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The Fast and the Furious: Golgi Contact Sites

Yotam David[1], Inês G. Castro[1] (iD) and Maya Schuldiner[1] (iD)

Abstract
Contact sites are areas of close apposition between two membranes that coordinate nonvesicular communication between organelles. Such interactions serve a wide range of cellular functions from regulating metabolic pathways to executing stress responses and coordinating organelle inheritance. The past decade has seen a dramatic increase in information on certain contact sites, mostly those involving the endoplasmic reticulum. However, despite its central role in the secretory pathway, the Golgi apparatus and its contact sites remain largely unexplored. In this review, we discuss the current knowledge of Golgi contact sites and share our thoughts as to why Golgi contact sites are understudied. We also highlight what exciting future directions may exist in this emerging field.

Keywords
Golgi, contact sites, cholesterol, phosphoinositide, PI4P, oxysterol-binding protein (OSBP)

Introduction

Eukaryotic cells have diversified their metabolism by isolating chemical niches within membrane-bound organelles. While the emergence of organelles enabled a higher control of specific biochemical reactions, it also raised the need to overcome a physical barrier, driving the evolution of various pathways to coordinate metabolic fluxes and the transfer of information across membranes. Three main methods of cellular communication exist and can be likened to modern technological systems (Figure 1): (1) vesicular traffic, offering long-range data transfer similar to Wi-Fi, (2) diffusion of molecules, such as ion fluxes or kinase cascades, which is usually local and shorter-ranged, analogous to today's Bluetooth, and (3) contact sites (or simply, contacts), which are fast, regulated (or secure) physical connections for transferring data, just like the Ethernet.

Contacts are areas of proximity (as close as 10 nm) between two organelles that do not underlie consequent membrane fusion. Contacts allow organelles to control the flux of lipids, proteins, and metabolites between their membranes as well as to communicate while maintaining their distinct identities.

First described in the 1950s (Bernhard & Rouiller, 1956), contacts are suggested to have emerged early in eukaryotic evolution, potentially even before vesicular trafficking (Jain & Holthuis, 2017). To date, contacts between most pairs of organelles have been described (Shai et al. 2018; Valm et al., 2017), and yet one organelle is rarely featured and much less explored —the Golgi apparatus (from here on termed, Golgi).

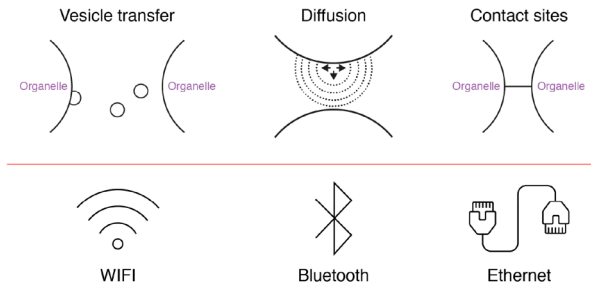The Golgi was discovered over 120 years ago (Golgi, 1898) and is a metabolic and trafficking hub, playing a major part in the secretory pathway. In most eukaryotic cells, the Golgi is composed of structures called cisternae: the *cis* cisterna being the first stop for vesicles exiting the endoplasmic reticulum (ER); followed by a medial cisterna in which cargo maturation occurs; last a *trans* cisterna, which functions as an exit gate for cellular cargo distribution. The *trans* cisternae are also often connected to a complex network of membranes and associated vesicles collectively dubbed the *trans*-Golgi network (TGN) (Farquhar & Palade, 1981). Each cisterna type is characterized by a specific protein composition, which in turn defines a specialized set of functions (Losev et al., 2006; Matsuura-Tokita et al., 2006; Weill et al., 2018). It is still not fully understood whether cisternae are independent entities or exist in a continuous maturation flux from one another (Glick & Luini, 2011; Mironov & Beznoussenko, 2019; Pantazopoulou & Glick, 2019; Pfeffer, 2010); however, this aspect of the Golgi will not be explored in this review. Regardless of how they emerge and are maintained, having three distinct compartments allows for a greater functional diversity and potentially for the emergence of multiple dedicated types of contacts with other organelles.

[1]Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

Corresponding Author:
Maya Schuldiner, Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, 7610001, Israel.
Email: maya.schuldiner@weizmann.ac.il

Figure 1. Analogy of cellular communication methods to modern communication technologies.

Figure 2. Components of Golgi–ER contacts in mammals, *Arabidopsis thaliana* and *Saccharomyces cerevisiae*. Lipid transfer proteins (LTPs) are shown in light blue and their interacting partners are shown in dark blue. Regulators are shown in magenta and scaffold proteins are in green.

The main function of the Golgi is the posttranslational modification (PTM) and trafficking of proteins and lipids. Cargo proteins arriving from the ER follow a step by step maturation pathway achieved by interacting with chaperones and PTM enzymes, before being trafficked to their final destination. If these maturation steps do not succeed, quality control mechanisms exist to enable selective degradation of membrane proteins (Schmidt et al., 2019).

Glycosylation is one example of a PTM that expands both protein and lipid diversity and is carried out inside Golgi cisterna. It is important for correct protein folding—an action that is crucial to execute diverse physiological activities such as the cellular response to insulin and accurate immune responses (Reily et al., 2019). Defects in glycosylation have been associated with numerous neurodevelopmental and muscular disorders, and are frequently lethal at the embryonic stage, demonstrating that correct execution of cargo modifications is crucial for cellular and tissue homeostasis (Reily et al., 2019).

Another central function of the Golgi is lipid remodeling. Most lipids are synthesized in the ER and then transferred to the Golgi for further processing and sorting to their target destinations. The presence of multiple lipid remodeling enzymes in the Golgi defines it as an important regulator of lipid homeostasis and membrane integrity (Liu et al., 2017; Mesmin et al., 2017; Schmidt et al., 2019). Lipid species regulated at Golgi such as sphingolipids and phosphatidylinositol (PI) mediate signaling mechanisms for a variety of functions such as sensing of nitrogen, pH, and glucose levels as well as cell adhesion and migration (Hannun & Obeid, 2018; Mousley et al., 2012; Shin et al., 2020).

The wide spectrum of Golgi functions demonstrates how this metabolic virtuoso is intertwined in a myriad of cellular processes. To shuttle both proteins and lipids, the Golgi takes part in vesicular trafficking both by anterograde and retrograde vesicular pathways (Hanada et al., 2003; Rothman & Wieland, 1996; Shin et al., 2020). However, vesicular trafficking cannot account for all lipid movement requirements of organelles as some do not engage in vesicular traffic (Wong et al., 2019). Moreover, vesicular trafficking is bound by limitations of membrane dynamics and speed as well as coupling the transfer of lipids with changes in the
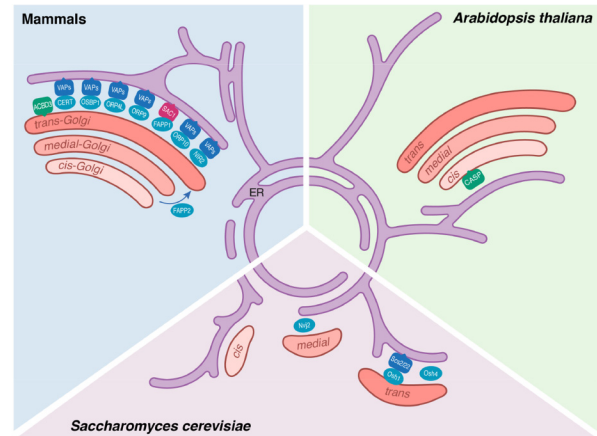
proteome of the acceptor membrane—all issues circumvented by contacts (Mesmin et al., 2019; Wong et al., 2019). Hence, the Golgi would clearly benefit from having additional communication pathways to other organelles through contacts. To this end, the sparsity of information on contacts of the Golgi, a central metabolic hub, is puzzling.

In this review, we will discuss what has already been gleaned about Golgi contacts (Masone et al. 2019; Mesmin et al. 2019) and what is still unknown. We will also discuss why we believe that the study and discovery of Golgi contacts have lagged behind those of other organelles, debating whether the Golgi really mediates fewer contacts or whether this phenomenon results from technical hindrances that have contributed to its underrepresentation in the contact site literature. Finally, we will suggest which exciting future directions this field is likely to take.

Golgi–ER Contacts and Their Known Functions

Golgi contacts with the ER were described as early as the 1960s (Novikoff, 1964) and are currently the most characterized Golgi contacts (Figure 2). The ER and the Golgi have extensive cross talk to ensure the synthesis and homeostasis of a variety of biomolecules. Below are the central events that occur in these contacts.

Ceramide Transfer

Discovery of Ceramide Transfer Protein (CERT). In eukaryotes, from fungi to mammals, ceramide is synthesized in the ER and is transferred to the *trans*-Golgi for maturation into complex sphingolipids. Transfer of ceramide can occur through vesicular transport or directly to the *trans*-Golgi

through lipid transfer at contacts (Funato & Riezman, 2001), depending on chain length (Funato et al., 2020; Hanada et al., 2003). In human cells, ceramide is converted to sphingomyelin (SM) in the *trans*-Golgi. The human protein CERT (for all acronyms of protein and domain names as well as compounds, see Table 1) is the lipid transfer protein (LTP) responsible for the transfer of ceramide at ER–*trans*-Golgi contacts (Hanada et al., 2003). The CERT gene was initially identified by the ability to restore SM levels in SM-deficient cells (Hanada et al., 2003). Further enzyme assays and metabolic labeling experiments showed increased ceramide to SM conversion as a result of CERT expression without upregulation of SM synthesis enzymes. Ceramide transfer assays in lipid vesicles revealed that CERT transports ceramide in a concentration and time-dependent manner, via its START domain (Hanada et al., 2003).

Structural Determinants Dictate CERT's Localization and Regulation. CERT localization to ER–*trans*-Golgi contacts is a result of its pleckstrin homology (PH) domain binding to PI-4-phosphate (PI4P) on Golgi membranes (Hanada et al., 2003) and its FFAT domain interaction with ER-resident VAP proteins (Peretti et al., 2008). The three-dimensional structure of CERT's PH domain revealed two binding sites which enable codetection and enhanced affinity to membranes that have both PI4P and phosphatidylserine (PS), a combination found in Golgi membranes (Kumagai & Hanada, 2019). Given that no other interacting components were identified for CERT and since it forms oligomers, it may be that the two binding sites of CERT are sufficient for positioning it at ER–Golgi contacts (Hanada, 2018).

CERT's START domain specifically binds ceramide and diacylglycerol (DAG; formed during the conversion of ceramide to SM) but not SM or sphingosine (a breakdown product of ceramide) (Kumagai et al., 2005). This could be the result of CERT–SM coevolution (Hanada, 2014) and a regulatory feature for tuning ceramide flow from ER to Golgi while inhibiting "back-flow." Elevated DAG levels, a co-product of SM synthesis, were shown to inhibit CERT activity (Fugmann et al., 2007). In addition, CERT's START domain was also suggested to have an inhibitory regulatory role by interacting with the PH domain and competing for PI4P recognition. This suggests an intrinsic regulatory loop—in the presence of higher levels of ceramides, these would occupy CERT's START domain and induce lipid transport, while low levels would cause CERT to disengage from Golgi membranes. Indeed, mutants whose START and PH domains cannot interact show higher PI4P binding and ceramide transfer (Prashek et al., 2017).

Physiological Importance of CERT. The importance of CERT's lipid transfer activity is highlighted by the range of cellular and physiological processes in which its function is central. CERT was suggested to be involved in directing membrane trafficking during neurogenesis (Xie et al., 2018). Indeed, a

Table 1. All Acronyms of Proteins, Domain Names And Chemicals Used in This Manuscript.

Names written in upper-case are mammalian proteins while yeast proteins are written in sentence-case.

ABCD(#) = ATP binding cassette subfamily D member (#)
ACBD3 = acyl-CoA binding domain-containing protein 3
ALPS = amphipathic lipid packing sensor
AMPK = AMP-activated protein kinase
ApoB100 = apolipoprotein B100
ARF1 = ADP-ribosylation factor 1
BirA* = a nonspecific biotin ligase mutant of the *Escherichia coli* biotin ligase BirA
CARTS = carriers of the *trans*-Golgi network to the cell surface
CERT = ceramide transfer protein
DHE = dehydroergosterol
FAPP1 = four-phosphate adaptor protein 1
FAPP2 = four-phosphate adaptor protein 2
FLCN = folliculin
FRET = Forster resonance energy transfer
GFP = green fluorescent protein
GTP = guanosine triphosphate
Kes1 = Kre11-1 suppressor
mTORC1 = mammalian target of rapamycin complex 1
NIR2 = PYK2 N-terminal domain-interacting receptor 2
Nvj2 = nucleus–vacuole junction protein 2
Opi1 = overproducer of inositol 1
Orp(#) = oxysterol-binding protein-related protein (#)
Orp4L = oxysterol-binding protein-related protein 4L
OSBP = oxysterol-binding protein
Osh(#) = OSBP homolog (#)
OSW-1 = 3beta,16beta,17alpha-trihydroxycholest-5-en-22-one 16-O-(2-O-4-methoxybenzoyl-beta-D-xylopyranosyl)-(1–>3)-(2-O-acetyl-alpha-L-arabinopyranoside)
PAUF = pancreatic adenocarcinoma up-regulated factor
Pex35 = peroxin (peroxisome biogenesis factor) 35
PH = pleckstrin homology
PI4KIIIβ = phosphatidylinositol-4 kinase β
PI4KIIα = phosphatidylinositol-4 kinase α
Pik1 = phosphatidylinositol kinase 1
PKD = protein kinase D
PP2Cε = protein phosphatase 2Cε
RAB(#) = Ras-associated binding (#)
RELCH = RAB11-binding protein containing LisH, CC, and HEAT repeats
RHEB = Ras homolog enriched in brain
RILP = Rab-interacting lysosomal protein
RNAi = RNA interference
SAC1 = suppressor of actin 1
SCAP = sterol regulatory element-binding protein cleavage-activating protein
Snf1 = sucrose nonfermenting 1
Tat2 = tryptophan amino acid transporter 2
VAPA = vesicle-associated membrane protein-associated protein A
VAP = vesicle-associated membrane protein-associated protein A and B
Vps53 = vacuolar protein sorting-associated 53 homolog

mutation in the gene *CERT1*, which encodes CERT, has been identified as a potential driver for development disorders (Fitzgerald & Gerety, 2015). Moreover, a variant of *CERT1* with a gain-of-function mutation has been identified in patients with severe intellectual disability (Murakami et al., 2021). CERT is also a target of the intracellular pathogen, *Chlamydia trachomatis*, which hijacks CERT to form a contact between its own inclusion bodies and ER tubules harboring VAP proteins. This specialized contact is thought to mediate the formation of a specialized microenvironment that supports bacterial growth (Derré et al., 2011). In addition, CERT's ceramide transfer at ER–Golgi contacts is important for insulin signaling in muscle cells, loss of which can lead to type II diabetes (Bandet et al., 2018).

CERT etc. – Additional Ceramide Transporters at Play. Fungi lack a CERT homologue (Kumagai & Hanada, 2019; Liu et al., 2017). However, in the budding yeast *Saccharomyces cerevisiae* (from here on called yeast), Nvj2 mediates the nonvesicular transfer of ceramide at ER–medial-Golgi contacts (Liu et al., 2017). While Nvj2 is predominantly localized to the nuclear–vacuolar junction contact, it moves to ER–medial-Golgi contacts during ER stress or upon toxic accumulation of ceramide at the ER, tethering both organelles and transferring ceramide to the Golgi (Liu et al., 2017).

Roles for PI4P in Golgi Contacts

Determining Subcellular Localization and Enabling Counter-Exchange of Lipids. PI4P is a lipid that characterizes TGN membranes. One central role of PI4P is therefore enabling the specific localization of cytosolic proteins to the external surface of the Golgi. Two examples of these, in addition to CERT mentioned above, are the contacts proteins OSBP and FAPP1 that bind PI4P via their PH domains (Hanada, 2018).

Another role of PI4P, which is carried out specifically at contacts, is to regulate the levels of other membrane lipids by counter-transfer. PI4P-sterol exchange is the best-studied example of lipid counter-transfer at Golgi contacts. In mammalian cells, this is performed by OSBP. Using an *in vitro* system with ER and Golgi-like membranes, it was shown that OSBP can transfer PI4P and the cholesterol analogue DHE between these membranes (Mesmin et al., 2013; Ngo & Ridgway, 2009). In cells, overexpression of OSBP caused a decrease in Golgi PI4P levels and prevented the accumulation of DHE in lipid droplets (LDs) (where it is stored to maintain low levels in the ER) (Mesmin et al., 2013). Reciprocally, the sterol biosynthesis inhibitor, lovastatin, caused PI4P accumulation at the Golgi (Mesmin et al., 2013). Altogether, these findings suggest that OSBP is regulating the equilibrium between PI4P at the Golgi and sterols on ER membranes. OSBP localizes to distinct ER and Golgi apposed regions by binding PI4P and ARF1–GTP through its PH domain in addition to interacting via its FFAT motif with VAPA, an ER membrane protein (Mesmin et al., 2013). Liposome assays revealed that the interaction of OSBP and VAPA is necessary for OSBP to transfer lipids (Mesmin et al., 2013).

The formation of PI4P at Golgi contacts seems to be governed by the PI kinase PI4KIIIβ, which catalyzes the formation of PI4P by phosphorylation of PI (Mesmin et al., 2017; Venditti et al., 2019a). As PI4P levels increase on ER-like liposomes, OSBP's activity decreases, suggesting a negative regulatory role for PI4P by competing with sterols for OSBP binding (Mesmin et al., 2013). Indeed, introducing SAC1, a PI4P phosphatase, which localizes to ER–Golgi contacts (Venditti et al., 2019a; Wakana et al., 2015), decreases the inhibition on OSBP by decreasing the levels of PI4P on ER-like membranes (Mesmin et al., 2013). This demonstrates that the energy for the transfer of sterol against its gradient, from the ER to the TGN, is generated by the action of SAC1 (Mesmin et al., 2013). Therefore, OSBP transports PI4P along its gradient, from the TGN to the ER, and in return counter-transfers sterol against its gradient as a result of SAC1, which maintains low levels of PI4P in the ER to enable the cycle of lipid transfer (Mesmin et al., 2013).

OSBP-Related PI4P Transporters at Play. In addition to OSBP, several OSBP-related proteins (ORPs) have been suggested to play a role in the transport of PI4P and sterols across contacts. ORP4L, a paralogue of OSBP was shown to bind sterol and PI4P as well as mediate sterol transfer in liposomes (Charman et al., 2014). OSBP's PI4P transfer activity as well as VAPA presence was shown to be important for ORP4L localization to ER–Golgi contacts (Pietrangelo & Ridgway, 2018). ORP4L silencing triggered growth arrest and apoptosis, demonstrating for the first time a regulatory role for a mammalian ORP on cell proliferation (Charman et al., 2014). One suggested function of ORP4L is to maintain the Golgi structure, however, its exact role as an LTP at ER–Golgi contacts is yet to be determined (Pietrangelo & Ridgway, 2018).

A similar mechanism has been proposed for ORP9 after demonstrating PI4P-sterol counter-transfer *in vitro* (Ngo and Ridgway, 2009). ORP9 depleted cells accumulate sterol at endosomal/lysosomal compartments (Ngo & Ridgway, 2009) whereas in OSBP depleted cells it accumulates at the ER and LDs (Mesmin et al., 2017). In addition, ORP9 did not affect SM synthesis similarly to OSBP, nor did it colocalize with PI4KIIIβ (Mesmin et al., 2017; Perry & Ridgway, 2006). In accordance with these discrepancies between OSBP and ORP9 localization, ORP9 was proposed to be functionally different from OSBP (Ngo & Ridgway, 2009). ORP9's lipid transfer ability is suggested to maintain the flux of endocytosed cholesterol to the TGN by transferring sterols to the ER. Moreover, ORP9 is important for Golgi organization (Ngo & Ridgway, 2009) and stabilization

of ER–Golgi contact structures independent of its lipid transfer ability (Venditti et al., 2019b).

In yeast, it has been suggested that PI4P-sterol exchange is carried out by the OSBP homologue Kes1/Osh4 (Von Filseck et al., 2015b). Similarly to OSBP, Osh4 has been shown (by Forster resonance energy transfer [FRET] assays) to transfer PI4P and DHE in vitro, between liposomes mimicking the Golgi and the ER. This lipid transfer also occurs in favor of the PI4P gradient and against that of DHE. In agreement with the mammalian model, the presence of Sac1 on ER-like liposomes increases the counter-transfer of DHE and PI4P by Osh4 (Von Filseck et al., 2015b). However, it is not clear if Osh4 can sustain the bulk lipid transfer between the Golgi and ER in vivo. Cells deleted for osh4 have shown little effect on the lipid composition along the secretory pathway (Georgiev et al., 2011).

The yeast Osh1, another homologue of OSBP, was also suggested to be a PI4P-sterol counter-transfer protein at ER–Golgi contacts (Shin et al., 2020). Osh1 mutants that lack PI4P binding ability have reduced Golgi localization. This was also seen for GFP–Osh1 in mutants that have reduced Golgi PI4P levels, supporting the hypothesis that Osh1 localization to the Golgi depends on PI4P (Shin et al., 2020).

The Role of PI4P in Regulating Protein Trafficking. A central function regulated by PI4P counter-transfer is the trafficking of proteins. It has been shown that OSBP and CERT mediated lipid exchange is necessary for processing, trafficking, and secretion of the PAUF protein by CARTS, a subclass of TGN-to-plasma membrane (PM) carriers (Wakana et al., 2015). Knock-out of VAP proteins as well as disruption of OSBP or CERT function, impaired PAUF secretion. The same phenotype was observed in cells overexpressing SAC1 (Wakana et al., 2015) and its interactor SCAP, a sterol sensor protein localized to the ER (Wakana et al., 2021). When cholesterol levels are sufficient, the interaction between SAC1 and SCAP promotes the formation of ER–Golgi contacts and the biogenesis of CARTS at specific *trans*-Golgi subdomains (Wakana et al., 2021). Hence, OSBP and CERT activity result in the establishment of a specific lipid composition that enables the location and fission of specific vesicles directed to the PM (Wakana et al., 2021).

ApoB100 secretion was shown to be affected by two components acting at the ER–TGN contact, ORP10, an LTP suggested to control PS levels at the TGN (Venditti et al., 2019b), and FAPP1, an activator of SAC1 (Nissilä et al., 2012). ORP10 was shown to bind and extract PS in vitro (Maeda et al., 2013). Its lipid transfer function is important for stabilizing the ER–TGN contact and was suggested to mediate a PS-PI4P counter-exchange, similarly to ORP5 and ORP8, which transfer PS at ER–PM contacts (Venditti et al., 2019b). Accordingly, hepatocytes depleted for ORP10 or FAPP1 demonstrated increased PI4P levels at the Golgi and hypersecretion of ApoB100 (Nissilä et al.,

2012; Venditti et al., 2019b). However, when focusing on ORP10, it was not clear whether the increased PI4P levels or reduced levels of PS were the cause for the increased ApoB100 secretion. Since the depletion of ORP9, ORP10, OSBP or VAP proteins all increased both TGN PI4P and ER–TGN contact-extent, there was no way to pinpoint the direct reason for the effect on secretion. In contrast, depletion of FAPP1 increased TGN PI4P levels but did not affect the extent of the contact (Venditti et al., 2019b), placing FAPP1 as a more direct regulator of PI4P (Venditti et al., 2019a). Indeed, FAPP1 was shown to form a tripartite complex with SAC1 and the VAP proteins, and activate SAC1 by binding to its N-terminal regulatory domain thus promoting PI4P dephosphorylation (Nissilä et al., 2012). Hence, using FAPP1 enabled to disentangle the roles of contact-extent versus PI4P levels in secretion. Indeed, altering PI4P levels by depleting FAPP1, also showed an increase in the number of post-Golgi carriers containing ApoB100 and accelerated the rate of ApoB100 exit from the TGN. This accelerated exit rate of ApoB100 from the TGN could be reduced by depleting PI4KIIIβ, suggesting that it was, in fact, the increased PI4P levels at the TGN that directly regulated ApoB100 secretion (Venditti et al., 2019a).

Osh1 in yeast was also shown to be important for trafficking (Shin et al., 2020). Under tryptophan limiting conditions, sterol is required for trafficking of the amino acid permease Tat2, from the Golgi to the PM, where it resides in sterol-abundant domains (Shin et al., 2020). Therefore, sterol transfer from the ER to the Golgi is required for yeast survival under tryptophan limiting conditions (Shin et al., 2020). Δosh1 cells have reduced levels of Tat2 at the PM and display growth defects in media lacking tryptophan. Growth on medium lacking tryptophan could not be rescued by expressing Osh1 mutants that cannot bind PI4P or in conditions of reduced PI4P at the TGN (Shin et al., 2020) emphasizing the role of PI4P-sterol counter-transfer on Tat2 trafficking. Importantly, Osh1 expression as well as its interaction with Scs2/22 (the yeast VAP homologues) is essential for yeast survival in tryptophan deprived media (Shin et al., 2020). Osh1 homology to OSBP, taken together with Osh1's competitive sterol-PI4P binding and the requirement of Scs2/22 binding for tryptophan uptake, place Osh1 as a potential counter-transfer protein (Shin et al., 2020) similar to other sterol binding proteins (Mesmin et al. 2013; Von Filseck et al. 2015a, 2015b).

Regulatory Principles of PI4P Counter Exchange. The importance of PI4P and the counter-transfer mechanisms that rely on it are emphasized by the fact that OSBP activity is important, indirectly, for the distribution of additional lipids throughout the secretory pathway. OSBP can use up to half of the cellular PI4P depending on the requirement for cholesterol transfer (Mesmin et al., 2017). RNA interference (RNAi) experiments in mammalian cells revealed the involvement of OSBP in steady-state SM synthesis (Perry & Ridgway,

2006). Monitoring cholesterol flow in live mammalian cells following treatment by a specific inhibitor of OSBP (OSW-1) caused a drop of DHE at both the Golgi and the PM alongside an accumulation at the ER and LDs (Mesmin et al., 2017). In addition, OSW-1 treatment showed a reduction in PM lipid order, suggesting that OSBP's inhibition disrupted the lipid gradient along the secretory compartments (Mesmin et al., 2017).

Since counter-transfer is so important it is clear that it must be regulated at multiple levels. One way of regulating it is the local formation of PI4P. Indeed, a dedicated PI kinase, PI4KIIIβ, is enriched at ER–TGN contacts (Mesmin et al., 2017). In contrast, PI4P synthesis in other areas of the Golgi membrane is mainly carried out by PI-4 kinase α (PI4KIIα; Mesmin et al., 2017; Minogue et al., 2010). This idea of tight cooperation between OSBP and PI4KIIIβ activity is further supported by their colocalization as well as by their preference for loosely packed membranes (Mesmin et al., 2017). Not surprisingly, therefore, viruses were shown to hijack both PI4KIIIβ and OSBP as a functional couple to mediate the formation of viral replication organelles (Antonny et al., 2018).

An alternative way of regulating counter-transfer to specific areas is by careful recruitment of LTPs to specific domains that have unique biophysical properties. For example, Osh4 has an ALPS motif, an amino acid sequence that forms amphipathic helices, which allows it to dock on the membrane surface in membranes with higher levels of saturated acyl chains, with a neutral charge or with lipid packing defects and high curvature (Von Filseck et al., 2015b). Indeed, in an *in vitro* system, Osh4 was shown to exchange PI4P for sterols more efficiently in such membranes. Similarly, mammalian PI4KIIIβ also has an ALPS motif that enables its binding to membrane areas enriched for unsaturated lipids (Mesmin et al., 2017). Another example is Sec14, a yeast phosphatidylcholine–PI transfer protein that binds specifically to areas of high membrane curvature *in vitro* (Sugiura et al., 2019). Osh4 acts as an antagonist of Sec14 signaling by sequestering PI4P from Golgi membranes. Moreover, these two LTPs were suggested to coordinate cell growth during cellular division (Huang et al., 2018). The similar membrane preference of Osh4 and Sec14 further strengthens the idea that the biophysical properties of membranes regulate LTP activity by providing a suitable setting for them to function.

Coordinating the Function of LTPs. While specific lipids may affect discrete Golgi functions, it is clear that multiple lipids must be coordinated to maintain the accurate composition of Golgi membranes. Therefore, it is not surprising that LTPs at Golgi contacts work in a coordinated manner. For example, OSBP and VAPA are required to stimulate ceramide transport by CERT (Perry & Ridgway, 2006). Depletion of VAP proteins altered PI4P levels in Golgi membranes and reduced DAG and SM levels resulting in reduced outward traffic from the Golgi (Peretti et al., 2008). This suggests that VAP proteins enable coordinated lipid transfer by OSBP (sterols), NIR2 (PI), and CERT (ceramide). NIR2 was shown to be required for Golgi targeting of OSBP and CERT (Peretti et al., 2008), probably due to its PI–PI4P exchange activity at ER–Golgi contacts. However, NIR2's contribution to PI transport at ER–Golgi contacts is still under debate (Hanada, 2020; Masone et al., 2019).

Another example of lipid coregulation is by the kinase PKD, which was shown to be involved in regulating ceramide abundance by linking PI4P and SM levels (Capasso et al., 2017; Fugmann et al., 2007). In the presence of high levels of DAG, PKCη activates PKD (Baron & Malhotra, 2002), which in turn inhibits CERT by phosphorylation of its serine-rich region, preventing PI4P binding (Fugmann et al., 2007). As a result, reduced CERT activity stops DAG accumulation at the Golgi creating a negative feedback loop. This loop can be modulated by the phosphatase PP2Cε, which dephosphorylates CERT. This process is dependent on the presence of VAPA (Saito et al., 2008) and the recruitment of PP2Cε to ER–Golgi contacts by the Golgi scaffold protein ACBD3 (Shinoda et al., 2012). It is important to note that turnover of sphingolipids is probably too low to sustain the DAG levels in the TGN and that other mechanisms, perhaps triggered by ceramide, might contribute strongly to the synthesis of DAG. Additionally, PKD is also part of a broader homeostatic regulatory model to maintain the constant synthesis rates of sphingolipids such as SM and glycosphingolipids at the *trans*-Golgi (Capasso et al., 2017). Upon activation, PKD also phosphorylates PI4KIIIβ and OSBP to encourage nonvesicular transport of PI4P to the ER. This results in a decrease in overall cellular PI4P, as ER PI4P is rapidly converted back to PI by SAC1. Lack of PI4P at the TGN negatively regulates the localization of CERT, FAPP2, and OSBP1 and causes a reduction in glucosylceramide and cholesterol transport to the TGN (Capasso et al., 2017), thus connecting in a negative feedback loop sphingolipid flow and PI4P turnover at the TGN (Capasso et al., 2017).

The Role of PI4P in Coupling Cellular State With Golgi Functions. Several signaling lipids, including PIs, have an interesting biophysical property: the pKa of their phosphomonoester lipid head group lies within the physiological range of the cytosol. Since the interaction of these lipids with binding proteins is mediated by electrostatic forces and hydrogen bonding between the lipid head group and the protein's binding pocket (Shin et al., 2020), changes in cytosolic pH will affect the strength of these interactions. For example, under glucose starvation, the cytosolic pH decreases, leading to protonation of lipids and to a reduction in the affinity of proteins that bind them thus connecting glucose levels (as a proxy for cellular energy levels) to protein localization (Shin et al., 2020). This has been previously shown for Opi1, a yeast transcription factor that binds to phosphatidic acid (PA) (Young et al., 2010). During glucose starvation, PA is protonated and Opi1 is released from the ER to the nucleus, where it represses the

transcription of phospholipid biosynthesis genes, and therefore restricts cell growth (Young et al., 2010). Recently, a similar mechanism has been described for Osh1 (Shin et al., 2020). Reduction in cytosolic pH reduces the affinity of Osh1's PH domain to PI4P leading to its dissociation from the Golgi (Shin et al., 2020). In fact, this property of PI4P turns it into a very sensitive pH biosensor for the Golgi.

Why is such pH sensing important for Golgi functions? During glucose starvation, Pik1, the yeast PI-4 kinase activity is regulated to maintain enough PI4P at the TGN to allow proteins, such as Osh1, to rapidly reassociate with the TGN when glucose is restored and cytosolic pH increases (Shin et al., 2020). Hence, pH biosensing by PI4P enables the recruitment of proteins to Golgi contacts in response to changes in cellular energy status and regulated cargo sorting. In the future, it will be interesting to find additional examples of lipids that use chemical changes (such as pH or ionic strength) to alter their interactions with proteins.

Hurdles for Discovering Golgi Contact Sites

While several resident proteins and functions have already been described for the ER–Golgi contact, very little is known about Golgi contacts with other organelles. This raises the question—does the Golgi not form contacts with other organelles or have they evaded discovery? It is our opinion that the Golgi, like any other organelle, should create contacts with all organelles and that we are yet to discover multiple Golgi contacts and their cellular functions.

However, to characterize a contact, one needs to visualize proximity between the two participating organelles, to identify the contact resident proteins and specific tethers and, ultimately, uncover the specific function of the proximity. Below are several inherent characteristics of the Golgi that may have made the above more difficult to achieve for the Golgi, and therefore underlie the reasons for the limited discovery of additional Golgi contacts to date (Figure 3).

Golgi Cisternae are Small and Sparse

It is not surprising that the first contact to be discovered was between the membranes of the ER and those of mitochondria—the two most abundant cellular membranes. In contrast, Golgi cisternae are small and rare and can be an order of magnitude less abundant in cells relative to the ER (Wei et al., 2012). This creates objective difficulties in findings rare events of organelle–organelle contacts.

Golgi Shape and Composition are Highly Dynamic

The dynamic character of the Golgi is another obstacle in discovering Golgi contacts. Cisternae rapidly change their composition with approximate maturation rates of <1 min (Ishii et al., 2016; Kurokawa et al., 2019; Losev et al., 2006; Matsuura-Tokita et al., 2006). This in turn implies that the
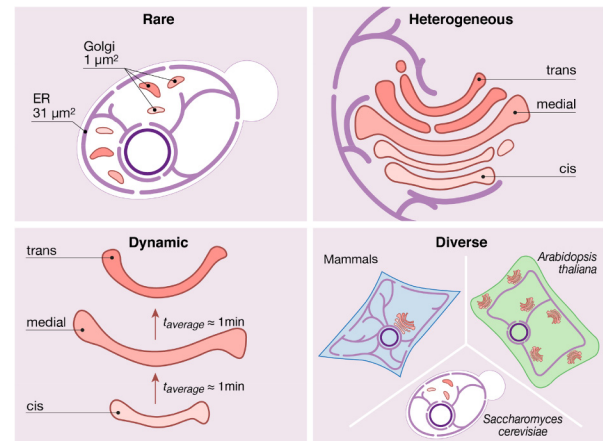
Figure 3. Golgi properties that hinder the discovery of novel contacts. *Rare*, the surface area of all Golgi cisternae combined is only about 1 μm^2—more than 30 times smaller than the ER surface area (Wei et al., 2012). *Dynamic*, the Golgi cisterna are constantly morphing with a maturation rate of about 1 min (Ishii et al., 2016; Kurokawa et al., 2019). *Heterogeneous*, although all called "Golgi" each cisterna type is unique and may form distinct contacts making it difficult to study this organelle as one cellular structure. *Diverse*, due to structural differences of the Golgi between kingdoms, methods and proteins that are relevant to one kingdom may be irrelevant in another.

formation of a specific contact destined to transfer one substrate may not be relevant after cisternae alteration. Hence, contacts must be constantly modulated and by nature will be extremely short-lived.

Each Golgi Cisterna Type has a Unique Lipid and Protein Composition

The division of the Golgi into three different cisternae, each with a unique proteome and function but still some overlapping proteins between them (Klumperman, 2011; Weill et al., 2018), may have added to the complexity of identifying contacts. This is because each cisterna could be viewed as a distinct organelle but many of the Golgi proteins are found in more than one cisterna type. Hence, there may be different contacts between each organelle and each Golgi cisterna increasing the complexity of discovery. Choice of marker proteins and misinterpretation of resulting proximities could have therefore slowed down detection of contacts specific to one type of cisterna or function.

Golgi Structure is Diverse in Different Kingdoms

While in all eukaryotes the Golgi is composed of cisternae, the morphology and architecture of these cisternae are extremely variable between organisms, therefore a discovery in one kingdom may not be relevant to another. The mammalian Golgi is characterized by disc-like, stacked, cisternae.

While the number of cisternae within a stack can differ depending on cell type (Ito et al., 2014; Klumperman, 2011) they are usually radially organized around the centromere via interactions with dynein proteins and microtubules. The plant Golgi is also made of stacked cisternae but, unlike the mammalian Golgi, the stacks (up to hundreds in number) are dispersed in the cytosol and in constant movement rather than centralized to one location (Ito et al., 2014). In the yeast *Pichia pastoris* the Golgi is stacked similarly to plants but in the most common yeast model, *S. cerevisiae*, the cisternae are scattered throughout the cytosol (Suda & Nakano, 2012) mostly in an unstacked form (Klumperman, 2011) unless glucose is depleted in which case they can form stacked structures (Rambourg et al., 1993).

The striking structural and dynamic differences in Golgi properties make it complicated to apply discoveries from one kingdom to another or to identify commonalities between them. For example, optical tweezers (a tool used for organelle trapping (Andersson et al., 2007; Gao et al., 2016; Pérez-Sancho et al., 2015; Wang et al., 2014) have been used to study the ER–Golgi contact of *Arabidopsis thaliana,* revealing that the golgin AtCASP (working with AtGolgin 84A (Osterrieder et al., 2017; Vieira et al., 2020), physically links the ER and *cis*-Golgi (Osterrieder et al., 2017), possibly maintaining Golgi orientation (Vieira et al., 2020). However, this method cannot be used to trap the mammalian Golgi due to its large size and ribbon-like structure, highlighting the technical challenges of studying Golgi contacts in various organisms.

Methodological Limitations

The above limitations due to the Golgi structure and composition should be seen as a stimulus to define what new technological principles would be necessary to identify Golgi contacts. To date, the above issues are all aggravated by some of the global methodological limitations in the contact site field. Resolution is the main limitation in visualizing contacts. Light microscopy, with its resolution limit of 250 nm, cannot always provide the necessary resolution to directly detect the presence of contacts even when using super-resolution platforms (Scorrano et al., 2019). Since the Golgi in mammals is always located in the dense perinuclear region, this hinders visualization of contacts with this organelle by light microscopy. Indeed, it was shown that even for the well-studied ER–Golgi contacts, light microscopy was challenging to use due to their perinuclear location and small size (in the range of 5–20 nm) (Venditti et al., 2019b).

One way to overcome this problem is by using split-fluorescence reporters. In this approach, each organelle surface is marked with one, nonfluorescent, part of a fluorophore. Only in areas where the two organelles come into extremely close proximity will the fluorophore be formed and a fluorescent signal emitted (Eisenberg-Bord et al.,

2016; Shai et al., 2018). While this method works extremely well for most contacts it is quite complicated to use for Golgi ones due to the small number of protein molecules that can be on any single cisterna and the fact that no single protein uniquely defines a specific cisterna (Tojima et al., 2019). Another fluorescence-based approach is FRET which allows the determination of proximity between two proteins at contacts by measuring the energy transfer from one protein tagged with a donor fluorescent probe to a second protein tagged with an acceptor probe. However, FRET experiments are generally intricate and require more specialized microscopes (Scorrano et al., 2019).

Electron microscopy (EM) can provide the required resolution, yet harbors drawbacks of its own, such as the lack of ability in visualizing live cells, the small number of samples that can be captured, the inability to scan large cellular populations for a rare phenotype, and the fixation techniques that can lead to changes of cellular phenomena (Ladinsky et al., 1999). For the yeast Golgi, this is of a particular hindrance since the various cisternae often look indistinguishable from each other by EM, as well as from many other organelles, and hence are difficult to track for the formation of specific contacts. Hence, since Golgi contacts are rare and short-lived, conventional EM is of little use in detecting them except for ER–Golgi contacts—the most abundant of Golgi contacts (Kurokawa et al., 2014; Rambourg et al., 2001; Venditti et al., 2019b).

A combined approach of FRET-based methods and EM was used to visualize and identify potential components of ER–Golgi contacts (Venditti et al., 2019b). However, a much more powerful approach than using each method independently is to use them together by harnessing correlative light and EM (CLEM) procedures, which are used to align fluorescent signals with EM imaging. CLEM allows the high-resolution study of rare events found by fluorescence microscopy and may reveal functional as well as structural information on Golgi contacts simultaneously. Recently, a high-throughput EM approach has been developed called multiCLEM (Bykov et al., 2019) and may enable more rapid scanning of physiological conditions or genetic backgrounds for Golgi contacts.

While imaging approaches are challenging, biochemical approaches may be the solution. Proximity labeling using biotinylation is an emerging tool used to identify potential contact proteins. Proximity-dependent biotin identification (BioID) is a method that utilizes a modified nonspecific biotin ligase BirA* (mutant of the *Escherichia coli* biotin ligase BirA; Roux et al., 2012). Another approach for proximity uses an engineered ascorbate peroxidase (APEX) (Lam et al., 2014). In both methods, the modified enzyme is fused to a protein of interest and then potential interactors found in proximity to the fused complex are identified by streptavidin pull-downs and peptide identification by mass spectrometry. Both methods have been used to identify molecular components involved in contacts (Cho et al., 2017; van Vliet et al.,

2017). Moreover, split versions of both BioID and APEX have been developed and may be utilized to investigate proteins that mediate contacts by tagging of two proteins that are on opposing membranes (De Munter et al., 2017; Han et al., 2019). One limitation of APEX is the low sensitivity caused by low expression levels. Overexpressing APEX may solve this problem, however, this strategy may cause organelle aggregation (Lam et al., 2014).

Emerging Insights Into New Golgi Contacts

The Golgi's multiple roles place it as a central hub in numerous cellular functions. Thinking about these various roles warrants the discovery or further characterization of contacts with other organelles. While many clues exist to suggest that other Golgi contacts should be present, the report on the Golgi–mitochondria contact seems to be the most established and based on direct experimental data. All others, as of yet, remain hypothetical.

Golgi–Mitochondria Contacts

Mitochondria are the cellular power plant and the source of multiple metabolites including some unique lipids. A Golgi–mitochondria interaction was visualized in mammalian cells (Valm et al., 2017) and recently, a Golgi–mitochondria contact has been suggested in yeast (Wycislo et al., 2020). In yeast, during respiratory conditions, the contact formation was shown to depend on phosphorylation of Vps53 by Snf1, the yeast homologue of AMPK (Wycislo et al., 2020), a kinase that is involved in cellular energy homeostasis. Vps53 is a member of the Golgi-associated retrograde protein complex, which is required for retrograde vacuole to Golgi trafficking, mitochondrial morphology, autophagy and lipid metabolism (Wycislo et al., 2020).

What is the function of such a contact? During respiration, mitochondria have a reduced abundance of lipid biosynthesis proteins and an increase of proteins functioning in the citric acid cycle (Di Bartolomeo et al., 2020). The requirement of mitochondria to shift resources to ATP production during respiratory conditions could raise the need for lipid import, which may be provided via the Golgi–mitochondria contacts that form more readily in such media (Wycislo et al., 2020). Lipids supplied by the Golgi–mitochondria contacts may regulate mitochondrial morphology as well as the adjustment of mitochondrial dynamics upon changes in carbon source. In support of this hypothesis, yeast strains expressing only a nonphosphorylatable mutant of Vps53 demonstrated fragmented mitochondria (Wycislo et al., 2020). Moreover, PI4P was shown to be essential for the final steps in mitochondrial fission (Nagashima et al., 2020), further supporting the idea that mitochondria dynamics is regulated by Golgi-derived lipids. Indeed it has been suggested that Golgi vesicles harboring PI4P are recruited to the ER–

mitochondria contact to form a three-way contact with the Golgi (Nagashima et al., 2020; Valm et al., 2017).

Potential Golgi–Endosome contacts

In mammalians cells, endocytosed sterols travel in both a vesicular and a nonvesicular manner from lysosomes to different cellular destinations, including the TGN (Sobajima et al., 2018; Wong et al., 2019). A potential recycling endosome (RE)–Golgi contact has been suggested in *Drosophila* and mammalian cells after the RE–Golgi association were observed by fluorescent microscopy and EM (Sobajima et al. 2018; Fujii et al., 2020). In mammalian cells, this contact seems to be mediated by OSBP, RAB11, and RELCH, a newly identified RAB11-binding protein (Sobajima, 2018). RELCH links OSBP to RAB11 and is required for RE relocation to the TGN. This model is based on the fact that OSBP was shown to mediate nonvesicular sterol transport between liposomes that recapitulated RE and TGN membranes (Sobajima (2018). Moreover, OSBP, RELCH, and RAB11 depleted cells suffered from reduced sterols in the TGN, while an increase was observed in late endosomes/lysosomes. The suggested mechanism may control the cholesterol flux from late endosomes/lysosomes through RE to the TGN (Sobajima, 2018).

Potential Golgi–Lysosome Contacts

Microtubule and actin interactions anchor the Golgi to the perinuclear area in mammalian cells. Nonetheless, Golgi architecture can be modulated in response to a variety of cues such as DNA damage, apoptosis and stress (Kulkarni-Gosavi et al., 2019). A possible Golgi–lysosome contact was shown to form upon amino acid stress. Under these conditions, lysosomes exhibit perinuclear clustering with a restricted movement. This perinuclear localization is dependent on the interaction of RAB34, a GTPase mainly localized to the Golgi, and RILP, an effector of the tumor suppressor protein FLCN, which localizes to lysosomes (Starling et al., 2016). However, contacts have also been suggested during amino acid repletion by RHEB localizing to Golgi membranes and activating mTORC1 at lysosomes (Hao et al., 2018). Nevertheless, the molecular components of this suggested contact remain elusive. Indeed, in mouse embryonic fibroblasts, when amino acids are added to the media, Golgi cisterna and lysosomes comigrate on microtubules toward the centrosomes (Hao et al., 2018). Whether the differences in dependence on amino acids are due to cell type (Hao et al., 2018) or whether they reflect a single contact with different tethers under different conditions remains to be explored.

A Golgi–lysosome contact may regulate signaling in addition to modulating the comovement of these dynamic organelles. For example, the protein FLCN can regulate localization of lysosome-associated transcription factors as

well as of mTORC1 (Starling et al., 2016). A better understanding of Golgi–lysosome and Golgi–endosome contacts and their regulatory components will be of great importance since their interplay was shown to play a role in neurodegenerative diseases and cancer (Wallings et al., 2019; Waugh, 2019).

Potential Golgi–PM Contacts

The Golgi is the cellular supplier of complex lipids to various destinations, most notably the PM (Van Meer et al., 2008). Beyond expanding the PM during cell growth or division, lipids transported to the PM serve as structural components of endocytic vesicles, form lipid rafts, act as signaling molecules and enable docking of specific proteins. A Golgi–PM contact may act in parallel to vesicular transport to meet the vast lipid demands of the PM, or to regulate the levels of signaling lipids.

Indeed it was shown that the Golgi PI4P pool is important for maintaining the PM pool of PI(4,5)P$_2$, which is important for PM integrity, and mediates a wide range of events such as exocytosis, cytoskeletal reorganization, and regulation of ion channels (Dickson et al., 2014). How the PM pool of PI(4,5)P$_2$ is affected by the Golgi is still not understood, but it could occur through a vesicular pathway or alternatively by the direct removal of PI4P from Golgi compartments by Osh proteins and direct transfer to the PM, likely at a close contact site (Nishimura et al., 2019). Hence, the presence of such a contact and its resident LTPs must be explored.

Potential Golgi–Lipid Droplet Contacts

LDs store neutral lipids that can be utilized as an energy source or for membrane assembly during times of need (Schuldiner & Bohnert, 2017). The Golgi is a main stop in lipid metabolism serving to modify lipids and coordinate their levels along the secretory pathway. Golgi–LD interactions have been observed in mammalian cells (Krahmer et al., 2018; Valm et al., 2017), and Golgi was the second most common observed association for LDs (only second to LDs association with mitochondria) (Valm et al., 2017). In addition, under a high-fat diet when Golgi and LD associations were observed, OSBP was shown to relocalize to LDs (Krahmer et al., 2018). Given the lipid monolayer membrane of LDs, tethering between Golgi and LDs would require a specialized monolayer-binding protein, such as proteins harboring an ALPS motif. ARF1, a binding partner of OSBP and FAPP1, has an ALPS motif and was shown to be targeted to LDs, similarly to OSBP (Bouvet et al., 2013; Krahmer et al., 2018). The two proteins could act together to form a Golgi–LD contact. Interestingly, blocking OSBP caused the accumulation of sterols at LDs (Mesmin et al., 2017). This could be due to inactivity of a potential Golgi–LD contact that provides LDs with PI or due to the sterol accumulation

at the ER where LDs are formed (Schuldiner & Bohnert, 2017).

More generally, a Golgi–LD contact could act to mediate a functional response to modulations in lipid levels as well as serve as a coordinator of lipid metabolism. Further research is required to authenticate the presence of such a contact and understand its function, regulators and the components enabling its formation.

Potential Golgi–Peroxisome Contacts

Peroxisomes execute essential cellular functions such as the breakdown (beta-oxidation) of fatty acids and the metabolism of reactive oxygen species (Castro et al., 2018). An interaction and cross talk between the Golgi and peroxisomes were previously suggested in both yeast and mammalian cells (Ferrer et al., 2005; Valm et al., 2017; Yofe et al., 2017). While PI4P at Golgi contacts serves as a biosensor for glucose levels and regulates the trafficking of nutrient transporters (Shin et al., 2020), peroxisome abundance was also shown to be regulated by the available carbon source (Yofe et al., 2017). Thus, one possible function for a Golgi–peroxisome contact is to transmit information regarding cellular energy levels. A possible link between the two organelles could be the Golgi GTPase Arf1, which was shown to regulate peroxisome biogenesis, possibly through a mechanism involving Pex35 in yeast (Yofe et al., 2017). An additional role for a Golgi–peroxisome contact could be in cholesterol and very-long-chain-fatty-acid (VLCFA) metabolism. The expression of ABCD2, a peroxisomal VLCFA transporter, is affected by cholesterol levels (Raas et al., 2019), in turn, regulated at Golgi contacts. Deletion of ABCD2 in mice caused accumulation of VLCFAs and disruption of the Golgi architecture which resulted in neurodegeneration (Ferrer et al., 2005). Moreover, ABCD2 is a homolog of ABCD1 (Ferrer et al., 2005), a peroxisome–LD contact protein (Chang et al., 2019), and although the two share partial functional redundancy, it seems that ABCD2 mediates another function that is yet to be discovered (Ferrer et al., 2005). Despite the lack of information that directly links the Golgi to peroxisomes, their involvement in similar metabolic pathways suggests such a contact would be beneficial. Further studies to confirm the existence of this contact require the identification of its molecular components. It may give a better understating of VLCFA metabolism in neurodegenerative diseases and of the signaling mechanisms which mediate the energy status of the cell.

Perspective

As new Golgi functions are discovered, it becomes clear that there is more than meets the eye to this dynamic and elusive, yet important organelle. Technological advances in visualization techniques, biochemical and lipid-related assays (Scorrano et al., 2019) will extend our knowledge and

facilitate the discovery of Golgi contacts, their molecular components and their function within the cellular metabolic atlas.

It will be intriguing to see what roles such contacts play on the cellular level in processes such as lipid biosynthesis, Golgi proteome integrity, cellular signaling and on the physiological level in diverse diseases ranging from tumor development to neurodegenerative diseases and infections of intracellular pathogens. From an evolutionary point of view, it will be exciting to see which Golgi contacts are conserved across kingdoms. Are there unique contacts that exist in only certain phyla, cell type or condition? Do the subdomains of a Golgi cisterna form multiple contacts with various organelles? And which molecular entities and physiological conditions regulate Golgi contacts? Given the highly connected character of the Golgi and its central role in key cellular functions, it is clear that many exciting discoveries await for this fascinating organelle and its communication with other cellular structures.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID iDs

Inês G. Castro https://orcid.org/0000-0003-4669-985X
Maya Schuldiner https://orcid.org/0000-0001-9947-115X

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