

# Elucidation of Cell Secretion: Pancreas Led the Way

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## Key Words

Porosomes · Fusion pores · SNARE-induced membrane fusion · SNAREs · Cell secretion, molecular level

## Abstract

Secretion is a basic process in all cells and is required for several important functions such as neurotransmission, the secretion of digestive juices from the exocrine pancreas and the release of hormones from endocrine and neuroendocrine cells. Due to these important functions, the mechanism of cell secretion has been intensely investigated for over half a century. However, it is only in the last decade, with the discovery of a new cellular structure, the 'porosome' or 'fusion pore', and the elucidation of SNARE-induced membrane fusion, that has finally provided us with an understanding of cell secretion at the molecular level. The 'porosome', a supramolecular structure at the cell plasma membrane, was first discovered in the exocrine pancreas, and subsequently in endocrine/neuroendocrine cells and in neurons. The structure and dynamics of the 'porosome' in live cells at nanometer resolution and in real-time, its composition and functional reconstitution in lipid membrane, have all been determined. These findings have fundamentally changed our understanding of cell secretion and provide a clear understanding of this highly regulated process in cells.

Cell secretion is a key process occurring in all cells and is involved in the physiology of neurotransmission, and the release of hormones and enzymes. A number of diseases are known to result from secretory defects. The area of intracellular transport and secretion has been intensely investigated for over half a century. Until recently, it was commonly accepted that the final step in secretion is the total incorporation of secretory vesicle membrane into the cell plasma membrane leading to the release of intravesicular contents by diffusion, and the compensatory retrieval of excess membrane by endocytosis at a later time [1–10]. Studies within the past decade have finally revealed a completely different molecular mechanism of secretion and membrane fusion in cells [11–22]. Contrary to accepted belief, the mechanism of cell secretion is quite different, and a highly regulated process. These studies [11–16, 23] demonstrate that membrane-bound secretory vesicles dock and transiently fuse at the base of specialized plasma membrane structures called porosomes or fusion pores, to expel vesicular contents. Contrary to what was previously suggested, there is no incorporation of the vesicle membrane at the cell plasma membrane. Recent studies further demonstrate that during secretion, secretory vesicles swell, enabling the expulsion of intravesicular contents through porosomes [24–26]. These seminal findings [11–16, 23] have given rise to a new understanding of cell secretion, and were confirmed by other laboratories [27–30].

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The presence of fusion pores had been suggested earlier, both from freeze-fracture electron microscopy [31] and electrophysiological studies on mast cells [32]. In both studies however, it was assumed that fusion pores are transiently formed as a result of plasma membrane invagination during secretion. The pore formed, either closed, termed 'flicker fusion' or completely distended as a result of total incorporation of the vesicle membrane with the cell plasma membrane. The later model, however, failed to account for the appearance of empty and partially empty vesicles following cell secretion. Further, contrary to this model, no change in vesicle number is demonstrated following secretion. So the only possibility left was 'flicker fusion'. In the mid 90s, atomic force microscopy on live pancreatic acinar cells demonstrated for the first time at nanometer resolution the presence of pores, 100–150 nm in diameter and 25–40 nm in depth at the apical plasma membrane, where secretory vesicles are known to dock and fuse to secrete digestive enzymes [11]. When the cell was stimulated to secrete, both the depth and opening of the pore increased by 35–50%, returning to resting size following completion of secretion [11]. Exposure of the cell to cytochalasin B resulted in a decrease in pore size and a significant loss (60–70%) in stimulatory secretion. These studies suggested that the pore may be the fusion pore, where secretory vesicles fuse to extrude their contents from the cell. Further, it demonstrated that actin may be an important component of the pore structure-function and the pore to be a stable structural entity at the cell plasma membrane. The actual release of secretory products through the pore was demonstrated when immuno-atomic force microscopy was performed on live pancreatic acinar cells [12]. An antibody against amylase (a major secretory enzyme within zymogen granules) was gold conjugated, and specifically decorated the opening of the pore [12]. Subsequently, similar secretory pores were identified in chromaffin cells [13], growth hormone cells of the pituitary gland [14], mast cells [23], the  $\beta$  cells of the endocrine pancreas [23], and in neurons [23]. Acknowledging the universal presence of the pores, from the exocrine pancreas through endocrine and neuroendocrine cells to neurons, the structure was named the 'porosome'. The next breakthrough in these seminal findings were the isolation of the porosomes, determination of their biochemical composition, and their structural and functional reconstitution into artificial lipid membrane [15, 16]. Electron microscopy subsequently has confirmed the presence of porosomes in cells [16, 23].

To understand the molecular mechanism of cell secretion, the next burning problem was how membrane fusion occurs in the cell. The discovery of SNAREs [17–20] led the way. Three soluble N-ethylmaleimide-sensitive factors (NSF)-attachment protein receptors (SNAREs) had been identified to be involved in membrane fusion in cells. Plasma membrane proteins SNAP-25 and syntaxin, collectively called t-SNARE or target SNARE, and secretory vesicle-associated membrane protein v-SNARE, were found to be involved in fusion of opposing bilayers. However, the detail of the molecular mechanism of SNARE-induced membrane fusion remained unknown until 2 years ago [21]. t-SNAREs were identified to associate at the base of the cup-shaped porosome [16]. It was demonstrated that in the presence of calcium, t-SNAREs and v-SNAREs in opposing bilayers interact in a circular array to form conducting pores. Later, it was further demonstrated that SNARE proteins are instrumental in bringing the opposing bilayers within a few ångströms of each other, allowing appropriate calcium bridging, leading to membrane fusion [22]. Thus, the molecular mechanism of SNARE-induced membrane fusion was solved. Following fusion of secretory vesicles at the base of porosomes, the molecular mechanism of secretory vesicle swelling for the expulsion of vesicular contents has also been determined [24–26]. With these findings the area of cell secretion has finally been resolved.

As mentioned above, cell secretion is a universal cellular process which regulates a number of processes, both at the cellular and physiological level. Its understanding has greatly impacted both biology and medicine. Although major discoveries in cellular transport and secretion are complete (as outlined in this minireview), what triggers the budding and fusion of intracellular transport vesicles, and how empty secretory vesicles are recycled, is not clearly understood. Future studies will address these questions, to provide an understanding of these cellular events.

## References

- 1 Ceccarelli B, Hurlbut WP, Mauro A: Depletion of vesicles from frog neuromuscular junctions by prolonged titanic stimulation. *J Cell Biol* 1972;54:30–38.
- 2 Dreifuss JJ: A review on neurosecretory granules: Their contents and mechanisms of release. *Ann NY Acad Sci* 1975;248:184–201.
- 3 Saras MP, Maylie-Pfenninger MF, Manzi RM, Jamieson JD: The effect of tunicamycin on development of the mammalian embryonic pancreas. *Dev Biol* 1981;87:1–15.
- 4 Ryan TA, Smith SJ, Reuter H: The timing of synaptic vesicle endocytosis. *Proc Natl Acad Sci USA* 1996;93:5567–5571.
- 5 Valentijn K, Gumkowski FD, Jamieson JD: The subapical actin cytoskeleton regulates secretion and membrane retrieval in pancreatic acinar cells. *J Cell Sci* 1999;112:81–96.
- 6 Zenisek D, Steyer JA, Feldman ME, Almers W: A membrane marker leaves synaptic vesicles in milliseconds after exocytosis in retinal bipolar cells. *Neuron* 2002;35:1085–1097.
- 7 Heidelberger R: ATP is required at an early step in compensatory endocytosis in synaptic terminals. *J Neuroscience* 2001;21:6467–6474.
- 8 Sudhof TC: The synaptic vesicle cycle: A cascade of protein-protein interactions. *Nature* 1995;375:645–653.
- 9 Fischer von Mollard G, Stahl B, Walch-Solimena C, Takei K, Daniels L, Khokhlatchev A, De Camilli P, Sudhof TC, Jahn R: Localization of Rab5 to synaptic vesicles identifies endosomal intermediate in synaptic vesicle recycling pathway. *Eur J Cell Biol* 1994;65(2):319–326.
- 10 Walch-Solimena C, Blasi J, Edelmann L, Chapman ER, Fischer von Mollard G, Jahn R: The t-SNAREs syntaxin 1 and SNAP-25 are present on organelles that participate in synaptic vesicle recycling. *J Cell Biol* 1995;128:637–645.
- 11 Schneider SW, Sritharan KC, Geibel JP, Oberleithner H, Jena BP: Surface dynamics in living acinar cells imaged by atomic force microscopy: Identification of plasma membrane structures involved in exocytosis. *Proc Natl Acad Sci USA* 1997;94:316–321.
- 12 Cho SJ, Quinn AS, Stromer MH, Dash S, Cho J, Taatjes DJ, Jena BP: Structure and dynamics of the fusion pore in live cells. *Cell Biol Int* 2002;26:35–42.
- 13 Cho SJ, Wakade A, Pappas GD, Jena BP: New structure involved in transient membrane fusion and exocytosis. *Ann NY Acad Sci* 2002; 971:254–256.
- 14 Cho SJ, Jeftinija K, Glavaski A, Jeftinija S, Jena BP, Anderson LL: Structure and dynamics of the fusion pores in live GH-secreting cells revealed using atomic force microscopy. *Endocrinology* 2002;143:1144–1148.
- 15 Jena BP, Cho SJ, Jeremic A, Stromer MH, Abu-Hamdah R: Structure and composition of the fusion pore. *Biophys J* 2003;84:1337–1343.
- 16 Jeremic A, Kelly M, Cho SJ, Stromer MH, Jena BP: Reconstituted fusion pore. *Biophys J* 2003; 85:2035–2043.
- 17 Clary DO, Griff IC, Rothman JE: SNAREs, a family of NSF attachment proteins involved in intracellular membrane fusion in animals and yeast. *Cell* 1990;61:709–721.
- 18 Söllner T, Whiteheart SW, Brunner M, Erdjument-Bromage H, Geromanos S, Tempest P, Rothman JE: SNAP receptors implicated in vesicle targeting and fusion. *Nature* 1993;362: 318–324.
- 19 Rothman JE, Söllner T: Throttles and dampers: Controlling the engine of membrane fusion. *Science* 1997;276:1212–1213.
- 20 Weber T, Zemelman BV, McNew JA, Westerman B, Gmachl M, Parlati F, Sollner TH, Rothman JE: SNAREpins: minimal machinery for membrane fusion. *Cell* 1998;92:759–772.
- 21 Cho SJ, Kelly M, Rognlien KT, Cho J, Hoerber JKH, Jena BP: SNAREs in opposing bilayers interact in a circular array to form conducting pores. *Biophys J* 2002;83:2522–2527.
- 22 Jeremic A, Kelly M, Cho JH, Cho SJ, Horber JKH, Jena BP: Calcium drives fusion of SNARE-apposed bilayers. *Cell Biol Int* 2004; 28:19–31.
- 23 Jena BP: Discovery of the porosome: Revealing the molecular mechanism of secretion and membrane fusion in cells. *J Cell Mol Med* 2004;8:1–21.
- 24 Jena BP, Schneider SW, Geibel JP, Webster P, Oberleithner H, Sritharan KC: Gi regulation of secretory vesicle swelling examined by atomic force microscopy. *Proc Natl Acad Sci USA* 1997;94:13317–13322.
- 25 Cho SJ, Sattar AK, Jeong EH, Satchi M, Cho JA, Dash S, Mayes MS, Stromer MH, Jena BP: Aquaporin 1 regulates GTP-induced rapid gating of water in secretory vesicles. *Proc Natl Acad Sci USA* 2002;99:4720–4724.
- 26 Abu-Hamdah R, Cho WJ, Cho SJ, Jeremic A, Kelly M, Ilie AE, Jena BP: Regulation of the water channel aquaporin-1: Isolation and reconstitution of the regulatory complex. *Cell Biol Int* 2004;28:7–17.
- 27 Taraska JW, Perrais D, Ohara-Imaizumi M, Nagamatsu S, Almers W: Secretory granules are recaptured largely intact after stimulated exocytosis in cultured endocrine cells. *Proc Natl Acad Sci USA* 2003;100:2070–2075.
- 28 Aravanis AM, Pyle JL, Tsien RW: Single synaptic vesicles fusing transiently and successively without loss of identity. *Nature* 2003;423: 643–647.
- 29 Tojima T, Yamane Y, Takagi H, Takeshita T, Sugiyama T, Haga H, Kawabata K, Ushiki T, Abe K, Yoshioka T, Ito E: Three-dimensional characterization of interior structures of exocytotic apertures of nerve cells using atomic force microscopy. *Neuroscience* 2000;101:471–481.
- 30 Thorn P, Fogarty KE, Parker I: Zymogen granule exocytosis is characterized by long fusion pore openings and preservation of vesicle lipid identity. *Proc Natl Acad Sci USA* 2004;101: 6774–6779.
- 31 Chandler DE, Heuser JE: Arrest of membrane fusion events in mast cells by quick-freezing. *J Cell Biol* 1980;86:666–674.
- 32 Alvarez De Toledo G, Fernandez-Chacon R, Fernandez JM: Release of secretory products during transient vesicle fusion. *Nature* 1993; 363:554–558.