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Mini-review

Discovery of a new cellular structure—the porosome: elucidation of the molecular mechanism of secretion

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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 nanometer 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. None the less, using electron microscopy, it appeared we knew just about all there is to know of cellular structure. After nearly half a century, nature has surprised us once more, with the discovery of a new cellular structure, the ‘porosome’, by Bhanu Jena and his research team (Jena et al., 2003; Jeremic et al., 2003; see Figs. 1 and 2).

During 1995–96, Jena’s group 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 1980s. In a series of elegant studies on *live* cells, his research team utilized the power and scope of the AFM to discover a new structure at the plasma membrane where membrane-bounded secretory vesicles dock and fuse to release their vesicular contents. The structure was found to be located in the plasma membrane of cells where secretion occurs. Circular ‘pits’ containing 150 nm diameter ‘depressions’ (now identified as porosomes) were present at the apical plasma membrane in living pancreatic acinar cells (Schneider et al., 1997). Stimulation of secretion caused the ‘depressions’ to dilate and then return to their resting size following completion of the process. Exposure of cells to actin depolymerizing agents resulted in collapse of ‘depressions’ and a consequent loss in secretion.

Zymogen granules, the membrane-bounded secretory vesicles in exocrine pancreas, contain the starch digesting enzyme amylase. Using amylase-specific immunogold-AFM studies, localization of amylase at ‘depressions’ following stimulation of secretion was demonstrated (Cho et al., 2002c; Jena, 2002). These studies demonstrate that these ‘depressions’ are the fusion pores of pancreatic acinar cells, where membrane-bound secretory vesicles dock and fuse to release their vesicular contents. Subsequently, a similar presence and operation of fusion pores was confirmed in growth hormone secreting cells of the pituitary gland (Cho et al., 2002a) and in chromaffin cells (Cho et al., 2002e), suggesting their universal presence in secretory cells. Recently, the detailed morphology of the porosome was also revealed using electron microscopy, showing a cup-shaped basket-like morphology (Jena et al., 2003; Jeremic et al., 2003). Using immuno-AFM and biochemical approaches, further studies revealed the composition of the fusion pores, and demonstrated t-SNAREs associated with the base of the cup (Jeremic

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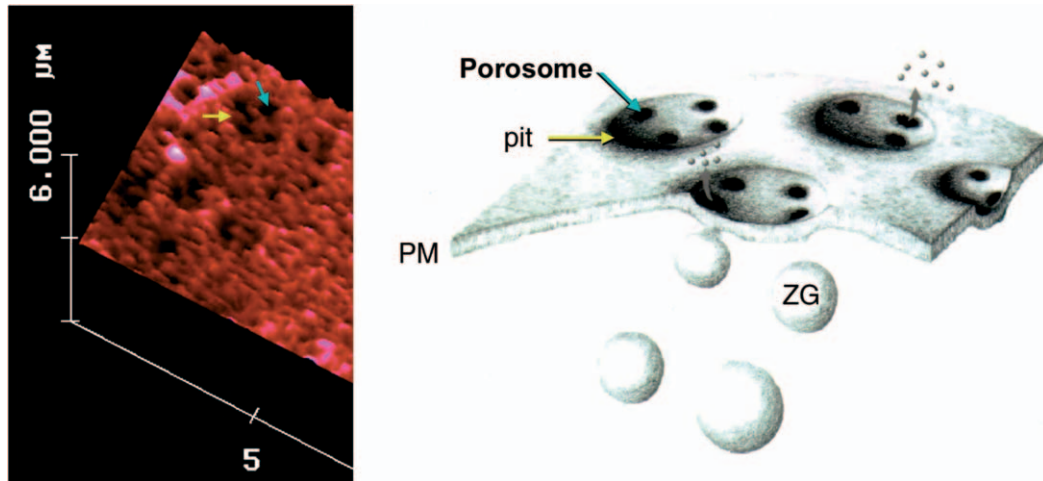


Fig. 1. Porosome 1 demonstrates an AFM micrograph of the plasma membrane at the apical end of live pancreatic acinar cells demonstrating the presence of circular PITS (yellow arrow) and the 100–150 nm in diameter porosomes (blue arrow) within. The right panel is a schematic diagram depicting the porosomes within PITS, at the base of which secretory vesicles (ZG) dock and fuse to release vesicular contents. Courtesy of Bhanu P. Jena.

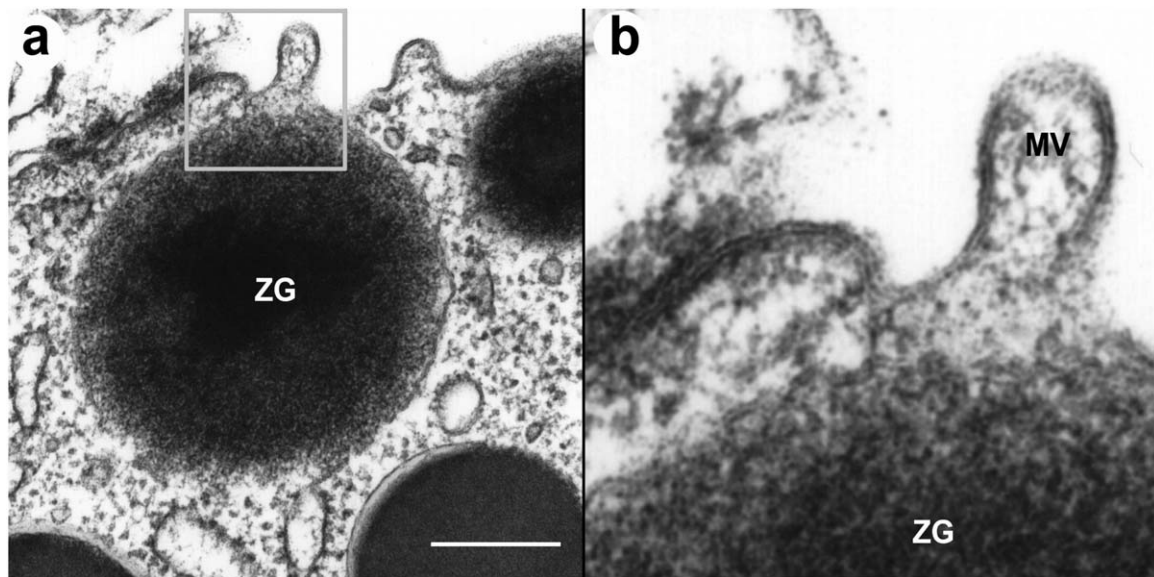


Fig. 2. Transmission electron micrograph of a porosome associated with a docked secretory vesicle at the apical end of a pancreatic acinar cell. (a) Part of the apical end of a pancreatic acinar cell demonstrating within the green bordered square, the presence of a fusion pore or porosome and an associated zymogen granule (ZG), the electron dense secretory vesicle of the exocrine pancreas, (bar=400 nm). 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. Courtesy of Bhanu P. Jena.

et al., 2003). Recently, Jena and his research team have reconstituted in artificial liposomes, both the structure and function of the porosome (Jena et al., 2003). For the first time, the molecular composition and architecture of exocytotic machinery at the cell plasma membrane has been revealed at nanometer resolution and its dynamics shown in real time, providing a new understanding of cellular secretion. The existence of the fusion pore, suggested a number of years ago from electrophysiological measurements, is now fully confirmed. 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.

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-bounded secretory vesicles fuse at plasma membrane-associated porosomes has also been elucidated by Jena's team (Cho

et al., 2002b). The work of James Rothman has identified a number of proteins involved in transport and membrane fusion in cells (Rothman, 1994). Among these proteins, the *N*-ethylmaleimide-sensitive factor (NSF)-attachment protein receptors (SNAREs) have been implicated as the minimal fusion machinery capable of fusing opposing bilayers (Weber et al., 1998). Target SNAREs or t-SNAREs (involving two proteins) are located at the cell plasma membrane (t-SNARE present at the base of porosomes), and vesicle SNARE or v-SNARE (a single protein) is present at the secretory vesicle membrane. Although the crystal structure of the core complex of t-/v-SNARE is known (Sutton et al., 1988) the molecular mechanism of SNARE participation in membrane remained a mystery. Employing AFM and electrophysiological measurements, the Jena team was able to resolve the molecular mechanism of SNARE-induced membrane fusion (Cho et al., 2002b). These studies also showed that t- and v-SNAREs in opposing bilayers interact in a circular array to form conducting pores. However, when any one of the two types of SNAREs was present in solution and exposed to the other SNARE in membrane, the interaction between the SNAREs failed to form such pores. Thus, these AFM studies 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 and analysis.

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 vesicular contents? This important question has also been addressed (Cho and Jena, 2002; Cho et al., 2002d; Jena et al., 1997). 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. The Jena group found that following stimulation of secretion, secretory vesicles swell (Cho and Jena, 2002). This swelling results in a build up of intravesicular pressure, dictating the amount of vesicular contents to be expelled. Using isolated secretory vesicles, Jena and his team was able to further confirm vesicle swelling and reveal its molecular mechanism (Cho et al., 2002d; Jena et al., 1997).

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. Understanding cellular secretion and membrane fusion will help in amelioration of secretory defects, provide insight into our understanding of cellular entry and exit of viruses, and in the development of smart drug delivery systems. Thus the role of secretion and membrane fusion in health and disease is profound.

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