Membranes and sorting
Editorial overview
Marino Zerial

Addresses
European Molecular Biology Laboratory, EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany; e-mail: marino.zerial@EMBL-heidelberg.de

Current Opinion in Cell Biology 1998, 10:475–476

http://biomednet.com/elecref/0955067401000475

Abbreviations

AP	adaptor protein complex
COP	coat protein or coatomer
NSF	*N*-ethylmaleimide sensitive factor
SNAP	soluble NSF attachment protein
SNARE	SNAP receptor

Can we say with confidence that we have gained a good understanding of the fundamental molecular principles of intracellular transport? Undoubtedly, progress has been enormous in the past years and concepts have emerged which explain the biochemical properties of several important factors of the trafficking machinery. Nevertheless, judging from the number of new molecules that are being discovered in yeast and higher eukaryotes, novel features of organelle structure and function are continuously unravelled. One of the problems we face is that the molecules that were first identified dominate our thinking and the way we portray the basic molecular principles. On the one hand reducing a mechanism to its minimal components is helpful to construct a working scheme, but on the other hand we must be ready to modify the scheme and question fundamental assumptions. For young scientists entering this field it is important to realize that the mechanisms currently proposed are by no means definitive. The challenge is to integrate the wealth of information from genetics, biochemistry and the genome sequencing projects into a coherent scheme. For example the complex process of coat assembly and vesicle budding requires multiple molecular interactions. Coat proteins selectively bind to signals exposed on the cytoplasmic tail of cargo molecules. The recruitment of these proteins is not restricted to those interactions, however, and several other factors, including the lipid composition of the membrane and the function of the cytoskeleton, must be incorporated to explain how vesicles form. The same is true for vesicle docking and fusion which is not simply brought about by SNARE (soluble *N*-ethylmaleimide sensitive factor attachment protein receptor) pairing. We now know the identity of all SNAREs, GTPases, ATPases, and so on, of yeast, and yet our opinions diverge about how vesicle targeting, docking and fusion are controlled molecularly. The diversity of problems discussed in this series of reviews reflects the complexity we are facing. This is an exciting phase and I am grateful to the authors who have agreed to contribute their ideas to this discussion. This section of *Current Opinion in Cell Biology* has assembled contributions from leading groups engaged in various areas of cell biology with the intention to deal both with controversial issues and rising new concepts in the molecular mechanisms of membrane organization and sorting.

The first two reviews deal with the mechanisms whereby transport vesicles bud and fuse with target membranes. Advances in vesicle budding are discussed by Kaiser and Ferro-Novick (pp 477–482) who focus on endoplasmic reticulum-to-Golgi transport. By analogy with clathrin coated vesicles, cargo proteins play an important role in initiating coat protein (COP) II vesicle coat assembly. Exciting progress has come with the reconstitution of synthetic COPII vesicles combining liposomes of defined composition and purified proteins. How pre-Golgi vesiculo-tubular clusters form by exchanging COPII for COPI coats, move along microtubules and are delivered to the Golgi complex is an enigma which promises exciting developments. This review also deals with the mechanisms of membrane docking and fusion describing a novel multiprotein complex implicated in endoplasmic reticulum-to-Golgi transport . This aspect is further discussed by Robinson and Martin (pp 483–492) who provide an excellent critical assessment of the mechanism of synaptic vesicle transport. Vesicle transport requires SNARE pairing which is regulated by the activities of *N*-ethylmaleimide sensitive factor (NSF) and SNAP (soluble NSF attachment protein). A controversial issue has been whether NSF activity on SNAREs is required for membrane fusion. A number of studies in several systems summarized here suggest that NSF disrupts SNARE complexes and primes SNAREs prior to fusion. Recent experiments *in vitro* with proteoliposomes support the idea that SNAREs may be sufficient for membrane docking and fusion. The low efficiency of this reaction, however, and other lines of evidence which are inconsistent with a role for SNAREs in docking question this interpretation. Given that other candidate docking molecules are implicated in this process it seems clear that elucidation of this mechanism will require additional eforts.

The year 1998 marks the hundredth anniversary of the discovery by Camillo Golgi of the "Apparato Reticolare Interno", now known as the Golgi apparatus. This issue could not miss the opportunity to discuss the current state of our understanding of the structural and functional organization of this organelle. The dilemma of whether transport through the Golgi complex occurs via vesicular transport or by cisternal maturation remains unsolved; however, new clues are expected from novel molecules and experimental approaches. Warren and Malhotra (pp 493–498) report on the mechanisms of Golgi disassembly and assembly in the context of the vesicle transport model. They describe tethering, an early step in vesicle docking, which precedes SNARE pairing and ensures efficiency of transport through the stacked cisternae by preventing Golgi vesicles from diffusing into the cytoplasm. This tethering system appears to be regulated by phosphorylation during mitosis when Golgi membranes fragment.

Besides the mitotic kinase $p34^{cdc2}$, the mitogen activated protein kinase pathway is involved, suggesting an interesting connection with the signal transduction pathway. Clues into the mechanism of cisternal stacking are expected from the characterization of a novel protein called GRASP65. Other molecular networks, however, may operate in cisternal stacking as well as the complex structural properties of the Golgi. De Matteis and Morrow (pp 542–549) review exciting work on Golgi spectrin and ankyrin which play a role in trafficking at precise stages of the secretory pathway. An interesting possibility is that these proteins may constitute a dynamic scaffold in the Golgi for tethering incoming vesicles and interacting with inter-cisternal matrix proteins. This hypothesis supports the maturation model as the spectrin and ankyrin scaffold would accompany cisternal maturation up to the *trans*-Golgi-network.

The clathrin coated vesicle pathway has provided a paradigm for the role of cargo molecules in driving the assembly of coat components on the membrane. Le Borgne and Hoflack (pp 499–503) review the function of the novel adaptor protein complex (AP)3. In yeast, AP3 is necessary for the delivery of alkaline phosphatase to the vacuole. Elegant work in mammalian cells has shown that while the mannose 6-phosphate receptors are sorted to endosomes via the AP1 complex, other proteins (e.g. LampI) require the AP3 complex. *Drosophila* and mouse mutants displaying pigment abnormalities exhibit deficiencies in AP3, suggesting that proteins involved in pigmentation are transported to melanosomes (functionally equivalent to lysosomes) via the AP3 route. It is intriguing that synaptic vesicles can also be produced from endosomes *in vitro* in a process that requires the AP3 coat, implicating AP3 in two apparently unrelated events. An important unanswered question is what determines the specificity of adaptor recruitment by apparently common sorting signals. A number of possibilities are envisaged including the interaction with locally produced phosphoinositides.

Dynamin is a GTPase which has been considered a hallmark for the clathrin-dependent pathway of endocytosis. Recent evidence reviewed by Schmid, McNiven and De Camilli (pp 504–512) suggests that dynamin also operates in budding of vesicles from the Golgi and caveolae from the plasma membrane. Recent data from the Schmid's laboratory provide interesting ideas for how dynamin self-assembly could coordinate the formation of helical collars around the neck of clathrin-coated pits and how GTP hydrolysis would lead to vesicles pinching off. The dynamin proline-rich domain binds various Src homology 3 domain (SH3)-containing molecules, such as amphiphysin which may link dynamin to the clathrin coat. Among other SH3-containing molecules are Grb2, PI3 kinase and Src. The functional relevance of this potential link with signal transduction molecules could be of extreme importance. The pleckstrin homology domain of dynamin binds phosphatidyl inositol-4,5-bisphosphate, again linking this class of lipids to the regulation of membrane transport.

Yeast has proven to be an excellent model system to uncover factors functioning in endocytosis and in transport to the vacuole. The article by Wendland, Emr and Riezman (pp 513–522) reviews recent progress in this area and traces a parallel between yeast and higher eukaryotes. Ubiquitination of proteins is an established signal for the proteasome-dependent degradation of cytosolic proteins and is now recognized also as a signal for endocytosis. Actin, actin binding proteins and the type I myosin Myo5 play an important role in endocytosis, perhaps contributing the necessary force to bend the membrane and cause the scission of endocytic vesicles. It will be interesting to clarify the potential link between dynamin and the actin cytoskeleton. The activity and the binding of these proteins depend on the levels of phosphatidylinositol-4,5-bisphosphate which can be modulated by phosphatases such as synaptojanin. Scott and Klionsky (pp 523–529) review non-conventional vacuolar targeting pathways of cytosolic components in yeast. Macroautophagy is a process induced by starvation and consists of the engulfment of cytoplasmic organelles and cytosol by autophagosomes which subsequently fuse with the vacuole delivering the autophagic body into the lumen where it is degraded. Interestingly, the vacuolar protease aminopeptidase I does not follow the conventional biosynthetic route to the vacuole but rather uses a mechanism which shares many of the molecular and morphological features of macroautophagy. Why different mechanisms for protein targeting to the vacuole have evolved is not clear at present and a molecular dissection is required to establish differences between the autophagic and protein import mechanisms.

Protein toxins have excelled as tools to uncover key regulatory elements of cellular structure and function. Montecucco (pp 530–536) reviews the intracellular route of certain toxins such as ricin and Shiga. Toxins can act by blocking exocytosis, for example tetanus neurotoxin and botulinum neurotoxin in the nerve terminals, or by stimulating exocytosis, for example spider or stone fish venoms. The *Helicobacter pylori* toxin recently came to the limelight as a novel potent membrane trafficking interfering agent. Toxigenic strains of *Helicobacter pylori* are implicated in the pathogenesis of gastroduodenal ulcers and are important targets in biomedical research. In sparsely cultured cells the cytotoxin VacA produced by *Helicobacter pylori* induces the formation of intracellular vacuoles due to a traffic jam in the late endocytic pathway. The molecular target of VacA is mysterious at present but will be an interesting tool for cell biologists and immunologists, since the toxin also interferes with the process of antigen presentation by MHC-II molecules.

Cell locomotion is generally considered to be mainly an actin-driven process. In an inspiring review Bretscher and Aguado-Velasco (pp 537–541) attract the reader's attention to the contribution of the endocytic and recycling membrane traffic to cell motility. The authors consider a number of studies in different experimental systems, including Physarum plasmodia, where the advancing edge of the cell is supplied with a flow of membrane vesicles, providing material for a new protrusion. The actin cytoskeleton is required to support the targeted deposition of new membranes, but, as the authors point out, major differences between cell types exist. Small GTPases could locally organize the cytoskeleton and polarize the trafficking route. Future studies should provide the molecular tools to examine the extent by which the membrane traffic apparatus contributes to cell motility. Yet, this is another area where membrane-cytoskeleton interactions deserve greater emphasis.