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REVIEW

Secretogranin Ⅲ: a Bridge between Core Hormone Aggregates and the Secretory Granule Membrane

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Abstract. Secretory granules in endocrine cells selectively store bioactive peptide hormones and amines, which are secreted in a regulated manner upon appropriate stimulation. In addition to bioactive substances, various proteins and lipids characteristic of secretory granules are likely recruited to a restricted space at the trans-Golgi Network (TGN), and the space then matures to the secretory granule. Although experimental findings so far have strongly suggested that aggregation- and receptor-mediated processes are essential for the formation of secretory granules, the putative link between these two processes remains to be clarified. Recently, secretogranin III (SgIII) has been identified as a specific binding protein for chromogranin A (CgA), a representative constituent of the core aggregate within secretory granules, and it was later revealed that SgIII can also bind to the cholesterol-rich membrane domain at the TGN. Based on its multifaceted binding properties, SgIII may act as a central player in the formation of cholesterol-rich membrane platforms. Upon these platforms, essential processes for secretory granule biogenesis coordinately occur; that is, selective recruitment of prohormones, processing and modifying of prohormones, and condensation of mature hormones as an aggregate. This review summarizes the findings and theoretical concepts on the issue to date and then focuses on the putative role of SgIII in secretory granule biogenesis in endocrine cells.

Key words: Secretory granule, Secretogranin Ⅲ, Cholesterol, Sorting, Secretory granule formation

SECRETORY GRANULES in endocrine cells are organelles that store bioactive peptides (peptide hormones and neuropeptides) and amines, which are exocytosed to the extracellular space in a calcium-dependent manner upon specific stimulation. Formation of secretory granules is initiated at the trans-Golgi network (TGN), and two processes have been emphasized for the sorting of soluble proteins to secretory granules, including aggregation- and receptor-mediated sorting processes [for reviews, see 1, 2].

The importance of the aggregation-mediated sorting process in secretory granule biogenesis, first proposed by Kelly [3], was evidenced by the experimental findings that granin proteins, such as chromogranin A (CgA), chromogranin B (CgB), and secretogranin II (SgII), could aggregate under conditions mimicking the intra-TGN milieu [4-6]. These aggregates significantly facilitate the condensation of bioactive peptides and amines, leading to the formation of secretory granules [4, 7, 8; for reviews see 9-13].

Even if aggregate formation at the TGN plays an important role in secretory granule formation, alternatively, certain soluble proteins might be recognized by the specific peripheral or transmembrane receptor molecules destined for the secretory granule. Membrane-associated carboxypeptidase E (CPE) was proposed by Loh and colleagues as a candidate for sorting receptor molecule based on the findings that proopiomelanocortin (POMC) is mis-sorted to the constitutive pathway both in CPE-down-regulated Neuro-2a cells and in the pituitary intermediate lobe of mice lacking CPE (Cpe^{fat} mice) [14]. Other transmembrane or peripheral enzymes, such as peptidylglycine alpha-amidating monooxygenase (PAM) and prohormone converting enzymes (PC2, PC1/3), which are associated with cholesterol-rich lipid microdomains, might also carry their own substrates together to the secretory granule [for a review, see 15].

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Although both the aggregation- and the receptor-mediated processes play important roles in the sorting mechanism, secretory granules could not be efficiently formed if the two processes work independently. The aggregates should be anchored to the specific membrane destined for the secretory granule, while the prohormones transported by corresponding receptors/enzymes on the membrane should be transferred to the core when they are stored in a concentrated form. The functional linkage between the aggregate core and the secretory granule membrane remained to be clarified until recently; no appropriate sorting signal motif in the aggregates and corresponding receptor on the secretory granule membrane could be identified [for a review, see 16].

At the beginning of the century, secretogranin III (SgIII) was identified as a specific binding partner protein for CgA [17], and it was later revealed that SgIII could also interact directly with the cholesterol-rich secretory granule membrane and CPE [18,19]. The multifaceted interactions of SgIII with both proteins and lipid(s) destined for secretory granules suggest that SgIII plays a central role in secretory granule biogenesis. In this review, we will first describe the experimental findings and theoretical concepts on secretory granule biogenesis to date. We will then focus on the putative roles of SgIII in the formation of the sorting platform on the cholesterol-rich lipid microdomain destined for the secretory granule.

1. Biosynthetic pathway and diverse destinations after exiting the Golgi apparatus

In endocrine cells, peptide-hormones and neuropeptides, synthesized as larger precursors on the rough endoplasmic reticulum (rER), are immediately translocated to the luminal spaces, where they are properly folded by ER-resident chaperones [for a review, see 20]. After passing the quality control checkpoint, the soluble secretory proteins and lysosomal enzymes are transported to the cis-face of the Golgi apparatus via the ER-Golgi interface compartment (ERGIC), where the leaked ER-resident chaperones are returned back to the ER, based on the specific interaction between the KDEL motif in the chaperones and the corresponding sorting receptor [for a review, see 21].

The soluble proteins transported to the Golgi apparatus are then sequentially modified by the Golgi-resident enzymes and finally reach the TGN. In the TGN, the soluble lysosomal enzymes and secretory proteins are sorted and proceed to at least three distinct destinations, including the late endosomes/lysosomes, secretory granules for regulated secretion, and tubulovesicular carriers mediating constitutive secretion directly to the plasma membrane [for a review, see 22]. Of these three destinations, the molecular basis for the sorting of lysosomal enzymes to late endosomes/lysosomes has been relatively well elucidated. Specifically, mannose 6-phosphate moieties (M6Ps) on the soluble lysosomal hydrolases, which are added by the Golgi-resident enzymes, are recognized at the TGN by the corresponding transmembrane receptors (M6PRs), which transport the hydrolases to late endosomes/lysosomes [for a review, see 23].

The sorting mechanisms of soluble secretory proteins at the TGN remain a central issue for secretory granule formation. Constitutive secretion is regarded as a fundamental secretory process commonly seen in eukaryotic cells, whereas regulated secretion mediated by secretory granules is assumed to be a specialized process occurring only in exocrine and neuroendocrine cells [24]. Based on the findings that the intracellular destination of constitutively secreted proteins fused with regulated secretory proteins can be shifted to secretory granules, secretory proteins stored in the secretory granules must carry some active targeting information to their destination [25, 26].

In addition to soluble cargo proteins, such as bioactive peptides and amines, a wide variety of proteins and lipids selectively come together at the TGN to form the secretory granule. As reported previously, the secretory granules contain granins [for a review, see 27], processing and modifying enzymes (e.g., CPE, PC2, PC1/3, PAM) [28, 29; for reviews, see 9, 30, 31], H^+ V-ATPase [32], phogrin, and IA-2 [33-35]. Molecules regulating membrane traffic and fusion for exocytosis, such as synaptotagmin [36], synaptobrevin-2 [37], Rab3 [38], granuphillin, and Rab27 [39], are also localized or recruited to the cytosolic face of the secretory granule. Although these molecules are specifically sorted or targeted to secretory granules in endocrine cells, the mechanisms for their sorting or retention to secretory granules remain to be elucidated.

2. Key processes for secretory granule biogenesis

2-1 Conditional aggregation of soluble secretory constituents

Secretory granules contain a wide variety of soluble proteins, and aggregation of these proteins is a key step for both sorting and concentrating the granule contents. In particular, self-aggregation of granin proteins at the TGN has been suggested to be crucial for proper and efficient sorting of peptide-hormones and bioactive amines.

Three representative granins, named CgA, CgB, and SgII, were first discovered as major soluble constituents of secretory granules in the adrenal medulla, parathyroid, and pituitary gland. Later, SgIII and 7B2 were included in the family based on their biochemical properties and tissue distribution. Granins are commonly rich in acidic amino acids, exhibit heat stability, possess a high binding capacity for calcium, and are restrictedly localized to secretory granules [for reviews, see 27, 40-42]. Granins self-aggregate at a mildly acidic pH (pH 5-6) and a high calcium concentration (10-40 mM), conditions that mimic the luminal milieu of the TGN [4, 7, 44]. Although the molecular basis of the selectivity is not yet elucidated, bioactive peptides and amines can be efficiently incorporated into the aggregates, whereas constitutively secreted proteins (such as ovalbumin, albumin, IgG, angiotensinogen, and heparan sulfate proteoglycan) are excluded from the aggregates [4, 5, 7, 44]. These findings suggest that aggregate formation is a necessary step in the elaborate sorting mechanisms to secretory granules.

The expression levels of granins in endocrine cells are affected by hormonal treatments. When the GH_4C_1 cells derived from a rat pituitary tumor are treated with a combination of 17-beta-estradiol, insulin, and epidermal growth factor (EGF), the number of the secretory granules and prolactin (PRL) content are prominently increased [45]. Under this inducible condition, expression of CgB and SgII is markedly up-regulated, indicating that the up-regulation of granins possibly correlates with an increased number of secretory granules [46, 47]. In contrast, treatment with 17-beta-estradiol significantly down-regulates the expression of CgA in the pituitary gland [48, 49]. Under the influence of 17-beta-estradiol, CgA-positive secretory granules disappear from the pituitary gonadotropes of castrated male rats, suggesting that the expression of granins ensures the formation of certain type(s) of secretory granules [49].

The putative relationship between the expression levels of granins and secretory granule biogenesis has also been examined using mice and cells lacking expression of a certain granin. When the expression of CgA was genetically ablated [50] or down-regulated by expressing antisense RNA against CgA [51], the storage level of catecholamines in adrenal chromaffin cells was significantly reduced although not depleted. Partial reduction of catecholamine storage was also observed in adrenal chromaffin cells of a CgB-knockout mouse [52]. The intracellular quantity of dense core granules in adrenal chromaffin cells is somewhat controversial; some groups demonstrated a significant reduction of dense core granules [50, 51], whereas another group reported that the dense core granules in the adrenal medulla are normal in both size and number [53]. Judging from the findings that CgA knockout mice exhibited no apparent defects in viability, fertility or neural/endocrine functions except abnormal leakage of catecholamines from the adrenal medulla, a single ablation of CgA or CgB could be largely compensated for in most endocrine tissues/cells by other granins [53]. The increased expression of the other granins in endocrine tissues of CgA- [50, 53, 54] and CgB- [52] knockout mice supports the possibility of compensation.

Although conditional aggregation of granins largely contributes to the formation of the core aggregate in most endocrine cells, endocrine cells that do not require granins to generate secretory granules may also exist. PRL and atrial natriuretic peptide potentially self-aggregate without granins under the appropriate condition [7, 55]. Whether the granins seize the initiative in the aggregation process or not, conditional aggregation of soluble constituents plays a pivotal role in secretory granule biogenesis, especially in the selective condensation of secretory materials.

2-2 Selective accumulation of cholesterol on the secretory granule membrane

In parallel with the selective aggregation of a certain set of soluble secretory proteins, the lipid composition of the corresponding membrane must be converted to one characteristic of the secretory granule membrane.

Regarding the lipid composition, the abundance of cholesterol in TGN membranes and the secretory granules was first demonstrated in the pancreatic exocrine

acinar cells and endocrine beta cells by labeling with filipin, a probe molecule that forms a specific complex with cholesterol recognizable by electron microscopy as a characteristic round deformation of the membrane [56, 57]. These findings have been confirmed by biochemical analyses of the lipid composition of secretory granule membranes isolated from endocrine cells [58]. Recent analyses have estimated the cholesterol content of secretory granule membranes as 65 mol% in the bovine pituitary neural lobe [59] and 37-45 mol% in PC12, AtT-20, and MIN6 cells [60]. When cholesterol biosynthesis was reduced by a HMG-CoA reductase inhibitor (lovastatin) in endocrine cells, the relative number of secretory granules and hormone content were significantly reduced [59, 61], suggesting that cholesterol is an essential component of secretory granules. A similar defect in the secretory granule formation was also observed in various exocrine and endocrine cells of two types of cholesterol-deficient mutant mice (Dhcr7-/- and Sc5d-/- mice lacking 7-dehydrocholesterol reductase and lathosterol-5-desaturase in cholesterol biogenesis pathway), confirming the importance of cholesterol in secretory granule biogenesis [62].

In addition to cholesterol being an essential component of the secretory granule membrane, cholesterol may contribute to the localization of certain processing enzymes and other transmembrane proteins to the secretory granule by forming raft-like lipid microdomains on the membrane. Selective transport of endocrine processing enzymes, such as CPE [59, 63], PC2 [64], and PC1/3 [65, 66], to secretory granules is likely to be dependent on their association with raft-like lipid microdomains. Loh and colleagues assigned a vital role in the sorting of prohormones to secretory granules based on the finding that sorting of POMC to secretory granules is impaired in obese Cpe^{fat} mice lacking CPE [14]. Depletion of CPE in Neuro-2a cells by expressing CPE antisense RNA resulted in the missorting of intrinsic proenkephalin and transfected proinsulin to the constitutive pathway [67]. However, the role of CPE in the sorting of proinsulin remained controversial, because proinsulin could be correctly sorted to the regulated pathway in pancreatic islets isolated from Cpe^{fat} mice [68] and pancreatic beta-cells of Cpe^{fat} mice [69].

Whether CPE functions as a sorting receptor or simply acts as a processing enzyme, the selective accumulation of cholesterol on the membrane destined for the secretory granules contributes to recruiting the raft-associated processing enzymes together with their substrates, prohormones [15, 44]. However, the ability of processing enzymes, including CPE, to bind to their own prohormones is not enough to explain how they can sort remarkably concentrated prohormones to secretory granules, because the binding ratio of enzymes to their substrates is theoretically expected to be 1:1 according to stoichiometry [70]. To efficiently form secretory granules with a dense core aggregate, molecules that can bind to both the core aggregates and the cholesterol-rich membrane domain are required.

2-3 Quest for the sorting motif(s) within granins destined for the secretory granule

Because granins are ubiquitously expressed in diverse endocrine cells, the quest for the definitive motifs within granins specifically interacting with the putative sorting receptor(s) on the membrane has begun.

As a candidate for the putative sorting motif within the granin family proteins, an N-terminal disulfide-bonded loop that is highly conserved both in CgA and in CgB has attracted initial attention. Reductive disruption of disulfide-bonds with dithiothreitol (DTT) resulted in the missorting of CgB to the constitutive secretory pathway in rat pheochromocytoma-derived PC12 cells, whereas the storage of SgII in secretory granules was not affected by DTT treatment [71, 72]. In conflict with these findings, DTT treatment did not affect the sorting of newly synthesized CgB to secretory granules in GH_4C_1 cells or the sorting of proinsulin/insulin in isolated mouse pancreatic islets, suggesting that the influence of reductive disruption of disulfide loops by DTT varies depending on the cell types [73]. The experimental findings on the intracellular trafficking of deletion mutants of CgA and CgB lacking the N-terminal disulfide loop are similarly confusing; mutant CgB lacking the N-terminal disulfide loop was mis-sorted to constitutive secretory vesicles in PC12 cells when endogenous protein synthesis was shut down [74], whereas mutant CgA lacking the N-terminal loop was properly sorted to the regulated secretory pathway in GH_4C_1 cells [75]. Because the addition of the disulfide-bonded loop of CgB to the constitutively secreted protein alpha1-antitrypsin (AT) could shift the localization of AT with CgB loop structure to the secretory granule, the loop structure might contain some targeting information to the secretory granule [26]. In any case, it still remains to be concluded whether and how the conserved disulfide-bonded loop structure contributes to the sorting of CgA and

CgB to secretory granules [76].

Apart from the disulfide-bonded loop structure, analysis of a truncated mutant of CgA indicate that the targeting information of CgA to the secretory granule is located on the N-terminal half of the molecule [77]. Later, an SgIII-binding domain (rat CgA 41-109) that is crucial for the sorting of CgA to the secretory granule was identified in the region [17]. The domain of CgA responsible for the proper sorting to the secretory granules was confirmed by other investigators, although the partner molecule like SgIII could not be identified [78, 79].

3. SgIII: a bridge between the core aggregate and cholesterol-rich microdomain

Although it was first identified as a product of the mouse 1B1075 gene that is abundantly expressed in the brain, SgIII is now regarded as a member of the granin family proteins because of its hydrophilic acidic amino acid composition and characteristic localization to the secretory granule in endocrine tissues [17, 19, 80-82]. Because mice missing the 1B1075 gene exhibited no apparent defects in viability, fertility, or locomotor behavior [83], functional analyses of SgIII were relatively overlooked in 1990s. Only Martens and colleagues reported that the mRNA levels of both POMC, a precursor of MSH, and SgIII in the Xenopus pituitary intermediate lobe were coordinately elevated when Xenopus was placed on a black background from a white background, suggesting the involvement of SgIII in the regulation of secretory processes [84].

At the beginning of the century, SgIII was rediscovered as a specific binding partner for CgA by screening a rat brain cDNA library using a yeast two-hybrid system [17]. SgIII strongly binds to CgA at pH 5.5 and 10 mM Ca^{2+}, mimicking the intragranular milieu, and deletion of the SgIII-binding domain found in the middle region of the CgA molecule results in the missorting of CgA to the constitutive pathway in AtT-20 cells, indicating that SgIII functions as a sorting/retention receptor for CgA to the secretory granule [17]. Although SgIII is essential for the sorting of CgA to the secretory granule, the agglomeration rate of CgA also influences transport of CgA and prohormones to the secretory granules. For example, in PC12 cells, in which the expression level of SgIII is relatively low, the aggregated form of CgA molecules, which includes other soluble peptides, such as adrenomedullin, could be recognized by a small amount of SgIII (the molar binding ratio of 11 (CgA) to 1 (SgIII) at the maximum) and transported efficiently to the secretory granule [8].

In contrast to the dependence of CgA on SgIII for proper sorting to the secretory granule, deletion of the CgA-binding domain from the SgIII molecule does not affect the intracellular fate of the SgIII; the deleted form of the SgIII expressed in AtT-20 cells could be transported to the secretory granule [17]. Within some endocrine cells, SgIII is preferentially localized to the periphery of the secretory granules despite lacking the transmembrane region [81, 82]. Biochemical analyses demonstrated that SgIII directly binds to the liposome mimicking the secretory granule membrane in a cholesterol-dependent manner, and binding of SgIII and CgA to the secretory granule membrane purified from AtT-20 cells was drastically impaired after treatment with methyl-β-cyclodextrin to deplete cholesterol from the membrane [18]. The SgIII domain responsible for binding to the secretory granule membrane was further identified to be in the N-terminal region because deletion of this domain resulted both in a dissociation of SgIII and CgA from the secretory granule membrane and in a shift from regulated to constitutive secretion of SgIII in AtT-20 cells [18].

In addition to bimodally binding to CgA and cholesterol-rich membrane, SgIII also binds to CPE [19], which is assumed to be a putative sorting/retention receptor for the secretory granule [for a review see 85]. Both CPE and SgIII tightly bind to the secretory granule membrane in a cholesterol-dependent manner and are localized similarly to the periphery of the secretory granule in pituitary endocrine cells. Although these proteins can laterally associate with each other on the secretory granule membrane, transport of SgIII to secretory granules is not impaired in pituitary endocrine cells of mutant mice lacking CPE [19]. These findings suggest that SgIII could be sorted to the secretory granule independently of CPE, even if the biochemical interaction between these two cholesterol-associated proteins increases the efficiency of transport of soluble secretory proteins to the secretory granule.

In summary, the SgIII molecule consists of at least the following three functional regions: a cholesterol-binding domain located on the N-terminal side of SgIII (rat SgIII 23-186), a CgA-binding domain in the middle region of SgIII (SgIII 214-373), and a domain responsible for the binding to CPE on the C-terminal side of SgIII (SgIII 374-471) (Fig. 1). By utilizing these domains, SgIII could function as a molecular

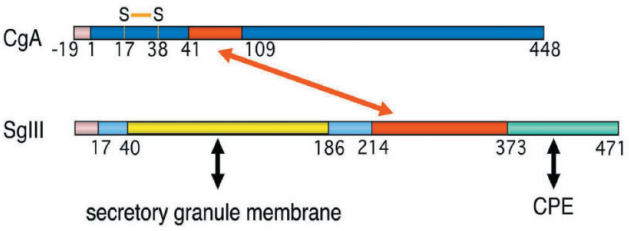


Fig. 1. Schematic representation of SgIII (lower) and CgA (upper) molecules.
The rat SgIII molecule (471 aa) contains at least three functional domains, including a cholesterol-binding domain (SgIII 23-186), a CgA-binding domain (SgIII 214-373), and a CPE-binding domain (SgIII 374-471). The CgA-binding domain of SgIII is specifically associated with an SgIII-binding domain within the rat CgA molecule (CgA 41-109).

bridge between the core aggregate and the cholesterol-rich secretory granule membrane, as discussed in the next section.

4. Theoretical models for secretory granule biogenesis

Since the aggregation-mediated process was first formulated by Kelly [3], self-aggregation of granins and other hormones/prohormones in the luminal milieu at the TGN have provided experimental evidence supporting the concept [for a review, see 11]. However, aggregation alone was not sufficient to explain how the aggregates could be properly retained in secretory granules. Therefore, some membrane-associated proteins, such as CPE, have been putatively assigned to a multi-potential sorting receptor for soluble secretory proteins, analogous to the KDEL receptor for retrograde transport to the rER and the M6P receptor for the endosomal/lysosomal compartment [1].

Based on accumulated findings, the sorting mechanism for secretory granules was classified into two hypothetical models, the "sorting for entry" and "sorting by retention" models [10, 86]. In the "sorting for entry" model, receptor-mediated recognition of the putative ligands, such as prohormones and granins, at the TGN is regarded as a key process for sorting. In contrast, the "sorting by retention" model emphasizes conditional aggregation of regulated secretory proteins at the TGN as well as subsequent retrieval of lysosomal enzymes and constitutively secreted proteins from the immature secretory granule. While distinct, these two models are not mutually exclusive. Proteins transported by the putative sorting receptor(s) must be condensed within the granule for efficient storage, while the aggregates must be anchored to the secretory granule membrane to be stably retained [16].

In addition to soluble proteins and bioactive amines, various membrane-associated proteins and lipids of the characteristic composition join to the secretory granule to ensure proper function [87]. To explain the localization of these functional molecules and the soluble cargo molecules to the secretory granule, a more appropriate model for sorting has been proposed by Dikeakos and Reudelhuber [15]. In their model, transmembrane and peripheral proteins associated with raft-like lipid microdomains converge on a restricted area at the TGN, and each protein brings their binding partners, such as prohormones. As hypothetically proposed by Pike [88], diverse raft-like lipid microdomains repeatedly converge and diverge, exchanging microdomain compositions with each other until a set of lipids and proteins is established as a united membrane patch bearing a specialized function. The proteins and lipids recruited to the restricted area at the TGN may form a functional platform on which the prohormones are easily transferred to other enzymes and further processed because of their proximity. If the diverse processing enzymes or sorting receptors were to carry the cargo molecules in a less specific manner, then this model ensures redundancy in the receptor-mediated sorting processes. For example, if a single enzyme or receptor is defected, then other intact enzymes/receptors may compensate to facilitate the overall transport of the secretory cargo molecules to the secretory granule. The experimental findings that secretory granules are not depleted in most endocrine cells of CPE- or granin-deficient mice [19, 50, 51, 53] are consistent with this hypothetical model.

Although the hypothetical model for selective recruitment of secretory granule contents on the specialized membrane patch at the TGN is sophisticated, the link between receptor- and aggregation- mediated processes is still required to elucidate the entire mechanism of secretory granule biogenesis. Taken together with the multifaceted binding capacity of SgIII, the model discussed above will be updated, as described in Fig. 2. First, micro-aggregates including CgA and prohormones are formed in the milieu at the TGN, and SgIII binds to the surface of the micro-aggregates through specific protein-protein interactions. Then, the SgIII tethers the micro-aggregates to the cholester-

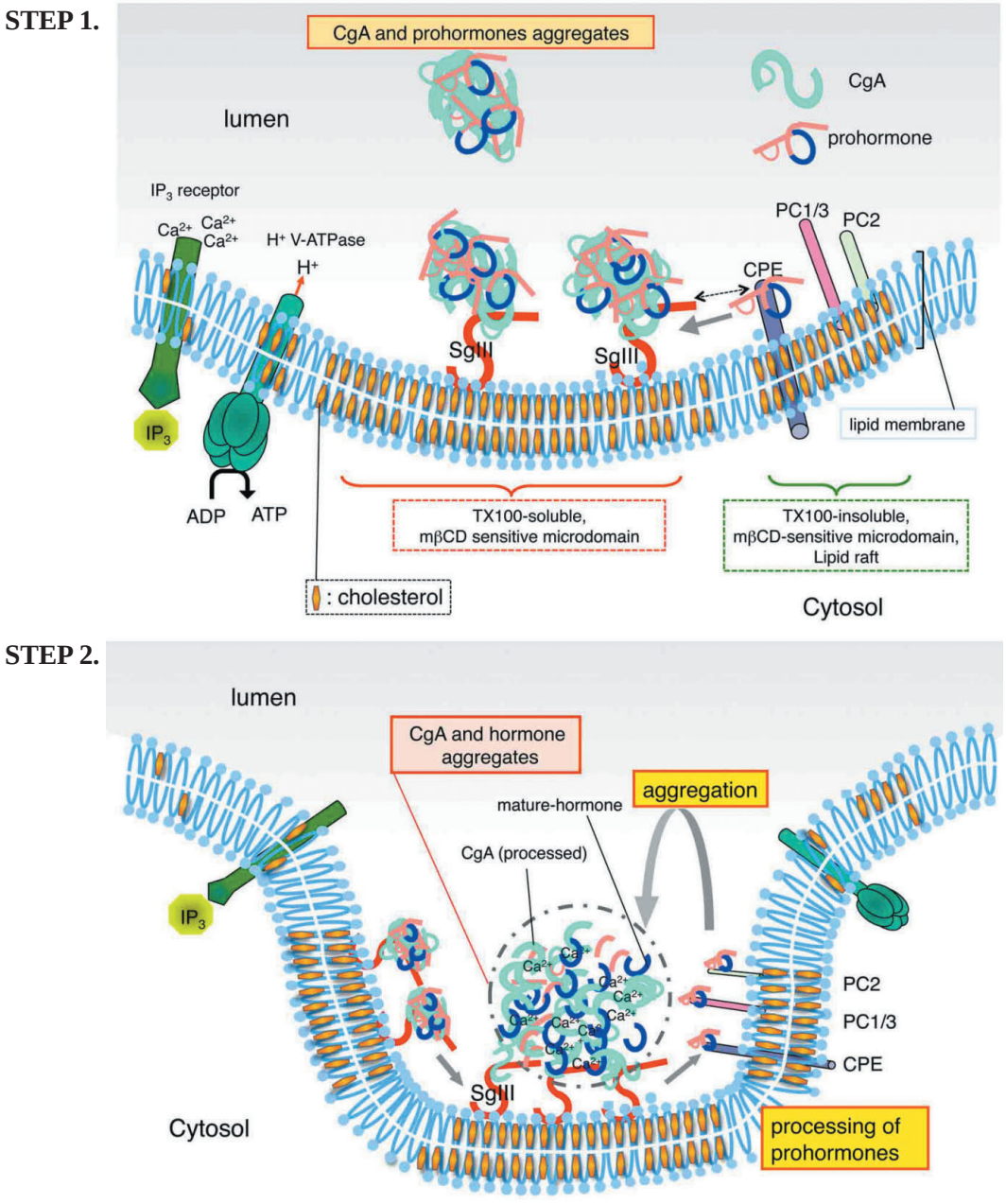


Fig.2. Key steps for prohormone sorting mediated by SgIII and high cholesterol-membrane.

STEP 1. Micro-aggregates, including prohormones and CgA (putatively small in size and less concentrated), can be tethered to a cholesterol-rich membrane domain at the TGN or the immature secretory granule with SgIII, which can bind to both CgA and cholesterol via its CgA- (SgIII 214-373) and cholesterol- (SgIII 23-186) binding domains, respectively. In parallel, processing enzymes (CPE, PC1/3, PC2, etc.) are also recruited to the raft-like microdomains. These cholesterol-rich domains easily join each other to form a membrane platform, on which the processing enzymes and a large amount of prohormones in the micro-aggregate are closely gathered. The specific interaction between SgIII and CPE possibly helps to strengthen this assembly of the membrane domains.

STEP 2. On the cholesterol-rich membrane platform, prohormones in the premature aggregates are transferred to the neighboring processing enzymes and then processed to small fragments. In turn, processed mature hormones are incorporated into the neighboring premature aggregates, including CgA, tethered to the membrane domain with SgIII. Depending on the cell type, CgA and SgIII might also be processed by the enzymes and the resulting fragments incorporated into the aggregate. As the processed mature hormones and granin fragments are incorporated, the aggregate matures to be larger in size and concentrated with hormones.

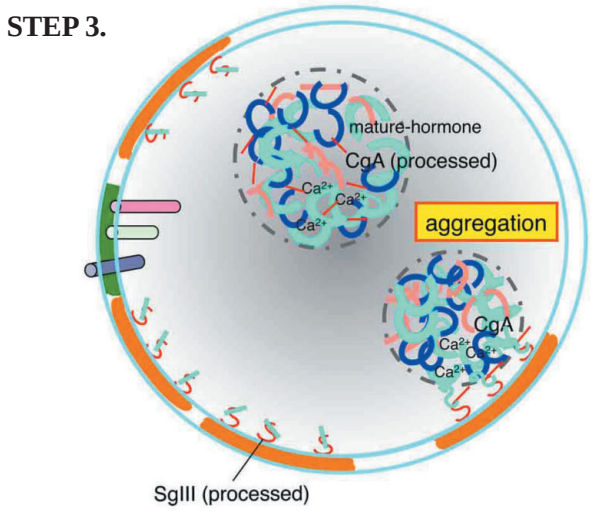


Fig.2. Key steps for prohormone sorting mediated by SgIII and high cholesterol-membrane.
STEP 3. After the secretory granule is established as an independent compartment, mature hormones and granin fragments move from the periphery to the inside of the secretory granules where they are further concentrated to form a dense core aggregate. Within the mature aggregate, hormones are ready to be released upon a membrane fusion event.

ol-rich membrane via its N-terminal cholesterol-binding domain (Fig. 2, STEP 1). In parallel, raft-like lipid microdomains with processing enzymes converge on the cholesterol-rich membrane, forming a functional platform for sorting and processing of prohormones. Once the functional platform is established, the prohormones are easily transferred from the premature aggregate to the neighboring processing enzymes. After being processed by the enzymes, the mature hormones are, in turn, transferred back and incorporated into the neighboring aggregates tethered to the cholesterol-rich membrane platform (Fig. 2, STEP 2). As more processed hormones are incorporated, the aggregate matures in size and density. After the secretory granule is established as an independent compartment, mature peptide hormones and other processed fragments of secretory proteins are further concentrated inside the granule, forming a dense core aggregate (Fig. 2, STEP 3). Within the mature aggregate, peptide hormones are ready to be released upon a membrane fusion event.

5. Concluding remarks and perspective

Although both receptor- and aggregation-mediated processes are essential for secretory granule biogenesis, the link between the sorting/processing platform on the cholesterol-rich membrane patch and the core aggregate located inside of the secretory granule remained unidentified. SgIII, a granin protein with multifaceted binding properties, functions as a molecular bridge linking these two essential processes and allowing them to work cooperatively. Currently, CgA is thought to be a unique binding partner for SgIII within the aggregate. However, other soluble binding cargo proteins that specifically bind to SgIII may be identified in the future, because SgIII is more ubiquitously expressed in the neuroendocrine cells than CgA [80-83].

In addition to the mechanism mediated by SgIII, similar mechanisms connecting aggregates of soluble cargo molecules with the sorting/processing platform on the membrane likely exist. As we previously demonstrated, two distinct subsets of secretory granules, CgA-positive and SgII-positive granules, co-exist within the pituitary gonadotrope [17, 49, 89]. These findings indicate the existence of an additional sorting mechanism for SgII localized specifically to one subset of secretory granules, at least within gonadotropes. Alternative mechanism(s) are also required to explain the redundancy in secretory granule biogenesis described above.

The abundance of cholesterol in the secretory granule membrane, which is required for SgIII to bind to the membrane [18], is essential for both formation of the secretory granule membrane and selective transport of certain sets of peripheral processing enzymes with their substrates. A cholesterol gradient is formed from the endoplasmic reticulum, where cholesterol is

synthesized, to the plasma membrane by way of the Golgi apparatus [90, 91], but it is unclear how cholesterol is selectively accumulated to the membrane patch destined for the secretory granule. One possible mechanism is that development of the aggregate(s) of soluble cargo molecules facilitates accumulation of cholesterol in the restricted area of the membrane at the TGN through the physical connection between CgA and cholesterol mediated by SgIII. As the aggregate become larger and more concentrated, the surface density of the SgIII bound to CgA in the aggregate increases, resulting in a high density of cholesterol in the corresponding membrane patch. This possibility should be addressed in the future.

Recently, according to genetic analysis on SgIII, it has been reported that two single-nucleotide polymorphisms affect the transcription activity of the SgIII gene in obese patients [92]. In their study, expression of SgIII in appetite-related neuropeptide-producing regions of the hypothalamus was demonstrated. Although one report showed that mice lacking the SgIII gene exhibited no major defects in viability, fertility, or locomotor behavior [83], the influence of ablation or mutation of the SgIII gene should be further considered in endocrine cells and patients.

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