Cellular postal services through vesicles – control system for the transport and delivery of cellular cargo: the 2013 Nobel Prize for Physiology or Medicine

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Living organisms are prokaryotic or eukaryotic. Cells are the building blocks of the organisms. Unlike prokaryotic cells, eukaryotic cells have a well-defined outer membrane called the cell wall and a variety of organelles. Till date, many findings have been reported on organelles such as the nucleus, mitochondria, ribosomes, endoplasmic reticulum (ER), etc. This biological organization is responsible for all biochemical processes in a eukaryotic cell. One such organelle, often overlooked, stole the limelight on 7 October 2013, when three scientists were honoured with the highest recognition in the scientific community – the Nobel Prize for Physiology or Medicine; they were acknowledged for their work in the field of vesicle trafficking.

We often compare a cell to a city, where the cell membrane signifies the city wall, mitochondria is the power house, nucleus is the town hall, lysosomes the scrap yard, vacuoles represent the water tank, etc. If the cell is a city, then evidently there must be some postal services to communicate between the cities. While Golgi apparatus plays the role of a post office, vesicles act as the envelope that carries information like insulin, neurotransmitters, cytokines and enzymes. This envelope should be transported and delivered to its destination postal address at the exact time and location. Formed by pinching of cell membrane, these vesicles are simple compartments that carry cellular cargo from one place to other, inside and outside the cell. Travelling across the cytoplasm, they release their cargo by the mechanism of membrane fusion (Figure 1). The precise procedure of budding and fusion of vesicles and the machinery required for operation and organization within the cell were, however, ambiguous.

Mystery of cell swipes Nobel again

Several scientists have been awarded the Nobel Prize for their seminal work in unravelling the structure and functions of cells. In 2009, the Nobel Prize for Physiology or Medicine was awarded for the discoveries in chromosome defence provided by the telomere and how through the enzyme telomerase, chromosomes are protected against degradation[1]. In the same year, the Nobel Prize in Chemistry was awarded for the study of structure and functions of the ribosome[2]. Other discoveries that assisted us in knowing a cell better were RNA interference – gene silencing by double-stranded RNA; key regulators of the cell cycle; split genes; protein phosphorylation: a biological regulatory mechanism; intrinsic signals in proteins that govern their transport and localization in the cell; function of single ion channels in cells; structural and functional organization of the cell; molecular structure of nucleic acids and its significance for information transfer in living materials and so on.

This year the Nobel Prize for Physiology or Medicine is shared by three scientists for unscrambling the vesicle puzzle. Different approaches to study vesicle trafficking have successfully revealed the machinery that organizes the transport system in the process of vesicle budding, how these packages know where to go, and how they identify, deliver and fuse with the target membrane[3]. Their discoveries added a new dimension to the understandings of the cell. Today we know about the cell, nucleus, its organization, the processes carried out in the cell which help us breathe, feel, think, inherit, grow, etc. though many secrets are yet to be revealed.

Scientific journey of these Nobel laureates

Randy Wayne Schekman (65 years) was born in St Paul, Minnesota, USA. He graduated from the University of California in Los Angeles and obtained his Ph D in 1974 from Stanford University. In 1976, he joined the faculty of the University of California at Berkeley, where he is currently a Professor in the Department of Molecular and Cell Biology. Also, he is an investigator and Editor-in-Chief of the journal *elife* at Howard Hughes Medical Institute (www.elifesciences.org). Schekman received numerous honours, including the Albert Lasker Award for Basic Medical Research, the Louisa Gross Horwitz Prize of Columbia University and Massry Prize from the Keck School of Medicine, University of Southern California.

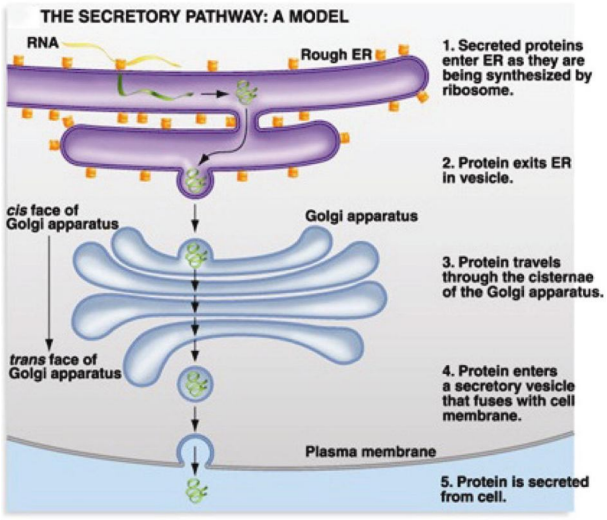

Figure 1. Secretory pathway in a eukaryotic cell (source: www.studyblue.com).

Table 1. Summary of important breakthroughs in vesicular trafficking in chronological order

Year	Breakthrough
1963	Bernard Katz's seminal work revealed that calcium triggers neurotransmitter release by stimulating ultrafast synaptic vesicle fusion.
1970	George Emil Palade proved that secreted proteins are carried from the endoplasmic reticulum (ER) to the cell surface in vesicles, that bud from one membrane and fuse with the next, transiting the Golgi stack en route.
1980	Schekman identified 23 complementation groups required for post-translational events in the yeast secretory pathway.
1980–1990	Rothman studied vesicle transport in mammalian cells
1986	Rothman discovered coat protein complex (COP) I, and its assembly protomer (coatomer), responsible for vesicle traffic within the Golgi complex that appears not to contain clathrin.
1987	Rothman and colleagues discovered NSF at Stanford University.
1987–1994	Principle of vesicle budding by Schekman and Rothman.
1989	Schekman and Rothman showed that mammalian NSF is encoded by *sec18*.
1992	α-SNAP is encoded by *sec17* as proved by Rothman and Schekman.
1993	Identification of the membrane receptors for SNAP called SNARES.
1993	Südhof showed synaptic vesicle fusion complex contains Munc-18 homologue bound to syntaxin.
2001	Schekmen showed COP II vesicles assemble at specialized regions of the ER that are dedicated to sorting proteins for export to the Golgi apparatus.
2002	Major puzzles of secretory pathway cracked.
2009	The SNARE hypothesis and the principle of membrane fusion.

James Edward Rothman (63 years) was born in Haverhill, Massachusetts, USA. He graduated from Yale College in 1971 with a degree in physics. He received his Ph D degree in biological chemistry from Harvard Medical School in 1976. He spent two years as a postdoctoral associate in the laboratory of Harvey F. Lodish. In 1978, he moved to the Department of Biochemistry at Stanford School of Medicine as an Assistant Professor, where he started research on vesicles. Rothman is currently serving as the Fergus F. Wallace Professor of Biomedical Sciences at Yale University, the Chairman of the Department of Cell Biology at Yale School of Medicine, and the Director of the Nanobiology Institute at the Yale West Campus. He has received the Louisa Gross Horwitz Prize, the Lasker Award for Basic Medical Research, the King Faisal International Prize and the Kavli Prize in Neuroscience.

Thomas Christian Südhof was born in 1955 in Göttingen, Germany. He studied at the Georg-August-Universität in Göttingen, where he received an MD in 1982 and a doctorate in neurochemistry in the same year. In 1983, he moved to the University of Texas Southwestern Medical Center in Dallas, Texas, USA, as a postdoctoral fellow. He became an investigator at Howard Hughes Medical Institute in 1991 and was appointed Professor of Molecular and Cellular Physiology at Stanford University in 2008. Currently, he is Professor in the School of Medicine in the Department of Molecular and Cellular Physiology at Stanford University. He received the National Academy Award in Molecular Biology in 1997, Kavli Prize with Rothman in 2010 and the Albert Lasker Award for Basic Medical Research in 2010.

Elucidation of their Nobel-winning work

After the discovery of cell membrane trafficking, the three scientists along with their teams studied the vesicles and their functions from different angles to solve the mystery of the cell (Table 1).

Contribution of Schekman – genetics of protein transport in the cell

Inspired by the work done by George Emil Palade in the assay of secretory pathway, Schekman realized the lack of molecular machinery which initiates and regulates the process of protein transport. Determined to explore the genetic basis of vesicle trafficking, he mutated strains of the yeast *Saccharomyces cerevisiae* cells and compared them with normal cells. He screened the temperature-sensitive mutants *sec*1 and *sec*2 that do not transport secretory intracellular enzymes. These enzymes were accumulated on the cell surface due to defects in the transportation system. The cause was found to be genetic. Further, through enrichment technique he successfully identified 23 secretory genes out of which one encoded for the protein *N*-ethylmaleimide-sensitive factor (NSF). NSF is a cytosolic protein that binds to membranes by means of the soluble NSF attachment protein (SNAP). He explained the role of SEC proteins and based on accumulation of membranes, he classified them in three classes based on blocking secretions from ER, Golgi bodies or with *sec*1 to the cell surface, as shown in Figure 2. In collaboration with Rothman's lab, he continued to characterize the isolated mutants and reveal their functions explaining the role of SEC proteins in the yeast cell that helps in the generation and transport of vesicles in the eukaryotic cells[4].

Contribution of Rothman – vesicle transport in the cell

Fascinated by the process of secretion, Rothman's curiosity drove him to uncover the secrets of protein docking – How could each vesicle identify where to go and deliver its cargo with precision?

Many viruses use vesicle transport system to drift from one point to another. To study the process, Rothman created a virtual environment in a test tube by organizing different pathways of the mechanism. He infected mammalian cells with vesicular stomatitis virus (VSV) and used the viral G protein as an indicator to trace its transportation pathway in the Golgi. Then, he isolated the proteins and protein complexes that are responsible for their identification and fusion with

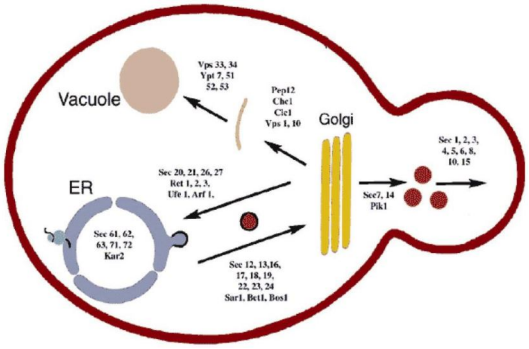

Figure 2. Yeast secretory pathway[4].

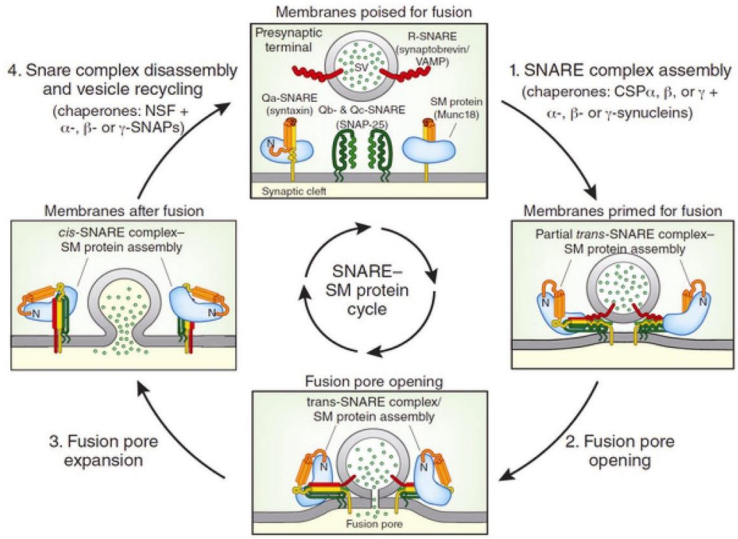

Figure 3. Model of the SNARE–SM protein cycle during synaptic vesicle fusion[6].

destination cells. Thus, he reconstituted the fundamental principle that transport depends on the intrinsic chemical specificity of the membranes involved, and not on the proximity of compartments in the cell. The first identified protein was NSF, associated with the process of transport vesicle targeting and fusion. He showed that mammalian NSF is encoded by *sec18*. Schekman and Rothman worked together on the degree of evolutionary conservation between yeast and mammalian transport system. After identification of the target membrane, the vesicle binds itself with the complementary proteins on the membrane through lock and key mechanism and fuses to release its content. Rothman revealed how a vesicle delivers the right cargo, at the right place, at the right time efficiently and proved that these proteins act at every stage where a vesicle docks at a target membrane, and directs the transport to target organelles and cells[5].

Contribution of Südhof – signals that instruct vesicles

Südhof was intrigued by the classic work of Bernard Katz, who showed that active synaptic transmission of neurotransmitters is triggered by calcium triggers. He started his experiments to find answers to the following questions – How does vesicles fusion occur in the synapse? How is calcium involved in the process? What are the mechanisms and machineries that facilitate the course and how?

Südhof isolated and cloned the major proteins present in presynaptic terminals and synaptic proteins like complexins, synaptotagmins, synaptophysin, synaptobrevin, synapsins, Munc18s, Munc13s, RIMs, RIM-BPs, neurexins and neuroligins. His team identified syntaxin-associated SM protein called Munc18-1, and explained how Munc18-1 and the SNARE proteins constitute the fusion mechanism of synaptic vesicles, demonstrated in Figure 3, for signal transmission from one nerve cell to another in the brain. He showed that calcium-binding synaptotagmins act as Ca^{2+} sensors which mediate the calcium-dependent exocytosis by shedding complexin from the SNARE complex, thereby elucidating how neurons communicate through vesicles that carry neurotransmitter chemicals and the role of calcium in this process[6,7].

Future implications of the discovery

This research has set the stage for a deep understanding of the complexities of vesicle trafficking and the machineries associated with it. The interdisciplinary nature of the research makes it beneficial for further studies in many areas like disease diagnosis and control, drug discovery, hormonal imbalance, growth factors, nutrient transportation, systemic mediators, etc. Emphasizing on the disturbances and defects in the process can help us control certain diseases like Alzheimer's, immunological disorders and diabetes. The cause of such diseases, and possible drugs and therapies that can regulate them can be the new interest of researchers. Many questions are yet unanswered, for example, what regulates vesicle speed and direction? What is the guiding machinery associated with them, their mode of action and regulation? How fusion influence brain diseases, and what are the precise physico-chemical mechanisms underlying fusion? New investigators can address these questions through scientific knowledge.

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2. Kumar, V. *et al.*, *IJAPBT*, 2010, **1**, 95–103.
3. Callaway, E., *Nature*, 2013, **502**, 149–150.
4. Schekman, R., *Nature Med.*, 2002, **8**, 1055–1058.
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