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Induction of membrane curvature by proteins involved in Golgi trafficking

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Abstract

The Golgi apparatus serves a key role in processing and sorting lipids and proteins for delivery to their final cellular destinations. Vesicle exit from the Golgi initiates with directional deformation of the lipid bilayer to produce a bulge. Several mechanisms have been described by which lipids and proteins can induce directional membrane curvature to promote vesicle budding. Here we review some of the mechanisms implicated in inducing membrane curvature at the Golgi to promote vesicular trafficking to various cellular destinations.

Keywords

Golgi; Phosphoinositides; GOLPH3; Membrane curvature; Vesicle budding; Trafficking

1. Introduction

Vesicle budding is a key step in the movement of proteins and lipids from one organelle to another. The initial formation of a vesicle bud depends on mechanisms that induce directional curvature of the lipid bilayer membrane. The *trans* Golgi is an important membrane compartment in the secretory pathway that functions in sorting proteins and lipids to their final cellular destinations. Vesicles exit from the Golgi on their way to various destinations, including the plasma membrane (PM), late endosomes or lysosomes, or back to the Golgi or the endoplasmic reticulum (ER). Different cargoes presumably follow different routes (although some cargoes may have several destinations). These diverse pathways of exit from the Golgi predict the existence of multiple mechanisms of vesicle budding from the Golgi. Indeed, many such mechanisms have been described. Here we review some of the proteins found at the *trans* Golgi reported to have the ability to induce membrane curvature, and thus, may act to help drive vesicle budding from the Golgi.

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Conflict of interest

We have no conflicts to report.

2. Mechanisms to induce membrane curvature

Membrane curvature can be achieved through many different mechanisms (Fig. 1), all with the common feature that they act to asymmetrically impart directional deformation of the lipid bilayer (Daumke et al., 2014; Hu et al., 2011; Jarsch et al., 2016; McMahon and Boucrot, 2015; Zimmerberg and Kozlov, 2006). For example, asymmetric insertion of lipids or proteins into one leaflet of the bilayer increases the surface area of one leaflet relative to the other, driving bilayer puckering to accommodate the strain. The phosphatidylserine (PS) flippase, Drs2p, provides an example at the Golgi of a mechanism to increase the lipid content of one leaflet at the expense of the other, thus driving membrane curvature (Fig. 1C) (Best et al., 2019). A related mechanism involves a change in the lipid composition of the membrane. Preferential accumulation of wedge-shaped or conical lipids in one leaflet will favor positive or negative curvature, respectively (McMahon and Boucrot, 2015; Raben and Barber, 2017). While biophysical experiments can demonstrate this effect *in vitro*, studies to definitively distinguish the importance of the biophysical properties of lipids versus their ability to recruit specific lipid-binding proteins are difficult and generally lacking.

There are several mechanisms by which proteins drive membrane curvature. Insertion of a protein helix or loop into the proximal leaflet but not the distal leaflet, by increasing the surface area of one leaflet relative to the other, will induce membrane curvature (Fig. 1A). Epsin N-terminal homology (ENTH) domains provide a classic example (Ford et al., 2002; Itoh and De Camilli, 2006). GOLPH3 is a recently described example of a protein that acts at the Golgi to insert a loop into the proximal leaflet of the bilayer to drive membrane curvature (Rahajeng et al., 2019). Another mechanism is employed by crescent-shaped proteins that bind tightly to the surface of the membrane (Fig. 1B). Since these proteins are relatively rigid, binding to the membrane forces the bilayer to adhere to, and mimic, the intrinsically curved shape of the protein. BAR (bin/amphiphysin/rvs) domains represent a classic example of a crescent-shaped protein domain that induces membrane curvature (Gallop et al., 2006; Gallop and McMahon, 2005; Peter et al., 2004). The Arfaptins are examples of Golgi-localized BAR domain proteins (Kanoh et al., 1997; Peter et al., 2004). A similar mechanism is used by larger multi-subunit assemblies of proteins (Fig. 1D). Clathrin cages provide an example of an intrinsically curved multi-part assembly (Dannhauser and Ungewickell, 2012; Saleem et al., 2015). While clathrin assemblies generally include a variety of proteins that have the ability to curve the membrane, the overall curvature of the cage imposes a physical barrier that forces membrane curvature. Molecular motors that apply a directional force to the membrane (e.g., pulling in a direction normal to the membrane) provide another means to induce deformation of the membrane (Chibalina et al., 2007; Roux et al., 2002). Finally, high concentrations of a membrane-associated protein on one face of the membrane, through repulsive forces associated with molecular crowding, are capable of promoting membrane curvature (Stachowiak et al., 2012). Real vesicle budding events *in vivo* appear to use multiple mechanisms for initiation, progression, and maturation of a budding vesicle. At the Golgi, there is evidence that many of these mechanisms play a role in various vesicle budding events.

3. Role of lipids to induce membrane curvature at the Golgi

At the Golgi, it has been proposed that the composition of the lipid bilayer may play a role in the process of vesicle budding for forward trafficking (Shemesh et al., 2003). While this model is understandably attractive, the data supporting it remain sparse. Rather, a large body of data supports the idea that effector proteins recruited by lipids play a dominant role in the process of vesicle budding at the Golgi.

The cytosolic leaflet of the *trans* Golgi is highly enriched in phosphatidylinositol 4-phosphate (PI4P), which serves to recruit several PI4P-binding proteins. Golgi PI4P is synthesized by two Golgi-localized PI-4-kinases, PI4K3 β and PI4K2 α (Balla et al., 1997; Matteis et al., 2013; Venditti et al., 2016; Wang et al., 2003; Weixel et al., 2005; Wong et al., 1997). Many proteins have been reported to bind specifically to PI4P, which helps to recruit them to the Golgi (Wang et al., 2019; Waugh, 2019; Wattenberg, 2019). Several of these PI4P-binding proteins are capable of inducing membrane curvature themselves or recruit other proteins with this ability. These Golgi-localized, membrane-sculpting proteins are the main focus of this review.

3.1 Flippase Drs2p

There is good evidence that flippases, enzymes that transfer lipid from one leaflet of the bilayer to the other, play a role in inducing membrane curvature to promote vesicle budding (Fig. 1C) (Best et al., 2019; Devaux et al., 2008; Sebastian et al., 2012; Takada et al., 2018). Drs2p is a flippase that localizes to the late Golgi in *S. cerevisiae* (equivalent to the metazoan *trans* Golgi). It catalyzes the transfer of PS from the luminal leaflet to the cytosolic leaflet, similar to the activity of other type IV P-type ATPases (Devaux et al., 2008; Natarajan et al., 2004; Sebastian et al., 2012). By asymmetrically expanding one leaflet at the expense of the other, the membrane must pucker to relieve the strain (Takada et al., 2018; Xu et al., 2013). Drs2p flippase activity is activated by PI4P in the membrane (Natarajan et al., 2009). In *S. cerevisiae*, Drs2p is required for AP-1 and clathrin-dependent trafficking of Chs3p from the Golgi to endosomes (Chen et al., 1999; Gall et al., 2002). Loss of Drs2p also impairs trafficking of Pma1p and Can1p to the plasma membrane (Hankins et al., 2015). In mammals, other type IV P-type ATPases have been shown to act at the PM to promote membrane curvature (Takada et al., 2018; Tanaka et al., 2016). However, in mammals, only limited data exist as to their role at the Golgi (Xu et al., 2009).

4. ARF

The ADP-ribosylation factor (ARF) family proteins are small GTPases related to the well known COPII ER secretory protein, Sar1 (Kahn and Gilman, 1984, 1986; Lee et al., 2005; Sztul et al., 2019; Tan and Gleeson, 2019). The ARFs bind GTP/GDP under the influence of various ARF guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) (Sztul et al., 2019; Tan and Gleeson, 2019). ARFs are cytosolic proteins, but the GTP-loaded form preferentially binds to membrane via insertion of a myristoylated N-terminal amphipathic helix (Walker et al., 1992; Antonny et al., 1997; Cohen and Donaldson, 2010). Given that, predictably, high concentrations of ARF1 *in vitro* are able to induce membrane curvature, observed as tubulation of lipid sheets or vesicles (Beck et al.,

2008; Lundmark et al., 2008). Tagged ARF1, expressed from the endogenous locus, is observed on Golgi-derived membrane tubules in cells (Bottanelli et al., 2017). Furthermore, overexpression of ARF1, but not the GTPase-defective mutant, drives the formation of these Golgi tubules. It remains unknown, however, whether endogenous levels of ARF1 are sufficient or necessary to induce curvature of Golgi membranes *in vitro* or *in vivo*.

The ARF family of proteins includes multiple paralogs (Sztul et al., 2019; Tan and Gleeson, 2019). ARF1, ARF3, ARF4, ARF5, and the related protein ARFRP1 are all found at the Golgi. Other family members are found on other cellular membranes. Many of the studies that implicate ARFs in Golgi trafficking have relied upon dominant-negative or constitutively-active mutants or small molecule inhibitors of the ARF-GEFs, such as Brefeldin A or Golgicide A, methods with serious concerns as to their specificity. The evidence supports roles for ARFs in trafficking from the Golgi to lysosomes, to the PM, and in retrograde trafficking. In addition to a role in inducing membrane curvature, ARFs have also been found to bind to a large number of other Golgi proteins. These include COPI proteins that function in retrograde trafficking (Palmer et al., 1993; Serafini et al., 1991), AP-1 (Dittie et al., 1996; Ren et al., 2013; Zhu et al., 1998) and GGA (Dell'Angelica et al., 2000; Kametaka et al., 2010; Puertollano et al., 2001b) proteins (discussed below) that function primarily in trafficking to late endosomes and lysosomes, and exomer (in yeast) (Paczkowski and Fromme, 2014; Wang et al., 2006) that functions in trafficking to the PM. Interestingly, ARF1 has also been seen to recruit and activate PI4K3 β at the Golgi and thus maintain Golgi PI4P levels (Godi et al., 1999; Haynes et al., 2005), potentially placing ARF1 functionally upstream of PI4P-binding proteins.

In HeLa cells, siRNA knockdown of individual ARFs (1, 3, 4, or 5) has no detectable effect on Golgi secretory trafficking or Golgi morphology (Volpicelli-Daley et al., 2005). Simultaneous knockdown of pairs of ARFs reveals mild defects with knockdown of ARF1+3 or ARF1+4. Depletion of ARFRP1 alone reduces trafficking of VSVG (vesicular stomatitis virus G glycoprotein, a commonly used experimental cargo), E-cadherin, and Vangl2 to the PM (Guo et al., 2013; Nishimoto-Morita et al., 2009; Zahn et al., 2008). Knockout of ARF1 or ARFRP1 in mice results in embryonic lethality, a strong phenotype that can be difficult to interpret mechanistically (Hayakawa et al., 2014; Zahn et al., 2008).

5. Arfaptin

Arfaptins, as the name implies, are proteins that were identified to bind to ARF family proteins (Kano et al., 1997). However, they also exhibit comparable affinity for other small GTPases, such as Rac (Tarricone et al., 2001). Arfaptins-1 and -2 both localize to the Golgi (Kano et al., 1997). Notably, the structure of Arfaptins reveals the presence of a crescent-shaped BAR domain (Fig. 1B) (Peter et al., 2004; Tarricone et al., 2001). Arfaptins have been shown to induce tubulation of liposomes *in vitro* (Peter et al., 2004). The extent to which they drive membrane curvature *in vivo* remains untested, although other BAR domain-containing proteins do drive membrane curvature in cells (Mim and Unger, 2012). Knockdown experiments indicate that Arfaptins are not required for general secretion (Cruz-Garcia et al., 2013). However, Arfaptin-1 was found to be phosphorylated by protein kinase

D, and to be required for secretion of some regulated cargoes, such as insulin and chromogranin A (Cruz-Garcia et al., 2013; Gehart et al., 2012).

6. Exomer

Exomer is a cargo adaptor complex found only in yeast, where it is required for trafficking a subset of specialized cargo, including Chs3p, Fus1p and Pin2p, directly from the late Golgi to the PM (Barfield et al., 2009; Guo et al., 2014; Paczkowski et al., 2015; Ritz et al., 2014; Sanchatjate and Schekman, 2006; Santos and Snyder, 2003, 1997; Trautwein et al., 2006; Wang et al., 2006). Exomer is composed of a homodimer of the core scaffolding subunit, Chs5p, and two of four cargo-binding paralogs called ChAPs (Chs5p-Arf1p binding proteins), Chs6p, Bch2p, Bud7p, and Bch1p (Paczkowski et al., 2012; Richardson and Fromme, 2013). Homologs of exomer have not been identified in metazoans (Ramirez-Macias et al., 2018). Binding to Arf1p and the lipid bilayer together recruits exomer to the Golgi (Paczkowski and Fromme, 2014; Wang et al., 2006). By electron microscopy, exomer is observed to coat synthetic liposomes (Wang et al., 2006). While exomer appeared insufficient to deform the membrane into buds or vesicles in earlier studies (Wang et al., 2006), more recent data from membrane vesiculation assays, dynamic light scattering, and electron microscopy (EM) suggest membrane remodeling activity through a hydrophobic element in Bch1p and coordination of Arf1p membrane insertion (Paczkowski and Fromme, 2014).

7. EpsinR

EpsinR (also known as CLINT1 or Enthoprotin) is an epsin family protein that localizes to the Golgi, and, like other epsin family members, contains an ENTH domain and interacts with AP family proteins, in this case, AP-1 (Ford et al., 2002; Hirst et al., 2003; Kalthoff et al., 2002; Mills et al., 2003; Wasiak et al., 2002). Other ENTH domains, for example from Epsin1, are well-known to induce membrane curvature *in vitro* and in cells via insertion of an amphipathic alpha helix into the proximal leaflet of the lipid bilayer (Ford et al., 2002). By analogy, a similar function has been proposed for EpsinR, but its weak interaction with membrane *in vitro* has precluded evidence as to whether EpsinR is capable of driving membrane curvature *in vitro* or *in vivo* (Mills et al., 2003). Epsin1 displays physiological binding to PI(4,5)P₂. However, while EpsinR can bind to PI4P (and to other phosphoinositides, as well), the physiological significance of this binding remains untested. Knockdown of EpsinR has no effect on AP-1-mediated trafficking of cathepsin D to lysosomes (Hirst et al., 2003). However, it does partially reduce trafficking of Frizzled6 to the PM (Ma et al., 2018). In *S. cerevisiae*, individual deletion of the EpsinR homologs ENT3 and ENT5 is innocuous, but deletion of both together impairs Golgi to endosome trafficking (Costaguta et al., 2006; Duncan and Payne, 2003).

8. Clathrin

Clathrin-coated vesicles are one of the major classes of transport vesicles, most thoroughly studied for their role in receptor-mediated endocytosis. However, clathrin is also observed at the Golgi and in vesicles that bud from the Golgi (Hinnens and Tooze, 2003; Jaiswal et al.,

2009). Several proteins that associate with the *trans* Golgi and are involved in vesicle budding interact directly or indirectly with clathrin. The AP and GGA family proteins are notable examples, and are discussed in more detail, below. Clathrin assembles into triskelions that interact to form curved polyhedra (Fig. 1D) (Kaksonen and Roux, 2018; Kirchhausen et al., 2014; Pearse, 1976). *In vitro*, clathrin polymerization alone is sufficient to generate spherical buds in a membrane (Dannhauser and Ungewickell, 2012; Saleem et al., 2015). *In vivo*, however, clathrin is always bound to a panoply of clathrin adaptor proteins, many of which have additional mechanisms to induce membrane curvature. Interplay of clathrin polymerization and adaptor complex proteins is likely to dictate membrane curvature for clathrin-mediated vesicle budding (Avinoam et al., 2015; Bucher et al., 2018; Leyton-Puig et al., 2017). Although clathrin can be found at the Golgi, it appears to not be involved in Golgi-to-PM trafficking. Examination of VSVG exit from the Golgi by EM reveals exit from sites that are devoid of clathrin (Griffiths et al., 1985). In *S. cerevisiae*, knockout of clathrin heavy or light chain genes produces viable yeast that still carry out protein secretion (Payne, 1990; Payne et al., 1987). Instead, clathrin is implicated predominantly in trafficking from the Golgi to late endosomes or lysosomes (Doray et al., 2002; Mardones et al., 2007; Progida and Bakke, 2016).

9. Adaptor Proteins (APs)

AP complex proteins produce a family of multisubunit complexes that generally serve to link membranes to clathrin. While AP proteins themselves are not known to induce membrane curvature, they serve as a hub for interaction with other proteins that do. Five AP complexes have been identified and they all exhibit similar organization, consisting of two large subunits, a medium subunit, and a small subunit. However, they display differences in their cellular localization and mediate distinct vesicle formation (Bonifacino, 2014; Guo et al., 2014). AP-1, AP-3 and AP-4 are generally believed to function at the *trans* Golgi and/or endosomes, whereas AP-2 functions at the PM (Lewin et al., 1998). In addition to these ubiquitously expressed complexes, AP-1 and AP-3 have cell type-specific isoforms, AP-1B in epithelia and AP-3B in neurons (Bonifacino, 2014; Grabner et al., 2006; Hase et al., 2013; Park and Guo, 2014). AP-1, AP-2 and AP-3 contain a clathrin binding sequence in the beta chain (Dell'Angelica et al., 1998; Gallusser and Kirchhausen, 1993), but AP-3 association with clathrin remains controversial (Newell-Litwa et al., 2007). AP-1 recruitment to the *trans* Golgi is PI4P-dependent and also requires ARF1 (Bonifacino, 2014; Farías et al., 2012; Guo et al., 2014; Ooi et al., 1998; Owen and Evans, 1998; Stamnes and Rothman, 1993; Traub et al., 1993; Wang et al., 2003). AP-1 is a multi-subunit complex and binds to cargo via one or more of several cargo binding motifs (Bonifacino, 2014; Guo et al., 2014; Paczkowski et al., 2015). The APs bind to proteins such as EpsinR, ARF1, and clathrin, all of which are capable of inducing membrane curvature. Presumably, in a complex each contributes to driving membrane curvature to promote vesicle budding.

Most studies have found a prominent role for AP-1 in trafficking from the Golgi to late endosomes and lysosomes (Bonifacino, 2014; Canuel et al., 2008; Chen et al., 2017; Polishchuk et al., 2006; Stahlschmidt et al., 2014) and in membrane recycling at the Golgi (Casler et al., 2019; Day et al., 2018; Papanikou et al., 2015). However, a few studies have reported defects in Golgi-to-PM trafficking with loss of AP-1 (Carvajal-Gonzalez et al.,

2012; Farías et al., 2012; Parmar and Duncan, 2016). Knockout of AP-1 in mice is embryonic lethal, a strong phenotype that is difficult to interpret mechanistically (Ohno, 2006).

AP-4 is homologous to AP-1, but differs in that it does not recruit clathrin (Bonifacino, 2014; Davies et al., 2018; Guo et al., 2014; Hirst et al., 2013; Paczkowski et al., 2015; Park and Guo, 2014). AP-4 may play a role in the sorting of amyloid precursor protein (APP) from the Golgi to endosomes (Burgos et al., 2010). AP-4 has also been reported to mediate transport of some lysosomal proteins from the *trans* Golgi to lysosomes (Aguilar et al., 2001). AP-5 is also related to AP-1 but does not associate with clathrin, and it appears to function primarily in retrograde trafficking from late endosomes to the Golgi (Hirst et al., 2011,2018).

10. GGAs

Golgi-localized, γ -ear-containing, ARF-binding proteins (GGAs) are another family of proteins that are not known to directly impart membrane curvature, but recruit other proteins that do. Three GGAs are found in humans (GGA1, 2, and 3). Their association with the *trans* Golgi depends on PI4P along with an interaction with ARF1 (Dell'Angelica et al., 2000; Kametaka et al., 2010; Puertollano et al., 2001b; Wang et al., 2007). They further interact with APs, EpsinR, and clathrin, providing multiple means to induce membrane curvature. The GGAs function primarily in trafficking from the Golgi to late endosomes or lysosomes. In *S. cerevisiae*, GGA knockout results in impaired transport of carboxypeptidase Y and Pep12p from late Golgi compartments to vacuoles (Black and Pelham, 2000; Dell'Angelica et al., 2000; Hirst et al., 2000). In mammals, GGAs play a notable role in trafficking of the mannose-6-phosphate receptor from the Golgi to lysosomes (Doray et al., 2002; Puertollano et al., 2001a). A few studies also provide examples of GGA-dependent trafficking to the PM (Kakhlon et al., 2006; Lamb et al., 2010; Zhang et al., 2016). Knockout of GGA1 or GGA3 results in mice that are grossly normal, and even combined knockout of GGA1 and GGA3 produces some viable mice (Govero et al., 2012). Loss of GGA2 causes embryonic lethality in some mouse strains, but not in others.

11. GOLPH3

GOLPH3 is an abundant peripheral membrane protein, highly localized to the *trans* Golgi and to vesicles budding from the *trans* Golgi (Bell et al., 2001; Dippold et al., 2009; Kuna and Field, 2019; Snyder et al., 2006; Wu et al., 2000). GOLPH3 binds tightly and specifically to PI4P both *in vitro* and in cells, which promotes GOLPH3 localization to the *trans* Golgi in humans and in *S. cerevisiae* (for the ortholog Vps74p) (Dippold et al., 2009). Upon binding to PI4P-containing membranes, GOLPH3 inserts a hydrophobic beta-loop into the cytosolic leaflet of the lipid bilayer, thus inducing membrane curvature (Fig. 1A) (Rahajeng et al., 2019). GOLPH3, at physiological concentrations, induces tubulation of PI4P-containing liposomes *in vitro*. Furthermore, endogenous GOLPH3 drives tubulation of the *trans* Golgi in cells. GOLPH3, its ability to bind PI4P, and its ability to induce membrane curvature, are all required for efficient trafficking of VSVG from the Golgi to the PM.

GOLPH3 further interacts with the unconventional myosin, MYO18A (Buschman and Field, 2018; Dippold et al., 2009; Ng et al., 2013; Taft et al., 2013). GOLPH3/MYO18A serves to link the Golgi to the F-actin cytoskeleton (Dippold et al., 2009; Lázaro-Diéguez et al., 2006; Ng et al., 2013). MYO18A applies a tensile force to the Golgi via GOLPH3, generating the characteristic shape and ultrastructure of the Golgi (Dippold et al., 2009; Ng et al., 2013; Xie et al., 2018; Xing et al., 2016). MYO18A and its interaction with GOLPH3 are required for efficient Golgi-to-PM secretory trafficking (Bishé et al., 2012; Dippold et al., 2009; Ng et al., 2013; Rahajeng et al., 2019; Xing et al., 2016). Knockdown of GOLPH3 or MYO18A results in ~60% reduction in Golgi-to-PM trafficking of VSVG (with no impairment in ER-to-Golgi trafficking) (Dippold et al., 2009; Rahajeng et al., 2019; Xing et al., 2016). Furthermore, overall endogenous secretion, measured by ³⁵S-amino acid pulse-chase experiments, is reduced >75% by knockdown of GOLPH3 or MYO18A (Ng et al., 2013). Likewise, secretion of hepatitis C viral particles from infected cells is reduced by >75% by knockdown of GOLPH3 or MYO18A. Thus, the GOLPH3/MYO18A complex appears to play a significant role in promoting vesicle exit from the *trans* Golgi for trafficking to the PM.

12. Conclusion

Many proteins have been implicated in driving vesicle exit from the Golgi, employing a variety of mechanisms to deform the membrane to initiate budding. Less clear are which cargoes and which trafficking routes to different destinations depend on which vesicle trafficking mechanisms. Approaches to systematically disentangle the relationships will be needed to more fully understand the secretory pathway.

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Fig. 1.

Examples of mechanisms to impart curvature to the Golgi membrane. **A)** Insertion of a loop or helix. GOLPH3 provides an example of a protein that inserts a loop into the proximal (cytosolic) leaflet, expanding that leaflet relative to the distal (luminal) leaflet, thus driving membrane curvature. Shown is a model of GOLPH3 (surface map by amino acid hydrophobicity on the Kyte-Doolittle scale; PDB: 3KN1 (Pettersen et al., 2004; Rahajeng et al., 2019; Wood et al., 2009) binding to a PI4P-containing lipid membrane. GOLPH3 inserts a hydrophobic beta-loop (amino acids 190-201) into the cytosolic leaflet of the lipid bilayer.

A similar mechanism is used by ENTH domains, as found in EpsinR, which insert an amphipathic alpha helix into the proximal leaflet of the lipid bilayer. ARFs induce membrane curvature by insertion of a myristoylated N-terminal amphipathic helix into the proximal leaflet. **B)** Intrinsically-curved, membrane-binding scaffold proteins induce curvature through binding, forcing the membrane to mimic the curve of the protein. Crescent-shaped BAR domains, as found in the Arfaptins, provide an example. Shown here is a model of the Arfaptin-2 BAR domain (PDB: 1I49 (Tarricone et al., 2001)) as a ribbon diagram of the antiparallel homodimer (green and purple) bound to a lipid bilayer. **C)** A lipid flippase expands one leaflet at the expense of the other, driving membrane curvature. Shown is a model of Drs2p (PDB: 6ROJ (Timcenko et al., 2019)) schematically catalyzing the transfer of phosphatidylserine (PS, in purple) from the luminal leaflet to the cytosolic leaflet. **D)** A large multi-subunit scaffold, such as clathrin, provides a physical barrier to sculpt the membrane. Clathrin triskelions (pink) assemble into a polygonal lattice that envelopes the membrane.