Facilitating Cellular Secretion

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.
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 past five decades, the release or quanta was shown by the nerve packet may activate vesicle content and then trigger nonneuronal secretory vesicles to transmit “kiss-and-run” to the vesicle membrane during secretion, greatly helping to manage to prevent the cell plasma membrane contents during growing body fusion to be the variety of cells.

Porosome Dynamics

Reconstituted Porosomes in Exocrine Pancreatic Acinar Cells

Pancreatic acinar cells have branous sacs

FIGURE 1. Porosomes in exocrine pancreatic acinar cells having branous sacs.

A. a high-resolution image of a live plasma membrane (ZG head) (60). B. a section of exocrine pancreas shown at zero time point following stimulation with a synthetic agonist in the presence of mag and stored the neuronal p order of magnitude of magnified ridges. C. some protein complexes at the rim of the structure (117) in pancreatic acini, the nucleus-like structure, and some complexes added to the cell increase in cap determined using 10.220.33.5 on June 18, 2017 http://physiologyonline.physiology.org/ Downloaded from
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; how-
ever, subsequent early experimental data concerning
neurotransmitter release mechanisms in the 60s, Katz
(64) and Follok (32) brilliantly hypothesized and pro-
pose 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 demon-
strated the accumulation of partially spent vesicles fol-
lowing cell secretion (12, 73, 76). Partial discharge from
secretory vesicles could only be explained if vesicles were
to transiently dock and fuse (44, 50), 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, 108, 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 mem-
branous sacs or vesicles, called zymogen 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
nerve, 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 resolu-
tion, the surface topology of the apical plasma mem-
brane in both resting and stimulated cells. Circular
“pits” measuring 0.4–1.2 μm, and typically 3–4 “depres-
sions” or porosomes (FIGURE 1, A–C) measuring
100–180 nm in diameter within each pit, are found at
the apical end of the cell (97). The basolateral mem-
brane 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 mor-
phology, with the depth of each cone measuring 15–35
nm. Similarly, examination of resting GH-secreting
cells of the pituitary gland (13), neurons (21), astro-
cytes (75), β-cells of the endocrine pancreas, mast
cells, and chromaffin cells of the adrenal medulla (53)
al have demonstrated the presence of porosomes as
permanent structures at the cell plasma membrane.

Exposure of pancreatic acinar cells to the secreta-
gogue mastoparan (Mast 7) results in a time-depend-
et 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

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**FIGURE 1** Porosome: the secretory portal in mammalian cells

A: a high-resolution AFM micrograph shows a single pit with four 100– to 180-nm porosomes within (blue arrowhead) at the apical plasma mem-
brane 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 arrowhead) of a disked ZG (inset). C: a scheme diagram of pits (yellow arrow) and porosomes (blue arrow) at the cell plasma membrane. ZGs, the secretory vesicles in
exocrine pancreas, called dock and transiently fuse at the porosome base to expel intravesicular contents (97). D: isolated zymogen granules 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 dilatation 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
Mast 7. Note the increase in porosome diameter, correlating with an increase in total cellular amylase release at 5 min following stimulation of secre-
tion. At 30 min following a secretory stimulus, there is a decrease in porosome diameter and no further increase in amylase secretion beyond the 5-
minute 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 (Mast 17) (97). E: a scheme cartoon of a porosome (blue arrowhead) at the nerve terminal, in association
with a synaptic vesicle (SV) at the pre synaptic membrane (Pre-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 intercon-
ected ridges within the porosome complex (20). H: electron density maps of negatively stained electron micrographs of isolated neuronal poro-
some protein complex. Note the ~12-nm complex exhibiting a circular profile and having a central plug, with eight interconnected protein densi-
ties at the rim of the complex (22). Bars = 5 nm. I: atomic force micrograph of a pit and three porosomes within (row 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 (10). J: electron micrograph of liposome-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 poro-
some 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).

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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 significant change in porosome height (13). The direct determination that porosomes are the portals via which secretory products 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 starch 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 found 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 to 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 reveal 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 (96) and syntaxin (t-SNARE) (7) and secretory vesicle-associated membrane protein v-SNARE or VAMP (199) are part of the conserved protein complex involved in the fusion of opposing bilayers in the presence of calcium (14). In the past decade, a number of studies demonstrated the involvement of cytoskeletal proteins in cell secretion, some implicating their direct interaction with SNAREs (8,18,28,41,87,88). Furthermore, actin- and microtubule-based cytoskeleton have been implicated in intracellular vesicle traffic. Fodrin, which was previously implicated in exocytosis, has also been shown to directly interact with SNAREs (87). Studies demonstrate α-fodrin to regulate exocytosis via its interaction with the t-SNARE syntaxin family of proteins, perhaps through interaction with the COOH-terminal region of syntaxin, which is known to interact with α-fodrin. Similarly, vimentin filaments interact with SNAP-23/25 and hence are able to control the availability of free SNAP23/25 for assembly of the t-/v-SNARE complex (41). Collectively, these findings suggested that vimentin, α-fodrin, actin, and SNAREs may be part of the porosome complex. Additional proteins such as v-SNARE, synaptoophysin, and myosin may associate when the porosome establishes continuity with the secretory vesicle membrane. The globular tail domain of myosin V contains a binding site for VAMP, which is bound 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 synaptoophysin and myosin V had also been reported (88). In agreement with these earlier findings, our studies (20,21,51,66) demonstrate the association of SNAP-23, syntaxin 2, cytoskeletal proteins actin, α-fodrin, vimentin, and calcium channels β3 and α1c, together with the SNARE regulatory protein NSF, at the porosome complex (20,21,51,66). 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 porosomes. Using yeast two hybrid confirmed the porosome association with t-SNARE complexes (21).

The size and complexity of porosomes and bilayers in pancreatic porosomes exhibits a characteristic negative stain morphology and native state with functional excocrine pancreatic enzyme secretion, as observed in bilayer vesicle preparations in near physiological conditions. Results from the study of these isolated lipid membrane fragments confirmed the presence of functional (21) vesicle docking and fusion(s), vesicle docking and fusion(s), and vesicle docking and fusion(s). Interactions between the t-SNARE syntaxin and v-SNARE VAMP are functional (21) and may be part of the porosome complex. In addition, studies demonstrated the involvement of cytoskeletal proteins in cell secretion, some implicating their direct interaction with SNAREs (8,18,28,41,87,88). Furthermore, actin- and microtubule-based cytoskeleton have been implicated in intracellular vesicle traffic. Fodrin, which was previously implicated in exocytosis, has also been shown to directly interact with SNAREs (87). Studies demonstrate α-fodrin to regulate exocytosis via its interaction with the t-SNARE syntaxin family of proteins, perhaps through interaction with the COOH-terminal region of syntaxin, which is known to interact with α-fodrin. Similarly, vimentin filaments interact with SNAP-23/25 and hence are able to control the availability of free SNAP23/25 for assembly of the t-/v-SNARE complex (41). Collectively, these findings suggested that vimentin, α-fodrin, actin, and SNAREs may be part of the porosome complex. Additional proteins such as v-SNARE, synaptoophysin, and myosin may associate when the porosome establishes continuity with the secretory vesicle membrane. The globular tail domain of myosin V contains a binding site for VAMP, which is bound 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 synaptoophysin and myosin V had also been reported (88). In agreement with these earlier findings, our studies (20,21,51,66) demonstrate the association of SNAP-23, syntaxin 2, cytoskeletal proteins actin, α-fodrin, vimentin, and calcium channels β3 and α1c, together with the SNARE regulatory protein NSF, at the porosome complex (20,21,51,66). 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 porosomes. Using yeast two hybrid confirmed the porosome association with t-SNARE complexes (21).
vesicle transmission studies confirm the structure of a neuron's porosome complex (44). AFM measurements demonstrate that the complex's intracellular loops intercalate with the membrane bilayer, and may associate with the t-SNARE tail domain (21, 22, 51, 60). The morphology of immunolocalized porosomes obtained using EM and AFM is almost identical and superimposable (51). The immunolocalized porosome complex has also been structurally characterized (FIGURE 1C) and functionally reconstructed into liposomes (21, 22, 51, 60). TEM of pancreatic porosome complex reconstituted into liposomes exhibit a 150- to 200-nm cup-shaped basket-like morphology, similar to what is observed in its native state when co-isolated with ZGs. To test the functionality of isolated porosomes obtained from exocrine pancreas or neurons, they were reconstituted into the lipid membrane of the electrophysiological bilayer apparatus (EPC9) and exposed to isolated ZGs or synaptic vesicles. Electrical activity of the reconstituted membrane was measured following stimulation of cell secretion in live cells and vesicle deflation following partial discharge of vesicular contents. A similar mechanism of synaptic vesicle swelling for neurotransmitter release has also been demonstrated. This direct estimation of vesicle size dynamics at nanometer resolution under various experimental conditions has enabled determination of some of the molecular players in secretory vesicle swelling. To be able to determine the molecular mechanism of vesicle swelling, isolated secretory vesicles have frequently been utilized. Early studies using isolated ZG demonstrated the presence of K+ and Ca2+ selective ion channels in the ZG membrane, whose activities were implicated in ZG swelling (35, 36, 38, 39, 94, 103, 105, 106). These studies further demonstrated that secretion of ZG contents from pancreatic acinar cells requires the presence of both K+ and Ca2+ selective ion channels in a number of tissues (44, 64, 98, 100). Analogous to the regulation of K+ and Ca2+ ion channels at the cell plasma membrane, the regulation of these channels at the ZG membrane by a G protein at the synaptic vesicle membrane (59), and AQP6 and Gq protein at the synaptic vesicle membrane (99), and their involvement in GTP-mediated vesicle water gating and swelling has been demonstrated (11, 59). The secretagog mastoparan (Mast 7) is an amphiphilic tetradecapeptide from wasp venom and is known to potentiate the GTPase activity of Gi/Ga proteins (45, 69, 113). Stimulation of G proteins is believed to occur by the peptide inserting into the phospholipid membrane and forming a highly structured α-helix that resembles the intracellular loops of G-protein-coupled receptors. Analogous to receptor activation, mastoparan is thought to interact with the COOH-terminal domain of the G protein α subunit (112). Active

identified in porosomes of the exocrine pancreas (51). Using yeast two-hybrid analysis, studies further confirmed the presence and interaction of some of these proteins with t-SNAREs within the porosome complex (46). The size and shape of the immunolocalized porosome complex has also been determined using both negative staining EM and AFM studies (21, 22, 51, 60). The morphology of immunolocalized porosomes obtained using EM and AFM is almost identical and superimposable (51). The immunolocalized porosome complex has also been structurally characterized (FIGURE 1C) and functionally reconstructed into liposomes (21, 22, 51, 60). TEM of pancreatic porosome complex reconstituted into liposomes exhibit a 150- to 200-nm cup-shaped basket-like morphology, similar to what is observed in its native state when co-isolated with ZGs. To test the functionality of isolated porosomes obtained from exocrine pancreas or neurons, they were reconstituted into the lipid membrane of the electrophysiological bilayer apparatus (EPC9) and exposed to isolated ZGs or synaptic vesicles. Electrical activity of the reconstituted membrane was measured following stimulation of cell secretion in live cells and vesicle deflation following partial discharge of vesicular contents. A similar mechanism of synaptic vesicle swelling for neurotransmitter release has also been demonstrated. This direct estimation of vesicle size dynamics at nanometer resolution under various experimental conditions has enabled determination of some of the molecular players in secretory vesicle swelling. To be able to determine the molecular mechanism of vesicle swelling, isolated secretory vesicles have frequently been utilized. Early studies using isolated ZG demonstrated the presence of K+ and Ca2+ selective ion channels in the ZG membrane, whose activities were implicated in ZG swelling (35, 36, 38, 39, 94, 103, 105, 106). These studies further demonstrated that secretion of ZG contents from pancreatic acinar cells requires the presence of both K+ and Ca2+ selective ion channels in a number of tissues (44, 64, 98, 100). Analogous to the regulation of K+ and Ca2+ ion channels at the cell plasma membrane, the regulation of these channels at the ZG membrane by a G protein at the synaptic vesicle membrane (59), and AQP6 and Gq protein at the synaptic vesicle membrane (99), and their involvement in GTP-mediated vesicle water gating and swelling has been demonstrated (11, 59). The secretagog mastoparan (Mast 7) is an amphiphilic tetradecapeptide from wasp venom and is known to potentiate the GTPase activity of Gi/Ga proteins (45, 69, 113). Stimulation of G proteins is believed to occur by the peptide inserting into the phospholipid membrane and forming a highly structured α-helix that resembles the intracellular loops of G-protein-coupled receptors. Analogous to receptor activation, mastoparan is thought to interact with the COOH-terminal domain of the G protein α subunit (112). Active
mastoparan (Mas7) in the presence of [γ-32P]GTP demonstrates a significant increase in swelling and GTase 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 a significant increase in swelling and GTase 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).

Furthermore, [γ-32P]GTP demonstrates a significant increase in swelling and GTase 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).

FIGURE 2. Porosome-like structures and other specialized processes in cells: (A) electron micrograph of a freeze-fractured discoid platelet. The organization and intercommunication of open canalicular system (OCS) demonstrating docked and fused secretory vesicles. Several apertures (T) of the OCS open into a communicating web of fenestrated channels that stretches across the cytoplasm to openings (t) at the cell membrane. Elements of the dense tubular system (DTS) are seen in channels of the OCS in the platelet (Reprinted from Ref. 115 with permission from the Rockefeller University Press). (B) electron micrograph revealing the type III secretion system (needle complex) spanning the inner and outer membranes of Salmonella typhimurium. Note the depressions at the insertion point of the needle complex (open arrowhead). This system helps the bacteria inject proteins into host cell (70). Bar = 100 nm. (C) electron micrograph of isolated needle complexes demonstrating the supramolecular structure of this type III protein secretion system of S. typhimurium. Bar = 100 nm (70). D. T. gondii parasite possesses a stable polarized secretory apparatus and a micropore. The secretory apparatus contains several morphologically distinct organelles, namely micronemes, rhoptries (Rh), and dense granules, which are present at the apical end of the parasite. The micropore is continuous with the plasma membrane (PM) of the parasite. The parasite also possesses an inner membrane complex (IMC). Copyright, Joiner and Ross, 2002. Originally published in J Cell Biol 157: 557–563 (63). (E–L): electron micrograph and schematic drawing of specialized structures found in various synapse, used with permission from Physiology (118). E and H are diagrams of the active zone structure in the synaptic terminal electron micrographs shown at right. F: a neuromuscular junction (NMJ) terminal in Drosophila (82) with a dense projection called a T bar (blue arrow). G. a tetrad synapse between photoreceptor and lamina monopolar cells in Drosophila. The T bar consists of a platform (double arrows) and a pedestal (single arrow). H. electronmicroscope in skate (77), with a long ribbon-like dense projection (blue arrow) and a halo of synaptic vesicles (yellow arrow). J. a triadic photoreceptor ribbon synapse between rod photoreceptor and lamina monopolar cells in Skate. I. a tetrad synapse between photoreceptor and lamina monopolar cells in Drosophila. The T bar consists of a platform (double arrows) and a pedestal (single arrow).
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 Ca2+, 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 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 intravesicular contents and the generation of partially empty vesicles. In compound secretion, there is secretion of vesicles fused with each other) should transiently fuse to enable the generation of empty and partially empty vesicles following cell secretion. Similarly, as pointed out in the beginning of this article, specialized structures such as the T bars at the Drosophila synapse (82) or the barbs, ribs, and pegs at the frog neuromuscular junction (42), and the long ribbon-like dense projections in skate and in rat have evolved, each organized to facilitate a certain specialized secretory activity in different cell types (FIGURE 2). The suggestion that these specialized structures merit study as docking sites for the availability of synaptic vesicles for neurotransmitter release at the nerve terminal is over simplification of its function that remains to be fully resolved.

In summary, poresomes are specialized plasma membrane structures universally present in secretory cells, from exocrine and endocrine cells to neurons, and exocrine and neuroendocrine cells and neurons. The fact that poresomes in exocrine and neuroendocrine cells measure 100–150 nm and that there is a 20–35% increase in its diameter following the docking and fusion of secretory vesicles highlights that vesicles transiently dock and fuse at the base of the poresome complex to release their contents. The discovery of the poresome and an understanding of its structure and dynamics at nanometer resolution and in real time in live cells, its composition, and its functional reconstitution in artificial lipid membrane has greatly advanced our understanding of cell secretion. It is evident that the secretory process in cells is a well coordinated, highly regulated, and finely tuned supramolecular orchestra.

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References


Molecular SNAREs and their role in secretion

The synaptic ribbon is a specialized nanoscale structure present in the nerve terminal of cholinergic synapses, which serves to facilitate the release of neurotransmitter vesicles. The synaptic ribbon consists of a circular array of vesicles, which are tethered to the membrane by a complex of proteins known as the SNARE (soluble NSF-attachment protein receptor) complex. The SNARE complex is composed of four distinct proteins:syntaxin, SNAP-25, and vesicle-associated membrane protein 2 (VAMP2), which form a protein complex that mediates the fusion of vesicles with the plasma membrane. The SNARE complex is essential for the release of neurotransmitter vesicles at synapses, and its disruption can lead to neurological disorders. The function of the synaptic ribbon and the SNARE complex have been extensively studied using various techniques, including electron microscopy, atomic force microscopy, and biochemical analysis. The understanding of the molecular mechanisms underlying synaptic function has important implications for the treatment of neurological disorders, such as Alzheimer's disease and Parkinson's disease.