Past times

Traffic in the rear-view mirror

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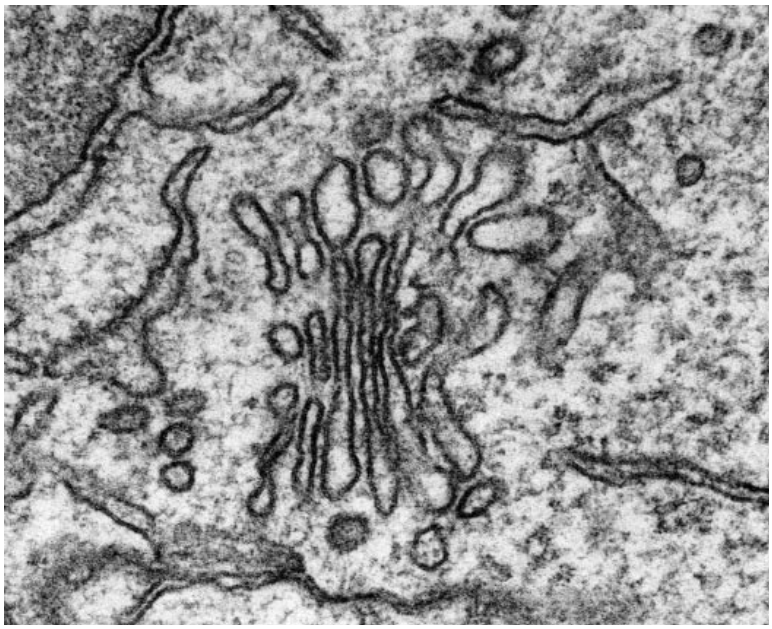
When I was a lad, the adage that "cells are not simply bags full of enzymes" was already popular in biology, and how true it turned out to be. We now know that eukaryotes comprise cellular compartments whose integrity and composition is maintained by specific mechanisms, including the membrane traffic between membrane-bound organelles. So what attracts cell biologists to the challenge of membrane traffic? One reason may be the complexity in composition and spatio-temporal dynamics — a complexity that manifests itself in the sheer beauty of the physical forms of the trafficking organelles. Another motivation may be the simple question of how complex mixtures of substances can be moved around selectively in membrane-bound vesicles while maintaining the compositional integrity of organelles. Whatever the attraction, it is clear that the full molecular inventory of traffic machinery will be known soon, and we stand now on the threshold of a deeper understanding. It is therefore a good time to look at what has been achieved so far. Interestingly, the focus of membrane trafficking research has come full circle. Initially, discrete organelles with specialized functions were described and then came a mass of molecular information. Now, we are back to the organelles, trying to work out how they are built and how they function in a dynamic way. As in any story of science, the road to discovery has been crucially dependent on clever insights, married with technical developments at both molecular and atomic resolution.

Early years: discovering organelles, function and pathways

A century ago, cytologists, such as Camillo Golgi, revealed, using light microscopy, intracellular structures that became a focus for much argument and speculation. Although the function of many structures remained uncertain for decades, the seminal works of Metchnikoff and others revealed that cells manufacture and store secretory substances and sample their environment by the uptake of extracellular particles. Such observations prepared the ground for the concept of vesicular transport between organelles. Without doubt, the single most significant technical advance in the early days of 'trafficking' biology was the development of the electron microscope. In 1933, Ernest Ruska built the first instrument, which achieved a modest, but at that time astounding, magnification of 12 000×. In the decades that followed, the cell-biology journals were replete with exciting and insightful ultrastructural descriptions of 'new' membrane-bound organelles and structures. Using this tool, Dalton and Felix[1] revealed the Golgi cisternal stack and associated masses of vesicles, which suggested the possibility for vesicular traffic between organelles. Later, Roth and Porter[2] identified clathrin-coated structures at the cell surface, which was a milestone in the characterization of the endocytic pathway.

During the 1970s, these discoveries fuelled huge interest in organelle function, which culminated in ground-breaking work using fractionation of cell components combined with electron microscopy. These studies revealed the integrity and function of membrane-bound lysosomes and the endoplasmic reticulum (ER)–Golgi–secretion-granule leitmotif, discoveries that resulted in Christian de Duve[3] and George Palade[4] being awarded the Nobel Prize for Medicine or Physiology in 1974. The work of Palade and Jamieson was all the more remarkable because it was done without the technical developments that were provided later by immuno-electron microscopy.

Immunoelectron microscopy was subsequently used to demonstrate distinct Golgi sub-compart-



ments, to show cargo entry into one side of the Golgi stack and to reveal the *trans*-Golgi network as an important sorting station for exit from the Golgi. Other studies around this time by Steinman, Brodie and Cohn[5] also provided insights into the membrane dynamics during endocytosis by showing that the internalized plasma membrane must be re-used and therefore recycled.

Modern era: molecular sorting, vesicle biogenesis, targeting and fusion

The next phase was marked by the comprehensive characterization of organelle composition, such as clathrin coats[6], and a breakthrough in the understanding of protein targeting to the ER by Blobel and Dobberstein[7]. This work suggested that signals might also sort cargo into trafficking vesicles. Indeed, Goldstein, Anderson and Brown[8] subsequently found that ligands bind to re-usable receptors that interact with cytoplasmic adaptors that are bound to the clathrin coat. The crucial signals were found to comprise tyrosine-containing motifs in the receptor tails, and in the years that followed, an extensive array of sorting signals were identified. These ranged from appropriate folding of proteins prior to export from the ER, to mannose-6 phosphate, which is required for the delivery of lysosomal enzymes to lysosomes. Interestingly, progress in characterizing sorting mechanisms into coats of the early secretory pathway has been relatively slow. For COPI-coated vesicles that function in retrograde (or maybe forward) transport to the ER, KKXX and RRXX signals are needed to recycle some escaped membrane proteins to the ER (soluble ER proteins contain the KDEL motif, which binds to a recycling receptor). For COPII-coated vesicles emerging from the ER, only limited sets of proteins have been found to contain signals.

In vitro assays and yeast

The work on clathrin-coated vesicles reinforced a second great theme of trafficking biology, namely that vesicles (mostly surrounded by a cytoplasmic coat) select their cargo, bud off from a donor compartment, uncoat and are transported to a target organelle where they become tethered, dock, fuse and deliver their cargo. In the late 1970s and early 1980s, Jim Rothman realized that the molecular analysis of vesicular transport could be tackled using *in vitro* assays that recapitulate *in vivo* function. From the knowledge of Golgi-stack compartments obtained by immunoelectron microscopy and cellular fractionation, his laboratory constructed an assay that measured the bringing together of cargo and cargo-modifying enzymes in a transport reaction that reflected membrane fusion within the Golgi stack. The assay was used to identify a number of important proteins involved in vesicle recognition and fusion including *N*-ethylmaleimide-sensitive fusion protein (NSF), and attachment proteins for NSF that are termed SNAPs (soluble NSF attachment proteins). The work highlighted roles of COPI coats in traffic and the importance of small GTPases in vesicle biogenesis. Small GTPases such as ARF-1 (ADP-ribosylation factor), in the Golgi, and Sar1, at the ER, are now known to be important switches that initiate coat assembly. Subsequent work in neurons identified receptors for NSF and SNAPs known as SNAREs (SNAP receptors) that can form complementary pairs on vesicles and target organelles, bringing their membranes close together for fusion. These proteins are now a major focus of interest in trafficking research.

Clearly, the *in vitro* approach identified important proteins, but a test of function *in vivo* was provided elegantly by work in yeast. Sheckman and colleagues[9] gener-

ated a large 'library' of mutants that were defective in secretion and known appropriately as 'sec' mutants. These assigned proteins to functions at specific stages of the secretory pathway and, by homology, identified important trafficking proteins in mammals. For example, this work identified COPII coat proteins and the first of the small Rab family GTPases, sec4 (Rabs contribute to vesicle targeting by forming large complexes with SNARE proteins to ensure specificity during vesicle tethering and fusion).

Future perspectives

With all the molecular details available now, one might get the impression that the pathways and compartments are now well worked out. Not at all: there are still important questions to be answered. For example, despite over 50 years of study, we are still not certain of the mode of transport through the Golgi and it is unclear whether proteins traverse the stack in small transport vesicles or remain in the cisternae (maturation/progression). Part of the problem is the complexity of these membranes and the final answer will depend on methods that can reliably trace the provenance of cargo molecules throughout the system.

Genome sequencing will be of significant help in future research on membrane trafficking, but only if it is combined with knowledge of the functional components that are present within trafficking structures (their proteomes). Hopefully, this information will enable us to make a holistic synthesis that will culminate in understanding the structure and function of trafficking organelles from cellular to atomic levels. To achieve this goal, the major advances are likely to capitalize on methods that afford higher resolution in both time and space, and provide a shift to understanding *in vivo*. Higher resolutions will increase the numbers of identifiable functional states/intermediates in the processes of vesicle budding, targeting and fusion, and will improve the ability to follow the dynamics of trafficking-related processes, either by *in vitro* assays or by microscopy.

In microscopy, the new areas are likely to be sub-wavelength light microscopy, atomic force microscopy (a technique with as yet unexplored potential) and high-resolution tomographic cryo-electron microscopy, which provides an opportunity to view the near-native state of whole-cell assemblies. There will also be the challenge to develop *in vitro* assays that can follow small, but significant, changes in processes tracking the conformation of proteins within molecular machines in the microsecond range. Hopefully, all of these advances will then help to answer the 'second order' problems such as, how organelles are made and maintained, why organelles are positioned in the way they are and what the regulatory networks that govern the relationship between the amounts and functions of different organelles under different environmental and growth conditions are. Encouragingly, bioinformatics is already providing, not only important tools for integrating the data obtained, but also quantitative methods for measuring outcomes. Another area that is ripe for development is the role of lipid organization: how these molecules co-operate in concentrating, sorting and recruiting proteins and how proteins mould them during vesicle budding and fusion are major challenges. There is also a burgeoning interest in the pathobiology of membrane traffic (not least in response to the need for translational research), with a focus on how gene mutations affect trafficking organelle proteins and cell function in human disease. Finally, the new challenge of comparative genomics in membrane traffic may help us to unravel how and why membrane traffic evolved, allowing humans to occupy its arguably ascendant position in the eukaryotic pecking order. Whatever the future holds, the work of pioneers in this field has already ensured that, at 50-plus years, the 'budding' discipline of membrane traffic has finally come of age.

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