle inhibitors for adult and pediatric cancer therapy. Cancer Res. 78(2):320–25 [PubMed: 29311160]

Möckl L, Pedram K, Roy AR, Krishnan V, Gustavsson A-K, et al. 2019. Quantitative super-resolution microscopy of the mammalian glycocalyx. Dev. Cell 50(1):57–72.e6 [PubMed: 31105009]

Murphy CR. 2004. Uterine receptivity and the plasma membrane transformation. Cell Res. 14(4):259–67 [PubMed: 15353123]

Mustonen A-M, Nieminen P, Joukainen A, Jaroma A, Kääriäinen T, et al. 2016. First in vivo detection and characterization of hyaluronan-coated extracellular vesicles in human synovial fluid. J. Orthop. Res 34(11):1960–68 [PubMed: 26919117]

Narimatsu Y, Joshi HJ, Nason R, Van Coillie J, Karlsson R, et al. 2019. An atlas of human glycosylation pathways enables display of the human glycome by gene engineered cells. Mol. Cell 75(2):394–407.e5 [PubMed: 31227230]

Narimatsu Y, Joshi HJ, Yang Z, Gomes C, Chen Y-H, et al. 2018. A validated gRNA library for CRISPR/Cas9 targeting of the human glycosyltransferase genome. Glycobiology 28(5):295–305 [PubMed: 29315387]

Nawrocki G, Im W, Sugita Y, Feig M. 2019. Clustering and dynamics of crowded proteins near membranes and their influence on membrane bending. PNAS 116(49):24562–67 [PubMed: 31740611]

Needham SR, Roberts SK, Arkhipov A, Mysore VP, Tynan CJ, et al. 2016. EGFR oligomerization organizes kinase-active dimers into competent signalling platforms. Nat. Commun 7:13307 [PubMed: 27796308]

Neves SR, Tsokas P, Sarkar A, Grace EA, Rangamani P, et al. 2008. Cell shape and negative links in regulatory motifs together control spatial information flow in signaling networks. Cell 133(4):666–80 [PubMed: 18485874]

Nielsen JS, Graves ML, Chelliah S, Vogl AW, Roskelley CD, McNagny KM. 2007. The CD34-related molecule podocalyxin is a potent inducer of microvillus formation. PLOS ONE 2(2):e237 [PubMed: 17311105]

Nijenhuis N, Mizuno D, Spaan JAE, Schmidt CF 2012. High-resolution microrheology in the pericellular matrix of prostate cancer cells. J. R. Soc. Interface 9(73):1733–44 [PubMed: 22319113]

Noble JM, Roberts LM, Vidavsky N, Chiou AE, Fischbach C, et al. 2020. Direct comparison of optical and electron microscopy methods for structural characterization of extracellular vesicles. J. Struct. Biol 210(1):107474 [PubMed: 32032755]

Ochs M, Hegermann J, Lopez-Rodriguez E, Timm S, Nouailles G, et al. 2020. On top of the alveolar epithelium: surfactant and the glycocalyx. Int. J. Mol. Sci 21(9):3075

Orbach R, Su X. 2020. Surfing on membrane waves: microvilli, curved membranes, and immune signaling. Front. Immunol 11. 10.3389/fimmu.2020.02187

Osswald M, Jung E, Sahm F, Solecki G, Venkataramani V, et al. 2015. Brain tumour cells interconnect to a functional and resistant network. Nature 528(7580):93–98 [PubMed: 26536111]

Palmieri V, Bozzi M, Signorino G, Papi M, De Spirito M, et al. 2017. α-Dystroglycan hypoglycosylation affects cell migration by influencing β-dystroglycan membrane clustering and filopodia length: a multiscale confocal microscopy analysis. Biochim. Biophys. Acta Mol. Basis Dis 1863(9):2182–91 [PubMed: 28572004]

Pan D, Chen J, Feng C, Wu W, Wang Y, et al. 2019. Preferential localization of MUC1 glycoprotein in exosomes secreted by non-small cell lung carcinoma cells. Int. J. Mol. Sci 20(2):323

Pan H, Colville MJ, Supekar NT, Azadi P, Paszek MJ. 2019. Sequence-specific mucins for glycocalyx engineering. ACS Synth. Biol 8(10):2315–26 [PubMed: 31500407]

Paszek MJ, DuFort CC, Rossier O, Bainer R, Mouw JK, et al. 2014.The cancer glycocalyx mechanically primes integrin-mediated growth and survival. Nature 511(7509):319–25 [PubMed: 25030168]

Patel DS, Park S, Wu EL, Yeom MS, Widmalm G, et al. 2016. Influence of ganglioside GM1 concentration on lipid clustering and membrane properties and curvature. Biophys. J 111(9):1987–99 [PubMed: 27806280]

Pei B, Chen J-W 2003. More ordered, convex ganglioside-enriched membrane domains: the effects of GM1 on sphingomyelin bilayers containing a low level of cholesterol. J. Biochem 134(4):575–81 [PubMed: 14607985]

Pelaseyed T, Hansson GC. 2020. Membrane mucins of the intestine at a glance. J. Cell Sci 133(5):jcs240929 [PubMed: 32169835]

Pezeshkian W, Gao H, Arumugam S, Becken U, Bassereau P, et al. 2017a. Mechanism of Shiga toxin clustering on membranes. ACS Nano 11(1):314–24 [PubMed: 27943675]

Pezeshkian W, Hansen AG, Johannes L, Khandelia H, Shillcock JC, et al. 2016. Membrane invagination induced by Shiga toxin B-subunit: from molecular structure to tube formation. Soft Matter 12(23):5164–71 [PubMed: 27070906]

Pezeshkian W, Nåbo LJ, Ipsen JH. 2017b. Cholera toxin B subunit induces local curvature on lipid bilayers. FEBS Open Biol. 7(11):1638–45

Pinho SS, Reis CA. 2015. Glycosylation in cancer: mechanisms and clinical implications. Nat. Rev. Cancer 15(9):540–55 [PubMed: 26289314]

Refaeli I, Hughes MR, McNagny KM. 2019. The first identified heterozygous nonsense mutations in podocalyxin offer new perspectives on the biology of podocytopathies. Clin. Sci 133(3):443–47

Refaeli I, Hughes MR, Wong AK-W, Bissonnette MLZ, Roskelley CD, et al. 2020. Distinct functional requirements for podocalyxin in immature and mature podocytes reveal mechanisms of human kidney disease. Sci. Rep 10:9419 [PubMed: 32523052]

Reily C, Stewart TJ, Renfrow MB, Novak J. 2019. Glycosylation in health and disease. Nat. Rev. Nephrol 15(6):346–66 [PubMed: 30858582]

Renard H-F, Simunovic M, Lemière J, Boucrot E, Garcia-Castillo MD, et al. 2015. Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. Nature 517(7535):493–96 [PubMed: 25517096]

Renard H-F, Tyckaert F, Lo Giudice C, Hirsch T, Valades-Cruz CA, et al. 2020. Endophilin-A3 and Galectin-8 control the clathrin-independent endocytosis of CD166. Nat. Commun 11:1457 [PubMed: 32193381]

Rice WL, Hoek ANV, Păunescu TG, Huynh C, Goetze B, et al. 2013. High resolution helium ion scanning microscopy of the rat kidney. PLOS ONE 8(3):e57051 [PubMed: 23505418]

Rilla K, Pasonen-Seppänen S, Deen AJ, Koistinen VVT, Wojciechowski S, et al. 2013. Hyaluronan production enhances shedding of plasma membrane-derived microvesicles. Exp. Cell Res 319(13):2006–18 [PubMed: 23732660]

Römer W, Berland L, Chambon V, Gaus K, Windschiegl B, et al. 2007. Shiga toxin induces tubular membrane invaginations for its uptake into cells. Nature 450(7170):670–75 [PubMed: 18046403]

Rosholm KR, Leijnse N, Mantsiou A, Tkach V, Pedersen SL, et al. 2017. Membrane curvature regulates ligand-specific membrane sorting of GPCRs in living cells. Nat. Chem. Biol 13(7):724–29 [PubMed: 28481347]

Russo D, Capolupo L, Loomba JS, Sticco L, D'Angelo G. 2018. Glycosphingolipid metabolism in cell fate specification. J. Cell Sci 131(24):jcs219204 [PubMed: 30559216]

Saito F, Moore SA, Barresi R, Henry MD, Messing A, et al. 2003. Unique role of dystroglycan in peripheral nerve myelination, nodal structure, and sodium channel stabilization. Neuron 38(5):747–58 [PubMed: 12797959]

Salustri A, Campagnolo L, Klinger FG, Camaioni A. 2019. Molecular organization and mechanical properties of the hyaluronan matrix surrounding the mammalian oocyte. Matrix Biol. 78–79:11–23

Sauvanet C, Wayt J, Pelaseyed T, Bretscher A. 2015. Structure, regulation, and functional diversity of microvilli on the apical domain of epithelial cells. Annu. Rev. Cell Dev. Biol 31:593–621 [PubMed: 26566117]

Schmick M, Bastiaens PIH. 2014. The interdependence of membrane shape and cellular signal processing. Cell 156(6):1132–38 [PubMed: 24630717]

Scholl FG, Gamallo C, Vilaró S, Quintanilla M. 1999. Identification of PA2.26 antigen as a novel cell-surface mucin-type glycoprotein that induces plasma membrane extensions and increased motility in keratinocytes. J. Cell Sci 112(24):4601–13 [PubMed: 10574709]

Schubert T, Sych T, Madl J, Xu M, Omidvar R, et al. 2020. Differential recognition of lipid domains by two Gb3-binding lectins. Sci. Rep 10:9752 [PubMed: 32546842]

Sens P, Turner MS. 2004. Theoretical model for the formation of caveolae and similar membrane invaginations. Biophys.J 86(4):2049–57 [PubMed: 15041647]

Sheetz MP, Singer SJ. 1974. Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. PNAS 71(11):4457–61 [PubMed: 4530994]

Shibue T, Brooks MW, Inan MF, Reinhardt F, Weinberg RA. 2012. The outgrowth of micrometastases is enabled by the formation of filopodium-like protrusions. Cancer Discov. 2(8):706–21 [PubMed: 22609699]

Shikichi M, Kitamura HP, Yanase H, Konno A, Takahashi-Iwanaga H, Iwanaga T. 1999. Three-dimensional ultrastructure of synoviocytes in the horse joint as revealed by the scanning electron microscope. Arch. Histol. Cytol 62(3):219–29 [PubMed: 10495876]

Shin I-S, Ishii S, Shin J-S, Sung K-I, Park B-S, et al. 2009. Globotriaosylceramide (Gb3) content in HeLa cells is correlated to Shiga toxin-induced cytotoxicity and Gb3 synthase expression. BMB Rep. 42(5):310–14 [PubMed: 19470247]

Shurer CR, Colville MJ, Gupta VK, Head SE, Kai F, et al. 2017. Genetically encoded toolbox for glycocalyx engineering: tunable control of cell adhesion, survival, and cancer cell behaviors. ACS Biomater. Sci. Eng 4(2):388–99 [PubMed: 29805991]

Shurer CR, Kuo JC-H, Roberts LM, Gandhi JG, Colville MJ, et al. 2019. Physical principles of membrane shape regulation by the glycocalyx. Cell 177(7):1757–70.e21 [PubMed: 31056282]

Simons M, Schwarz K, Kriz W, Miettinen A, Reiser J, et al. 2001. Involvement of lipid rafts in nephrin phosphorylation and organization of the glomerular slit diaphragm. Am. J. Pathol 159(3):1069–77 [PubMed: 11549599]

Sipione S, Monyror J, Galleguillos D, Steinberg N, Kadam V 2020. Gangliosides in the brain: physiology, pathophysiology and therapeutic applications. Front. Neurosci 14. 10.3389/fnins.2020.572965

Sirviö E, Mikkonen JJW, Koistinen AP, Miinalainen I, Kullaa AM. 2019. Localization of transmembrane mucin MUC1 on the apical surface of oral mucosal cells. Ultrastruct. Pathol 43(4–5):184–89 [PubMed: 31680599]

Sitarska E, Diz-Muñoz A. 2020. Pay attention to membrane tension: mechanobiology of the cell surface. Curr. Opin. Cell Biol 66:11–18 [PubMed: 32416466]

Son S, Takatori SC, Belardi B, Podolski M, Bakalar MH, Fletcher DA. 2020. Molecular height measurement by cell surface optical profilometry (CSOP). PNAS 117(25):14209–19 [PubMed: 32513731]

Song K, Fu J, Song J, Herzog BH, Bergstrom K, et al. 2017. Loss of mucin-type O-glycans impairs the integrity of the glomerular filtration barrier in the mouse kidney. J. Biol. Chem 292(40):16491–97 [PubMed: 28842487]

Sonnino S, Chiricozzi E, Grassi S, Mauri L, Prioni S, Prinetti A. 2018. Gangliosides in membrane organization. In Progress in Molecular Biology and Translational Science, Vol. 156, ed. Schnaar RL, Lopez PHH, pp. 83–120. New York: Academic/Elsevier [PubMed: 29747825]

Sreekumari A, Lipowsky R. 2018. Lipids with bulky head groups generate large membrane curvatures by small compositional asymmetries. J. Chem. Phys 149(8):084901 [PubMed: 30193489]

Stachowiak JC, Brodsky FM, Miller EA. 2013. A cost-benefit analysis of the physical mechanisms of membrane curvature. Nat. Cell Biol 15(9):1019–27 [PubMed: 23999615]

Stachowiak JC, Hayden CC, Sasaki DY. 2010. Steric confinement of proteins on lipid membranes can drive curvature and tubulation. PNAS 107(17):7781–86 [PubMed: 20385839]

Stachowiak JC, Schmid EM, Ryan CJ, Ann HS, Sasaki DY, et al. 2012. Membrane bending by protein-protein crowding. Nat. Cell Biol 14(9):944–49 [PubMed: 22902598]

Stotter BR, Talbot BE, Capen DE, Artelt N, Zeng J, et al. 2020. Cosmc-dependent mucin-type *O*-linked glycosylation is essential for podocyte function. Am. J. Physiol. Ren. Physiol 318(2):F518–30

Stowell SR, Ju T, Cummings RD. 2015. Protein glycosylation in cancer. Annu. Rev. Pathol. Mech. Dis 10:473–510

Tsafrir I, Sagi D, Arzi T, Guedeau-Boudeville M-A, Frette V, et al. 2001. Pearling instabilities of membrane tubes with anchored polymers. Phys. Rev. Lett 86(6):1138–41 [PubMed: 11178029]

Tsai F-C, Bertin A, Bousquet H, Manzi J, Senju Y, et al. 2018. Ezrin enrichment on curved membranes requires a specific conformation or interaction with a curvature-sensitive partner. eLife 7:e37262 [PubMed: 30234483]

Tukaj C, Bohdanowicz J, Kubasik-Juraniec J. 2002. A scanning electron microscopic study of phenotypic plasticity and surface structural changes of aortal smooth muscle cells in primary culture. Folia Morphol. 61(4):191–98

Twarock S, Tammi MI, Savani RC, Fischer JW. 2010. Hyaluronan stabilizes focal adhesions, filopodia, and the proliferative phenotype in esophageal squamous carcinoma cells. J. Biol. Chem 285(30):23276–84 [PubMed: 20463012]

Uyar A, Torrealday S, Seli E. 2013. Cumulus and granulosa cell markers of oocyte and embryo quality. Fertil. Steril 99(4):979–97 [PubMed: 23498999]

Wang H, Sency V, McJarrow P, Bright A, Huang Q, et al. 2019. Oral ganglioside supplement improves growth and development in patients with ganglioside GM3 synthase deficiency. In JIMD Reports, Vol. 45, ed. Morava E, Baumgartner M, Patterson M, Rahman S, Zschocke J, Peters V, pp. 9–20. Berlin: Springer [PubMed: 30209782]

Watkins EB, Majewski J, Chi EY, Gao H, Florent J-C, Johannes L. 2019. Shiga toxin induces lipid compression: a mechanism for generating membrane curvature. Nano Lett. 19(10):7365–69 [PubMed: 31538793]

Welf ES, Miles CE, Huh J, Sapoznik E, Chi J, et al. 2020. Actin-membrane release initiates cell protrusions. Dev. Cell 55(6):723–36.e8 [PubMed: 33308479]

Williams G, Wood A, Williams E-J, Gao Y, Mercado ML, et al. 2008. Ganglioside inhibition of neurite outgrowth requires Nogo receptor function: IDENTIFICATION OF INTERACTION SITES AND DEVELOPMENT OF NOVEL ANTAGONISTS. J.Biol. Chem 283(24):16641–52 [PubMed: 18411262]

Willysson A, Ståhl A, Gillet D, Barbier J, Cintrat J-C, et al. 2020. Shiga toxin uptake and sequestration in extracellular vesicles is mediated by its B-subunit. Toxins 12(7):449

Woods EC, Kai F, Barnes JM, Pedram K, Pickup MW, et al. 2017. A bulky glycocalyx fosters metastasis formation by promoting G1 cell cycle progression. eLife 6:e25752 [PubMed: 29266001]

Wrede C, Hegermann J, Mühlfeld C. 2020. Novel cell contact between podocyte microprojections and parietal epithelial cells analyzed by volume electron microscopy. Am.J. Physiol. Ren. Physiol 318(5):F1246–51

Wu G, Lu Z-H, André S, Gabius H-J, Ledeen RW 2016. Functional interplay between ganglioside GM1 and cross-linking galectin-1 induces axon-like neuritogenesis via integrin-based signaling and TRPC5-dependent Ca^{2+} influx. J. Neurochem 136(3):550–63 [PubMed: 26526326]

Xu R, Rai A, Chen M, Suwakulsiri W, Greening DW, Simpson RJ. 2018. Extracellular vesicles in cancer—implications for future improvements in cancer care. Nat. Rev. Clin. Oncol 15(10):617–38 [PubMed: 29795272]

Ying H, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC, et al. 2012. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell 149(3):656–70 [PubMed: 22541435]

Yoshikawa M, Go S, Suzuki S, Suzuki A, Katori Y, et al. 2015. Ganglioside GM3 is essential for the structural integrity and function of cochlear hair cells. Hum. Mol. Genet 24(10):2796–807 [PubMed: 25652401]

Zhang Y, Yan Z, Qin Q, Nisenblat V, Chang H-M, et al. 2018. Transcriptome landscape of human folliculo-genesis reveals oocyte and granulosa cell interactions. Mol. Cell 72(6):1021–34.e4 [PubMed: 30472193]

Zhu Y, Groth T, Kelkar A, Zhou Y, Neelamegham S. 2021. A GlycoGene CRISPR-Cas9 lentiviral library to study lectin binding and human glycan biosynthesis pathways. Glycobiology 31(3):173–80 [PubMed: 32776087]

NEW TECHNIQUES USED TO STUDY THE GLYCOCALYX

Emerging tools are aiding our understanding of glycocalyx-mediated processes. Chemically defined synthetic glycopolymers and genetic engineering can specifically modify the cell glycocalyx (Godula et al. 2009, Kramer et al. 2015, Narimatsu et al. 2019, H. Pan et al. 2019, Woods et al. 2017). Super-resolution microscopy techniques are being developed to visualize the glycocalyx and its effect on membrane regulation. For example, membrane topology imposed by glycocalyx thickness can be observed by fluorescence interference microscopy (Ajo-Franklin et al. 2005, Braun & Fromherz 1997, Godula et al. 2009, Shurer et al. 2017). Cell surface optical profilometry can report glycoprotein height dependence on molecular crowding (Son et al. 2020), making this technique potentially invaluable for delineating similar effects on the glycocalyx. Remarkably, nanoscale curvatures due to glycolipid-toxin interactions have been directly observed on live cells using novel polarized localization microscopy (Kabbani et al. 2020). In addition, electron microscopy (EM) can visualize nanoscale membrane structures. For instance, scanning EM and correlative light EM can detect tubular protrusions and vesicular structures induced by the glycocalyx (Koistinen et al. 2015). Cryogenic EM also preserves glycocalyx-coated microvesicles for investigation (Noble et al. 2020). Finally, molecular sensors should provide invaluable biophysical insights into glycocalyx effects on membrane properties including tension and steric pressures (Colom et al. 2018, Houser et al. 2020).

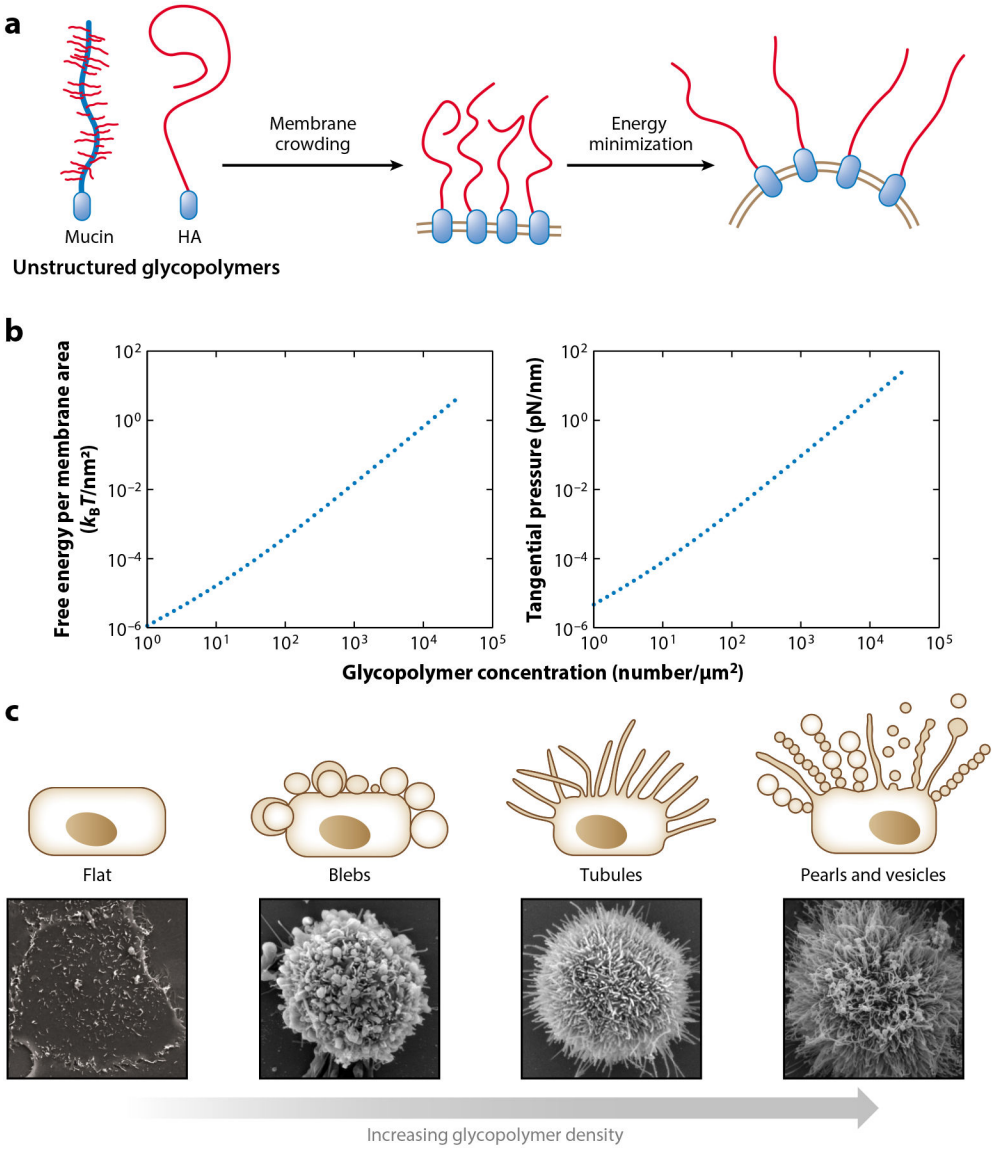

Figure 1.

The molecular crowding of glycopolymers bends membranes outward into a plethora of shapes.(*a*) Unstructured glycopolymers such as mucin and hyaluronan (HA) are anchored to the cell surface. Increasing polymer density achieves the coverage necessary for molecular crowding. (*b*) Estimates of the energetic driving forces and two-dimensional tangential pressures in a theoretical mucin 1 (MUC1) brush on a flat segment of membrane. The free energy per area of the brush is normalized by the thermal energy, k_BT. Complete details of the theoretical model and parameter estimates are in Gandhi et al. (2019). (c, *top*) Steric interactions between crowded polymers exert increasing pressure to bend a flat surface into blebs and tubular membrane protrusions that pearl or vesiculate tips to release microvesicles. (*Bottom*) Micrographs showing examples of these cell morphologies. Figure adapted with permission from Shurer et al. (2019).

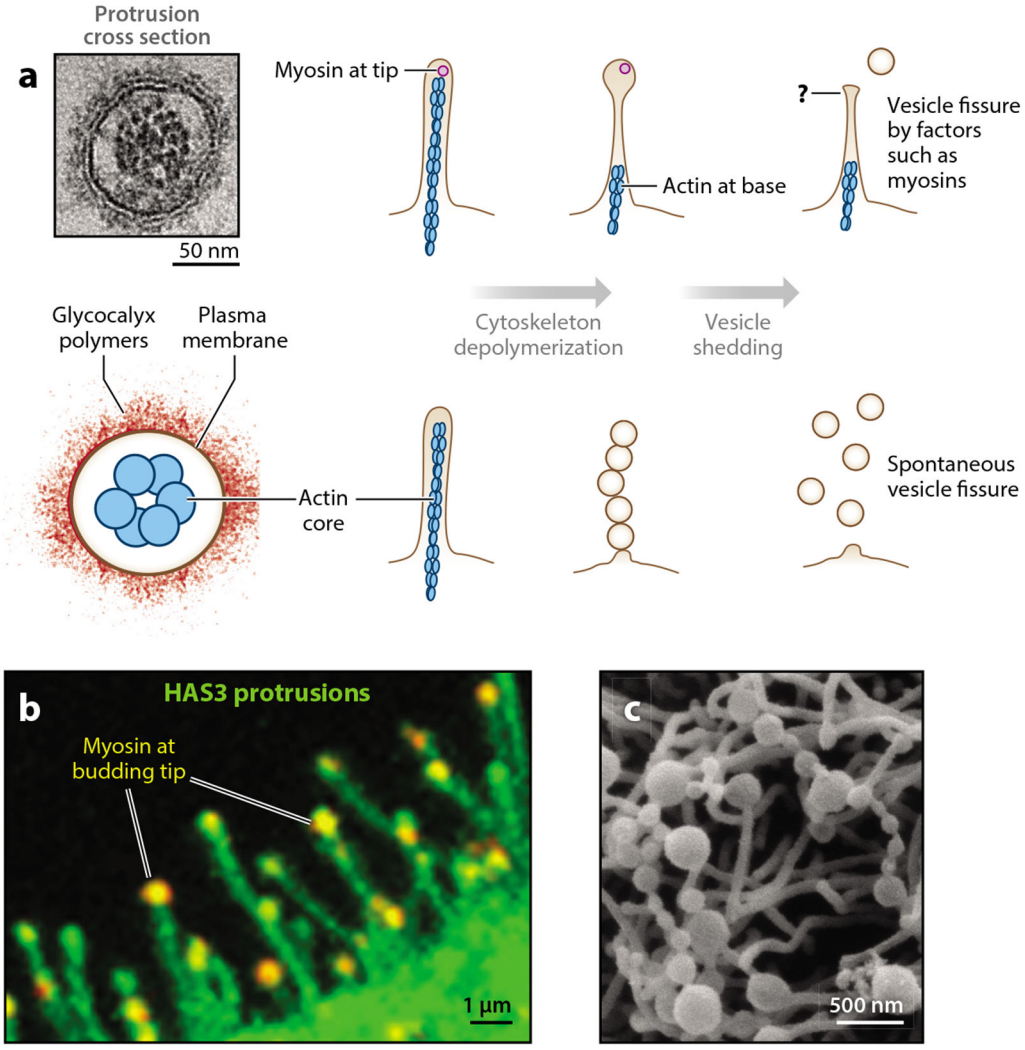

Figure 2.

Microvesicle release from membrane protrusions. (*a*) Electron micrograph and diagrams depict the presence of an actin core in glycocalyx-coated tubular membrane protrusions. Upon cytoskeletal depolymerization, pressures from the glycocalyx can vesiculate protrusion tips or undulate protrusions. Protrusions with vesiculated tips may be stabilized by actin retained at the base. Myosin accumulation at protrusion tips can mediate membrane fissure for microvesicle release. Alternatively, the spontaneous fissure of undulating protrusions can lead to microvesicle release. (*b*) Fluorescence image shows myosin accumulation at the vesiculated tips of hyaluronan synthase 3 (HAS3)-induced tubular protrusions. (*c*) Protrusion undulation and pearled morphologies have been observed for mucin 1 (MUC1)-mediated processes, as depicted in this scanning electron micrograph. Figure adapted with permission from Koistinen et al. (2015) and Shurer et al. (2019).

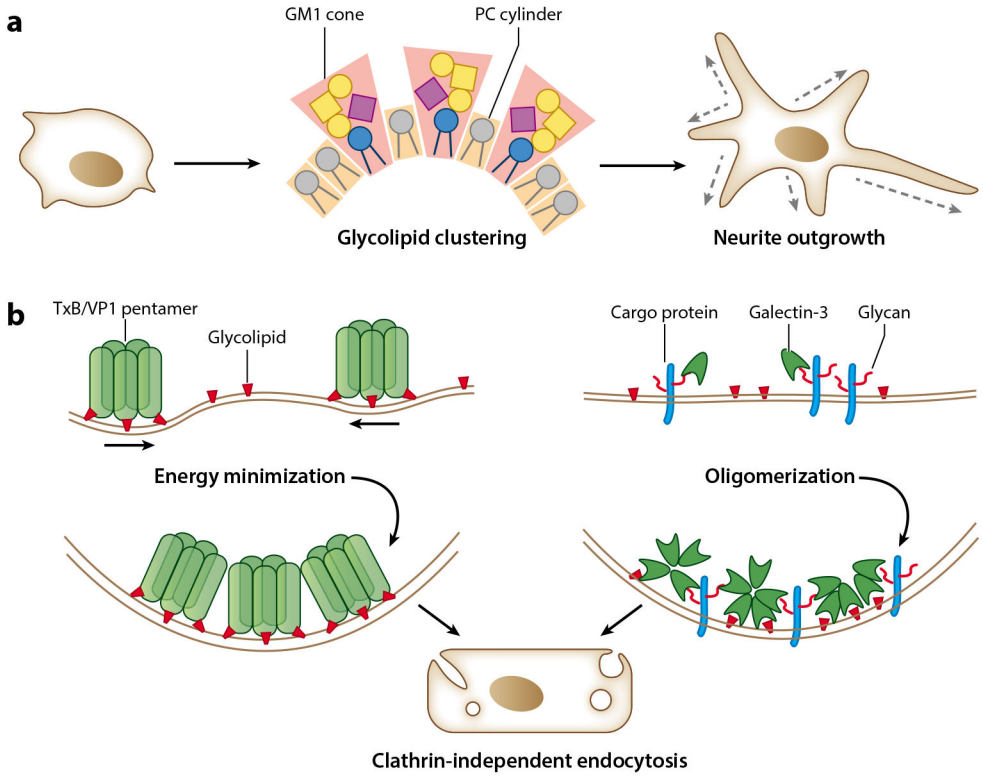

Figure 3.

Glycolipid-induced membrane structures. (*a*) Glycolipid clustering induces outward membrane protrusions for neurite outgrowth. Major bilayer-forming lipids such as phosphatidylcholine (PC) have a cylindrical shape (*orange*). The membrane bends away from large headgroups that shape glycolipids such as GM1 into a cone (*pink*). Factors such as matrix proteins bind glycolipids to induce curvature. PC is depicted with a choline (*gray*) headgroup, while GM1 is depicted with a headgroup of five sugar monomers (*blue, yellow,* and *purple*). (*b*) Glycolipids organized by carbohydrate-binding proteins (lectins) can bend membranes inward to form clathrin-independent endocytic buds and tubules. (*Left*) Glycolipid binding to the pentameric toxin subunit B (TxB) of Shiga and cholera toxins and polyomavirus SV40 VP1 causes membrane curvature. Glycolipid-TxB/VP1 complexes are clustered by membrane fluctuation and induce lipid compression and reorganization for tubulation. (*Right*) Endogenous galectin-3 first binds cargo glycoproteins before oligomerization occurs for binding glycolipids and inducing membrane bending for endocytosis.

Table 1

Examples of glycocalyx-coated membrane structures

Glycocalyx	Membrane structures	Reference(s)
Unstructured long-chain glycopolymers		
MUC1	Microvilli (lung and uterus), cilia and uterodome (uterus), microplicae (corneal cell sheet and mouth), tubular protrusions (tumor cells)	Bennett et al. 2001; Gipson et al. 2008, 2014; Kelleher et al. 2016; Kesimer et al. 2013;Shurer et al. 2019; Sirviö et al. 2019
MUC4	Cilia (lung)	Kesimer et al. 2013
MUC16	Uterodome (uterus), microplicae (cornea)	Blalock et al. 2007, Gipson et al. 2008
MUC17	Microvilli (intestine)	Pelaseyed & Hansson 2020
MUC20	Cilia (lung)	Kesimer et al. 2013
Enteric mucins	Microvesicle (intestine)	McConnell et al. 2009
Podocalyxin	Microvilli (kidney organoid), foot processes and microvesicles (kidney), foot process–like protrusions (tumor cells)	Fröse et al. 2018, Hara et al. 2010, Kim et al. 2017, Kerjaschki et al. 1984
CD34	Microvilli (bone marrow), tubular protrusions (tumor cells)	AbuSamra et al. 2017
Hyaluronan	Microvilli (cartilage, mesothelium, synovium, alveoli, oocytes), dendritic processes (bone), transzonal projections (granulosa cells), microvesicles (synovium), tubular protrusions (tumor cells)	Burra et al. 2010, Cohen et al. 2003, El-Hayek et al. 2018, Koistinen et al. 2016, Kyykallio et al. 2020, Makabe et al. 2006, Mustonen et al. 2016, Ochs et al. 2020, Twarock et al. 2010
Glycolipids		
GM3	Stereocilia (cochlea), foot processes (kidney)	Jin et al. 2012, Yoshikawa et al. 2015
GM1	Microvilli (intestine), stereocilia (cochlea), neurite (nervous system), spiculae and exovesicles (erythrocytes)	Hägerstrand et al. 2006, Jennemann et al. 2012, Ledeen & Wu 2015, Sipione et al. 2020b, Yoshikawa et al. 2015
GA1	Microvilli (intestine)	Jennemann et al. 2012
GD1a	Neurite (nervous system)	Sipione et al. 2020