Bhanu P. Jena: Research Summary

In the past 30 years, we have gained much understanding of the molecular machinery and mechanism of cell secretion. Our studies demonstrate that cells possess cup-shaped supramolecular structures called ‘porosomes’ at specific plasma membrane locations, where membrane-bound secretory vesicles dock and fuse to release their contents from the cell. We have isolated the porosome from several cells including neurons, endocrine, neuroendocrine, and exocrine cells, determined its composition, and succeeded both in its structural and functional reconstitution into artificial lipid membrane. Our studies further provide at the molecular level, an understanding of the assembly and disassembly of proteins (SNAREs) present in opposing bilayers, involved in membrane fusion in cells. Thus an understanding of the molecular assembly and disassembly of the SNARE complex, has progressed our understanding of secretory vesicle fusion at the porosome during cell secretion. Finally, our studies provide an understanding of secretory vesicle swelling, which has been determined to be a requirement for the regulated release of vesicular contents during cell secretion. These findings therefore, provide the unifying principle governing cell secretion, such as neurotransmission, and the release of hormones and digestive enzymes. Defects in cell secretion results in numerous debilitating diseases like diabetes, neurological disorders, growth defects, etc. Diseases resulting from secretory defects can now be managed and treated, by the design and use of various regulators, such as stimulators or blockers of the cells secretion machinery. We continue to work in the field, to gain further understanding of secretion and membrane fusion in cells.

'molecular understanding of the machinery and mechanism of cell secretion'

Secretion is a fundamental cellular process responsible for numerous physiological functions in living organisms, such as neurotransmission and the secretion of hormones and digestive enzymes. Secretory defects in cells are responsible for a host of debilitating diseases, and hence this field has been the subject of intense investigation for over half a century. In the past decade, our studies on cell secretion, has provided an understanding of the molecular machinery and mechanism of the process. **Our unexpected discovery of the ‘porosome’, a new cellular structure at the cell plasma membrane, has been determined to be the universal secretory machinery in cells, providing a molecular understanding of cell secretion.** Our findings can be summarized as follows: (1) Discovery of a new supramolecular structure ‘the porosome’ at the cell plasma membrane, and the determination that it is the universal secretory machinery in cells [Figure 1]. (2) Determination of the structure and dynamics of the porosome in live cells, at nanometer resolution and in real time, its composition, and its structural and functional reconstitution in artificial lipid membrane. (3). Determination of the molecular assembly and disassembly of the t-/v-SNARE complex involved in membrane fusion, resulting in the establishment of continuity between the secretory vesicle membrane and the plasma membrane at the base of porosomes [Figure 2]. (4) Determination that secretory vesicle swelling is a requirement for the regulated release of intravesicular contents to the outside, and the molecular mechanism of vesicle swelling [Figure 3,4].

Research papers supporting contributions 1 & 2:


dynamics of the fusion pores in live GH-secreting cells revealed using atomic force microscopy.  
*Endocrinology* 143(3):1144-1148.


**Research papers supporting contribution 3:**


**Research papers supporting contribution 4:**


**REVIEWS**


Figure 1. Prosome: the universal secretion machinery in cells. Electron and AFM micrographs of prosome in the exocrine pancreas (top panels) and neuron (bottom panels). The top left panel, is an electron micrograph of a single prosome at the apical plasma membrane (PM) of a pancreatic acinar cell. Note the prosome membrane (POM) associated with the membrane of a secretory vesicle (ZGM). Cross section through a ring structure (blue arrowhead) is found at the prosome neck region. The top right panel is an AFM micrograph of four proosomes, each 100-180 nm (yellow arrowhead), at the apical plasma membrane of a live pancreatic acinar cell. The bottom left panel shows an electron micrograph of a prosome (red arrowhead) at the nerve terminal, in association with a synaptic vesicle (SV) at the presynaptic membrane (Pre-SM). Note a central plug in the neuronal prosome complex. The bottom right panel is an AFM micrograph of a neuronal prosome in physiological buffer, also showing the central plug (red arrowhead). The central plug in neuronal proosomes may regulate its rapid closing and opening during neurotransmitter release. Note that the neuronal prosome is an order of magnitude smaller (10-12 nm) in comparison to prosome in the exocrine pancreas.

Figure 2. t-SNAREs and v-SNARE in opposing bilayers interact in a circular array, result in the formation of conducting channels in presence of calcium. (A) Unfused v-SNARE vesicles on t-SNARE reconstituted lipid membrane. (B) Dislodgement of v-SNARE-reconstituted vesicles from the t-SNARE-reconstituted lipid membrane exposes the pore-like (C,D) t/v-SNARE ring complexes. (C,D) Three dimension AFM micrographs of SNARE-ring complexes, at low (C) and high resolution (D). A channel at the center of the t/v-SNARE ring complex is clearly visible (Scale bar=100nm). In presence of calcium, recombinant t-SNAREs and v-SNARE in opposing bilayers drive membrane fusion. (E) When t-SNARE vesicles are exposed to v-SNARE reconstituted bilayers in presence of calcium, vesicles fused. Vesicles containing nystatin/ergosterol and t-SNAREs when added to the cis compartment of a bilayer chamber, fusion of t-SNARE containing vesicles with the v-SNARE membrane bilayer, is demonstrated as current spikes that collapse as the nystatin spreads into the bilayer membrane. To confirm membrane stability, the transmembrane gradient of KCl is increased to 3M, allowing gradient driven fusion of nystatin-associated vesicles. (F-H) The size of the t-v-SNARE complex is directly proportional to the size of the SNARE-reconstituted vesicles. (F) Schematic diagram depicting the interaction of t-SNARE- and v-SNARE-reconstituted liposomes. (G) AFM images of vesicle
before and after their removal using the AFM cantilever tip, exposing the t-/v-SNARE ring complex at the center.

(H) Note the high correlation coefficient between vesicle diameter and size of the SNARE complex.

Figure 3. Zymogen granule swelling in live pancreatic acinar cells. (A) Electron micrograph of pancreatic acinar cells showing the basolaterally located nucleus (N) and the apically located ZGs. The apical end of the cell faces the acinar lumen (L). Bar = 2.5 µm. (B-D) The apical ends of live pancreatic acinar cells were imaged by AFM, showing ZGs (red and green arrowheads) lying just below the apical plasma membrane. Exposure of the cell to a secretory stimulus using 1 µM carbamylcholine, resulted in ZG swelling within 2.5 min, followed by a decrease in ZG size after 5 min. (n=8). The decrease in size of ZGs after 5 min is due to the release of secretory products such as α-amylase, as demonstrated by the immunoblot assay (E).

Figure 4. Fusion of isolated ZGs at porosome-reconstituted bilayer and GTP-induced expulsion of α-amylase. (A) Schematic diagram of the EPC9 bilayer apparatus showing the cis and trans chambers. Isolated ZGs when added to the cis chamber, fuse at the bilayers-reconstituted porosome. Addition of GTP to the cis chamber induces ZG swelling and expulsion of its contents such as α-amylase to the trans bilayers chamber. (B) Capacitance traces of the lipid bilayer from three separate experiments following reconstitution of porosomes (green arrowhead), addition of ZGs to the cis chamber (blue arrowhead), and the red arrowhead represents the 5 min time point after ZG addition. Note the small increase in membrane capacitance following porosome reconstitution, and a greater increase when ZGs fuse at the bilayers. (C) In a separate experiment, 15 min after addition of ZGs to the cis chamber, 20 µM GTP was introduced. Note the increase in capacitance, demonstrating potentiation of ZG fusion. Flickers in current trace represent current activity. (D) Immunoblot analysis of α-amylase in the trans chamber fluid at different times following exposure to ZGs and GTP. Note the undetectable levels of α-amylase even up to 15 min following ZG fusion at the bilayer. However, following exposure to GTP, significant amounts of α-amylase from within ZGs were expelled into the trans bilayers chamber. (n=6). Ref: Kelly, M., Cho, W-J., Jeremic, A., Abu-Hamdah, R., Jena, B.P. (2004). Vesicle swelling regulates content expulsion during secretion. Cell Biol. Int. 28:709-716.

These findings have contributed to a molecular understanding of cell secretion. It is now understood that membrane-bound secretory vesicles can transiently dock at the porosome base, establish continuity with the porosome membrane via SNAREs, and expel intravesicular contents through the porosome opening to the outside, following vesicle swelling.