Porosomes, the universal secretory machinery at the cell plasma membrane, are cup-shaped supramolecular lipoprotein structures, where membrane-bound vesicles transiently dock and fuse to release intravesicular contents during cell secretion. In this review, the discovery of the porosome and its structure, dynamics, composition, and functional reconstitution are outlined. Furthermore, the architecture of porosome-like structures such as the "canaliculi system" in human platelets and various associated structures such as the T-bars at the Drosophila synapse or the "beams," "ribs," and "pegs" at the frog neuromuscular junction, each organized to facilitate a certain specialized secretory activity, are briefly discussed.

Cells synthesize and release products such as hormones, growth factors, neurotransmitters, or digestive enzymes by the process of secretion and do so in a highly regulated manner. Cellular cargo destined for secretion are packaged and stored in membranous sacs or vesicles that, when required by cells to be released to the outside, dock and establish continuity at the base of specialized plasma membrane structures called porosomes (FIGURE 1) (13, 15, 16, 18, 20–22, 51, 53, 56, 66, 75, 97). In the past decade, the isolation of the "porosome complex" and determination of its composition, its structure, and its dynamics at nm resolution and in real time, and its functional reconstitution into artificial lipid membrane (13, 15, 16, 18, 20–22, 51, 53, 56, 66, 75, 97) has greatly advanced our understanding of the secretory process in cells (1, 3–5, 23, 27, 30, 46–48, 50, 62, 71, 74, 84, 91, 92, 101, 113, 114, 119, 120). Interestingly in nature, even single-cell organisms have developed specialized and sophisticated secretory machinery, such as the secretion apparatus of Escherichia coli (FIGURE 2) (63), the contractile vacuoles in paramecium (43), or the various types of secretory structures in bacteria (70). Therefore, it is not surprising that mammalian cells too have evolved such highly sophisticated and specialized cup-shaped supramolecular lipoprotein structures—the porosome complexes and porosome-like "canaliculi system" in human platelets (FIGURE 2) (115, 116), for the precise and regulated docking, fusion, and release of intravesicular contents from cells. In addition to the presence of porosome as the universal secretory machinery at the cell plasma membrane, various forms of specialized structures, such as the T-bars at the Drosophila synapse (FIGURE 2) (82) or the "beams," "ribs," and "pegs" at the frog neuromuscular junction (FIGURE 2) (42), have evolved, each organized to facilitate a certain specialized secretory activity in different cell types.

Porosomes were first discovered in the mid 1990s (97) in acinar cells of the exocrine pancreas and subsequently in growth hormone (GH)-secreting cells of the pituitary gland (13), in chromaffin cells (16), in β-cells of the endocrine pancreas (53), in neurons (21), and in astrocytes (75). Porosome size ranges from ~100 to 180 nm in pancreatic acinar cells to ~12 to 17 nm in neurons and astrocytes. Several proteins constitute the porosome complex (51), including structural proteins such as actin, ion channels such as the chloride and calcium channels (51, 18), and the t-SNARE membrane fusion proteins located at its base (60), where secretory vesicles transiently dock and fuse to expel their contents. During cell secretion, the porosome opening dilates to allow release of secretory products, returning to its resting size following completion of the process (13, 15, 97). Accordingly, secretory vesicles can undergo multiple rounds of docking-fusion-release cycles, generating partially empty and empty vesicles as observed in electron micrographs. The empty vesicles can then be recycled via endosomes and the Golgi complex. In contrast to transient fusion, if secretory vesicles were to completely merge at the porosome base, it would result in a total obliteration of the structure, since the surface area of secretory vesicles are several-fold larger than that of the porosome complex. In human platelets, where a one-time massive and complete degranulation need to occur following cell activation, the porosome has become invaginated into the cell giving rise to the "canaliculi system" (115, 116) and, as a consequence, greatly increased the surface area for a large population of secretory vesicles to simultaneously dock and fuse for release. Similarly, in a variety of specialized nerve endings, electron-dense structures like the "beams," "ribs," and "pegs" at the frog neuromuscular junction (42), the T-bars at the Drosophila neuromuscular junction (82), the long projections in skate
electroceptor (77), and in rat photoreceptor ribbon synapse (26) have all developed, and it has been suggested that they function primarily as vesicle docking platforms (118) and to provide a large, readily available pool of synaptic vesicles at the release site (118). In the past five decades, our understanding of the secretory process.

In the 1950s, the releasable quanta was ever, subsequently neurotransmitter (64) and Folkow (65) proposed that limits by the nerve packet may act vesicle content nonneuronal substrate to the accreting cell secreting vesicle vesicle was to transmit “kiss-and-run” the vesicle model during secretion greatly helped manage to project the cell plasma contents during growing body fusion to become variety of cells.

Porosome Dynamics
Reconstituted

Porosomes were exocrine pancreas cells having Pancreatic acini and stored the branous sacs.

FIGURE 1
A: a high-resolution image in a live plasma membrane (ZG head) (60). C: an exocrine pancreas shown at zero arrowhead pointing following stimulation with a synaptic vesicles for increasing the neuronal protein content (115). B: a synaptic AFM micrograph of the neuronal protein content (115). C: a synaptic AFM micrograph of the neuronal protein content (115).

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secretory process in cells has greatly advanced. In the 1950s, the release of neurotransmitters of constant size or quanta was proposed by Katz and coworkers; however, subsequent experimental data concerning neurotransmitter release mechanisms in the 60s, Katz (64) and Folkow (32) brilliantly hypothesized and proposed that limitation of the quantal packet may be set by the nerve membrane, in which case the size of the packet may actually correspond to just a fraction of the vesicle content (31-33). In confirmation, studies on nonneuronal cells using electron microscopy demonstrated the accumulation of partially spent vesicles following cell secretion (12, 73, 76). Partial discharge from secretory vesicles could only be explained if vesicles were to transiently dock and fuse (64, 55), also termed “kiss-and-run” (9), instead of the complete merger of the vesicle membrane at the cell plasma membrane during secretion. The discovery of the porosome has greatly helped in understanding how secretory vesicles manage to precisely and transiently dock and fuse at the cell plasma membrane to release intravesicular contents during cell secretion (56-58). There is now a growing body of evidence demonstrating transient fusion to be the primary method of secretion in a wide variety of cells (6, 10, 72, 96, 104, 110).

Porosome: Discovery, Structure, Dynamics, Composition, Reconstitution

Porosomes were first discovered in acinar cells of the exocrine pancreas (97), which are polarized secretory cells having an apical and a basolateral end. Pancreatic acinar cells synthesize digestive enzymes and stored them within 0.2-1.2 μm in diameter membrane-bound sacs or vesicles, calledzymogen granules (ZGs), located apically. Following a secretory stimulus, ZGs dock and fuse with the apical plasma membrane to release their contents to the outside. In contrast to neurons, where secretion of neurotransmitters occurs in milliseconds, pancreatic acinar cells secrete digestive enzymes over minutes following a secretory stimulus and therefore were ideal for investigating the molecular steps involved in cell secretion. In the mid 1990s, atomic force microscopy (AFM) studies on live pancreatic acinar cells were performed to evaluate, at high resolution, the surface topology of the apical plasma membrane in both resting and stimulated cells. Circular “pits” measuring 0.4-1.2 μm, and typically 3-4 “depressions” or porosomes were observed following mastoparan (Mast 7) results in a time-dependent increase in total cellular amylase release (100-180 nm in diameter within each pit, are found at the apical end of the cell (97). The basolateral membrane in acinar cells is devoid of such pit or porosome structures. High-resolution AFM images of porosomes in live acinar cells further reveal a cone-shaped morphology, with the depth of each cone measuring 15-35 nm. Similarly, examination of resting GTH-secreting cells of the pituitary gland (13), neurons (21), astrocytes (75), β-cells of the endocrine pancreas, mast cells, and chromatin cells of the adrenal medulla (53) all have demonstrated the presence of porosomes as permanent structures at the cell plasma membrane.

Exposure of pancreatic acinar cells to the secretagogue mastoparan (Mast 7) results in a time-dependent increase (25-45%) in both the diameter and relative depth of porosomes (FIGURE 1D). Studies demonstrate that porosomes return to their resting size on completion of cell secretion (FIGURE 1D) with no demonstrable change in pit size (15, 97). Enlargement of porosome diameter and relative depth follows exposure to the secretagogue and correlates with cell secretion (FIGURE 1D). Exposure of acinar

FIGURE 1: Porosome: the secretory portal in mammalian cells
A. A high-resolution AFM micrograph shows a single pit with four 100–180 nm porosomes within (blue arrow) at the apical plasma membrane in a live pancreatic acinar cell (97). B: An electron micrograph depicting a porosome (blue arrowhead) close to a microvilli (MV) at the apical plasma membrane (PM) of a pancreatic acinar cell. Note the association of the porosome membrane (yellow arrowhead) and the zymogen granule membrane (ZGM) (red arrow head) of a docked ZG (red). Cross section of a circular complex at the mouth of the porosome is seen (blue arrowhead) (60). C: Schematic diagram of pits (yellow arrow) and porosomes (blue arrow) at the cell plasma membrane. ZGs, the secretory vesicles in exocrine pancreas, docked and transiently fuse at the porosome base to expel intravesicular contents (77). D: Several porosomes within a pit are shown at zero time and 5 min and 30 min following stimulation of secretion. Section analysis across three porosomes is shown at top, with the blue arrowhead pointing at a porosome (97). Note the dilation of the porosome at the 5 min time point and its return to near resting size after 30 min following stimulation of secretion. E: % total cellular amylase release in the presence (yellow bars) and absence (blue bars) of the secretagogue. Max -7. Note the increase in porosome diameter, correlating with an increase in total cellular amylase release at 5 min following stimulation of secretion. At 30 min following a secretory stimulus, there is a decrease in porosome diameter and no further increase in amylase secretion beyond the 5 min time point. No significant changes in amylase secretion or porosome diameter were observed in control cells in either the presence or absence of the nonstimulatory mastoparan analog (Max-17) (97). F: Electron micrograph of a porosome (blue arrowhead) at the nerve terminal, in association with a synaptic vesicle (SV) at the presynaptic membrane (Pres-SM). Notice the central plug-like structure at the neuronal porosome opening (21). G: AFM micrograph of a neuronal porosome in physiological buffer, also showing the central plug (blue arrowhead) at its opening. The central plug in the neuronal porosome complex may regulate its rapid close-open conformation during neurotransmitter release. The neuronal porosome is an order of magnitude smaller (10–15 nm) compared with porosome in the exocrine pancreas (100–180 nm). Note the central plug and eight interconnected ridges within the porosome complex (20). H: Electron density maps of negatively stained electron micrographs of isolated neuronal porosome protein complexes. Note the ~12 nm complex exhibiting a circular profile and having a central plug, with eight interconnected protein densities at the rim of the complex (22). Bar = 5 nm. I: Atomic force micrograph of a pit and three porosomes within (one shown by the blue arrowhead) in pancreatic acinar cell and the specific immunolocalization of amylase-specific immunoglobulin (yellow spots) demonstrating amylase secretion through the structure (15). J: Electron micrograph of liposome- and mastoparan-reconstituted porosome complex isolated from pancreatic acinar cell. Note the cup-shaped basket-like morphology of the porosome complex reconstituted in a 500-nm lipid vesicle. Bar = 100 nm (60). K: The lipid bilayer-reconstituted porosome complex is functional. Top shows a schematic drawing of the EPC9 setup for electrophysiological measurements. Isolated zymogen granules added to the cis compartment of the bilayer chamber dock and fuse with the reconstituted porosomes at the bilayer and are detected as an increase in capacitance and current activity and a concomitant time-dependent release of amylase to the trans compartment of the bilayer chamber determined using immunoblot assay (60).
cells to cytochalasin B, a fungal toxin that inhibits actin polymerization and cell secretion, results in a 15–20% decrease in porosome size and a consequent 50–60% loss in secretion (97). Results from these studies suggested porosomes to be the secretory portal in cells. These studies further demonstrated the involvement of actin in regulation of both the structure and function of porosomes. Similarly, following stimulation of secretion, porosomes in resting GH cells measuring 154 ± 4.5 nm (mean ± SE) in diameter result in a 40% increase in porosome diameter (215 ± 4.6 nm; P < 0.01), with no appreciable change in pit depth (13). The direct determination that porosomes are the portals via which secretory proteins are discharged from cells finally came from immuno-AFM studies (13, 15). Localization at porosomes of gold-conjugated antibody to secretory proteins provided direct evidence that secretion occurs through them (FIGURE 1I) (13, 15). ZOs contain the stach digesting enzyme amylase. AFM micrographs of the localization of gold-tagged amylase-specific antibodies at the porosome-opening following stimulation of cell secretion (15, 66) demonstrate porosomes to be the cells’ secretory portals. Similarly, gold-tagged growth hormone-specific antibody bound to selectively localize at porosomes following stimulation of secretion (13) establishes them to be the secretory portals in GH cells. The morphology of the porosome complex facing the cytosolic compartment in exocrine pancreas (51), and in neurons (21), has also been determined at near-nm resolution in subcellular fractions. AFM studies on isolated plasma membrane preparations in near physiological conditions revealed scattered circular disks measuring 8.5–1 μm in diameter, with inverted cup-shaped structures (51). The inverted cups at the cytosolic compartment of isolated pancreatic plasma membrane preparations range in height from 10 to 15 nm, and on several occasions ZGs ranging in size from 0.4–1 μm in diameter are observed in association with one or more of these inverted cups, suggesting them to be porosomes. To further confirm that the cup-shaped structures are indeed porosomes, immuno-AFM studies were carried out. Target membrane proteins SNAP-23/-25 (90) and syntaxin (t-SNARE) (7) and secretory vesicle-associated membrane protein v-SNARE or VAMP (109) are part of the conserved protein complex involved in the fusion of opposing bilayers in the presence of calcium (14, 17, 19, 61, 79, 117). If porosomes are the secretory sites for vesicle docking and fusion, then plasma membrane-associated t-SNAREs should localize at the base of the structure facing the cytosol. The t-SNARE protein SNAP-23 had previously been reported in pancreatic acinar cells (37). In agreement, a polyclonal mouse-specific SNAP-23 antibody recognizing a single 23-kDa protein in immunoblots of resolved pancreatic plasma membrane fraction demonstrates selective localization to the porosome base, confirming the inverted cups to be porosomes (51).

The morphology of the pancreatic porosome complex has been further evaluated using transmission electron microscopy (TEM) (60). TEM studies confirm the porosome to possess a cup-shaped structure, with similar dimensions as determined from AFM measurement. TEM micrographs further demonstrate pancreatic porosomes to possess a basket-like morphology, with three lateral and several vertically arranged rings. A ring at the base of the complex is further identified (22, 60) and hypothesized to represent t-SNAREs or t-/v-SNARE complex organized in a circular array, since these proteins have been previously shown to require both calcium and diisothiocyanate for binding in a calcium-independent manner (88). Further interaction of myosin V with syntaxin had previously been shown to require both calcium and calmodulin. It had also been suggested that VAMP acts as a myosin V receptor on secretory vesicles and may regulate formation of the SNARE complex (87). Interaction of VAMP with syntaxin and myosin V had also been reported (88). In agreement with these earlier findings, our studies (20, 21, 51, 60) demonstrate the association of SNAP-23, syntaxin 2, v-SNARE complex, actin, vimentin, and calcium channels b3 and α1c, together with the SNARE regulatory protein NSF, at the porosome complex (20, 21, 31, 60). Additionally, chloride ion channels CIC2 and CIC3 were also identified as part of the porosome complex. Isoforms of a number of other proteins identified as components of the porosome complex have also been reported using 2D-BAC gels electrophoresis (51). For example, three isoforms each of the calcium ion channel and vimentin have been identified in pig. Using yeast two-hybrid methods, porosome complex has been found to interact with porosomes and bilayer structures such as synaptic vesicles from the exocrine pancreas (21, 41), and in neurons (21), and in neurons (21). The morphology of the porosome complex has also been proposed to regulate membrane fusion and regulation of secretion of synaptic vesicles from the exocrine pancreas (21, 41), and in neurons (21), and in neurons (21).

Studies demonstrated that the requirement for calcium channel activity increases following stimulation of secretion (97). Results from these studies further demonstrated the involvement of calcium in regulation of both the structure and function of porosomes. Similarly, following stimulation of secretion, porosomes in resting GH cells measuring 154 ± 4.5 nm (mean ± SE) in diameter result in a 40% increase in porosome diameter (215 ± 4.6 nm; P < 0.01), with no appreciable change in pit depth (13). The direct determination that porosomes are the portals via which secretory proteins are discharged from cells finally came from immuno-AFM studies (13, 15). Localization at porosomes of gold-conjugated antibody to secretory proteins provided direct evidence that secretion occurs through them. Similarly, gold-tagged growth hormone-specific antibody bound to selectively localize at porosomes following stimulation of secretion establishes them to be the secretory portals in GH cells. The morphology of the porosome complex facing the cytosolic compartment in exocrine pancreas and neurons (21) has also been determined at near-nm resolution in subcellular fractions. AFM studies on isolated plasma membrane preparations in near physiological conditions revealed scattered circular disks measuring 8.5–1 μm in diameter, with inverted cup-shaped structures. The inverted cups at the cytosolic compartment of isolated pancreatic plasma membrane preparations range in height from 10 to 15 nm, and on several occasions ZGs ranging in size from 0.4–1 μm in diameter are observed in association with one or more of these inverted cups, suggesting them to be porosomes. To further confirm that the cup-shaped structures are indeed porosomes, immuno-AFM studies were carried out. Target membrane proteins SNAP-23/-25 and syntaxin (t-SNARE) and secretory vesicle-associated membrane protein v-SNARE or VAMP are part of the conserved protein complex involved in the fusion of opposing bilayers in the presence of calcium (14, 17, 19, 61, 79, 117). If porosomes are the secretory sites for vesicle docking and fusion, then plasma membrane-associated t-SNAREs should localize at the base of the structure facing the cytosol. The t-SNARE protein SNAP-23 had previously been reported in pancreatic acinar cells. In agreement, a polyclonal mouse-specific SNAP-23 antibody recognizing a single 23-kDa protein in immunoblots of resolved pancreatic plasma membrane fraction demonstrates selective localization to the porosome base, confirming the inverted cups to be porosomes.
Regulation of Intravesicle Content Expulsion at the Porosome Complex

Regulation of intravesicle contents at the porosome complex is a fundamental process in cell secretion. Recent studies have demonstrated the intricate mechanisms involved in the expulsion of intravesicular contents following cellular activation. This process is crucial for the proper functioning of secretory cells, as evidenced by the role of porosomes in neurotransmitter release from synaptic vesicles in neurons and the role of ATP-sensitive K+ channels at the ZG membrane in exocrine pancreas cells.

The porosome complex is a supramolecular structure that mediates vesicle exocytosis. It consists of multiple subunits, including t-SNAREs and ARFs, which are essential for vesicle docking and fusion. The regulation of porosome activity is critical for maintaining cellular homeostasis and is under tight control by various signaling pathways.

One of the key events in porosome function is the regulation of intravesicle content expulsion. This process is tightly regulated and involves a complex interplay between the mechanical forces generated at the vesicle-membrane interface and the biochemical signals that modulate porosome activity.

Intravesicle content expulsion is a two-step process: vesicle docking and fusion, followed by vesicle deflation. Vesicle docking involves the formation of a stable complex between the vesicle and the zymosome, which results in the temporary insertion of the vesicle membrane into the zymosome membrane. Fusion of the vesicle and zymosome membranes is then achieved, allowing the transfer of vesicle contents into the zymosome compartments.

Following fusion, vesicle deflation occurs, which is mediated by the activation of ATP-sensitive K+ channels. This process is essential for the proper expulsion of vesicle contents and the maintenance of cellular integrity.

Studies have shown that the regulation of vesicle deflation involves the activation of ATP-sensitive K+ channels, which are known to be modulated by various signaling pathways, including G protein-coupled receptors (GPCRs) and other intracellular signaling molecules.

The porosome complex is therefore a critical regulator of vesicle exocytosis, and its proper function is essential for the normal operation of secretory cells. Understanding the regulatory mechanisms that govern porosome function is crucial for advancing our knowledge of cell secretion and for developing new therapeutic strategies for diseases associated with altered secretion mechanisms.
mastoparan (Mas7) in the presence of [γ-32P]GTP demonstrates a significant increase in swelling and GAPase activity in ZG over control inactive mastoparan (Mas17), confirming the presence and activity of Gi proteins at the ZG membrane (52). Similarly, Mas7 has also been demonstrated to stimulate synaptic vesicle swelling (59).

It has been reported that vH+-ATPase present at the plasma membrane results in swelling of synaptic vesicles (60, 65). Swelling of synaptic vesicles is typically observed following GTP exchange on transducin (52). Mastoparan (Mas7) in the presence of [γ-32P]GTP demonstrates a significant increase in swelling and GAPase activity in ZG over control inactive mastoparan (Mas17), confirming the presence and activity of Gi proteins at the ZG membrane (52). Similarly, Mas7 has also been demonstrated to stimulate synaptic vesicle swelling (59).

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Furthermore, mastoparan (Mas7), in G0-G1, inhibitory effects on synaptic vesicle swelling are not required for vH+-ATPase activity (102). In agreement with this, vH+-ATPase has been reconstituted into liposomes and was found to contribute to the swelling of synaptic vesicles (60, 65)

The generation of neurotransmitter is a complex process that involves the release of vesicles containing neurotransmitter. The exocytosis of vesicles is facilitated by the activity of the vH+-ATPase, which drives the movement of ions across the membrane, thereby facilitating the release of neurotransmitter.

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sympathetic vesicle membrane (44, 102) is responsible for the generation of the electrochemical H$^+$ gradient (pH 5.2–5.5) within vesicles (34, 83), required for transport of neurotransmitters into the vesicle lumen. In addition to the established role of vH+-ATPase in neurotransmitter transport into synaptic vesicles, vH+-ATPase has also been suggested to participate in the secretion of the stored neurotransmitters (86, 93). Furthermore, guanine nucleosides have been reported to influence the glutamate-induced cellular response via diverse trophic, proliferative, and modulatory effects on neurons (95). Since synaptic vesicle swelling is G$_m$-mediated and is required for cell secretion (65), the involvement of vH+-ATPase at the synaptic vesicle membrane (44, 102), in G$_m$-mediated water gating through the AQP-6 channels resulting in vesicle swelling, was explored (100). In agreement, synaptic vesicle-associated vH+-ATPase is found to be required for GTP/G$_m$-mediated swelling of synaptic vesicles. The sequence of events, however, involving vH+-ATPase and all associated players, remains to be determined. Since mastoparan behaves like an activated β-adrenergic receptor that stimulates vesicle swelling, it is possible that secretory vesicle swelling occurs via an internal agonist-β-adrenergic receptor-PKA-G protein-PLA$_2$-mediated pathway, resulting in the elevation of intravesicular [H$^+$] concentration, which then leads to active water entry via water channels at the synaptic vesicle membrane.

Measurements of intracellular ZG size, further reveals that different vesicles swell differently following a secretory stimulus (65). This differential swelling among ZGs within the same cell may explain why, following stimulation of cell secretion, some intracellular ZGs express less vesicular content than others and hence have discharged more of their contents. To determine precisely the role of swelling in vesicle-plasma membrane fusion and in the expulsion of intravesicular contents, electrophysiological ZG-reconstituted lipid bilayer fusion assays (60, 65) have been performed. Similar to what is observed in live acinar cells, each isolated ZG responds differently to the same swelling stimulus (11, 52, 65). These studies demonstrate that, following stimulation of cell secretion, ZGs within pancreatic acinar cells swell, followed by a release of intravesicular contents through porosomes (60, 65) and a consequent shrinkage of the vesicle. On the contrary, isolated ZGs stay swollen following GTP exposure since there is no release of the intravesicular contents (11, 52, 65). The role of vesicle swelling in membrane fusion and in the expulsion of intravesicular contents has been determined using electrophysiological bilayer fusion assays (60, 65). Reconstitution of the porosome into the lipid membrane results in a small increase in capacitance, possibly due to the increase in membrane surface area contributed by incorporation of porosome complexes, ranging in size from 100 to 180 nm in diameter (60, 65).

Isolated ZGs, when added to the cis compartment of the bilayer chamber, fused at the porosome-reconstituted lipid membrane, and are detected as a step increase in membrane capacitance. However, even after 15 min of ZG addition to the cis compartment of the bilayer chamber, little or no release of the intravesicular enzyme α-amylase is detected in the trans compartment. On the contrary, exposure of ZGs to 20 μM GTP induced swelling (11, 52) and results both in the potentiation of fusion as well as a robust expulsion of α-amylase into the trans compartment of the bilayer chamber. These studies demonstrate that, during cell secretion, secretory vesicle swelling is required for the efficient discharge of intravesicular contents. Within minutes or even seconds following stimulation of secretion, empty and partially empty secretory vesicles accumulate within cells (12, 73). Following addition of ZGs to the cis compartment of the bilayer chamber, membrane capacitance continues to increase; however, little or no detectable secretion occurred even after 15 min, suggesting that either variable degrees of vesicle swelling or repetitive cycles of fusion and swelling of the same vesicle, or both, may operate during cell secretion. Under these circumstances, empty and partially empty vesicles could be generated within cells. To test this hypothesis, two key parameters were examined (65): 1) whether the extent of swelling is same for all ZGs exposed to a certain concentration of GTP and 2) whether there is a correlation between the extent of swelling and the quantity of intravesicular contents expelled. The answer to the first question was clearly yes; different ZGs respond to the same stimulus differently (65). Studies reveal that different ZGs within cells or in isolation undergo different degrees of swelling, even though they are exposed to the same stimuli (carbamylcholine for live pancreatic acinar cells or GTP for isolated ZGs). The requirement of ZG swelling for expulsion of vesicular contents was further confirmed when the GTP dose-dependent increase in ZG swelling (65) translated into increased secretion of α-amylase. Although higher GTP concentrations elicited an increased ZG swelling, the extent of swelling between ZGs once again varied. Similar to ZGs, exposure of synaptic vesicles to 20 μM GTP also results in an increase in synaptic vesicle swelling (65) and, following exposure to Ca$^{2+}$, results in the transient fusion of synaptic vesicles at the porosome, expulsion of intravesicular contents, and the consequent decrease in synaptic vesicle size (65). Additionally, as observed in ZG, not all synaptic vesicles respond to a swelling stimulus, and, if they do, they exhibit different swelling responses to the same stimuli. This differential response of synaptic vesicles within the same nerve ending may dictate and regulate the potency and efficacy of neurotransmitter release at the nerve terminal (65), explaining how partially empty vesicles are generated in cells (12, 73) following secretion. The generation of empty secretory vesicles contributes by incorporation of porosome complexes, ranging in size from 100 to 180 nm in diameter (60, 65).
vesicles could result from multiple rounds of fusion-swelling-expulsion cycles during the secretory process. Another process termed "compound" secretion (78, 81) may also contribute to partial release of synaptic vesicles measuring 0.2–1.2 μm in diameter following the docking and fusion of secretory machinery in cells. Exocytosis from excocrine and neuroendocrine cells measure 100–180 μm in diameter, demonstrating that vesicles transiently dock and fuse at the base of the porosome complex to release their contents. The discovery of the porosome and an understanding of its structure and function at nanoscale resolution and in real time in live cells, its composition, and its functional reconstitution in artifical lipid membrane has greatly advanced our understanding of cell secretion. It is evident that the secretory process in cells is well coordinated, highly regulated, and finely tuned supramolecular orchestra.

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A review of the secretory pathway: from molecular biology to cellular processes

A. Diez, J. Eferbeck, M. Jena, M. Jena, and B. Jena

The secretory pathway is a complex series of molecular and cellular events that allow cells to secrete proteins and other biological molecules. This process is critical for a wide range of cellular functions, including signal transduction, hormone release, and immune response. The review article highlights recent advances in our understanding of the secretory pathway, focusing on the molecular mechanisms that regulate vesicle trafficking and fusion.

Key Concepts

1. **Molecular SNAREs**
   - SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) are a family of proteins that mediate membrane fusion in the secretory pathway.

2. **SNAREpins**
   - SNAREpins are complexes of SNAREs that form upon the docking of vesicles to target membranes.

3. **Molecular Motors**
   - Molecular motors, such as myosin V, are involved in the transport of vesicles along microtubules and actin filaments.

4. **Regulatory Proteins**
   - Regulatory proteins, such assyntaxin 1A and synaptosomal-associated protein 23 (SNAP-23), modulate SNARE complex formation and disassembly.

5. **Calcium Signaling**
   - Calcium ions play a crucial role in the activation of exocytosis.

6. **G Protein-Coupled Receptors (GPCRs)**
   - GPCRs are a class of receptors that activate heterotrimeric G proteins, leading to the activation of effector proteins, such as adenylate cyclase and protein kinase A.

7. **Perspectives**
   - The integration of single-molecule techniques with other imaging methods, such as atomic force microscopy and super-resolution microscopy, offers new opportunities to study the dynamic processes of the secretory pathway in real time.

The review article emphasizes the importance of understanding the secretory pathway not only for basic research but also for the development of therapeutic strategies targeting this process in various diseases, such as diabetes, neurological disorders, and cancer.