Getting high on traffic

The biochemistry of intracellular transport

Catherine F. Latham,¹ Mary Munson² and Elizabeth A. Miller^{1,*}

¹Department of Biological Sciences; Columbia University; New York, NY USA; ²Department of Biochemistry and Molecular Pharmacology; University of Massachusetts Medical School; Worcester, MA USA

Key words: meeting report, intracellular traffic, organelle dynamics, membrane dynamics, Lake Tahoe

Molecular mechanisms of secretory and endocytic transport pathways were the focus of a recent ASBMB Special Symposium on Biochemistry of Membrane Traffic, held last October in the picturesque high-altitude setting of Lake Tahoe. An exciting and diverse array of research and discussion topics focused on recent advances in understanding the molecular basis of movement of protein and membrane through the eukaryotic secretory pathway. Highlights included discussions of the common structural and functional features of tethering complexes, the roles of the lipid PI4P in the late secretory pathway, organization of specific ER sub-domains and a new look at Golgi biogenesis.

Intracellular trafficking is an essential cellular process by which the interconnected compartments of the endomembrane system maintain communication with each other and facilitate the complex process of protein and lipid sorting. Newly synthesized proteins are translocated into the ER where these clients are folded, sorted and transported in vesicles to other organelles or secreted via exocytosis. Protein cargo can also be internalized by endocytosis and transported via vesicles to specific organelles. These transport processes are coordinated by specific protein-protein and protein-lipid interactions that ensure the protein cargo gets to the right place at the right time.

Biochemical, structural and high-resolution microscopy studies are providing significant insights into the key mechanistic questions in intracellular trafficking. How do the critical complexes that perform each individual step in the transport reaction—sorting, coating, budding, uncoating, tethering and fusion—coordinate with each other to ensure successful cargo delivery? Are there common features of these protein complexes that hint at evolutionary relationships despite unique functions at different cellular compartments? What roles do lipids play in this process; how is specificity for cargo achieved? How are the organelles—ER, Golgi, vacuoles—inherited during cell division? The dynamic molecular interactions that orchestrate these complex molecular events were the focal point of the recent ASBMB Special Symposium, Biochemistry of Membrane Traffic: Secretory and

Endocytic Pathways, held over three days in October in the stunning high sierra near Lake Tahoe in California. The captivating program, organized by Suzanne Pfeffer (Stanford University School of Medicine) and Vivek Malhotra (Centre for Genomic Regulation in Barcelona, Spain) was accompanied by lively scientific discussion and enthusiastic poster presentations. Here, in the fitting forum of *Cellular Logistics*, a new journal focusing on processes governing the flow of molecules and energy in the cell, we discuss the highlights of this recent meeting.

Moving Cargo: Vesicle Budding, Fission, Tethering and Fusion

Scott Emr (Cornell University) kicked off the meeting with a keynote address that described the role of the Arrestin-Related Trafficking adaptors (ARTs), proteins that function upstream of the ESCRT machinery in protein turnover. ESCRT complexes mediate vesicular budding into the lumen of multi-vesicular bodies (MVBs) that represent a waystation in protein trafficking to the lysosome/vacuole, either in a biosynthetic pathway from the Golgi or in an endocytic pathway from the plasma membrane. Art1p was first shown to act as an adapter between cargo and the ubiquitin ligase, Rsp5p.¹ More recently the Emr group has shown that phosphorylation of Art1p by the kinase, Npr1p, regulates its activity and its localization. Npr1p phosphorylation of Art1p inactivates Art1p at the plasma membrane, and in turn, shifts Art1p back to the Golgi. Emr proposed that the ART proteins mediate quality maintenance, to remove damaged proteins from the plasma membrane. Ubiquitination of these proteins subsequently directs them into the MVB sorting system via the ESCRT machinery.^{2–5}

Recent insights into the coat complexes that generate transport vesicles were discussed in several talks. Randy Schekman (University of California, Berkeley) described in vitro reconstitution of Pex19p-dependent trafficking of peroxisomal membrane proteins from their site of synthesis in the ER into a vesicle population that is distinct from canonical COPII ER-derived vesicles. Packaging of peroxisome-bound cargo, Pex3p and Pex15p, was defective when Pex19p-deficient cytosol drove the reaction, but recombinant Pex19p was not sufficient to generate these structures, suggesting additional cytosolic factors remain to be discovered.⁶ Maxence Nachury (Stanford University) presented new structural studies of the primary cilium-specific sorting complex involved in Bardet-Biedl Syndrome, the “BBSome.” Initial

*Correspondence to: Elizabeth A. Miller; Email: em2282@columbia.edu
Submitted: 12/10/10; Accepted: 12/14/10
DOI: 10.4161/cl.1.1.14466

characterization suggested that the BBSome is a coat protomer complex responsible for sculpting vesicles prior to delivery to the cilium,⁷ but recent analysis by cryo electron microscopy indicates that the BBSome bears a greater resemblance to an adapter layer, as no cage structure was present. This observation is consistent with previous reports by the Nachury lab showing no detectable vesicle formation from liposomes in vitro, unlike other coat complexes. Turning to more canonical coats, Liz Miller (Columbia University) described how yeast cells can survive without the normally essential COPII protein, Sec13p: she proposed that mutations that perturb biosynthesis of GPI-anchored proteins create a “bendy membrane” that can be more easily deformed without the structural support that is provided by this outer coat component. Frances Brodsky (University of California, San Francisco) discussed recent structural studies that shed light on the lesser-known of the two clathrin endocytic coat components, the clathrin light chain (CLC), which regulates coat formation and assembly of the triskelion hub.⁸ The structure of CLC bound to a central hub domain of clathrin heavy chain revealed two states of CLC that seem to act as a conformational switch to control cage formation.⁹ In a non-endocytic role, CLC was also found to control actin reorganization through interactions with Huntingtin interacting protein 1 (Hip1), which in turn influences cell migration.¹⁰ Additionally, a role for mammalian clathrin heavy chain isoform, CHC22, in trafficking of the insulin-regulated glucose transporter, GLUT4, from the TGN to the plasma membrane links clathrin function to the disease phenotype of Type 2 diabetes. Continuing the discussion of endocytic mechanisms, Sandy Schmid (Scripps Research Institute, San Diego) discussed recent insights into the endocytic membrane fission activity of the large atypical GTPase, dynamin. Characterization of a dynamin fusion protein consisting of only the GTPase domain and GTPase effector domain (GED) demonstrated that this minimal dynamin fusion protein was able to reconstitute fission in vitro, and this activity could be dependent on dimerization of the GED domain. This observation provides evidence for the coupling of GTP hydrolysis and dynamin post-fission disassembly.¹¹

A combination of crystallography, EM and biochemical studies suggests that the role of the large multisubunit tethering complexes is scaffolding, to organize the coats, Rabs, SNAREs and membranes for specific membrane fusion. Fred Hughson (Princeton University) discussed the structural similarities among multiple classes of these tethering complexes, uniting several into a new family, CATCHR (complexes associated with tethering containing helical rods). Subunits of the conserved oligomeric Golgi (COG)¹² and Dsl1 complexes,¹³ have similar helical bundle structures, and likely common evolutionary origins, with the exocyst complex subunits, determined by Mary Munson’s group (University of Massachusetts Medical School)¹⁴ and others^{15–17} as well as the endosomal GARP complex.¹⁸ Furthermore, the CATCHR complexes’ role in coordinating SNARE assembly for specific fusion may be facilitated by interactions with the Sec1/Munc18 family of SNARE regulators (Mary Munson; Vladimir Lupashin, University of Arkansas).

Since the protein clientele of the secretory pathway have highly diverse physical properties, faithful trafficking requires

complex and tightly-regulated sorting processes. Julia von Blume (Center for Genetic Regulation, Barcelona, Spain) discussed her recent work on cargo sorting at the trans-Golgi network (TGN) by the actin-depolymerizing factor (ADF)/cofilin complex. Knock-down of cofilin in mammalian and *Drosophila melanogaster* cells resulted in defective trafficking of secretory cargo at the TGN where secretory proteins were detained and TGN-resident proteins were secreted.¹⁹ Recent data suggests a model that defines discrete sorting domains within the TGN,¹⁹ where sorting of luminal proteins is mediated by the ADF/cofilin complex via its interaction with SPCA1, a calcium homeostasis protein at the TGN membrane. Carolyn Machamer (Johns Hopkins University) presented evidence showing that large viral cargo, like the coronaviruses, can manipulate trafficking machinery in mammalian cells to enhance viral exocytosis during infection. The coronavirus, IBV, was found to cause major structural rearrangements in the Golgi apparatus as well as greatly reducing trafficking of endogenous cargo to the plasma membrane.²⁰

Organelle and Lipid Dynamics

Discussions on spatial and structural organization of organelles were centred largely on the ER. Gia Voeltz (University of Colorado, Boulder) presented recent work visualizing peripheral ER domains in *Saccharomyces cerevisiae*. Using tomographic reconstruction of electron microscopy images, the Voeltz group characterized the ER into three distinct domains—plasma membrane-associated, central cisternal and tubular. Studies on the effects of membrane-shaping proteins, reticulons (Rtn1p and Rtn2p) and Yop1p on biogenesis and inheritance of tubular ER domains revealed that the reticulons are required for maintenance, rather than initiation, of membrane curvature, through a transmembrane domain ‘wedge’ inserted into the lipid bilayer.²¹ With a focus on structural factors governing forward trafficking from the ER, Akihiko Nakano (RIKEN ASI, Wako, Japan) presented high-resolution fluorescence microscopy images of sites of COPII assembly and ER export in *Saccharomyces cerevisiae*. ER exit sites (ERES) preferred to localize to concave edges of the ER domains, and displayed some dependence on the tubular ER, since cells that were deficient in tubular ER structures had fewer ERES than wild type cells. Evidence for a novel “kiss and run” interaction between ERES and the cis-Golgi provided additional food for thought in how traffic between these two organelles might be regulated.

Differing perspectives on Golgi function and biogenesis fuelled several spirited discussions. Yanzhuang Wang (University of Michigan) reported on studies dissecting the requirements for Golgi stack dynamics during mitosis. Using an in vitro assay that reconstitutes Golgi formation,²² the Wang group identified several factors that are required for reassembly including p47, specifically its ubiquitin-associated (UBA) domain. Other proteins involved in ubiquitin-related pathways including an E3 ligase, RUL, and a deubiquinating enzyme, VCIP135, were also identified, and the ubiquitin substrate and the precise role in Golgi reassembly are currently under investigation. Suzanne Pfeffer

presented structural and functional analyses from her lab on the GRIP-domain golgin, GCC185. The giant elongated coiled-coil of GCC185 can span multiple Golgi elements and binds to 14 different Rab GTPases at five sites along its lengthy structure.²³ Perhaps spurred by the complexity of these Golgi-associated Rab interactors, Suzanne also described an exciting new “cisternal progenitor” model as a mechanism for Golgi biogenesis,²⁴ whereby stable and tightly-packed Golgi compartments undergo constant fission and fusion, allowing protein transport through the Golgi, possibly regulated by Rab cascades.

Several talks accentuated different roles for the specific lipid, phosphatidylinositol-4-phosphate (PI4P), which is greatly enriched in the Golgi membrane. Two talks focused on the roles of Golgi proteins, mammalian GOLPH3 and its yeast counterpart, Vps74p, at the TGN. Chris Burd (University of Pennsylvania) presented structural and functional studies of GOLPH3 and Vps74p, which demonstrated that both are dependent on PI4P binding for localization to the Golgi and interactions with the cytosolic tails of Golgi resident membrane proteins, as part of a retention mechanism regulated by the PI4-kinase, Pik1p.²⁵ Seth Field (University of California, San Diego) described how the dependence of GOLPH3 on PI4P for localization may be a critical link between the actin cytoskeleton and Golgi morphology and trafficking.²⁶ Peter Novick (University of California, San Diego) demonstrated that the Rab guanine nucleotide exchange factor (GEF), Sec2p, is dependent on PI4P as a regulatory switch for activation of the Rab GTPase, Sec4p, on secretory vesicles, and subsequently its downstream effectors, Rab GTPase, Ypt32p and the exocyst subunit, Sec15p.²⁷ Also at the TGN, PI4P can influence assembly of clathrin adapter complexes, as outlined by Greg Payne (University of California, Los Angeles).

As a model for membrane fusion events, Bill Wickner (Dartmouth Medical School) described the minimal components required for vacuolar homotypic fusion in yeast. Using an

in vitro reconstituted system, the precise roles for each of these components—homotypic fusion and protein sorting complex (HOPS), SNARE proteins, SNARE disassembly chaperones Sec17/Sec18p, the Rab GTPase Ypt7p and five specific vacuolar lipids—in membrane fusion is becoming clearer. Their in vitro studies show that the HOPS tethering function is dependent on the Rab GTPase, Ypt7p^{28,29} and that specific lipids, diacylglycerol, ergosterol and phospholipids, play a critical role in coordinating protein-lipid interactions during fusion.³⁰

Techniques and Technology

Despite the diversity in research topics presented at this meeting, several emerging methodologies were clearly favored. Visualization of intracellular events using high-resolution microscopy deepened our understanding of ER morphology and local structure (Gia Voeltz, Akihiko Nakano) and will be invaluable for understanding coordination between molecular events and cellular dynamics at the organelle level. In vitro reconstitution to simulate protein-lipid trafficking events has long been established as a highly informative and effective method to determine the precise contributions and minimal functions of individual components. At this meeting, this methodology clearly showed great scope for modification to suit specific systems, including dynamin-mediated membrane fission using SUPER-templates (Sandy Schmid), the assembly cycle of Golgi stacks during mitosis (Yanzhuang Wang), regulatory effectors of SNARE-mediated membrane fusion (Jim Rothman, Yale University School of Medicine) and the subtleties of vacuolar fusion (Bill Wickner). Cleverly combining molecular and cell-based functional assays with information gained from atomic resolution protein crystal structures, as well as cryo electron microscopy, continues to prove highly effective for dissecting protein and lipid interplay in protein trafficking.

References

- Lin CH, MacGurn JA, Chu T, Stefan CJ, Emr SD. Arrestin-related ubiquitin-ligase adaptors regulate endocytosis and protein turnover at the cell surface. *Cell* 2008; 135:714-25.
- Babst M, Katzmann DJ, Snyder WB, Wendland B, Emr SD. Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body. *Dev Cell* 2002; 3:283-9.
- Katzmann DJ, Babst M, Emr SD. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* 2001; 106:145-55.
- Reggiori F, Pelham HR. Sorting of proteins into multivesicular bodies: ubiquitin-dependent and -independent targeting. *EMBO J* 2001; 20:5176-86.
- Urbanowski JL, Piper RC. Ubiquitin sorts proteins into the intraluminal degradative compartment of the late-endosome/vacuole. *Traffic* 2001; 2:622-30.
- Lam SK, Yoda N, Schekman R. A vesicle carrier that mediates peroxisome protein traffic from the endoplasmic reticulum. *Proc Natl Acad Sci USA* 2010; In press.
- Jin H, White SR, Shida T, Schulz S, Aguiar M, Gygi SP, et al. The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell* 2010; 141:1208-19.
- Ybe JA, Greene B, Liu SH, Pley U, Parham P, Brodsky FM. Clathrin self-assembly is regulated by three light-chain residues controlling the formation of critical salt bridges. *EMBO J* 1998; 17:1297-303.
- Wilbur JD, Hwang PK, Ybe JA, Lane M, Sellers BD, Jacobson MP, et al. Conformation switching of clathrin light chain regulates clathrin lattice assembly. *Dev Cell* 2010; 18:841-8.
- Wilbur JD, Chen CY, Manalo V, Hwang PK, Fletterick RJ, Brodsky FM. Actin binding by Hip1 (huntingtin-interacting protein 1) and Hip1R (Hip1-related protein) is regulated by clathrin light chain. *J Biol Chem* 2008; 283:32870-9.
- Chappie JS, Acharya S, Leonard M, Schmid SL, Dyda F. G domain dimerization controls dynamin's assembly-stimulated GTPase activity. *Nature* 2010; 465:435-40.
- Lees JA, Yip CK, Walz T, Hughson FM. Molecular organization of the COG vesicle tethering complex. *Nat Struct Mol Biol* 2010; 17:1292-7.
- Ren Y, Yip CK, Tripathi A, Huie D, Jeffrey PD, Walz T, et al. A structure-based mechanism for vesicle capture by the multisubunit tethering complex Dsl1. *Cell* 2009; 139:1119-29.
- Sivaram MV, Furgason ML, Brewer DN, Munson M. The structure of the exocyst subunit Sec6p defines a conserved architecture with diverse roles. *Nat Struct Mol Biol* 2006; 13:555-6.
- Dong G, Hutagalung AH, Fu C, Novick P, Reinisch KM. The structures of exocyst subunit Exo70p and the Exo84p C-terminal domains reveal a common motif. *Nat Struct Mol Biol* 2005; 12:1094-100.
- Moore BA, Robinson HH, Xu Z. The crystal structure of mouse Exo70 reveals unique features of the mammalian exocyst. *J Mol Biol* 2007; 371:410-21.
- Wu S, Mehta SQ, Pichaud F, Bellen HJ, Quirocho FA. Sec15 interacts with Rab11 via a novel domain and affects Rab11 localization in vivo. *Nat Struct Mol Biol* 2005; 12:879-85.
- Vasan N, Hutagalung A, Novick P, Reinisch KM. Structure of a C-terminal fragment of its Vps53 subunit suggests similarity of Golgi-associated retrograde protein (GARP) complex to a family of tethering complexes. *Proc Natl Acad Sci USA* 2010; 107:14176-81.
- von Blume J, Duran JM, Forlanelli E, Alleaume AM, Egorov M, Polishchuk R, et al. Actin remodeling by ADF/cofilin is required for cargo sorting at the trans-Golgi network. *J Cell Biol* 2009; 187:1055-69.
- Ruch TR, Machamer CE. The Hydrophobic domain of the infectious bronchitis virus e protein alters the host secretory pathway and is important for release of infectious virus. *J Virol* 2010; 85:675-85.
- Zurek N, Sparks L, Voeltz G. Reticulon short hairpin transmembrane domains are used to shape ER tubules. *Traffic* 2011; 12:28-41.

22. Tang D, Xiang Y, Wang Y. Reconstitution of the cell cycle-regulated Golgi disassembly and reassembly in a cell-free system. *Nat Protoc* 2010; 5:758-72.
23. Burguete AS, Fenn TD, Brunger AT, Pfeffer SR. Rab and Arl GTPase family members cooperate in the localization of the golgin GCC185. *Cell* 2008; 132:286-98.
24. Pfeffer SR. How the Golgi works: A cisternal progenitor model. *Proc Natl Acad Sci USA* 2010; 107:19614-8.
25. Wood CS, Schmitz KR, Bessman NJ, Setty TG, Ferguson KM, Burd CG. PtdIns4P recognition by Vps74/GOLPH3 links PtdIns 4-kinase signaling to retrograde Golgi trafficking. *J Cell Biol* 2009; 187:967-75.
26. Dippold HC, Ng MM, Farber-Katz SE, Lee SK, Kerr ML, Peterman MC, et al. GOLPH3 bridges phosphatidylinositol-4-phosphate and actomyosin to stretch and shape the Golgi to promote budding. *Cell* 2009; 139:337-51.
27. Mizuno-Yamasaki E, Medkova M, Coleman J, Novick P. Phosphatidylinositol 4-phosphate controls both membrane recruitment and a regulatory switch of the Rab GEF Sec2p. *Dev Cell*; 18:828-40.
28. Hickey CM, Stroupe C, Wickner W. The major role of the Rab Ypt7p in vacuole fusion is supporting HOPS membrane association. *J Biol Chem* 2009; 284:16118-25.
29. Hickey CM, Wickner W. HOPS initiates vacuole docking by tethering membranes before trans-SNARE complex assembly. *Mol Biol Cell* 2010; 21:2297-305.
30. Xu H, Wickner W. Phosphoinositides function asymmetrically for membrane fusion, promoting tethering and 3Q-SNARE subcomplex assembly. *J Biol Chem* 2010; 285:39359-65.