Fusion Pore in Live Cells

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Earlier electrophysiological measurements on live secretory cells suggested the presence of fusion pores at the plasma membrane, where secretory vesicles fuse to release vesicular contents. Recent studies using atomic force microscopy demonstrate for the first time the presence of the fusion pore and reveal its morphology and dynamics at near-nanometer resolution and in real time.

The fusion of membrane-bound secretory vesicles at the cell plasma membrane and consequent expulsion of vesicular contents is a fundamental cellular process regulating basic physiological functions such as neurotransmission, enzyme secretion, and hormone release. Secretory vesicles dock and fuse at specific plasma membrane locations following secretory stimuli. Earlier electrophysiological studies on mast cells suggested the existence of “fusion pores” at the cell plasma membrane, which become continuous with the secretory vesicle membrane following stimulation of secretion (13). By using atomic force microscopy (AFM), the existence of the fusion pore was confirmed, and its structure and dynamics in both exocrine (16, 19) and neuroendocrine cells (7, 9) were determined at near-nanometer resolution and in real time.

Why had this new cellular structure (the fusion pore) eluded visualization in live cells for so long? The answer lies simply in the resolution limit of the light microscope, which is dependent on the wavelength of the light used, and hence the resolving power would be at best 300–400 nm. The recently discovered fusion pore in live cells is cone shaped, measuring 100–150 nm at its wide end and 15–30 nm in relative depth. As a result, it had evaded visual detection. With the development of AFM (4) and its improved capabilities to image biological samples at near-nanometer resolution, cellular structures such as the fusion pore and its dynamics could be examined at nanometer resolution and in real time (1, 2, 18). In AFM, a probe tip microfabricated from silicon or silicon nitride and mounted on a cantilever spring is used to scan the surface of the sample at a constant force (1). Either the probe or the sample can be precisely moved in a raster pattern by using an xyz piezo tube to scan the surface of the sample (5). The deflection of the cantilever measured optically is used to generate an isoforce relief map of the sample (2). AFM therefore allows imaging at nanometer resolution and in real time of live cells, subcellular structures, or single molecules submerged in physiological buffer solutions. Structure and dynamics of the fusion pore at nanometer resolution is just the first of many structures waiting to be identified in the living cell. This finding has opened the window to a new understanding of the workings of a living cell. In this review, the structure and dynamics of the fusion pore in live cells, as determined by using the AFM, is presented.

New cellular structure

Isolated live pancreatic acinar cells in physiological buffer, when imaged by using AFM (8, 19), reveal at the apical plasma membrane a group of circular “pits” measuring 0.4–1.2 μm in diameter, punctuated by smaller “depressions” within. Each depression averages ~100–150 nm in diameter (Fig. 1), and typically three to four depressions are located within a pit. The basolateral membranes of acinar cells are, however, devoid of either pits or depressions. High-resolution AFM images of depressions in live cells further reveal a cone-shaped morphology (Fig. 2). The depth of each depression cone measures ~15–30 nm. Similarly, both growth hormone (GH)-secreting cells of the pituitary gland and the chromaffin cell also possess pits and depression structures on their plasma membranes (7, 9), suggesting the universal presence of fusion pores in secretory cells.

Regulation and dynamics of depressions

Exposure of pancreatic acinar cells to a secretagogue (mastoparan) results in a time-dependent increase (20–35%) in depression diameter, followed by a return to resting size following completion of secretion (Fig. 3). However, no demonstrable change in pit size is detected during this time. Enlargement of depression diameter and an increase in its relative depth following exposure to secretagogues correlated with increased secretion. Exposure of pancreatic acinar cells to cytochalasin B, a fungal toxin that inhibits actin polymerization, results in 15–20% decrease in depression size and a consequent 50–60% loss in secretagogue-induced secretion. Results from these studies suggested that depressions are the fusion pores in pancreatic acinar cells. Furthermore, these studies demonstrated the involvement of actin in regulation of the structure and function of depressions.
Analogous to pancreatic acinar cells, examination of resting GH-secreting cells of the pituitary (7) and chromaffin cells of the adrenal medulla (9) also reveal the presence of pits and depressions on the cell plasma membrane. Depressions in resting GH cells measure 154 ± 4.5 nm (mean ± SE) in diameter. Exposure of the GH cell to a secretagogue resulted in a 40% increase (215 ± 4.6 nm; *P* < 0.01) in depression diameter but no appreciable change in pit size.

**Depressions are fusion pores**

Enlargement of depression diameter following exposure of acinar cells to a secretagogue correlated with increased secretion. Additionally, actin-depolymerizing agents known to inhibit secretion (19) resulted in decreased depression size and accompanied loss in secretion. These studies suggested depressions to be the fusion pores. However, a more direct determination of the function of depressions was required. Combining the use of a gold-conjugated antibody to a specific vesicular secretory protein with AFM provides the means to determine if secretion occurs at depressions (8, 7).

![FIGURE 1](image1.png)

**FIGURE 1.** Topology of the apical cell surface of an isolated live pancreatic acinar cell, observed by using atomic force microscopy. Scattered pits (one shown with dotted outline) and depressions (arrowheads) are identified. See Schneider et al. (19).

![FIGURE 2](image2.png)

**FIGURE 2.** Nanometer resolution of a single depression or fusion pore in a live pancreatic acinar cell. Note the cone-shaped fusion pore, with a 100- to 150-nm opening. See Cho et al. (8).

![FIGURE 3](image3.png)

**FIGURE 3.** Dynamics of depressions following stimulation of secretion. Top: a number of depressions within a pit in a live pancreatic acinar cell. The scan line across 3 depressions is represented graphically at middle and defines the diameter and relative depth of the depressions; the middle depression is represented by red arrowheads. Bottom: percentage of total cellular amylase release in the presence and absence of the secretagogue Mas7. Notice an increase in the diameter and depth of depressions, correlating with an increase in total cellular amylase release at 5 min after stimulation of secretion. At 30 min after stimulation of secretion, there is a decrease in diameter and depth of depressions, with no further increase in amylase release over the 5-min time point. No significant increase in amylase secretion or depression diameter were observed in resting acini or those exposed to the nonstimulatory mastoparan analog Mas17. See Schneider et al. (19).
strated (Fig. 4) (8). These studies confirm depressions to be the fusion pores in pancreatic acinar cells, where membrane-bound secretory vesicles dock and fuse to release vesicular contents (Fig. 5). Similarly in somatotrophs of the pituitary, gold-tagged GH-specific antibodies were found to be selectively localized at depressions following stimulation of secretion (7), again confirming depressions to be fusion pores.

Composition of the fusion pore

Although the molecular composition of the fusion pore (depression) remains to be established, our studies on the role of actin in the regulation of depression structure and dynamics clearly suggests actin to be a major component of the fusion pore complex. Target membrane proteins SNAP25 and syntaxin (t-SNARE) and secretory vesicle-associated membrane protein (v-SNARE) are part of a conserved protein complex involved in fusion of opposing bilayers (17, 20). Since membrane-bound secretory vesicles dock and fuse at depressions to release vesicular contents, it is reasonable to suggest that plasma membrane-associated t-SNAREs are part of the fusion pore complex. In the past decade, a number of studies demonstrated the involvement of cytoskeletal proteins in exocytosis, some directly interacting with SNAREs (3, 8, 10, 11, 14, 15). Actin and microtubule-based cytoskeleton have been implicated in intracellular vesicle traffic (11). Fodrin, which
was previously implicated in exocytosis (3), has recently been shown to directly interact with SNAREs (14). Recent studies demonstrate that α-fodrin regulates exocytosis through its interaction with the syntaxin family of proteins (14). The COOH-terminal coiled coil region of syntaxin interacts with α-fodrin, a major component of the submembranous cytoskeleton. Similarly, vimentin filaments interact with SNAP23/25 and control the availability of free SNAP23/25 for assembly of the SNARE complex (10). Additionally, our recent studies (unpublished observations) demonstrate a direct interaction between actin and SNAREs. Results from these studies suggest that vimentin, α-fodrin, actin, and SNAREs may all be part of the fusion pore complex. However, purification and further biochemical characterization of the fusion pore are required to determine its composition. Additional proteins such as v-SNARE (VAMP or synaptobrevin), synaptophysin, and myosin may associate when the fusion pore establishes continuity with the secretory vesicle membrane. The globular tail domain of myosin V is its binding site, and VAMP is bound to myosin V in a calcium-independent manner (15). Further interaction of myosin V with syntaxin requires calcium and calmodulin. Studies suggest that VAMP acts as a myosin V receptor on secretory vesicles and regulates formation of the SNARE complex (15). Furthermore, interaction of VAMP with synaptophysin and myosin V has been demonstrated (8).

**Perspectives and conclusion**

Fusion pores or depressions in pancreatic acinar or GH-secreting cells are cone-shaped structures at the plasma membrane, with a 100- to 150-nm-diameter opening. Membrane-bound secretory vesicles ranging in size from 0.2 to 1.2 μm in diameter dock and fuse at depressions to release vesicular contents. Following fusion of secretory vesicles at depressions, only a 20–35% increase in depression diameter is demonstrated. It is therefore reasonable to conclude that secretory vesicles “transiently” dock and fuse at depressions. In contrast to accepted belief, if secretory vesicles were to completely incorporate at depressions, the fusion pore would distend much wider than what is observed. Furthermore, if secretory vesicles were to completely fuse at the plasma membrane, there would be a loss in vesicle number following secretion. Examination of secretory vesicles within cells before and after secretion demonstrates that, although the total number of secretory vesicles remains unchanged following secretion, the number of empty and partially empty vesicles increases significantly, supporting the occurrence of transient fusion (6). Earlier studies on mast cells also demonstrated an increase in the number of spent and partially spent vesicles following stimulation of secretion, without any demonstrable increase in cell size (12). Other supporting evidence favoring transient fusion is the presence of neurotransmitter transporters at the synaptic vesicle membrane. These vesicle-associated transporters would be of little use if vesicles were to fuse completely at the plasma membrane to be endocytosed at a later time. Although the fusion of secretory vesicles at the cell plasma membrane occurs transiently, complete incorporation of membrane at the cell plasma membrane takes place when cells need to incorporate signaling molecules like receptors, second messengers, and ion channels.

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**References**