The discovery of the molecular mechanism of cellular secretion

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The rôle of secretion and membrane fusion in health and disease is profound. The pioneering discoveries by Jena and Rothman of a new cellular structure, the porosome, have essentially explained this process. The elucidation of the porosome's morphology, composition and functional reconstitution in lipid membranes explain how cellular products destined for secretion undergo maturation by shuttling between subcellular compartments via budding and fusion of transport vesicles. Finally packaged in membranous sacs called secretory vesicles, which fuse at specific plasma membrane locations, they expel their contents. These and some other aspects of the discoveries of these scientists are described.

Keywords: molecular mechanism, porosome, secretory vesicle fusion

FUSION

The budding and fusion of transport vesicles [1–5], the fusion of secretory vesicles at the cell plasma membrane [5–12] and the release of intravesicular contents [13–15] have been elucidated primarily through the pioneering works of Bhanu P. Jena and James E. Rothman. The discovery of a new cellular structure called the porosome [16], and the elucidation of its morphology and dynamics at nm resolution in live cells, its composition and its functional reconstitution in lipid membranes have been of major significance. These important discoveries explain how cellular products destined for secretion undergo maturation by shuttling between subcellular compartments via budding and fusion of transport vesicles, and are finally packaged in membranous sacs called secretory vesicles, which fuse at specific plasma membrane locations to expel their contents. Important physiological processes like the pancreatic acinar cell secretion of digestive enzymes, the working of endocrine and neuroendocrine cells like the growth hormone cells of the pituitary that secrete growth hormone, the islet cells of the pancreas that secrete insulin or glucagons, and nerve cells which secrete neurotransmitters for nerve-nerve or nerve-muscle communication can all be explained by the discoveries of Jena and Rothman.

In mammalian cells, the proteins involved in vesicle budding and fusion were discovered by Rothman. In his studies, Rothman used mammalian cell lines lacking an enzyme necessary to complete the transport of secretory proteins. In these cell lines, secretory proteins accumulate within their organelles without being transported and therefore are unable to be subsequently secreted. Rothman isolated the organelles containing the proteins and when they were mixed with extracts from normal cells in test tubes, vesicle budding and fusion took place and the secretory protein could be transported to the next membrane compartment. He could track the destination of the secretory proteins by radiolabelling them prior to isolation of the organelle containing them. The transport of the radiolabelled proteins was further confirmed using electron microscopy. Using this assay system, Rothman was able to isolate and identify the first enzyme required for the fusion of a vesicle with its target membrane. This enabled the discovery of the three main proteins, two at the target membrane (syntaxin and SNAP or t-SNAREs) and one in the vesicle membrane (VAMP, vesicle associated membrane protein, or v-SNARE), required for membrane fusion. In his studies using electron microscopy, Rothman noticed a coat only on budding vesicles, suggesting a functional rôle of the coat in vesicle budding. Rothman's initial attempts to identify the composition of the coat failed since the coats rapidly dissociated from the vesicles once the vesicles budded off. He suspected the involvement of a GTP-binding protein. When he added a GTPase inhibitor, he found that the coat remained on the vesicles, but with the coat on, the vesicles failed to fuse with the target organelle. Rothman could isolate enough coated vesicles to be able to purify and analyse the composition of the coat. Seven proteins were identified. These studies unravelled the molecular composition and mechanism of the transport machinery, as well as the molecular components required for membrane fusion.

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SECRETION

The molecular mechanism of secretory vesicle fusion at the cell plasma membrane and the release of the vesicular contents were revealed by the discoveries of Jena. Once mature secretory vesicles are generated, they are stored and then secreted on demand by the cell. Where at the plasma membrane do the secretory vesicles dock and fuse? What is the structure and composition of the vesicle docking site at the plasma membrane? What is the molecular mechanism of membrane fusion? How do secretory vesicles release their contents? All these questions were answered by the work of Jena leading to the discovery of a new cellular structure, the porosome. It is present at the cell plasma membrane where secretory vesicles fuse to release their contents. Curious to understand the structure and dynamics of live secretory cells at near nm resolution, Jena used atomic force microscopy (then almost unknown to biologists) to study the structure and dynamics of live secretory cells placed in physiological buffer solutions at near nm resolution. Circular pits some 100–150 nm wide were observed as well as several nm deep pores, which he called porosomes. These were at specific plasma membrane locations where secretory vesicles fuse to release their contents. The first observations were made in pancreatic acinar cells, and subsequently in other secretory cells in mammals. When the cells are stimulated to secrete, the porosomes enlarge by about 20–35%, returning to their resting size following the completion of secretion. Earlier studies reported that cytochalasin B, a fungal toxin that inhibits actin polymerization, inhibits secretion. When Jena exposed the pancreatic acinar cells to cytochalasin, the porosomes collapsed and a loss in secretion was observed. Results from these studies strongly suggested that the porosome was the secretory pore. In a series of experiments using gold-conjugated antibody against an intravesicular secretory protein, Jena was able to demonstrate that secretion occurred through the porosome opening to the outside of the cell. This he also confirmed in other secretory cells. It was previously hypothesized that the secretory vesicles fuse and their membrane gets completely incorporated into the cell plasma membrane, allowing the passive release of the vesicular contents by diffusion. There were a number of unexplainable issues with such a mechanism, especially since one could not explain why empty and partially empty vesicles were generated following secretion. However, if the secretory vesicles transiently dock and fuse at the porosomes to release a portion of or all their contents, it becomes clear why empty and partially empty vesicles appear following secretion. Moreover, if 500–1000 nm diameter secretory vesicles in the acinar cells of the exocrine pancreas were to completely incorporate their membrane into the 100–150 nm porosome, it would enlarge much more than the observed 20–35% increase. Hence, the discovery of the porosome clarified the mechanism of cellular secretion. Also, transient fusion between synaptic vesicles and the presynaptic membrane has also been demonstrated in neurons. It is now clear that in a fast secretory cell (the neuron) there are neurotransmitter transporters in the synaptic vesicle membrane. Synaptic vesicles that have discharged their neurotransmitters can be rapidly refilled, so that they can undergo a new round of docking and release.

To further understand the structure, chemistry and function of the porosome, Jena carried out extensive electron microscopic studies on whole cells, the immunoisolated porosome complex, and the complex reconstituted in artificial liposomes. An intact porosome, associated with secretory vesicles, was imaged in the electron micrographs. Negative staining electron microscopy combined with atomic force microscopy revealed the detailed architecture of the proteinacious backbone of the immunoisolated porosome. Transmission electron micrographs of porosomes reconstituted into liposomes further confirmed their cup-shaped, basket-like morphology. To further examine if the immunoisolated porosomes were functional, they were reconstituted into a lipid bilayer membrane in an electrophysiological setup and exposed to isolated secretory vesicles. These studies revealed the reconstituted porosomes to be functional, exhibiting an increase in capacitance and current on exposure to secretory vesicles. Immunoassay of vesicular content transport across the bilayer confirmed vesicle fusion at the porosome and the transport of vesicular contents across the bilayer. Atomic force and immuno-atomic force microscopy further revealed the association of secretory vesicles at the base of the porosome, and also the presence of t-SNARE at the porosome base. Thus the discovery, knowledge of the structure, composition and function, and the method of reconstitution of the porosome were complete.

The molecular mechanism of secretory vesicle fusion at the cell plasma membrane and the release of vesicular contents were also established by Jena. Rothman had identified both the t- and v-SNAREs as the entities for minimal fusion but the molecular mechanism of how the SNAREs enable membrane

fusion was still unknown. By using atomic force microscopy and electrophysiological approaches, Jena was able to resolve the molecular mechanism of SNARE-induced membrane fusion. His studies revealed that, t- and v-SNAREs in opposing bilayers interact in a circular array to form conducting pores. However, when in solution, SNAREs interact differently and fail to form pores. Besides solving the molecular mechanism of membrane fusion, these studies demonstrate that membrane proteins in solution interact differently when they are associated with membranes. Jena's studies have also revealed the molecular mechanism of vesicle swelling, which is involved in the expulsion of vesicular contents.

CONCLUSION

These pioneering discoveries by Bhanu P. Jena and James E. Rothman have finally revealed the molecular mechanism of transport and cellular secretion. Discovery of the molecular mechanism of secretion allows not only an understanding of various cellular and physiological processes as discussed in the beginning of this article, but also enables an understanding and therefore the treatment of diseases such as diabetes, neurological ailments, and digestive problems. Although a number of other investigators have contributed to the field, the seminal and major contributions of these researchers clearly stand out.

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