Membrane trafficking heats up in Pavia

Golgi Meeting on Membrane Trafficking in Global Cellular Responses

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Introduction

Ever since its discovery by Camillo Golgi in 1898, the Golgi apparatus has been the subject of spirited scientific discourse. Over 300 cell biologists from around the world met in a hot Pavia (Italy)—which is where Golgi first discovered the “*apparato reticolare interno*” that now bears his name—to attend the 2008 Golgi meeting. Ten years ago, scientists met in Pavia for the first Golgi meeting to celebrate the centenary of Golgi’s discovery. At that time, scientists were grappling with a shift in the paradigm of how secretory proteins move through the Golgi complex on their way to the plasma membrane (Featherstone, 1998). The Golgi complex comprises a series of flattened, disc-shaped membranes or cisternae, in addition to vesicles that are associated with the rims of the cisternal stacks. In the ‘old’ model (Fig 1A), the cargo proteins were selectively packaged in vesicles that moved in a forward (anterograde) direction from one cisterna to the next, while the Golgi-resident proteins, such as glycosylation enzymes, remained static. The paradigm-shifting ‘new’ model of cisternal maturation (Fig 1B) maintains that the cargo moves or progresses within the cisternae, while the Golgi glycosylation enzymes are selectively packaged into vesicles for backward (retrograde) trafficking to regenerate earlier cisternae. In the intervening years, cisternal maturation has been generally accepted, and details of the roles played by coat proteins, membrane lipids and cytoskeletal elements at the Golgi complex are being defined.

The organizers of the 2008 meeting expanded the scope to encompass both biosynthetic and endosomal membrane trafficking. Notable among the presentations were those defining the roles of specific lipids and proteins in modelling membrane surfaces, the interplay between cytoskeletal and signalling elements in the regulation of trafficking events, the application of new imaging and systems biology approaches to cell biology, and the descriptions of how alterations in membrane trafficking in the secretory pathway contribute to disease.

Nonetheless, the organelle of honour was the Golgi, and its exact nature and operational properties were discussed and debated. A. Luini (Santa Maria Imbaro, Italy) presented a modified cisternal maturation model in which tubular connections between Golgi cisternae allow for rapid equilibration of soluble proteins—such as albumin—between the stacks (Fig 1C). The formation of these tubules is dependent on the activity of phospholipase A2, and inhibition of this enzyme prevents tubule formation and the rapid transport of

albumin through the Golgi. These tubular connections could explain why some cargo proteins transit rapidly, whereas larger cargo proteins such as procollagen—which would be confined to the cisternae—show slower rates of transit. In a more radical variation on cisternal maturation (Fig 1D), J. Lippincott-Schwartz (Bethesda, MD, USA) proposed that within all cisternae, glycolytic enzymes and transmembrane cargo are partitioned into either enzymatic-processing domains or Golgi-export domains, which are enriched in glycerophospholipids and cholesterol/sphingolipids, respectively. This model arose from the observation of a rapid segregation of VSV-G cargo from Golgi-resident enzymes within the cisternae. Moreover, as export to the plasma membrane can occur from any cisterna—in contrast to the assumption that export occurs exclusively at the *trans*-Golgi network (TGN)—this model can explain why the VSV-G appears at the plasma membrane without the lag expected from a cisternal maturation model. This model can also account for the self-association of Golgi enzymes and lipid gradients across the Golgi, and is compatible with some cisternal maturation processes. It will be interesting to watch the further development of ideas regarding how proteins travel through the Golgi.

Modelling of membranes

The past 10 years have seen remarkable progress in identifying and determining the functional roles of the protein machineries that facilitate membrane modelling. In particular, the realization that specific protein modules drive membrane curvature by the direct modification of lipids has emerged since the centenary meeting. H. McMahon (Cambridge, UK) provided an overview of these concepts. BAR and F-BAR domains have a positively charged concave side that stabilizes curved membranes; ENTH and C2 domains stimulate curvature by insertion of an amphipathic helix or hydrophobic loops into the phospholipid bilayer; and N-BAR domains use a combination of these mechanisms. When coat proteins control curvature, vesicles arise, whereas uncontrolled curvature leads to tubule formation. McMahon and V. Haucke (Berlin, Germany) showed that the Arf1 and Arf6 GTPases stimulate tubule formation at the Golgi and the plasma membrane, respectively, by membrane insertion of an amino-terminal amphipathic helix, thereby introducing new components to the curvature machinery. The Arf1 tubules that form at the Golgi might be those that allow the rapid transfer of cargo between Golgi cisternae. Interestingly, B. Goud (Paris, France) reported that Arf1 alone does not induce optically visible membrane deformation of giant unilamellar vesicles with a lipid composition mimicking that of the Golgi. However, Arf1 and COPI coat proteins do induce weak curvature when membranes are under high tension, and membrane deformation becomes extensive with lower membrane tension. One possible explanation for this discrepancy is that the experiments with the unilamellar vesicles were performed at lower protein/lipid ratios, and it is possible that Arf contributes to membrane deformation only at high surface concentrations, which would occur during coat polymerization (Manneville *et al*, 2008). The interplay between membrane-deforming proteins and coats in shaping membrane buds is clearly complex, and will continue to be an active area of investigation.

At the meeting, much attention was paid to the role of lipids in defining organelle identity, and in facilitating vesicle budding/fission and fusion. A. De Matteis (Santa Maria Imbaro, Italy) described her studies on the importance of PI(4)P at the Golgi. FAPP2 is recruited to the Golgi by its PH domain, which recognizes

Glossary

AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
APP	amyloid precursor protein
Arf	ADP ribosylation factor
Arf-GAP	Arf GTPase-activating protein
ARL	Arf-like
BAR	Bin1/amphiphysin/Rvs167
BARS	brefeldin A-ADP ribosylated substrate
C2	conserved domain 2
CFTR	Cystic Fibrosis transmembrane conductance regulator
COP	coat protein complex
EHD1	Eps15 homology-domain-containing 1
ENTH	epsin amino-terminal homology
FAPP2	four-phosphate adaptor protein 2
F-BAR	Fes/CIP4 homology and Bin1/amphiphysin/Rvs167
FIP2	family-interacting protein 2
G α q	α subunit of heterotrimeric Gq protein
GCC185	Golgi-localized coiled-coil protein of 185 kDa
GFP	green fluorescent protein
GRIP	golgin-97, RanBP2, Imh1p and <i>trans</i> -Golgi p230
KDEL	lysine/aspartic acid/glutamic acid/leucine
MINT3	munc-18-interacting protein 3
N-BAR	Bin1/amphiphysin/Rvs167 domain with an amino-terminal amphipathic helix
NMDA	N-methyl-D-aspartic acid
PALM	photoactivated localization microscopy
PH	pleckstrin homology
PI(4)P	phosphoinositide 4-phosphate
SEC24	secretory mutant 24
VANGL 2	Van Gogh-like 2
VSV	vesicular stomatitis virus
VSV-G	vesicular stomatitis virus protein G

both PI(4)P and the Arf GTPase. FAPP2 was previously found to be associated with the tubular precursors of the pleiomorphic carriers that transport cargo from the Golgi to the plasma membrane. De Matteis showed that FAPP2 is a glucosylceramide transfer protein that is required for glycosphingolipid synthesis. Depletion of FAPP2 inhibits Golgi-to-plasma membrane transport of VSV-G and impairs glycosphingolipid synthesis, suggesting that the formation of these lipids or glucosylceramide itself at the TGN has a role in the formation of transport carriers.

In studies designed to identify lipid components required for the formation of COPI-coated vesicles, V. Hsu (Harvard, MA, USA) reported that the generation of phosphatidic acid (PA) by phospholipase D2 is required for vesicle fission in the presence of COPI, Arf1, Arf-GAP1 and BARS in an *in vitro* vesicle-formation assay. However, others have claimed that diacylglycerol (DAG)—which can be formed from PA by a PA phosphatase—is the lipid required for vesicle fission (for a discussion of this topic, see Roth, 2008). T. Nilsson (Montreal, QC, Canada) presented data in support of the DAG requirement. Treating cells with propranolol, which blocks PA phosphatase, results in an elevation of PA at the Golgi, flattened Golgi morphology and an arrest in vesicle fission. As DAG is also required for Arf-GAP recruitment and functioning, both PA and DAG are probably required during COPI vesicle formation. The role of lipids in membrane modelling will continue to be an area of focus in the immediate future. In fact, the recent developments in mass spectrometry technologies, which allow a comprehensive analysis of the

Fig 1 | Evolving Golgi transport models. Schematic versions of how resident Golgi enzymes (denoted by the letter E) and transiting cargo (denoted by asterisks) move with respect to each other during cargo transport through the Golgi. (A) An early model featuring static cisternae with cargo moving in an anterograde direction between cisternae in vesicles. (B) A cisternal progression or maturation model, in which cargo remains within the cisternae and progresses through the Golgi as resident Golgi proteins, including enzymes, are carried in retrograde vesicles that move to reconstitute earlier cisternae. (C) A modified cisternal maturation model with tubular continuities that connect cisternae within a stack. Export from the *trans*-most cisterna occurs through tubular extensions. (D) A rapid-partitioning model of Golgi organization showing processing domains containing Golgi enzymes and export domains containing cargo within each cisterna. ER, endoplasmic reticulum.

ensemble of lipids present in a sample (lipidomics), will accelerate the pace of discovery in this area.

Protein-curvature modules work together with alterations in membrane lipids to drive membrane curvature; however, coat proteins are required to mold the membrane into transport vesicles. Clathrin coats are among the best studied of the coat systems: classical deep-etch electron-microscopy images reveal large and flat clathrin lattices on the plasma membrane. The large lattices are periodically associated with smaller curved coats (Heuser *et al*, 1987). The curved coats correspond to functional clathrin-coated pits (CCPs), whereas it has remained unclear whether the flat lattices are functional. T. Kirchhausen (Harvard, MA, USA) demonstrated that these lattices, which he calls plaques, are indeed active sites of endocytosis. Unlike standard CCPs, which have a lifetime in the range of 45 to 75s, the plaques are stable over extended periods of time. Treatment with latrunculin, which is an inhibitor of actin polymerization, freezes the plaques in place; it was therefore proposed that actin polymerization is required to lift the plaques from the membrane, driving their invagination. This relationship between

actin dynamics and plaque lifting might provide an answer to the question of how certain clathrin-mediated endocytic cargo, such as the VSV, which is larger than a standard CCP, can gain access to cells. When CCPs reach a deeply invaginated state, they undergo fission in order to pinch off the membrane. The dynamin family of large-molecular weight GTPases has been characterized extensively as the mediator of this fission reaction, and GTP hydrolysis-driven global conformational changes in dynamin generate the forces that are necessary for the fission process. S. Schmid (La Jolla, CA, USA) presented a new model system to measure dynamin-dependent fission directly, and revealed that the assembly state of dynamin regulates vesicle fission. P. De Camilli (New Haven, CT, USA) provided new insights into the role of dynamin in fission and endocytosis, as revealed by the study of fibroblasts that lack dynamin expression. His work emphasized the interconnections between the action of dynamin, actin polymerization and plasma-membrane tubulation mediated by BAR domain-containing proteins in the fission of CCPs from the plasma membrane.

In addition to the roles played by coat proteins and membrane lipids in facilitating membrane curvature and vesicle formation, another set of Golgi proteins—the golgins—is important for the maintenance of Golgi structure and function. These large coiled-coil GRIP-domain-containing proteins are thought to act as membrane tethers that help to maintain the Golgi stacks and bring transport vesicles close to their target membrane for fusion. The recruitment of golgins to the Golgi apparatus requires ARL proteins, which bind to the GRIP domain at the carboxy-terminal region of the golgins, and Rab proteins, which bind to the extended coiled-coil regions. S. Pfeffer (Stanford, CA, USA) presented the crystal structure of Rab6 bound to the golgin GCC185, which indicates how the Rabs—with their variable C termini—could extend out from a vesicle or Golgi membrane and interact with the extended portion of the golgin, whereas ARL1—which is closely associated with the Golgi membrane surface—binds to the juxtamembrane GRIP domain. Interestingly, it seems that several different Rabs can interact with these golgins. GCC185, which is important for the recycling of mannose 6-phosphate receptors back to the TGN, can interact with Rab6 and Rab9, among other factors. S. Munro (Cambridge, UK) also identified many Rab proteins—including Rab2, Rab6 and Rab30—that interact with golgins, using *Drosophila* as a model system, and showed that some Rabs bind to golgins from both the *cis* and the *trans* Golgi. The extent to which these golgins interact with Rabs on the same or other membranes, and how Rab binding would be regulated have yet to be determined. In any case, these findings confirm that the golgins could fulfil their tethering function by capturing various Rab-associated vesicles and reeling them in to the Golgi.

Connecting the cytoskeleton with cell signalling

Moving beyond vesicle formation, the exact manner in which membrane traffic is directed and coordinated has attracted the interest of many investigators. The importance of the microtubule and actin cytoskeletons, and their respective motor proteins, in facilitating trafficking in the secretory and endocytic pathways has been revealed over the past 10 years. J. Nelson (Stanford, CA, USA) spoke of the role played by septin 2 in the post-Golgi trafficking of membrane proteins to both the apical domains and the basolateral domains of polarized Madin–Darby canine kidney cells. Septins are unusual GTP-binding proteins, which form filaments that align with a subset of microtubules

made up of polyglutamylated tubulin. The knockdown of septin 2 or the loss of polyglutamylated microtubules impairs the delivery of vesicles to both apical and basolateral domains, while cell polarity remains intact.

Although most work on signal transduction has focused on growth factor and G-protein-coupled receptor signalling at the plasma membrane, there has been a growing interest in whether signalling also occurs at the Golgi. M. Sallèse (Santa Maria Imbaro, Italy) described a traffic-activated signalling pathway at the Golgi that leads to the activation of SRC family kinases. Surprisingly, the influx of cargo in the Golgi is sensed by the KDEL receptor, which binds to KDEL-containing endoplasmic reticulum-resident proteins that have escaped from the endoplasmic reticulum and takes them back. The KDEL receptor is a G-protein-coupled receptor that interacts with the α -subunits of heterotrimeric G proteins. Sallèse reported that the specific small-interfering RNA depletion of G α_q inhibits the cargo stimulation of SRC activation and impairs secretion. M. McNiven (Rochester, MN, USA) showed that cargo-activated SRC phosphorylates Golgi-associated dynamin 2 on Tyr231 and Tyr597, and that mutation of the SRC-phosphorylation sites reduces cargo traffic through the Golgi. It will be interesting to determine how SRC phosphorylation affects the function of dynamin 2, thereby altering trafficking, and to identify additional substrates for SRC and downstream effectors of G α_q .

M. Ehlers (Durham, NC, USA) provided a notable example in which both cytoskeletal factors and cell signalling jointly regulate membrane trafficking. He has been interested in the membrane trafficking of AMPA-type glutamate receptors in the dendritic spines of neurons. In this system, spine growth is stimulated on the activation of NMDA-type glutamate receptors and is mediated by the recycling of endosomal membranes that contain AMPA receptors, rather than by the secretory pathway. This recycling is dependent on Rab11a, EHD1, syntaxin 13 and myosin Vb. Upon stimulation of NMDA receptors, an influx of calcium leads to myosin Vb recruitment to the recycling endosomes by stimulating the interaction of myosin Vb with Rab11-FIP2. Once recruited, myosin Vb transports recycling endosomes from the dendritic shaft at the base of the spine into the actual spine head. This coupling of signalling to the functions of a molecular motor and to membrane trafficking could form the basis for the plasticity of dendritic spines during learning.

Trafficking and disease

Given their importance for cellular function, it is not surprising that membrane-trafficking pathways are targeted loci in many human diseases. However, it is surprising that alterations in trafficking pathways previously thought to be essential for cell survival—such as COPI-mediated and COPII-mediated pathways—are also targets in diseases such as neurodegenerative disorders (Burman *et al*, 2008), as it would be expected that alterations in such fundamental pathways would cause cell death. R. Schekman (Berkeley, CA, USA) provided important insights into this concept and described studies related to craniorachischisis, which is a rare malformation of the skull and spinal bones resulting from the failure of neural tube closure. In a screen for neural tube-closure defects, the David Ginty laboratory discovered a mutation in SEC24b, which is a member of the SEC24 family required for COPII trafficking. SEC24b was found to interact genetically with VANGL 2, which is a surface protein required for signalling pathways that control planar polarity. Schekman showed that SEC24b targets VANGL 2 to COPII vesicles, and that mutations in the C-terminal

cytoplasmic domain of VANGL 2 cause it to be retained in the endoplasmic reticulum. He also pointed out that there are four isoforms of SEC24; hence, most cargo is trafficked normally because other SEC24 isoforms are functional, whereas VANGL 2 is not packaged into COPII vesicles, planar polarity is disrupted and the neural tube fails to close.

The cleavage of the APP by β - and γ -secretases leads to the generation of amyloid- β peptide, which is the main component of senile plaques in Alzheimer disease. R. Kahn (Atlanta, GA, USA) reported that MINT3—which is recruited to the Golgi in an Arf-dependent manner—binds to APP and is required for APP export from the TGN towards its normal basolateral-sorting route. In the absence of MINT3, APP is redirected to endosomes; therefore, traffic-sorting decisions resulting from the binding of MINT3 to the tail of APP are probably crucial for APP processing by secretases, and defects in this pathway could contribute to the pathophysiology of Alzheimer disease.

One-third of the proteins in the human genome are estimated to be membrane or secreted proteins, all of which must be translocated into the endoplasmic reticulum and then correctly folded within its lumen. W. Balch (La Jolla, CA, USA) reported that incorrect folding and retention of mutant proteins in the endoplasmic reticulum leads to several diseases. For example, many cases of cystic fibrosis result from a common mutation in the chloride-channel protein, CFTR, which destabilizes the protein and blocks its exit from the endoplasmic reticulum through the endoplasmic reticulum quality-control pathway. There are more than 50 proteins involved in the folding of CFTR, and Balch spoke of several pharmacological approaches that have been taken either to help fold the CFTR or to override the folding requirement for endoplasmic reticulum export, given that mutant CFTR is functional when it reaches the plasma membrane. These studies highlight the importance of understanding how alterations in secretory-protein trafficking contribute to disease, in order to define therapeutic intervention points.

New approaches to cell-biology research

Discoveries in science are often accelerated by the development of new technologies and instrumentation. At the previous Golgi meeting, Lippincott-Schwartz imaged endoplasmic reticulum-to-Golgi traffic in live cells using GFP chimeric proteins. This year, she described a new light-microscopy approach called PALM, with a resolution below the diffraction limit of light. By using a photoactivatable form of GFP, her group selectively and repeatedly activated individual GFP-tagged proteins to generate a high-resolution image in the x/y-axis. H. Hess is developing a new generation of PALM, iPALM, which uses wave-particle interference to improve the resolution in the z-axis. These approaches are particularly suited for use with flattened cells that are grown on plastic. However, cells in the body exist as part of tissues that are surrounded by a matrix and the vascular system. R. Weigert (Bethesda, MD, USA) aims to study membrane trafficking in living animals using intravital two-photon microscopy. He demonstrated that the salivary glands of the rat could be imaged with sufficient subcellular resolution to observe the endocytosis of dextran and its delivery to lysosomes in fibroblasts. The ability to manipulate the gland both genetically and pharmacologically indicates that this technique will allow us to examine membrane traffic as it occurs in the cells of whole organisms.

Since the centenary meeting, cell biology has seen the advent and growth of systems-biology approaches. In fact, in 1998 the first draft of the human genome had not yet been released. Cell biologists have capitalized on the current rich genomic information with proteomics

and automated fluorescence microscopy-based screens, and this was reflected at the 2008 meeting. J. Bergeron (Montreal, QC, Canada) applied quantitative proteomics to the endoplasmic reticulum, Golgi and Golgi-derived COPI vesicles. Interestingly, the hierarchical clustering of the identified proteins revealed that Golgi fractions contain predominantly luminal proteins of the secretory pathway, whereas COPI vesicles contain Golgi-resident enzymes, supporting the cisternal maturation model (Fig 1B,C). R. Pepperkok (Heidelberg, Germany) described a fully-automated microscopy system that was used to screen for defects in the delivery of VSV-G to the cell surface following the knockdown of human proteins. The scale of the screen—in which approximately 55,000 small-interfering RNAs were used to screen 22,000 genes—was impressive. Interestingly, 93 of the 681 genes that were found to regulate VSV-G trafficking were ‘unknown’. Despite these successes, L. Pelkmans (Zurich, Switzerland) warned about the dangers of population context influencing phenotype. For example, depletion of a particular protein might influence the cell population (the cell density, for example) in a manner that would then alter the phenotype being studied. However, he indicated that most of the cell-to-cell variation can be predicted and corrected for if cell size, cell cycle, edge location and other properties are accounted for. Undoubtedly, new technologies and mathematical approaches for modelling membrane trafficking will continue to drive this field for many years to come.

Concluding remarks

The past 10 years have seen impressive advances in our understanding of membrane trafficking in the secretory and endocytic systems. The protein machineries that control the remodelling of membranes have been identified, and new genomic/proteomic strategies coupled to large-scale screens are being applied to trafficking systems to generate a systems-level understanding. Increased understanding of the contribution of membrane trafficking to human diseases will lead to intensified efforts to search for pharmaceuticals that modulate these pathways. Many challenges remain for the future and will guarantee a vibrant field in the years to come.

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