Abstract

The release of neurotransmitters at the nerve terminal for neurotransmission, release of insulin from β-cells of the endocrine pancreas for regulating blood glucose levels, the release of growth hormone from GH cells of the pituitary gland to regulate body growth, or the expulsion of zymogen from exocrine pancreas to digest food, are only a few examples of key physiological processes made possible by cell secretion. It comes as no surprise that defects in cell secretion are the cause for numerous diseases, and have been under intense investigation for over half a century. Only in the last decade, the molecular machinery and mechanism of cell secretion has become clear. Cell secretion involves the docking and transient fusion of membrane-bound secretory vesicles at the base of plasma membrane structures called porosomes, and the regulated expulsion of intravesicular contents to the outside, by vesicle swelling. The discovery of the porosome in live cells, its morphology and dynamics at nanometer resolution and in real time, its isolation, its composition, and its structural and functional reconstitution in lipid membrane, are complete. The molecular mechanism of secretory vesicle fusion at the base of porosomes, and the regulated expulsion of intravesicular contents during cell secretion, are also resolved. In this minireview, the monumental discovery of the porosome, a new cellular structure at the cell plasma membrane, is briefly discussed.

Keywords: fusion pore/porosome • cell secretion

Introduction

Cell secretion involves the fusion of membrane-bound vesicles at the cell plasma membrane, and the release of intravesicular contents to the cells exterior. This fundamental cellular process allows cell-cell communication, such as neurotransmission, release of hormones, immune response, histamine release,
and a variety of important physiological functions. Any disturbance in cell secretion is known to result in numerous diseases, and hence there has been a concerted effort for over half a century to understand the molecular machinery and mechanism of the process. Only in the past decade, seminal and groundbreaking discoveries [1–35], finally provide a clear understanding of secretion from cells. These discoveries have profound impact on our understanding of the cell, and on human health and disease.

Before atomic force microscopy

Invention of the light microscope some 300 years ago allowed discovery of the unit of life, the cell. Up until the early 1940s, the light microscope, so efficient in the 19th Century, reached the limits of its resolving power, of some 200 nanometers in lateral resolution and much less in its depth resolution. With the invention of the electron microscope and its use in biological research, a new era dawned for Cell Biology, when major subcellular organelles, and their organization and function, were elucidated. Pioneering cell biologists - George E. Palade, Keith Porter, and Albert Claude - made stellar contributions during this period. With a lateral resolving power of nearer a nanometer, the electron microscope matured into the ultimate imaging tool in the study of the cell. Unlike the light microscope, the electron microscope sacrificed live cell imaging for a considerable gain in resolution. Although, the electron microscope was capable of imaging biological samples at nanometer resolution, it required sample processing that included freezing, fixation, dehydration, and staining with heavy metal compounds to increase contrast. Hence, morphological changes due to freezing, tissue fixation, and processing for electron microscopy have always been a major concern. Nonetheless, using electron microscopy, it appeared we knew just about all there is to know of cellular structure. After nearly half a century, Nature surprised us, with the discovery of a new cellular structure, the 'porosome', [7, 14–16, 22, 23, 27; see Figs. 1–3].

‘Porosome’ discovery

During the early to mid 90’s, Professor Bhanu P. Jena and his research team at Yale University School of Medicine, circumvented the problems faced by both light and electron microscopy in their
pioneering studies, by using the Atomic Force Microscope (AFM), which had been developed in the mid 1980's. In a series of elegant studies on live cells, Jena and his research team utilized the power and scope of the AFM to discover a new supramolecular structure (porosome) at the cell plasma membrane, where membrane-bound secretory vesicles dock and fuse to release their contents. Cup shaped, 150 nm in diameter 'porosomes', were present at the apical plasma membrane in live pancreatic acinar cells, chromaffin cells, growth hormone cells of the pituitary, and β-cells of the endocrine pancreas [7, 14–16, 22, 23, 29]. Similarly, porosomes were also discovered at the nerve terminal, but were an order of magnitude smaller, measuring 10–12 nm in diameter [27].

Stimulation of secretion caused 'porosomes' to enlarge, returning to their resting size following completion of the process. Exposure of cells to actin depolymerizing agents like cytochalasin B or D, results in collapse of 'porosomes' and a consequent loss in secretion. These results suggest 'porosomes' to be actin-regulated dynamic structures at the cell plasma membrane, where membrane-bound secretory vesicles dock and fuse to release their contents [7]. Zymogen granules, the membrane-bound secretory vesicles in exocrine pancreas, contain the starch digesting enzyme amylase. Using amylase-specific immunogold-AFM studies, localization of amylase at 'porosomes' following stimulation of secretion has been demonstrated [14], confirming 'porosomes' to be the secretory apparatus. Subsequently, similar presence of 'porosomes' was confirmed in chromaffin cells [15], in growth hormone secreting cells of the pituitary gland [16] and in neurons [27], suggesting their universal presence in secretory cells. The detailed morphology of the 'porosome' has been further confirmed, using electron microscopy, demonstrating a cup-shaped basket-like morphology [22, 23, 27]. Using immunofluorescence and biochemical approaches, further studies revealed the composition of the 'porosome' [22, 27], and demonstrated t-SNAREs and Ca^{2+}-channels, associated with the base of the 'porosome' cup [22, 30]. The native 'porosome' has been isolated, and both its structure and function reconstituted in lipid bilayers [22, 30].

![Fig. 2](image-url) Transmission electron micrograph of a porosome associated with a docked secretory vesicle at the apical end of a pancreatic acinar cell. (a) Part of the apical end of a pancreatic acinar cell demonstrating within the green bordered square, the presence of a fusion pore or porosome and an associated zymogen granule (ZG), the electron dense secretory vesicle of the exocrine pancreas. (Bar = 400 nm). (b) The area within the green square in (a) has been enlarged to show the apical microvilli (MV) and a section through porosome and the ZG. Note the ZG membrane bilayer is attached directly to the base of the porosome cup.
membrane [23, 27]. For the first time, the molecular composition and architecture of the secretory machinery in cells has been revealed at nanometer resolution and its dynamics shown in real time, providing a new understanding of cellular secretion. This brilliant and pioneering discovery has propelled us into a new era in our understanding of the cell, and into its wonderful architecture and function. This discovery has also revealed how little we know about the cell, and thus heralds a new revolution in cell biology, i.e. the birth of Nano Cell Biology.
Molecular machinery of membrane fusion and cell secretion

The discovery of the porosome and elucidation of its morphology, dynamics, and composition, revealed where membrane-bound secretory vesicles dock and fuse to release their contents. How membrane-bound secretory vesicles fuse at plasma membrane-associated 'porosomes' has also been determined by Jena and his team [21, 25, 26]. Professor James E. Rothman and his research team have identified a number of proteins involved in vesicular budding and fusion in cells [36, 37]. Among these proteins, the N-ethylmaleimide-sensitive factor (NSF)-attachment protein receptors (SNAREs) were implicated as the minimal fusion machinery capable of fusing opposing bilayers [37]. Target SNAREs or t-SNAREs (syntaxin and SNAP-25/23) are located at the cell plasma membrane (present at the base of porosomes), and vesicle SNARE or v-SNARE, (vesicle associated membrane protein or VAMP) is present at the secretory vesicle membrane. However, the molecular mechanism of SNARE participation in membrane remained a mystery, until resolved by the Jena team. Employing a variety of approaches, including AFM and electrophysiology, Jena and his research group were able to resolve the molecular mechanism of SNARE-induced membrane fusion in cells [21, 25, 26]. Their studies demonstrate that in the presence of Ca^{2+} t- and v-SNAREs in opposing bilayers interact in a circular array to form conducting channels [21]. However, when any one of the two types of SNAREs is present in solution and exposed to the other SNARE in membrane, the interaction between the SNAREs failed to form such channels. Thus, these combined AFM and electrophysiological studies [21] on the structure and arrangement of SNAREs, further demonstrate for the first time that membrane proteins assemble and interact differently when membrane-associated, then when in solution. This is critical when conducting x-ray crystallographic studies on interacting membrane protein complexes. This in itself is an extremely important observation in the area of biomolecular structure-function. Studies by Jena and his research team further demonstrate that SNAREs bring opposing bilayers closer to within a distance of 2–3 Å, allowing Ca^{2+} to bridge them [25, 26]. The bound Ca^{2+} then leads to the expulsion of water between the bilayers at the bridging site, allowing lipid mixing and membrane fusion [26]. Hence SNAREs, besides bringing opposing bilayers closer, dictate the site and size of the fusion area during secretion. The size of the t-/v-SNARE ring complex is dictated by the curvature of the opposing membranes, hence depends on the size of t-/v-SNARE-reconstituted vesicles [34]. The smaller the vesicles, the smaller the pores formed [33]. Once formed, energy (NSF-ATP) is required for t-/v-SNARE complex disassembly [35]. Thus for the first time, we have an understanding of the molecular mechanism of SNARE-induced membrane fusion in cells.

Following fusion of secretory vesicles at the base of 'porosomes', the question remained, how are vesicular contents expelled? Is it mere diffusion, or is there some form of regulation in the release of intravesicular contents? This important question was also addressed by Jena and his research team [9, 18, 24, 28, 33]. Studies on secretory cells reveal that following secretion, empty and partially empty vesicles accumulate within the cell, suggesting regulation of some sort in the release of intravesicular contents. Jena and his team found that following stimulation of secretion, secretory vesicles swell [13]. This swelling results in a build up of intravesicular pressure, dictating the amount of vesicular contents to be expelled. Using isolated cells, secretory vesicles, and reconstituted liposomes, Jena and his team were able to resolve the molecular mechanism of secretory vesicle swelling and determine that the amount of secretion is directly proportional to the extent of secretory vesicle swelling [28].

Conclusion

Understanding cellular secretion and membrane fusion is critical, since important cellular events such as ER-Golgi transport in protein maturation, plasma membrane recycling, cell division, sexual reproduction, and the release of enzymes, hormones and neurotransmitters, all require fusion of opposing bilayers. Thus, the elucidation of cell secretion and membrane fusion, has profoundly impacted human health and medicine.
References