CURRENT SCIENCE

Volume 105 Number 12 25 December 2013

GUEST EDITORIAL

Vesicle traffic wins the Nobel

It is indeed been a long time since George Palade made a fundamental discovery on protein transport in the 1960s at the Rockefeller Institute of Medical Research, New York. Using electron microscopy and pulse chase experiments, Palade showed how proteins required for export were processed intracellularly. He demonstrated the transport of newly synthesized proteins in the form of small vesicles from the endoplasmic reticulum to the Golgi complex with eventual release at the cell surface[1]. These studies won him the 1974 Nobel Prize in Physiology or Medicine along with Albert Claude and Christian de Duve. As it happens during the early phases of discovery, the phenomenon was firmly established but the regulatory processes were barely understood. In the decades to come, Palade's creative studies on the transport of protein cargo captured the imagination of membrane biologists, biochemists and geneticists and a large repertoire of interesting observations on various aspects of regulation of vesicle transport were made. Forty years after the concept of intracellular processing of secretory proteins was described and awarded the Nobel, the 2013 Nobel Prize in Physiology or Medicine recognized the discovery of the molecular principles that govern the transport process. Three American scientists, James E. Rothman, Randy W. Schekman and Thomas C. Südhof were jointly awarded this year's Nobel Prize 'for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells'. Their observations shaped the picture of molecular mechanisms of protein transport and linked the anomalies in the processes to the origin of multiple diseases.

In the eukaryotic cell, systems are required to ensure correct transport of the synthesized proteins for secretion to the right destination at accurate time because proteins like neurotransmitters are required to be released at a precise time for nerve activation, hormones need to be delivered to distant targets, digestive enzymes are to be secreted and antimicrobial peptide secretion is required for self-defense. Obviously, defects in the intracellular protein trafficking would have huge effects on the physiology of an organism. Inspired by Palade's data and subsequent developments in membrane biology, Schekman who trained with enzymologist and Nobel laureate Arthur Kornberg at Stanford, changed his field of work to study how membranes helped in protein transport. Schekman pioneered the use of yeast (*Saccharomyces cerevisiae*) as a model system to study protein trafficking and made discoveries fundamental to the delivery of protein cargo[2,3]. He showed that the defects were genetic and anomalies in protein transport could be linked to mutations in the genes. This was important because similar genes in humans could be identified for tracking medical conditions. Studies from Schekman's laboratory identified coat proteins on the ER membrane like Coat protein complex II (COPII), consisting of a set of highly conserved proteins involved in the creation of transport vesicles. During the complex process of vesicle biogenesis, Sar1 is the first protein to be recruited to initiate the process of vesicle formation where it anchors the forming coat complex and recruits the next COPII sub-complex, the Sec23–Sec24 proteins. Sec23 and Sar1 interaction serves a structural as well as catalytic role. Sec24 binds to cargo proteins concentrating and packaging them into the vesicle and forming the pre-budding complex. For budding of the vesicle and movement towards the destination, Sec13 and Sec31 complex completes the process of membrane cargo sorting and vesicle fission following which transport of the vesicles begin.

For any study with lower eukaryotes, the primary aim is to translate these findings to higher organisms. Since vesicular protein transport is highly conserved from yeast to higher eukaryotes, many similar proteins could be detected in humans. This recognition helped associate diseases to mutations in human genes of the secretory pathway. Mutations in a Sar paralog (Sar1B) in the humans generate proteins associated with chylomicron retention and the Anderson's disease, both being fat-malabsorption diseases. A solitary missense mutation in the Sec paralog (Sec23A) in humans is the cause of cranial-lenticulo-sutural dysplasia marked by skeletal defects because mutated protein Sec23A is unable to recruit Sec31, leading to reduction of cargo protein packaging. Separate mutations in Sec paralog (Sec23B) are related to congenital dyserythropoietic anaemia type II. In addition, mutations in Sec24B were shown to produce major neural tube defects in animal models. Therefore, while Schekman's work provided new insights into the tightly

regulated machinery of vesicle formation, it also enabled the recognition of mutations in human genes that caused a variety of diseases.

In the late 1960s, Palade had concluded that secretory proteins pass through the Golgi apparatus after being released from the endoplasmic reticulum (ER). Rothman, who shared the 2013 Nobel Prize with Scheckman, and is currently the professor of cell biology and chemistry at Yale, started working on the role of the Golgi apparatus in protein transport. He developed a powerful biochemical approach by which cellular traffic could be reconstituted in a cell-free system[4]. These assays led to the isolation of a number of essential proteins with involvement in vesicle biogenesis and fusion. Using the cell-free assay, Rothman's laboratory successfully identified four proteins, the NEM-Sensitive Fusion protein (NSF), the Soluble NSF Attachment Factor (SNAP), a vesicle membrane protein named v-SNARE and a target membrane protein named t-SNARE[5]. The SNARE proteins are receptors for SNAP that incorporates NSF for vesicle docking. Thus, Rothman was able to define the regulatory elements for specificity of membrane fusion and transport of vesicles through the Golgi apparatus once they arrive from the ER for subsequent release outside the cell.

Cellular communication is vital for the survival of any organism and nerve cells or neurons in the brain must communicate for organisms to react. The neurotransmitters, vital for cellular communication, are released from a nerve cell by the machinery described by Schekman and Rothman, but how the cross-talk occurred was not known. Neurons communicate with each other through synapse and the plasma membrane of the signal-passing neuron (pre-synaptic neuron) comes in close contact with the signal-receiving neuron (post-synaptic neuron) at the synapse. A large array of molecular machinery links the two membranes together to execute signalling and neurotransmitters stored in vesicles within neuronal cells are released upon receiving the stimuli. Since the communication is achieved by transfer of chemical messengers in a millisecond timescale, the timing of signalling is critical. Südhof, who is currently a professor of molecular and cellular physiology at Stanford, defined the regulatory events that achieve the speedy and precise signalling required for all information processing. His collective findings have provided much of our current scientific understanding of pre-synaptic neuron behaviour in neurotransmission and synapse formation[6,7]. These studies were important in the context of human health because evidence links impairments in synaptic transmission to diseases such as Alzheimer's and autism. Although it was known in the 1990s that calcium was involved in neurotransmitter release, details of regulation were not available. In 20 years of research, Südhof established synaptotagmins as calcium-sensing proteins that mediate neurotransmitter release from presynaptic neurons.

He also discovered RIM proteins that serve as active zone scaffolding molecules mediating vesicle priming and interacting with most other essential pre-synaptic proteins. The interactions facilitate the fusion of synaptic vesicles containing neurotransmitters with the pre-synaptic plasma membrane, the process that ultimately causes neurotransmitter release. RIMs tether N- and P/Q-type Ca^{2+} channels to presynaptic active zones resulting in fast, synchronous triggering of neurotransmitter release at a synapse. His discoveries also identified neurexins in pre-synaptic neurons and neuroligins, proteins on the post-synaptic neurons that bind to each other at the synapse. There are many types of neuroligins and neurexins and the variable pairing between the two determines the wide variability in the types of synapses in the brain. Südhof's studies suggest that mutations in the genes encoding these proteins contribute to the pathogenesis of diseases such as autism and schizophrenia in humans.

Discoveries of Rothman, Schekman and Südhof revealing the precise control system for the transport and delivery of cellular cargo have immense implication in the understanding of diseases. In times to come, the involvement of the protein cargo transport in many human conditions will be established. Components of the pathway for vesicle movement could play a major role in cancer cell phenotype, although the mutations may or may not be directly involved. Through this award, the Nobel Committee has recognized the importance of basic discovery in molecular cell biology of protein trafficking in the fight against diseases.

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