Cellular Transport

DOI: 10.1002/anie.201308937

Intracellular Transport Mechanisms: Nobel Prize for Medicine 2013

*Kirsten Bacia**

membrane proteins · membranes · reconstitution · vesicles · yeast genetics

How are biomolecules transported between cellular compartments? For their achievements in deciphering the molecular mechanism of intracellular vesicle transport, James E. Rothman, Randy W. Schekman, and Thomas C. Südhof were awarded the Nobel Prize for Physiology or Medicine on October 7, 2013.

Apart from the plasma membrane, which forms an outer boundary, eukaryotic cells also contain inner membranes, which enclose compartments that are specialized in different metabolic processes. As early as 1897, Camillo Golgi (Nobel Prize 1906) discovered these inner membranes using light microscopy and a staining method that he had developed. These structures were subsequently described in more detail by George Palade, who won the Nobel Prize in 1974. Thanks to the increased spatial resolution that became attainable with the advent of electron microscopy, Palade and his colleagues were able to uncover the route taken by newly synthesized proteins in the so-called secretory pathway, which initiates at the endoplasmic reticulum (ER) and progresses through the cisternae of the Golgi apparatus to the plasma membrane. It became clear that the transfer of cargo between compartments is carried out by small membrane-enclosed containers called vesicles.

Building on this morphological observation, Randy Schekman, James Rothman, and Thomas Südhof in conjunction with other researchers were able to unravel the principles of this important transport mechanism on a molecular level. Randy Schekman used genetics to approach the problem. Building on the work of Leland H. Hartwell (Nobel Prize 2001), who in a similar way had identified proteins that drive cell division in the baker's yeast (*Saccharomyces cerevisiae*), Schekman chose the same model organism for his experiments on intracellular transport.

Even though he did not have experience with yeast genetics, he and his first PhD student, Peter Novick, started to look for mutations in genes that cause defects in protein secretion. Owing to the fact that mutants with a loss-of-function of an essential transport gene are non-viable, the two researchers looked for mutants with a conditional temperature-sensitive phenotype. Thus mutations that impart a moderate decrease in thermostability to an essential protein are viable at the permissive temperature of 25 °C while increasing the temperature to the restrictive temperature results in cell death.

Novick and Schekman described the phenotype of the first temperature-sensitive secretion mutant that they had discovered. At the restrictive temperature of 37 °C, the yeast strain *sec1* accumulates vesicles, which are not able to fuse with the plasma membrane (Figure 1). These accumulated vesicles contain enzyme molecules that would otherwise be secreted to the exterior.[1]

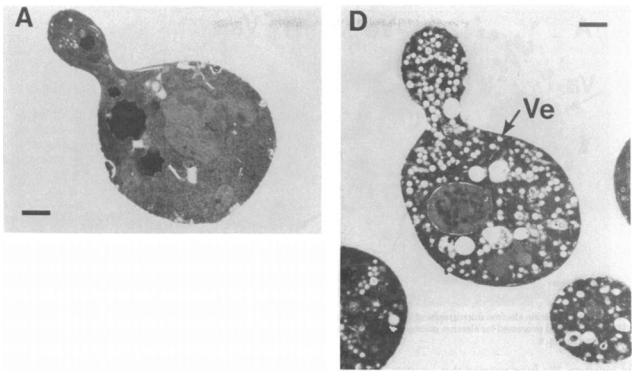

Figure 1. Electron micrographs of *S. cerevisiae* wild type (left) and *sec1-1* mutant (right). An accumulation of secretory vesicles is seen in the mutant. Scale bars = 500 nm. (Figure reprinted from Ref. [1] with kind permission of the authors.)

The development of a new centrifugation method, which allows enrichment of yeast cells that have accumulated ER-, Golgi-, or transport vesicle membranes owing to a block in secretion, led to the discovery of several other genes required on the secretory pathway.[2] Furthermore, the researchers identified gene products that are required for the insertion of transmembrane proteins into the ER membrane. These findings are relevant not only to yeast cells. As the mechanism of vesicular transport is remarkably conserved amongst eukaryotic species, homologues of the *SEC*-genes are found in plants, animals, and man.

James Rothman used a different approach to investigate the secretory pathway. Whereas Randy Schekman started out by studying intact, live yeast cells, Rothman and co-workers

[*] Jun.-Prof. Dr. K. Bacia
HALOmem, University of Halle
Kurt-Mothes-Strasse 3, 06120 Halle/Saale (Germany)
E-mail: kirsten.bacia@chemie.uni-halle.de
Homepage: http://www.halomem.de

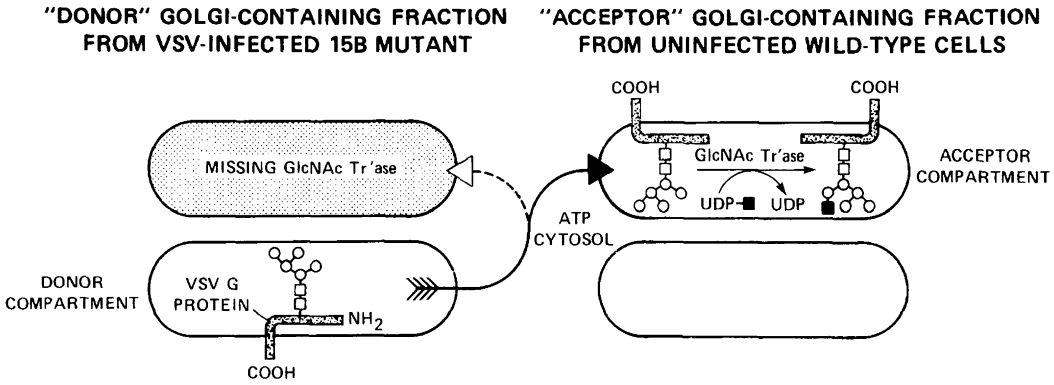

Figure 2. Representation of the cell-free transport assay using a mixture of purified Golgi compartments isolated from "donor" and "acceptor" cells. Donor cells lack the enzyme *N*-acetylglucosamine transferase ("GlcNAcTr'ase"). They have been infected with VSV (vesicular stomatitis virus) and therefore produce the VSV G protein. Modification of VSV G with *N*-acetylglucosamine (GlcNAc) occurs in the Golgi compartments isolated from acceptor cells that are not infected with VSV but contain the enzyme *N*-acetylglucosamine transferase. (Figure reprinted from Ref. [3] with permission from Elsevier.)

isolated Golgi compartments from mammalian cells and reconstituted transport between different types of compartments in a cell-free assay in the test tube. Utilizing the viral glycoprotein VSV G and its enzymatic modification with the sugar *N*-acetylglucosamine by the host cell, they were able to trace the transport of the viral protein between the Golgi compartments (Figure 2). Golgi membranes from virus-infected cells, which produce the viral protein but, owing to an enzyme defect, do not perform this protein modification step, were combined with Golgi membranes from uninfected cells, which do not produce the viral protein, but are able to perform the enzymatic modification step.[3] The *N*-acetylglucosamine modification of the viral protein was indeed detected in this reaction mixture, showing that vesicular transport between compartments takes place without the need for a particular spatial relationship between donor and acceptor compartments.

The cell-free system used by Rothman proved useful for some key biochemical experiments because of its high accessibility, and this led to the identification of two cytosolic proteins required in membrane fusion: namely NSF (*N*-ethylmaleimide sensitive factor) and SNAP (soluble NSF attachment protein). As it turned out, the NSF and SNAP genes were homologous to the yeast genes *SEC17* and *SEC18* identified by Schekman and Novick. The convergence of Schekman's and Rothman's initially independent lines of investigation underlines the relevance of Schekman's yeast system for mammalian cells. Moreover, it showed that Rothman's cell-free experiments are of significance for the natural process.

Following the discovery of NSF and SNAP, membrane-anchored SNAP-binding proteins were discovered and hence termed SNAREs (SNAP receptor proteins). These proteins were already known from other studies, including those of Richard H. Scheller and Thomas Südhof. The new pieces of information led to Rothman's pioneering 'SNARE hypothesis', which stated that SNAREs constitute an evolutionarily conserved fusion machinery for membranes, with matching sets of v-SNARE (vesicle-SNARE) and t-SNARE (target membrane-SNARE) proteins enabling the fusion of designated membranes. (The distinction of v-SNAREs and t-SNAREs was later replaced by a structure-based classification, which is also applicable to the fusion of vesicles of the same type.[4])

Initially, various notions existed about the exact function of NSF and SNAP during membrane fusion. Bill Wickner and colleagues later showed that these proteins are needed for the ATP-dependent separation and thus the recycling of SNARE proteins after membrane fusion.

Proteins of the SNARE family act at many different vesicular transport steps in the cell, for instance, in the ER-Golgi transport studied by Randy Schekman, the intra-Golgi transport studied by James Rothman, in the fusion of yeast vacuoles studied by Bill Wickner, and in the calcium-dependent process of synaptic vesicle fusion with the plasma membrane, during which neurotransmitters are released into the synaptic cleft, a process studied by Thomas Südhof, Reinhard Jahn, and others. The results of structural investigations of the SNARE-complex by means of electron microscopy and X-ray crystallography supported the concept that SNAREs, which are anchored to their respective vesicle and target membranes, promote fusion by zippering up into helix bundles, thereby bringing the membranes into close proximity for fusion.

To test the SNARE hypothesis, Rothman and colleagues reconstituted SNARE proteins into artificial liposomes. In this way, fusion could be studied under isolated conditions, that is, barring the influence of additional cellular factors. Indeed they observed that, even though the reaction rates in the artificial system were exceedingly slow, liposome fusion is mediated by matching sets of cellular SNAREs.[5] Nevertheless, the question remained as to how fusion is triggered within the cell and how neuronal cells achieve the rapid fusion of synaptic vesicles with the plasma membrane. Although it was known that the process is induced by calcium ions and takes less than a millisecond, it could not be explained on the molecular level.

Thomas Südhof made key contributions to the resolution of this problem.[6] Südhof had systematically extracted and characterized proteins from synaptic vesicles and their target membrane, among them the v-SNARE synaptobrevin, the calcium-binding protein synaptotagmin, complexin (which

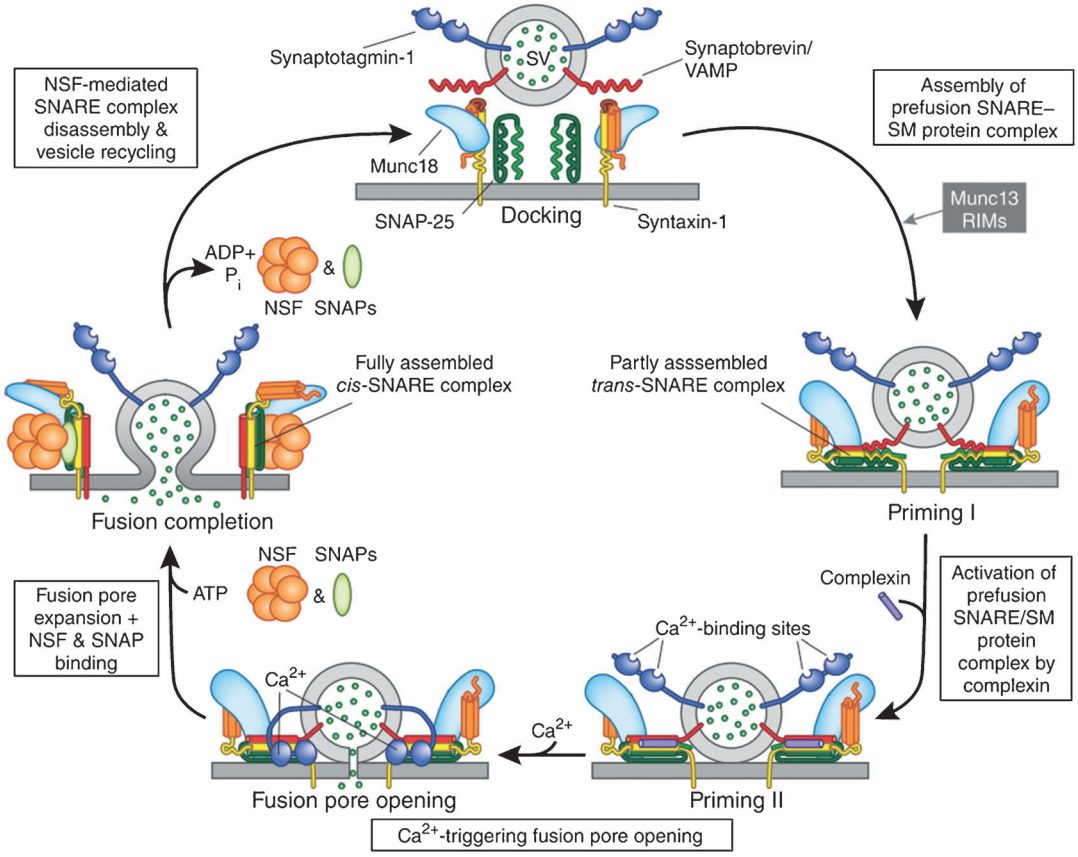

Figure 3. Current model of vesicle fusion. (Figure reprinted from Ref. [6] by permission from Macmillan Publishers Ltd.)

binds to the SNARE-complex), and Munc18-1, a homologue of the Sec1 protein identified by Schekman in yeast (proteins of the Sec1/Munc18-like family are now called SM proteins). Südhof showed that Munc18-1 is a vital constituent of the SNARE fusion machinery. Synaptotagmin, which is anchored to the vesicle by a transmembrane domain, binds to membrane lipids in a calcium-dependent manner and also to the SNARE complex. Evidently, synaptotagmin in conjunction with complexin plays an important regulatory role in the rapid, calcium-triggered synaptic vesicle fusion process (Figure 3).[7]

The investigations of intracellular transport that were highlighted by the Nobel Prize have not only improved our understanding of the fusion of transport vesicles with acceptor membranes, but have also provided insight into the formation of transport vesicles at donor compartments by the coat protein complexes coatomer (COPI), COPII, and clathrin.

The processes studied by Rothman, Schekman, and Südhof are of fundamental importance for cell biology in general, but also for medical applications and drug development. In some cases, defects in the intracellular transport system are responsible for metabolic diseases. In the pharmaceutical industry, a significant percentage of recombinant insulin used in the treatment of diabetes mellitus is produced by yeast cells, which synthesize and release the drug via the secretory pathway.

The fact that neuronal SNAREs are targets of clostridial toxins (botulinum toxins and tetanus toxin) is important both in fundamental research and in medicine. By cleaving SNARE proteins, botulinum toxins inhibit synaptic vesicle fusion and the release of neurotransmitter into the synaptic cleft. Muscular paralysis resulting from foodborne poisoning with botulinum toxins can be lethal. On the other hand, clostridial toxins have proven extremely useful as research methods in the exploration of the cellular fusion machinery. Moreover, botulinum toxin is used in the treatment of involuntary muscle contractions (dystonia) and cosmetically against facial wrinkles caused by the mimetic muscles.

Received: October 14, 2013
Published online: November 8, 2013

[1] P. Novick, R. Schekman, *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 1858 – 1862.
[2] P. Novick, C. Field, R. Schekman, *Cell* **1980**, *21*, 205 – 215.
[3] W. E. Balch, W. G. Dunphy, W. A. Braell, J. E. Rothman, *Cell* **1984**, *39*, 405 – 416.
[4] R. Jahn, R. H. Scheller, *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 631 – 643.
[5] T. Weber, B. V. Zemelman, J. A. McNew, B. Westermann, M. Gmachl, F. Parlati, T. H. Söllner, J. E. Rothman, *Cell* **1998**, *92*, 759 – 772.
[6] T. C. Südhof, *Nat. Med.* **2013**, *19*, 1227 – 1231.
[7] J. Tang, A. Maximov, O. H. Shin, H. Dai, J. Rizo, T. C. Südhof, *Cell* **2006**, *126*, 1175 – 1187.

