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Membranes and organelles

Editorial overview

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Current Opinion in Cell Biology 2003, 15:369–371

This review comes from a themed issue on
Membranes and organelles
Edited by Alice Dautry-Varsat and Alberto Luini

0955-0674/\$ – see front matter
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DOI 10.1016/S0955-0674(03)00079-6

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The field of intracellular membranes, like the rest of cell biology, is moving ahead in rapidly changing directions. No longer are the discovery of new molecular components and the elucidation of their structures (albeit still very important) the central engines of growth. It is, instead, the increasing emergence of the complexity, diversity and multiplicity of both the molecular machines and of the transport pathways themselves that has become the main agent of conceptual transformation. Closely related to this issue is the increasing weight in the field of the ways and mechanisms by which membrane traffic integrates with other cellular functions, such as signalling, apoptosis, mitosis, differentiation and development. And an interesting side effect of these changes has been to stimulate investigators to look critically at established principles, and to accept the possibility that some of them might lose their pristine simplicity and clarity.

A concept that is losing clarity is that of transport vesicles. These small and round membranes, 60–100 nm in diameter and covered with proteins coats such as clathrin or COP, have long been held to be the main or sole traffic intermediates. There is now evidence that the bulk of secretory traffic is carried by large (i.e. up to several microns) pleiomorphic tubular-saccular membrane carriers. Thus, the specific role of coated vesicles should be re-examined. Alternatively, we could consider the large pleiomorphic carriers as being vesicles, as they are often still termed, only with a different shape. Or should we instead call them tubulo-vesicular or tubular carriers? We propose that the latter term would be more useful because it reflect not only the structure of these intermediates but also the fact that they are most likely different objects, generated by different processes from those previously described for true vesicles. Possibly relevant to this issue, Farsad and De Camilli analyse a variety of mechanisms that can deform membranes in addition to the 'traditional' vesicular coats. These include the mechanical force exerted by cytoskeleton-based motors, the *trans*-bilayer transfer and the metabolism of specific lipids, and the effects of a growing group of proteins, including amphiphysin and endophilin, which can partially penetrate the lipid bilayer and, in doing so, generate tubules from flatter surfaces.

Conversely, an increasing number of lipid species is found to modulate the function of legions of proteins with key roles in traffic, cytoskeletal dynamics and signalling. This is a long-standing theme in membrane biology, but it is now becoming ever more pervasive in most exocytic and endocytic traffic segments. In endocytosis, as reviewed by Gruenberg, lipids not only contribute to the intrinsic properties of membranes; they participate in regulating membrane dynamics. Some lipids segregate in specialised membrane

microdomains that can be transient or more stable. Thus, different lipid territories are present in the various organelles. Many cytosolic proteins can interact with membranes by interacting with the lipids and proteins they contain, often through multiple interactions. Phosphoinositides appear as key player in the endocytic pathway, and the functions of proteins that interact with them are beginning to be unravelled. Bankaitis and Morris survey known and novel aspects of the roles of a variety of lipids and lipid metabolic enzymes in controlling the exit of carriers from the ER and from the Golgi complex. Given the large number of known lipid species not yet implicated in regulating cellular functions, the breadth and complexity of this area is likely to expand greatly in the near future.

Another case in which progress lies not in a single striking molecular finding, but in the gradual unravelling of the complexity of the overall picture, is provided by two families of Golgi proteins, the ARFs and the Golgins. Randazzo and colleagues review the ARF GTPase protein regulators of membrane traffic. The six ARF isoforms interact with 14 exchange factors and 16 GTPase-activating proteins, as well as with three functionally diverse classes of effectors. However, despite this rich molecular knowledge, the logic used by the ARFs to make choices within this maze of potential interactions is just beginning to be understood, although only in a few cases.

The Golgins, a numerous and diverse family of coiled-coil Golgi proteins (reviewed by Barr and Short), are undergoing a similar process of proliferation, and of acquisition of novel functions. Golgins were originally proposed to play a role in Golgi stacking, and as tethers between cisternae and vesicles. The most exciting new aspect here is the discovery of interactions between Golgins and Rab GTPase family members, hinting at the possible participation of the Golgins in Golgi dynamics and traffic, rather than simply in maintaining the structure of this organelle, as had been originally proposed.

Not only the molecular machines but also the transport pathways themselves are beginning to show their complexity and multiplicity. It turns out that there exist several, possibly five, endocytic pathways, as discussed by Pelkmans and Helenius. Viruses are important tools to study membrane trafficking and their study has recently uncovered novel organelles or previously uncharacterised intracellular trafficking. They make use of the existing endocytic pathways to enter host cells — clathrin-dependent, caveolae-mediated, lipid-rafts-mediated and macropinocytosis — and some of these are subject to regulation. Things are even more complicated in polarised cells, where basolateral endocytosis resembles the constitutive route in other cells, whereas the apical pathway is remarkably specialised in terms of both organelle organisation (e.g. the SAC compartment), and molecular machineries

involved in sorting and translocation (e.g. myosins V and VI), as discussed by Altschuler *et al.*

The establishment of apical–basal polarity is a fascinating issue in itself, which can serve as a model for the other important biological processes (e.g. the formation of neural and immunological synapses) where spatial control of transport plays a role. This is the area of traffic where the spatio-temporal coordination between signalling, cytoskeletal and secretory functions is more apparent, and where it has been more extensively studied and understood, as discussed by Spiliotis and Nelson.

In all cells, one of the functions of endocytosis is to support retrograde membrane transport by which cells counterbalance the flux of secretory traffic to the plasma membrane. Our knowledge of the retrograde routes is still limited, but it appears that these are multiple at each traffic segment. For instance, there seem to be at least four pathways from the endosomes to the *trans*-Golgi network, and at least two from the Golgi to the ER. Goud and colleagues review the organisational and molecular specificities of this transport. A related, rapidly developing issue is the biogenesis of multivesicular endosomes, discussed by Stenmark and co-workers. Here, a novel function has been found for ubiquitin: monoubiquitination serves as a signal to sort transmembrane proteins into intraluminal vesicles of multivesicular endosomes and their subsequent delivery to lysosomes. Also, the machineries involved in the formation of multivesicular endosomes have been discovered. These involve three multisubunit complexes, the so-called ESCRT-I to -III, which are also essential for sorting and are also used by some enveloped viruses such as HIV to bud.

Intracellular traffic has long been studied as a separate functional module, but in living cells it is closely coordinated with other cellular functions. The mechanism by which this happens is an area of great and growing interest. For instance, secretory organelles play a role in apoptosis and host many members of the death machinery, including caspases 2 and 12. These caspases can be activated by secretory stresses, such as an excessive accumulation of misfolded proteins or calcium depletion in the ER; moreover, the caspase-induced cleavage of Golgin-160 can serve as a further apoptotic signal. How secretory organelles might initiate apoptosis is discussed by Machamer and colleagues. Another example of integration is the role of the Golgi in mitosis. During mitosis, the Golgi becomes dispersed through a complex series of steps, to provide the daughter cells with equal complements of Golgi components. Conversely, Golgi dispersal controls the entry of the cell into the mitotic cycle. If the Golgi is not dispersed, the cells are arrested in the G2 phase. The mechanism and the significance of these fascinating observations are discussed by Malhotra and colleagues.

Integration is emerging also at a higher hierarchical level, between intracellular traffic and multicellular organisms functions. Gatti and Pierre review the role of endocytosis in the immune response, specifically in antigen presentation by dendritic cells. As dendritic cells mature, their proteolytic properties, which are critical for antigen presentation, are modified, and a general activation of endosomal proteolysis takes place. One of the remarkable features of dendritic cells is that, as they mature, MHC class II molecules are redistributed from lysosomes to the surface. This is a previously unknown pathway by which selected molecules can be rescued from degradation in lysosomes. Also development and morphogenesis are revealing crucial links with intracellular traffic. Piddini and Vincent discuss the different ways by which developmental signals are modulated by endocytosis. The classical negative role of endocytosis leading to degradation in lysosomes is important to downregulate some signals. However, recent data show that endocytosis can also have a positive role in signalling, either by activating a signal (in the Delta/Notch pathway) or by segregating a negative regulator. Also, some signals can be activated along the endocytic route, in endosomes. Finally, transcytosis might control the transport of morphogenic signals in epithelia.

Fascinating similarities are being unravelled between the organisation and biogenesis of trafficking membranes and that of other organelles, at both the molecular and supramolecular levels. The maintenance of mitochondrial structure requires both fusion and fission, two events that are fundamental also in traffic. Nunnari and co-workers review the recently characterised molecular components that direct these processes. They also discuss mitochondrial fission taking place during apoptosis and the specific proteins involved. The biogenesis of peroxisome and its evolutionary conservation is reviewed by Lazarow. Peroxisome division involves dynamin and regulated actin-dependent segregation. Protein translocation involves membrane peroxins that interact with

soluble receptors. A receptor enters the peroxisome carrying folded cargo and recycles back to the cytosol. Ubiquitination has been observed, suggesting again potential new roles for this reaction.

Phagosomes (reviewed by Desjardins and Griffiths) have long been known to exchange with the endocytic pathway. A recent surprise was that, at the initial stages of their formation, their membrane derives from direct fusion between the ER and the plasmalemma. Proteomics and mass spectrometry have shown that phagosomal membrane contains at least 600 identified proteins, as well as several dozen different lipids, a measure of the level of complexity of this relatively 'simple' organelle. Lipid signalling controls actin polymerisation and bacterial killing in phagosomes, and it has been proposed that the modulation of phagosomal lipids could be used for therapeutic purposes.

Thus, the multiple complexities of intracellular traffic and its links with other cellular functions have begun to be addressed, and glimpses of understanding have appeared here and there. Similar developments are occurring in almost all areas of cell biology, and it is clear that a profound cultural shift is taking place. Much less clear is how the new questions will be experimentally addressed. In the traffic field, it is likely that both approaches to monitor the physiology of processes *in vivo* and more sophisticated and faithful *in vitro* reconstitution assays will prove crucial. Combining these with the developing conceptual, computational and physical tools of systems biology, the community will hopefully continue to address the questions of membrane and organelle organisation in manners ever more interesting and productive.

Acknowledgements

A Luini is supported by the Italian Association for Cancer Research and Telethon Italia. A Dautry-Varsat is supported by Institut Pasteur, Centre National de la Recherche Scientifique and the Association pour la Recherche sur le Cancer.